

4/9/15 Preliminary Comments for review and deliberations by the CAAC Committee Augmented for the Review of EPA’s Draft IRIS Benzo[a]pyrene Assessment. Do Not Cite or Quote. These preliminary comments are draft and a work in progress. They do not reflect consensus advice or recommendations, have not been reviewed or approved by the chartered SAB and do not represent EPA policy.

**Preliminary Comments from Members of the Chemical Assessment Advisory Committee  
Augmented for the Review of the EPA’s Draft IRIS Benzo[a]pyrene Assessment  
(September, 2014)**

**Comments Received as of April 9, 2015 (Corrected Version)**

**Table of Contents**

Dr. Scott Bartell.....	2
Dr. Ronald Baynes.....	5
Dr. Annette Bunge.....	9
Dr. Scott Burchiel.....	21
Dr. Anna Choi.....	23
Dr. Joanne English.....	28
Dr. Michael Foster.....	35
Dr. Helen Goeden.....	39
Dr. Sean Hays.....	47
Dr. Ed Levin.....	50
Dr. Maureen Lichveld.....	53
Dr. Barry McIntyre.....	54
Dr. Bhagavatula Moorthy.....	60
Dr. Miriam Poirier.....	64
Dr. Kenneth Portier.....	72
Dr. Steven Roberts.....	79
Dr. Richard Schlesinger.....	82
Dr. Leslie Stayner.....	85
Dr. Alan Stern.....	98
Dr. Charles Vorhees.....	91
Cr. Christi Walter.....	99

## **Dr. Scott Bartell**

### **1. Literature search/study selection and Evaluation.**

*The process for identifying and selecting pertinent studies for consideration in developing the assessment is detailed in the Literature Search Strategy/Study Selection and Evaluation section. Please comment on whether the literature search approach, screening, evaluation, and selection of studies for inclusion in the assessment are clearly described and supported. Please comment on whether EPA has clearly identified the criteria (e.g. study quality, risk of bias) used for selection of studies to review and for the selection of key studies to include in the assessment. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of benzo[a]pyrene*

The criteria are clearly identified, and I am not aware of any additional studies that should be considered.

**2a. Developmental toxicity** (sections 1.1.1, 1.2.1). *The draft assessment concludes that developmental toxicity and developmental neurotoxicity are human hazards of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?*

Yes, but findings from the epidemiologic studies are only weakly (if at all) informative because they involved unmeasured exposures to mixtures of known developmental toxicants. It is not clear that the adverse health outcomes in these epidemiologic studies were due to benzo[a]pyrene exposure per se. Some of the wording in the assessment is too strong, e.g., "susceptibility to benzo(a)pyrene toxicity is indicated by epidemiological studies" on p. 1-20.

**3a. Oral reference dose for effects other than cancer** (section 2.1). *The draft assessment proposes an overall reference dose of  $3 \times 10^{-4}$  mg/kg-d based on developmental toxicity during a critical window of development. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.1.5) reflect the scientific considerations that are inherent for exposures during a critical window of development?*

This RfD value is mostly well supported, but further clarification is needed regarding the use of NOAELs/LOAELs for the IgM and IgA endpoints from De Jong et al., 1999. The assessment states that the data for these endpoints were "inconsistent and non-amenable to dose-response modeling," but it is not clear what aspects of the data were inconsistent and whether those inconsistencies also cast doubt on the use of the data for deriving NOAELs/LOAELs. In addition, is it unclear why the data were non amenable to dose-response modeling. Was that

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because of the inconsistencies, poor lack of fit, lack of convergence of the fitting algorithm, or some other reason?

**3b. Inhalation reference concentration for effects other than cancer** (section 2.2). *The draft assessment proposes an overall reference concentration of  $2 \times 10^{-6}$  mg/m<sup>3</sup> based on decreased fetal survival during a critical window of development. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.2.5) reflect the scientific considerations that are inherent for exposures during a critical window of development?*

The RfC is mostly well supported, but the reason for avoiding dose-response modeling is entirely unclear. "Not amenable to BMD modeling due to the pattern of variability in the data set" (p. 2-17, lines 15-17) is vague and insufficient justification.

**3c. Oral slope factor for cancer** (section 2.3). *The draft assessment proposes an oral slope factor of 1 per mg/kg-d based on alimentary tract tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?*

Although consistent with previous methods used by EPA, choosing the maximum slope factor from the available studies is not the most reliable metric for characterizing carcinogenic potency; a mean or median would better capture the totality of evidence. The oral slope factor is otherwise well supported.

**3d. Inhalation unit risk for cancer** (section 2.4). *The draft assessment proposes an inhalation unit risk of 0.6 per mg/m<sup>3</sup> based on a combination of several types of benign and malignant tumors in hamsters. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?*

Although consistent with previous methods used by EPA, choosing the maximum slope factor from the available studies is not the most reliable metric for characterizing carcinogenic potency; a mean or median would better capture the totality of evidence. The oral slope factor is otherwise well supported.

**3e. Dermal slope factor for cancer** (section 2.5). *The draft assessment proposes a dermal slope factor of 0.006 per ug/day based on skin tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and scaling from mice to humans? Does the method for cross-species scaling (section 2.5.4 and appendix E) reflect the appropriate scientific considerations?*

Yes, this value is well supported reflecting appropriate scientific considerations.

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## **OTHER COMMENTS**

p. 1-1, line 20, "Two?" should probably be "Two."

p. 1-1, lines 25-29 are unclear. What exactly is meant by "independent effect on birth weight was not observed" and how does that differ from the 8% reduction in birth weight with ETS exposure in utero? The assessment should make it clear that this is a WTC-related exposure, and that the 8% reduction in birth weight was per doubling of adducts.

Table 1-1, no mention of potential confounding by other WTC-related exposures?

p. 1-36, lines 19-20 apply to the epidemiologic evidence for all health outcomes--all of the epi studies appear to use benzo(a)pyrene as a surrogate for exposure to complex PAH mixtures.

p. 1-47, lines 4-5 give the false impression that other epidemiologic cohorts described in this review use higher quality study designs, but many of the other cited studies seem to use one biomarker measurement per person, e.g. BaP-DNA adducts with a half-life of about 3-4 months. In such cases a statistical exposure model based on good occupational records may actually result in more accurate exposure assignment than a single exposure biomarker.

p. 1-52, lines 26-38, several of the CIs reported in this section (for Gibbs, 2007, Hammond 1977, and Pukkala 1995) are asymmetric on the log scale, which is unlikely. Double check for accuracy.

p. 1-82, lines 15-17, "the exposure-response patterns seen with benzo(a)pyrene measures make it unlikely that these results represent confounding by other exposures." Existence of a dose-response pattern does not at all constitute evidence against confounding. If this is not a misstatement then EPA should explain the argument behind this claim very carefully. Otherwise it suggests a grave misunderstanding of confounding in observational studies, which can induce strong dose-response patterns with non-causal agents (when correlated with an unmeasured causal agent).

p. 2-6, check on whether correlation of rats from same litter was accounted for in the dose-response modeling

p. 2-6, lines 16-17, kudos to EPA for requesting raw data (here and in other parts of the assessment).

p. 2-13, line 18, change "a statistically significance" to "statistical significance"

Table 2-3, why "low" confidence in immunological toxicity RfD versus "medium" for other effects?

p. 2-32, why were Brune study and Neal and Rigdon study not used to produce slope factors in this assessment?

**Dr. Ronald Baynes**

### **Question #1. Literature Search Strategy / Study Selection**

This review appears to have implemented a comprehensive search strategy to identify studies that are relevant to our understanding of benzo[a]pyrene toxicology using various approaches that involved *in silico* and *in vitro* methods as well as human and animal *in vivo* exposures. There are however, several references listed below that could have been added to this review to support their dermal assessment. This review excluded studies describing the use of therapeutic products containing BaP. While all of these products are formulations/mixtures containing BaP, it would have been useful to tabulate or provide an estimate of human exposure to BaP in these products; providing this information will be consistent with the current data in this Toxicology Review describing the adverse effects in human occupational exposure to BaP mixtures although the relative contributions of BaP and of other PAHs cannot be established.

### **Question #3e. Dermal Slope Factor**

#### **2.5.1. Analysis of Carcinogenicity Data (Choice of studies)**

##### ***Principal Study***

The Sivak et al (1997) appears to be the appropriate principal study. Three dose levels (0.0001, 0.001, 0.01%) and controls were evaluated with BaP in cyclohexane/acetone (1:1) were administered twice a week for 104 weeks. A multi-stage models were used to fit the mouse data; a POD for mouse data was 0.06 µg/day and the slope factor was 1.7 per µg/day for mouse. The mouse POD was scaled to a human POD and dermal slope factor was 0.006 per µg/day. There may be some issues with this approach which are discussed below.

##### ***Supporting Studies.***

This toxicology review documents at least 10 carcinogenicity bioassay studies from 1959 to 1997 where they demonstrated a dose-response relationship and the document adequately presented why they were not selected as the principal study. Mice of various strains were the dominant species evaluated and various vehicles (e.g., acetone, toluene) were used as topical delivery of BaP. Mice appear to be the most sensitive species and should not be interpreted as a limitation of this dose response assessment. The slope factors ranged from 0.25 to 1.8 per µg/day compared to the 1.7 per µg/day for the principal study. These studies were not chosen for several good reasons such as limited dose response points, higher exposure levels, significant low-dose extrapolation, and incomplete exposure information.

#### **2.5.4. Dermal Slope Factor Cross-Species Scaling**

According to EPA guidance, if the fraction of the agent is absorbed from the diet for humans and animals differs, the U.S. EPA applies a correction when extrapolating the animal-derived value to humans. This needs to be taken into consideration when assessing dermal absorption especially when extrapolating from mice skin to human skin uptake.

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Modeling of mouse tumor data generated a POD of 0.06 µg/day and a POD<sub>HED</sub> of 17 µg/day. An interspecies scaling approach used  $\frac{3}{4}$  power of body weight that was claimed to account for the more rapid distribution, metabolism and clearance in mice compared to humans. For many drugs and chemicals, absorption and penetration is significantly greater in mice skin than human skin. This approach assumes that mouse skin disposition can be scaled to an equivalent human skin disposition. The literature has sufficient human and mouse skin BaP absorption data (Kp, flux, etc) and metabolism data that could have been used to arrive at the human equivalent POD. This would have been more physiologically relevant than scaling according to BW ratios. Furthermore, mice epidermal thickness is 0.0104 mm vs 0.052 mm for humans which provides an epidermal thickness factor of 0.2 (Knafla et al., 2011).

The Knafla et al (2011) paper also proposed a skin cancer slope factor 3.5 per µg/cm<sup>2</sup> -day derived on a per unit skin surface area that can be used to estimate risks as a function of exposed surface area. The review is correct in stating that exposure to 0.01 µg/day over 10 cm<sup>2</sup> or 19,000 cm<sup>2</sup> could result in risk of a tumor; however, increased surface area can result in increased chemical flux and increased dose to target sites in skin.

For interspecies scaling of the oral slope factor, a different approach was used; TWAs were used here and not mentioned in the skin slope factor adjustment.

#### **2.5.5. Uncertainties in the Derivation of the Dermal Slope factor**

The review adequately addresses the uncertainty associated with the principal study and the supporting studies. The seven-fold difference in PODs is not alarming especially as they span 10 independent studies across male and female mice of at least 6 strains. Several of the concerns with the data sets such as low-dose extrapolation adds to the uncertainty in computation of slope factors from some studies. The solvent mixture (cyclohexane/acetone) used in the principal study does not reflect the real world dermal exposure for BaP, but it does provide an optimal exposure scenario. There is also some uncertainty in the extrapolation from animal skin exposure to human exposure. The statement on page 2-46, line 13-14, is not accurate; the toxicokinetics and toxicodynamics in mouse skin and human skin are not similar. Dermal absorption data in the literature demonstrated that there is an almost a 2-3 fold difference. Appendix D (page D-3) does not present a complete picture of BaP skin absorption in spite of the many publications available. For example, Ng et al (1992) and Sanders et al (1986) could be useful. Metabolism has also been shown to occur in basal cells which are located in the epidermis and not the dermis.

#### **Question #5. Charge Question on Public Comments**

**Public Comment:** *“Scientifically inappropriate to base human health risk assessment of hundreds of differing complex mixtures on the basis of one PAH, BaP.....” from Brian Magee (ACCCI, AFPM, AI, AAR, etc).”*

**Response:** The principal study for the slope factor determination (Sivak et al., 1997) did evaluate “real world” mixtures (fractions of asphalt roofing fumes) with BaP but it also evaluated BaP topically applied in a simple binary solvent system. There was a dose response with fume fractions with BaP concentrations. This could have been explained in the review to address this concern.

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**Public Comment:** *“EPA review omitted entire literature on coal tar pharmaceutical products.”*  
.... *“..there is little evidence that humans are at risk of developing skin cancer following dermal exposure to BaP”* . *“persuasive studies have grafted human skin onto mouse backs than then dosed with BaP...and these studies have repeatedly shown that the functioning human skin does not develop skin cancer as does the mouse skin beyond the margins of the graph”*: from *Brian Magee (ACCCI, AFPM, AI, AAR, etc)* . *“Pharmaceutical uses – was given short shift...failing to identify literature on exposures not associated with adverse effects”* from *Ann LeHurray, Pavement Coatings Technology Council.....*

Response: This was adequately answered in the review, For example: “Acute studies of coal tar treated patients provide in vivo evidence of benzo[a]pyrene-specific genotoxicity (increased BPDE-23 DNA adducts) in human skin (Godschalk et al., 2001; Rojas et al., 2001; Zhang et al., 1990), an early 24 key event in the carcinogenic mode of action of benzo[a]pyrene (see Figure 1-6 of Section 1.1.5).”

However, the use of human-mouse skin xenographs in the review should be addressed. The review needs to address the weakness in these xenograph applications such as lack of positive controls, mouse life expectancy is 2 yrs and humans is 70 years, etc.

The presence of formulation additives in various pharmaceutical formulations (e.g., surfactant) are not often associated with increased dermal absorption but can more likely retain the chemical on the skin surface than cause epidermal penetration thereby limiting the effective dose to cause tumors.

Taken together, epidemiological studies that probe the link between skin cancer and topical exposure to pharmaceutical coal tar provided mixed results. In almost all cases the dose, duration and the appearance of skin tumor are not reported. The discovery of 50 mg/kg benzo[a]pyrene in cosmetic hair shampoos in Germany led the German government to ban coal tar products in 1992. Subsequently, German cosmetic manufacturers removed coal tar from their products. In 1997, the European Union placed refined coal tars on the list of substances that must be excluded from cosmetic products. There are epidemiological studies, also supported by anecdotal reports, that coal tar pharmaceutical can cause skin cancer.

- Sarto F, Zordan M, Tomanin R, et al. Chromosomal alterations in peripheral blood lymphocytes, urinary mutagenicity and excretion of polycyclic aromatic hydrocarbons in six psoriatic patients undergoing coal tar therapy. *Carcinogenesis*. 1989;10:329-334.
- Saperstein MD, Wheeler LA. Mutagenicity of coal tar preparations used in the treatment of psoriasis. *Toxicol Lett*. 1979;3:325-329.

A more recent 2015 study found no increase in skin or bladder cancer:

- Roelofzen JH, Aben KK, Van de Kerkhof PC, Van der Valk PG, Kiemeneij LA. [Dermatological exposure to coal tar and bladder cancer risk: a case-control study](#). *Urol Oncol*. 2015 Jan;33(1):20.e19-22. doi: 10.1016/j.urolonc.2013.12.006. Epub 2014 Mar 12.

FDA re-reviewed Coal Tar in 2001 in response to a citizens' petition. This review, including more recent epidemiology studies, confirmed Coal Tar as a Category I (safe and effective) OTC drug ingredient (FDA 2001a; 2001b). The International Cosmetic Ingredient Dictionary and Handbook (Gottschalck and McEwen 2004) gives a function of Coal Tar in cosmetics as an antidandruff

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agent, industry reports of Coal Tar use in cosmetics are actually in OTC preparations at concentrations from 0.06% to 7% (CTFA 2002). There needs to be an estimate of what fraction of the 0.06-7% coal tar is BaP and furthermore, from that fraction, what proportion of BaP in that cosmetic formulation is available for transdermal diffusion. It is very likely that that fraction is very small and not of significance

**Public Comment:** *“the true and relevant dose of BaP in the skin is cumulative and increases over time. Erroneously using daily dose to derive a DSF results in meaningless DSF that is artificially high”*

**Response:** Human skin is a living membrane and BaP is transported by passive diffusion and not by membrane transporters which could be a reason for “depot” formation. In essence, a multiple dose regimen as often with other routes of exposure will result in steady state pharmacokinetics.

**Public Comment:** *USEPA (2013) needs to clearly state that any DSF that it may finalize in the future is focused on dermally absorbed dose and not applied dose. The studies used as the basis for the proposed DSF used soluble BaP in solvents that ensured that the BaP was completely absorbed into the skin. Real world exposures to BaP and other potentially carcinogenic PAHs are to complex mixtures and matrices that would impede the dermal absorption of the BaP.*

**Response:** This is a plausible observation. However, this will have to be applied to other routes of exposure where there is uncertainty in the dosimetry. However, the basis for the assessment is protection of the more sensitive population and scenarios/formulations/mixtures that will deliver BaP to the effector site.

**Public Comment:** *“Study Selection. Despite the weight of evidence that humans are not sensitive to chemically induced skin tumorigenesis as is the mouse skin and that PAHs build up in mouse skin after repeated dose administrations, USEPA (2013) has reviewed the mouse skin literature and chosen ten published papers as Key Studies. They exclude several studies by an arbitrary criterion: Study Duration. The excluded studies include:*

- *Levin et al. (1977)*
- *Nesnow et al. (1983)*

**Response:** EPA did not consider these studies because of a 1-time/week (Nesnow et al 1977) or 1-time every 2 weeks. This needs to be explained as the principal study (Sivak et al 1997) dosed twice weekly. (note application to shaved skin is not the same as clipped hair skin; the former increases permeability).

## Dr. Annette Bunge

### 1. Literature search/study selection and evaluation.

The process for identifying and selecting pertinent studies for consideration in developing the assessment is detailed in the *Literature Search Strategy/Study Selection and Evaluation* section. Please comment on whether the literature search approach, screening, evaluation, and selection of studies for inclusion in the assessment are clearly described and supported. Please comment on whether EPA has clearly identified the criteria (e.g. study quality, risk of bias) used for selection of studies to review and for the selection of key studies to include in the assessment. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of benzo[a]pyrene.

The following paper presents results related to vehicle effects and DNA adduct formation at remote sites following dermal exposure.

Booth, E.D., Loose, R.W., Watson, W.P. (1999). Effects of Solvent on DNA Adduct Formation in Skin and Lung of Cd1 Mice Exposed Cutaneously to Benzo(a)Pyrene. *Arch. Toxicol.* 73, 316-322.

The following papers include results of dermal absorption from BaP contaminated soils.

Abdel-Rahman, M. S.; Skowronski, G. A.; Turkall, R. M. Assessment of the Dermal Bioavailability of Soil-Aged Benzo(a)Pyrene. *Hum. Ecol. Risk Assess.* 2002, 8, 429-441.

Yang, J. J.; Roy, T. A.; Krueger, A. J.; Neil, W.; Mackerer, C. R. Percutaneous Absorption of Benzo(a)Pyrene from Soils with and without Petroleum Crude Contamination. In *Petroleum Contaminated Soils*; Calabrese, E. J., Kostecki, P. T., Eds.; Lewis Publishers: Chelsea, MI, 1989; Vol. 2, pp 399-407.

Stroo, H. E.; Roy, T. A.; Liban, C. B.; Kreitinger, J. P. Dermal Bioavailability of Benzo[a]Pyrene on Lampblack: Implications for Risk Assessment. *Environ Toxicol Chem* 2005, 24, 1568-1572.

The following paper provides a critical review of data quality and compares results from several studies of BaP absorption from contaminated soil studies

Spalt, E.W., Kissel, J.C., Shirai, J.H., Bunge, A.L. (2009). Dermal Absorption of Environmental Contaminants from Soil and Sediment: A Critical Review. *J. Expo. Sci. Environ. Epidemiol.* 19, 119-148, doi:10.1038/jes.2008.57.

**3e. Dermal slope factor for cancer** (section 2.5). The draft assessment proposes a dermal slope factor of 0.006 per ug/day based on skin tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and scaling from mice to humans? Does the method for cross-species scaling (section 2.5.4 and appendix E) reflect the appropriate scientific considerations?

2.5.2. Dermal absorption and dosimetrics

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#### 2.5.4. Dermal Slope Factor Cross-Species Scaling

#### 2.5.5. Uncertainties in the Derivation of the Dermal Slope Factor

##### RESPONSE:

The discussion, analysis, recommendations and conclusions of the IRIS BaP document are extensive and involve a large number of studies that were (and were not) used in the document or in public comments about the document. It was not possible to thoughtfully examine all of this information within the time available. Therefore, comments presented here are preliminary and may be revised after additional review of the IRIS BaP document, public comments, related literature and discussions with expert scientists, including those on the review panel for this document.

The challenge of IRIS assessments is to “identify potential adverse health effects and characterize exposure-response relationships” by integrating a wide range of scientific data (which were collected for diverse purposes, have varying quality and quantity, include contradictory results, and do not address many questions) and then extrapolating the results to human lifetime exposures with incomplete understanding of the fundamental biological processes. The aim is to protect (but not over protect) human health through scientifically reasonable and justifiable recommendations of exposure-response relationships with incomplete information. This review of the IRIS benzo[a]pyrene (BaP) document is mindful of the goal to make a reasoned, scientific, “best guesses” from the present publically available information (with its limitations and flaws) to provide exposure-response relationships that could be used in risk assessments for identifying potential adverse health effects.

##### *Hypothetical framework of the dermal slope factor*

Skin cancer risk from skin exposure to BaP depends on the levels of BaP metabolites in the cellular epidermis (i.e., the viable epidermis layer). Except when skin is damaged, BaP in the viable epidermis is the dose that absorbed into and through the outermost skin layer, the stratum corneum. Because the stratum corneum functions as a barrier limiting chemical exposure to tissues beneath it, the absorbed dose is less, often much less than the exposed dose (i.e., the BaP amount on the skin surface). In a typical human exposure, it is likely that a significant fraction of the exposed BaP dose will never be absorbed.

Although skin cancer risk depends on the absorbed dose, the dermal slope factor is derived from the exposed dose (also called the applied or administered dose) because the exposed dose is known, whereas the absorbed dose is not, unless all the applied dose eventually absorbs. It is possible that the nearly all the exposed dose is absorbed in the skin cancer bioassays of BaP skin exposure. In human exposures the absorbed dose will be less than the exposed dose, which will reduce the cancer risk compared with that estimated using a dermal slope factor derived in experiments in which most of the exposed dose was absorbed. In addition, several factors can cause significant variation in the absorbed dose to exposed dose ratio, which would be expected to cause a corresponding variation in skin cancer risk. The IRIS BaP document should have a discussion about the relationship of the estimated dermal slope factor to exposed and absorbed doses and how this will affect the uncertainties in its use (e.g., biased towards over estimating cancer risk).

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The description of the development of the dermal slope factor for cancer in humans would benefit from an outline of the steps (as best they are currently understood or might be plausibly explained) that cause skin cancer. Justification of choices (e.g., in the dose-response analysis, methods for scaling from mice to humans, and adjustments for absorbed compared with exposed doses) could then be related to assumptions about the mechanisms. This mechanistic framework might include:

1. Skin exposure to BaP (Controlling quantity or factor: exposed dose)
2. Absorption through the stratum corneum to reach the cancer forming tissue; i.e., the viable epidermis. (Controlling quantity or factor: absorbed dose)
3. Local concentration of BaP in the cancer forming tissue; i.e., the viable epidermis (Controlling quantities or factors: cancer forming tissue mass, absorption to and clearance from cancer forming tissue)
4. Rates of metabolism and adduct formation within the cancer forming tissue (local concentration of BaP, BaP metabolite profile in mice and humans, metabolism rate, adduct formation rate)

Recognizing that my expertise is in dermal absorption and not in mechanisms of carcinogenesis, experts in carcinogenesis may have alternative recommendations for describing the steps related to cancer development.

#### *Scaling the dermal slope factor derived from mice to humans*

According to EPA guidance ([U.S. E.P.A. 1992](#)), the strategy when empirical data for developing a scaling factor are absent is to develop a “scientific rationale for a particular scaling factor by investigating the allometric variation of the biological features and processes that influence and underlie carcinogenic potency”. Therefore, lacking chemical specific data for oral exposures, the consensus for a typical or average chemical is that equal doses in units of mg/kg<sup>3/4</sup>/day, when experienced daily for a full lifetime, will produce equal lifetime cancer risks across mammalian species (U.S. E.P.A., 1992). Scaling by the <sup>3</sup>/<sub>4</sub> power of body weight is consistent with allometric variation of key physiological parameters across mammalian species (provided doses are low enough that saturation of enzyme activity is unlikely). For example daily intakes of food or water are approximately proportional to the <sup>3</sup>/<sub>4</sub> power of body weight.

In the IRIS BaP document the selected scaling using body weight to the <sup>3</sup>/<sub>4</sub> power is justified as follows (p. 2-44, lines 10-15):

“allometric scaling using body weight to the <sup>3</sup>/<sub>4</sub> power was selected based on known species difference in dermal metabolism and penetration of benzo[a]pyrene. In vitro skin permeation was highest in the mouse, compared to rat, rabbit, and human, and was enhanced by induction of CYP enzymes (Kao et al., 1985). Using this approach, rodents and humans exposed to the same daily dose of a carcinogen, adjusted for BW<sup>3/4</sup>, would be expected to have equal lifetime risks of cancer.”

This same explanation is given in the EPA response to reviewers' comments (p. G-11, lines 48-49). Unfortunately, the IRIS BaP document failed to mention that the same paper (Kao et al. 1985) reported that BaP permeation rates through rabbit and rat skins were smaller (by a small amount) than through human skin (despite greater BaP metabolism in rabbit and rat skin), which contradicts the conclusion that risk scales with body weight to the <sup>3</sup>/<sub>4</sub> power. As noted in Appendix E (p. E-112), Knafla et al. (2011) did review the biological features and processes that might affect extrapolating tumor potency in mice to humans. Based on their hypothesized

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mechanism of carcinogenesis and analysis of BaP metabolism to DNA adduct forming metabolites human and mice, Knafla et al. (2011) chose to adjust the dermal slope factor in mice by 0.2, which represents the ratio of epidermal thickness in the mice and humans. The Knafla et al. (2011) approach for scaling is mentioned in Appendix E to the IRIS BaP document, but no explanation was given for choosing to not use this approach. Instead, Appendix E presents four alternative approaches to cross-species scaling with minimal discussion on why scaling by body weight to the  $\frac{3}{4}$  power has been chosen instead of other alternatives.

I found the description of the four alternative approaches for cross-species (mouse to human) scaling of the dermal slope factor unnecessarily confusing. Since the scaling is only from mouse to human, it would be simpler and more consistent in its application to the four approaches (and less confusing) to start with the dermal scaling factor in the mouse (i.e.,  $DSF_M = 1.7 (\mu\text{g}/\text{day})^{-1}$ ), and then adjust it by the appropriate human to mouse ratio to obtain the dermal slope factor in humans ( $DSF_H$ ) for each approach as follows:

Approach 1. No interspecies adjustment:  $DSF_H = DSF_M / 1$

**$DSF_H = 1.7 (\mu\text{g}/\text{day})^{-1}$**

Approach 2. Scaling by surface area:  $DSF_H = DSF_M / (SA_H / SA_M) = DSF_M / (19,000 \text{ cm}^2 / 100 \text{ cm}^2) = (1.7 (\mu\text{g}/\text{day})^{-1}) / 190$

**$DSF_H = 0.0089 (\mu\text{g}/\text{day})^{-1}$**

Approach 3. Scaling by body weight:  $DSF_H = DSF_M / (BW_H / BW_M) = DSF_M / (70 \text{ kg} / 0.035 \text{ kg}) = (1.7 (\mu\text{g}/\text{day})^{-1}) / 2000$

**$DSF_H = 0.00085 (\mu\text{g}/\text{day})^{-1}$**

Approach 4. Scaling by body weight to the  $\frac{3}{4}$  power:  $DSF_H = DSF_M / (BW_H / BW_M)^{0.75} = DSF_M / (70 \text{ kg} / 0.035 \text{ kg})^{0.75} = (1.7 (\mu\text{g}/\text{day})^{-1}) / 2000^{0.75}$

**$DSF_H = 0.0057 (\mu\text{g}/\text{day})^{-1}$**

In the above calculations,  $SA_j$  = total skin surface area,  $BW_j$  = body weight, and the subscript  $j$  designates that the quantity is specified for either mouse (M) or human (H). Cancer risk predicted by all four approaches is then calculated by multiplying the  $DSF_H$  by the same lifetime averaged dermal dose (LADD), which has units of  $\mu\text{g}/\text{day}$ . For the example calculations listed in Table E-25, LADD is  $0.0004 \mu\text{g}/\text{day}$ . The DSF for Approach 4 is reported in Appendix E as listed above. However, the DSF for Approaches 2 and 3 are listed as  $170 (\mu\text{g}/\text{cm}^2 \text{ day})^{-1}$  and as  $0.058 (\mu\text{g}/\text{kg day})^{-1}$ , which requires that the LADD values used for calculating risk be divided by  $19,000 \text{ cm}^2$  surface area for Approach 2) and by  $70 \text{ kg}$  for Approach 3. This is confusing and these adjustments to LADD are not described clearly. It is recommended that Table E-25 be revised as follows: (1) the dose metric column should be deleted; (2) the dermal slope factor numbers should be listed as given above (i.e., 1.7, 0.0089, 0.00085, 0.0057 for Approaches 1 through 4, respectively); these numbers all have the same units, which can be listed in the column heading; (3) the risk numbers do not change; however the "nominal exposure" should be called the "lifetime averaged dermal dose" (LADD) instead to be consistent with the terminology elsewhere.

I found the Assumptions listed in Table E-25 for the four approaches confusing and sometimes incorrect. First, in all four approaches, the risk in a given species is assumed to depend on only the overall dose and not the dose per unit area or body weight. For a given LADD (e.g.,  $0.0004$

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µg/day listed in Table E-25), cancer risk is different for the different approaches. However, the cancer risk in a human is the same whether 0.0004 µg/day is applied to a skin area of 1 cm<sup>2</sup> (dose per area = 0.0004 µg/cm<sup>2</sup>-day) or 19,000 cm<sup>2</sup> (dose per area = 2.1E-8 µg/cm<sup>2</sup>-day). Thus, the last sentence of the Assumptions listed in Table E-25 for Approach 2:

“This approach implies that risk does not increase with area exposed as long as dose per area remains constant.”

is incorrect. If dose per exposed area is constant, then increasing the exposed area increases the LADD (which is calculated based on total skin area) and therefore, the cancer risk. I believe the Assumptions listed in Table E-25 for Approach 1 should be: Equal mass per area per day (µg/cm<sup>2</sup>-d), if applied to equal areas of skin (cm<sup>2</sup>), will affect similar numbers of cells across species.

I believe the Assumptions listed in Table E-25 for Approach 2 should be: Equal mass per area per day, if applied to equal fractions of total skin surface (cm<sup>2</sup>), will have similar cancer risks. That is, 1 µg/cm<sup>2</sup>-d applied to 190 cm<sup>2</sup> in a human (1% skin surface area, which corresponds to a whole-body exposure of 0.01 µg/cm<sup>2</sup>-d) will have the same cancer risk as 1 µg/cm<sup>2</sup>-d applied to 1 cm<sup>2</sup> in a mouse (also 1% skin surface area corresponding to a whole-body exposure of 0.01 µg/cm<sup>2</sup>-d). I found the above description confusing. I believe the assumptions of Approach 2 can be stated more clearly as: Equal mass distributions over the whole body skin area represent equal cancer risk. I believe that the last two sentences listed as Assumptions for Approach 2 are incorrect. Approach 2 assumes that risk is proportional to dose expressed as mass per total skin surface area per day, which is different from saying that risk is proportional to dose expressed as mass per area. For example, 1 µg/cm<sup>2</sup>-d applied to 19 cm<sup>2</sup> in a human (i.e., a whole-body exposure of 0.001 µg/cm<sup>2</sup>-d) would have 1/10<sup>th</sup> the cancer risk than the same 1 µg/cm<sup>2</sup>-d applied to 190 cm<sup>2</sup> (i.e., a whole-body exposure of 0.01 µg/cm<sup>2</sup>-d).

I find the following first 3 sentences of the Assumptions listed in Table E-25 for Approach 3 confusing.

“The skin is an organ with thickness and volume; benzo[a]pyrene is distributed within this volume of skin. Cancer risk is proportional to the concentration of benzo[a]pyrene in the exposed volume of skin. Equal mass per day (µg/d), if distributed within equal fractions of total body skin will have similar cancer risks. That is, whole-body lifetime exposure (e.g., 5%-of-the-body lifetime exposure) at the same loading rate (µg/cm<sup>2</sup>-d) gives similar cancer risks across species.”

I recommend deleting these three sentences. The next sentence:

“This approach assumes that risk is proportional to dose expressed as mass per kg body weight per day.”

is correct and could be explained as: (1) equal BaP skin concentrations, which correspond to mass per day (µg/d) per total skin mass, will have similar lifetime cancer risk, and (2) the mass of skin is approximately the same fraction of the body weight in all species.

The numbers listed as “Risk” in the right hand column of Table E-25 were calculated using the “nominal exposure” of 0.0004 µg/day. The description of the derivation of 0.0004 µg/day in provided in footnote “a” is inadequate of Table E-25 is inadequate. Users of this document should be able to understand readily the calculation of this number, and all numbers used in the calculation should be listed in footnote “a”. Also, as stated above, “nominal exposure” should be called the “lifetime averaged dermal dose”. I would further recommend that the equation for

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LADD, which is listed on p. G-14, be added to Table E-25 along with specification of the parameter values, similar to the way they are shown for an adult on p. G-16. As best as I can determine,  $0.0004 \mu\text{g}/\text{day}$  was calculated using  $C_{\text{soil}} = 100 \text{ ppb}$  (as listed in Table E-25), SA (surface area exposed, as listed on p. E-114, line 9) =  $950 \text{ cm}^2$ , AF (soil adherence factor) =  $10 \mu\text{g}/\text{cm}^2\text{-d}$ , ED (exposure duration, I think from (U.S. E.P.A. 2004)) = 30 years, EF (exposure frequency) = 350 days/yr, and AT (lifetime averaging time) =  $365 \text{ days}/\text{yr} * 70 \text{ years} = 25550 \text{ days}$ . Thus, this calculation differs from that shown on p. G-16, which is for a SA =  $5700 \text{ cm}^2$ , an exposure duration of 9 years and includes the soil-to-skin transfer coefficient ( $K_{\text{soil}} = 0.25$ ). I would recommend that example calculations for estimating cancer risk from soil exposures be the same in different places in the document and be consistent with a cited source (for Table E-25, this is (U.S. E.P.A. 2004)).

#### Relating BaP cancer risk derived from solvent deposited BaP to contaminated soils

The Preface to the IRIS BaP document (pages xii-xiii) explains that the IRIS Program's first dermal slope factor was motivated by the "Agency's need to estimate the potential for skin cancer from dermal exposure (U.S. EPA, 2004), especially in children exposed to contaminated soil...". Because cancer bioassays from skin contact to BaP contaminated soil are not available, the dermal slope factor was derived for BaP delivered to skin using volatile solvent solution (typically acetone or toluene) that evaporated leaving BaP in direct contact with skin. A similar BaP dose on soil will have a reduced cancer potency compared to the solvent delivered BaP. Moreover, in actual human exposures, some of the exposed dose is removed during daily activities such as bathing, which did not occur in the cancer bioassay experiments. Although skin exposures to BaP contaminated soil motivated the slope factor derivation, the IRIS BaP document provides little information on how large the overestimate of risk might be or factors for adjusting the risk. While there are no cancer studies of skin contact to BaP contaminated soil, there are dermal absorption studies from both soil and volatile solvents, which could be considered. One study (Wester et al. 1990) is mentioned on pages G-12 (lines 35-43) and G-16 in the EPA response to comments, along with a soil to skin transfer coefficient ( $K_{\text{soil}}$ ) of 0.25.

Skin cancer risk from skin exposure BaP depends on the levels of BaP metabolites in the cellular epidermis (i.e., the viable epidermis layer). Therefore, except when skin is damaged, BaP in the viable epidermis is the dose that absorbed into and through the outermost skin layer, the stratum corneum. This will be essentially the same as the systemically absorbed dose. In the *in vitro* experiment, the systemically absorbed dose will be represented by the amount that appears in the receptor fluid during the experiment plus the amount present in the washed skin (i.e., after excess, non-absorbed BaP has been cleaned from the skin surface) at the end of the experiment.

I agree with the comment from CH2M Hill (summarized on p. G-12, lines 9-11) suggesting that the IRIS BaP document could be strengthened with increased discussion of uncertainties in assessing risk from exposure BaP in soil. However, a brief mention of one study by Wester et al. (1990) and introduction of  $K_{\text{soil}}$  is insufficient. First, the IRIS BaP document needs to discuss the difference between absorbed dose and exposed dose and how this difference relates to the doses in the development of the dermal slope factor. In my understanding of the cancer bioassay experiments, almost all of the exposed dose may have absorbed. Therefore, the cancer risk from skin exposure to BaP contaminated soils would be reduced by the fraction of exposed soil dose

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that absorbs (and a ratio of soil absorption compared with absorption from acetone would be unnecessary). Further examination of the cancer bioassay data is needed to assess the likely absorbed dose and how this would impact estimates of cancer risk from BaP contaminated soils.

A thorough review of dermal absorption studies of BaP from contaminated soils should be added to the IRIS BaP. This review should also include dermal absorption measurements of BaP applied in a volatile solvent. If a soil-to-solvent absorption ratio is deemed a useful measure of reduced absorption from soil, then careful thought will be needed to produce a scientifically supportable comparison of the soil and solvent data. For example, the mass of soil applied in most soil studies was large enough that only a small fraction of the soil was in contact with the skin; i.e., the soil mass was larger than the mass required to cover the skin with a single tightly packed layer (a monolayer) of soil. A complication is that the mass of soil in a monolayer depends on the particle sizes of the soil (see Exhibit C-4 in (U.S. E.P.A. 2004)), which have varied drastically among the studies. At 40 mg of soil/cm<sup>2</sup>, the soil load in the Wester et al., (1990) experiments was larger than other soil studies (Spalt et al. 2009). Despite this, it is likely that the skin was covered with just one layer of particles because the particle size fraction they used was large (i.e., 180 - 320 μm) (Spalt et al. 2009). This makes direct comparison of their results in terms of percent absorption appropriate; this would not have been the case if there had been multiple soil layers in their experiments.

The study by Wester et al. (1990) includes both *in vitro* human skin and *in vivo* Rhesus monkey results. The  $K_{\text{soil}}$  value of 0.25, proposed on p. G-12 and G-16 of the Supplemental Information, was calculated from the *in vivo* results (13% from soil and 51% from acetone), with no mention of the *in vitro* results. Based on an examination of the experimental protocol used in the *in vivo* soil measurements, Spalt et al. (Spalt et al. 2009) questioned the reliability of the 13% absorption value reported by Wester et al. (1990). Certainly, in comparison with the other literature of BaP absorption from soils into human skin, the *in vivo* result from Wester et al. is significantly higher. However, the *in vitro* soil measurement (1.4 % BaP in the skin and receptor fluid in 24 h) is more consistent with the other literature on BaP absorption from soils (based on a recent comprehensive review that is being prepared presently for publication). Note also that the *in vitro* and *in vivo* measurements from acetone deposited BaP are more similar to each other than the soil results (only a factor of two different – 51% versus 24% when deposited from acetone compared a factor of almost 10 – 13% versus 1.4% from soil). Therefore, as a starting point,  $K_{\text{soil}} = 0.06$  calculated from the *in vitro* data reported by Wester et al. (1990) might be more representative than 0.25 skin exposure to BaP in soils compared with a acetone. Given that this factor is calculated from absorption measurements, it might be more appropriate to refer to it as the relative absorption factor, as did Knafla et al. (2011). Note that the Knafla et al. (2011) also considered studies by Moody et al. (Moody et al. 2007) and (Abdel-Rahman et al. 2002) in their examination of the relative absorption factor. It should be noted, however, that absorption measurements for BaP reported by Moody et al. (2007) and Abdel-Rahman et al. (2002) contaminated soils may not represent dermal absorption from soils. In the studies by Moody et al. (2007), soils were suspended in water, and thus, the results are pertinent to absorption from water in contact with soil but not to skin exposures to contaminated soil. In the studies of freshly contaminated soil by Abdel-Rahman et al. (2002) it appears that BaP solvent was added to the soil after it was placed on the skin (Spalt et al. 2009).

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#### Questions about the administered dose in skin cancer bioassays

The analysis of cancer bioassay following dermal exposure has included a few assumptions. First, it was assumed that risk at low doses of benzo[a]pyrene is dependent on absolute dermal dose and not dose per unit of skin. This means that a higher exposure concentration of benzo[a]pyrene contacting a smaller area of exposed skin could carry the same risk of skin tumors as a lower exposure concentration of benzo[a]pyrene that contacts a larger area of skin. From the perspective of dermal absorption, this assumption will be satisfied for doses that are smaller than the amount of BaP that would be required to cover an exposed skin area with a complete film of BaP. Thus, at low doses, piles of neat BaP will cover a fraction of the skin area to which BaP in the volatile solvent was applied. Increasing the amount of BaP applied to the same area proportionally increases the skin area fraction covered with neat BaP. Given this, I agree with the conclusion of the IRIS BaP document that the surface area over which the BaP dose is applied does not need to be considered.

It seems to this reviewer that a second assumption of the dermal exposure cancer bioassay is that most of the administered dose (which is applied periodically without cleaning any remaining residue from the skin surface prior to application) is absorbed. Therefore, it is a concern that most cancer bioassay studies do not mention how losses of the administered dose (e.g., transfer from the skin to the cage) are prevented. If losses are not prevented, then cancer risk is estimated from higher than delivered dose.

Related to this, it is not clear to this reviewer why studies that applied BaP 1-time/week (e.g., Nesnow et al., 1983) or 1-time every 2 weeks (e.g., Levin et al., 1977) are "less useful for extrapolating to daily human exposure" (see page D-62, lines 8-10) than studies that applied BaP 2-times or more per week. If the results of applying BaP 1-time/week differ from applications of 2-times or more per week, then continuous daily exposure, which has been assumed in the analysis for the dermal slope factor is inappropriate; i.e., there would be data indicating that dose-rate effects cannot be ignored (see lines 12-13, p. 2-41).

While 100% absorption of the repeated applications of known doses in the dermal cancer bioassay may occur, the same will not be true of actual exposures to humans, who will remove exposed doses by regular bathing and hand washing. Thus, only a fraction of the exposed dose will be absorbed, thereby reducing the risk of skin cancer compared with predictions from the dermal slope factor, which was derived from experiments with nearly complete absorption. This is a potentially important uncertainty is not mentioned in the IRIS BaP document and should be.

#### Comments about vehicle effects

In studies by Grimmer et al. (1983, 1984), BaP was applied using solvent solution of 1:3 v:v dimethyl sulfoxide:acetone. Acetone is more volatile than dimethyl sulfoxide (DMSO) and would rapidly evaporate leaving BaP in a DMSO solution. *In vitro* diffusion cell studies of n-alcohols (methanol, butanol and octanol) at 1 % concentrations in DMSO:water solutions varying from zero to 100% DMSO have shown that skin is damaged irreversibly at DMSO concentration of 50% or larger after less than 3 h of exposure ([Kurihara-Bergstrom et al. 1986](#)). (Similar findings have been observed by other authors for other permeants as well.) Thus, skin damage that might have promoted skin penetration and perhaps tumor development may have occurred in the Grimmer et al. studies. This possibility should be mentioned in the detailed

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review of the Grimmer et al. studies provided in the Supplemental Information to the IRIS BaP document.

Extrapolation of cancer risk estimated from experiments that deposited BaP from a volatile solvent to BaP in other vehicles (e.g., solutions that do not evaporate or PAH containing ointments) must consider how the vehicle affects the driving force for BaP transfer into and through the skin (i.e., the thermodynamic activity) as well how vehicle components could affect have on the skin. Generally, extrapolation to other vehicles will be inappropriate and could, although not always, overestimate cancer risk, sometimes significantly. An example of vehicle effects was observed in mice treated with 25- $\mu$ L applications of the same BaP concentrations (ranging from 0.4 to 280  $\mu$ g/mL); fewer DNA-adducts were observed from the lipophilic n-dodecane vehicle (0.067 to 3.5 fmol adducts/ $\mu$ g DNA) compared with the more polar tetrahydrofuran vehicle (0.089 to 16.9 fmol adducts/ $\mu$ g DNA) ([Booth et al. 1999](#)). This difference is most likely related to different BaP solubility in the two solvents, which affects thermodynamic activity.

Other specific comments (in no particular order):

The statement on p. D-3, lines 6-10:

“Studies of benzo[a]pyrene metabolites or DNA adducts measured in humans exposed dermally to benzo[a]pyrene-containing PAH mixtures demonstrate that benzo[a]pyrene is absorbed dermally. One study of dermal absorption in volunteers found absorption rate constants ranging from 0.036 to 0.135/hour over a 45-minute exposure, suggesting that 20–56% of the dose would be absorbed within 6 hours (VanRooij et al., 1993).”

is potentially misleading and should be revised. The absorption rate constants, which appear to be taken from Table 2 of VanRooij et al. (1993), are not for BaP as implied; they are PAHs with four or more fused ring PAHs after application of coal tar ointment. These absorption rates depend on concentrations of the various PAHs in the coal tar ointment as well as the amount applied, which are both unknown. Moreover, the assumption that the absorption rates for PAHs with four or more fused rings will represent BaP is not stated or justified.

I found two examples citations of incorrect citations. The first of these was also mentioned by Brian Magee in comments he submitted (p. 81) in November 2013 (on behalf of a consortium of trade groups). He noted that the Grimmer et al. (1984) citation for the mouse skin painting study is incorrect. He suggested that the correct citation is to Grimmer et al. (1985). However, there is also a different 1984 paper by Grimmer et al. that also contains mouse skin painting data. I have not examined these two papers, which are cited below, to determine which reference is the correct one. All of the numerous citations of this reference in the main IRIS BaP document and the Supplemental Information need to be checked.

G. Grimmer, H. Brune, R. Deutsch-Wenzel, G. Dettbarn, J. Misfeld, U. Abel, J. Timm (1984). The contribution of polycyclic aromatic hydrocarbons to the carcinogenic impact of emission condensate from coal-fired residential furnaces evaluated by topical application to the skin of mice. *Cancer Lett*, 23:167-176.

G. Grimmer, H. Brune, R. Deutsch-Wenzel, G. Dettbarn, J. Misfeld, U. Abel, J. Timm (1985). The contribution of polycyclic aromatic hydrocarbon fractions with different boiling ranges to the carcinogenic impact of emission condensate from coal fired

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residential furnaces as evaluated by topical application to the skin of mice, Cancer letters, 28, 203-211.

Godschalk et al. 1998a should be Godschalk et al., 1998b and the reference to Godschalk et al., 1998b should be 1998a.

There may be other citation errors. It is strongly recommended that the main and supporting information documents be thoroughly and carefully check all citations.

EPA states on p. G-12 (lines 22-24): the assumption of this dose metric is that risk at low doses of BaP is dependent on absolute dermal dose and not dose per unit of skin". Note that this should say "unit of skin area". Also, this idea is stated more clearly at the bottom of p. E-113 and continuing on to p. E-114:

"Risk at low doses of benzo[a]pyrene is dependent on absolute dermal dose and not dose per unit of skin, meaning that a higher exposure concentration of benzo[a]pyrene contacting a smaller area of exposed skin could carry the same risk of skin tumors as a lower exposure concentration of benzo[a]pyrene that contacts a larger area of skin."

Consider revising the text on p. G-12 to make it more similar to the clearer description on p. E-114.

Regarding the statement on p. 2-42, lines 16-21:

"Dermal slope factors calculated from the supporting studies (Sivak et al., 1997; Grimmer et al., 1984; Habs et al., 1984; Grimmer et al., 1983; Habs et al., 1980; Schmähl et al., 1977; Schmidt et al., 1973; Roe et al., 1970; Poel, 1963, 1959) using the multistage model and linear extrapolation from the BMDL<sub>10</sub> values ranged from 0.25 to 1.8 per µg/day, a roughly sevenfold range (Table 2-11). Values ranged from 0.9 to 1.7 per µg/day for male mice, and from 0.25 to 0.67 per µg/day for female mice."

How can the BMDL<sub>10</sub> for males and females range from 0.25 to 1.8 per µg/day if the maximum of the range is 1.7 per µg/day for males and 0.67 per µg/day for females. The numbers in the above statement do not seem to correspond to the numbers listed in Table 2-11. In my reading of Table 2-11, it seems that for males the range is 1.3 to 1.7 and for females the range is 0.25 to 1.8?

In Table 2-11, I believe that POD = BMDL should be POD = BMDL<sub>10</sub> to agree with the text describing Table 2=11.

Regarding the statement on p. 2-44, lines 33-35:

"Note that the dermal slope factor should only be used with lifetime human exposures <18 µg/day, the human equivalent of the POD<sub>M</sub>, because above this level the dose-response relationship is not expected to be proportional to the mass of benzo[a]pyrene applied."

I don't understand the recommendation that dermal slope factor should only be used with lifetime human exposures <18 µg/day. The explanation needs to be clarified and a reference supporting the explanation provided.

The statement on p. 2-44, line 38: "Secondly, it is assumed that the risk at low doses of benzo[a]pyrene is linear." needs to specify what that dose is the quantity that has a linear effect on risk (i.e., risk is linear with dose).

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The IRIS BaP document explains on p. 2-45 that the dermal slope factor has been developed for local effect and not systemic cancer risk. It cites Godschalk et al. (1998a), which should be (1998b), as a source of information suggesting that benzo[a]pyrene metabolites can enter the systemic circulation following dermal exposure in humans. Booth et al (1999) might also be cited as evidence that dermal exposure could cause systemic effects. They observed DNA adduct formation in the lungs of mice exposed to a single dermal dose of BaP.

The discussion on p. 2-45 continues by explaining that tumors have not been found at distal sites in lifetime skin cancer bioassays that included pathological examination of other organs. This is an expected observation because the concentration of BaP in skin (i.e., the portal of entry and a smaller tissue volume) will be larger than the systemic concentration, consisting of large tissue volume in which BaP is diluted. The explanation of skin binding and reactive metabolites in the skin (lines 10-17) is unnecessary and irrelevant. Experimentally, a significant amount of BaP is found in the skin at the end of the dermal absorption experiments (typically lasting 24-h or less) because it takes time for the BaP to absorb into and then transfer through the skin to enter the systemic circulation. Had the experiment continued for a longer time, almost all of the BaP would have been excreted or found in the systemic circulation (in the *in vivo* study) or in the receptor fluid (in the *in vitro* study if the time required were not too long to maintain skin integrity).

Adding a footnote to Tables E-20, E-21 and E-22 (which appear on pages E-79 and E-80) that explains how the average daily dose was adjusted to lifetime averaged dose would help readers who may not remember the explanation provided in the text on p. E-75.

p. D-3 (lines 20-28) states:

“The vehicle for benzo[a]pyrene exposure is an important factor in skin penetration. Exposure of female Sprague-Dawley rats and female rhesus monkeys topically to benzo[a]pyrene in crude oil or acetone caused approximately fourfold more extensive absorption than benzo[a]pyrene in soil (Wester et al., 1990; Yang et al., 1989). The viscosity of oil product used as a vehicle also changed skin penetration with increased uptake of benzo[a]pyrene for oils with decreased viscosity (Potter et al., 1999). Soil properties also greatly impact dermal absorption. Reduced absorption of benzo[a]pyrene occurs with increasing organic carbon content of the soil and increased soil aging (i.e., contact time between soil and chemical) (Turkall et al., 2008; Roy and Singh, 2001; Yang et al., 1989).”

This paragraph requires substantial revision. Several pieces of information are incorrect.

#### REFERENCE CITED IN REVIEW

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## **Dr. Scott Burchiel**

**Question 2c. Immunotoxicity (sections 1.1.3, 1.2.1). The draft assessment concludes that immunotoxicity is a potential human hazard of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?**

### **Key Points:**

- The evidence for human immunotoxicity is based on complex mixture exposures; while there is no doubt that BaP and other PAHs with specific SARs can cause suppression of human HPBMC at low concentrations in vitro (10-100 nM, Davila and Burchiel, 1996), it is unclear whether these levels of exposure can be achieved from in vivo environmental inhalation exposures or ingestion of cooked foods.
- Immunotoxicity is caused by a combination of genotoxic (DNA adducts and p53 –induced cell death) and non-genotoxicity (signaling due to AhR and oxidative stress); some of these mechanisms are similar to cancer initiation and promotion, but it is unclear whether there is a threshold effect for immunotoxicity.
- Effects of BaP can vary by dose and time and sometimes leads to biphasic (U-shaped) observations of increased or decreased immune parameters, which may be mechanistically explained by differing metabolites (e.g., diol-epoxides, vs quinones) or mechanisms of action.
- Most immunotoxicity animal studies utilize mouse models (not rat) and they rely upon sensitive functional assays, such as the T-dependent antibody response (TDAR); the dose required to produce thymic atrophy are quite high in mice and rats; EPA acknowledges that thymic atrophy may not be a reliable indicator of immunotoxicity (Luster et al 1992), page 2-5, line 19.
- It is recommended that EPA establish an Immunotoxicity Guidance Document to standardize risk assessment and to identify data gaps.

### Human Studies – all mixtures

- Szczeklik et al., 1994 reported decreased Ig's in serum in coke workers with mg/m<sup>3</sup> exposures
- Zhang , 2012 studied 129 coke oven workers compared to 37 warehouse controls) for early and late apoptosis (Annexin V/PI) in HPBMC; concentrations of BaP were 10-1,600 ng/m<sup>3</sup> in the working environment; 2,78-3.66 ng 1-OHP measured in urine
- Winker et al 1997 is an immune function and phenotype study of HPBMC comparing old and new coke facilities; results show depression of T cell activation – this study is most compelling
- Cigarette smoking - usually looking at immune suppression, but effects are complicated by the strong action of nicotine, which is immunosuppressive

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- Human PBMC In vitro studies should be included in risk assessment; some studies suggest that BaP is more toxic to human HPBMC (10-100 nm) than mouse spleen cells (Davila et al, 1996); compared to 1-20 uM for mouse spleen cells White and Holsapple, 1984)

### Animal Studies

- Important structure activity relationships established early on: Dean et al (1983) showed suppression of PHA response of mouse spleen at 50 mg/kg, but not by BeP
- Consistency of findings in mice and some rat strains: Temple/White (1993) showed decreased IgM response and PFC in mouse spleen at 5, 20, 40 mg/kg and F344 rats at 10 and 40 mg/kg 14 da s,c,
- Metabolism and PK very important in BaP and other PAH immunotoxicity: General points
  - Nebert (2013) importance of balance of Cyp1A1 and Cyp1B1
  - Uno – Cyp1A1 mostly protective
  - For immunotox Cyp1B1 important in lymphoid tissues
- Immune function tests indicate that BaP is suppressive and should result in increased risk of infections and perhaps cancer; this is evidenced by Munson et al 1985 showed decreased resistance to, Strep, Herpes, and B16 melanoma by BaP but not by BeP; influenza was not affected and Listeria resistance was increased
- EPA focuses on DeJong studies in rats with toxic endpoint being thymic atrophy at 90 mg/kg; Munson Kawabata and White (1987, 1989) have shown that BaP metabolites are responsible for suppression of TDAR in mouse spleen; immune function tests are more sensitive than changes in cell viability, lymphoid organs weights, and; PAHs produce immune suppression at concentrations that are not cytotoxic

### Developmental Immunotoxicity

- Since neurobehavioral endpoints are to be used for RfD calculation, I will defer to the neurotox people
- It is generally well known that developmental immunotoxicity is produced at much lower doses (10x?) than those required to produce immunotoxicity in adults; however this may not be well documented for BaP in the present literature citations used for assessment.

### Other Comments:

- BaP exposures are high in woodsmoke, but there are few immunotox studies (Burchiel et al, 2005)
- We should look for evidence of increased infections in cohorts as a demonstrated health effect
- EPA should consider developing Guidelines for immunotoxicity assessment, as have been done by WHO (2012)

## Dr. Anna Choi

### 1. Literature search/study selection and Evaluation.

**The process for identifying and selecting pertinent studies for consideration in developing the assessment is detailed in the *Literature Search Strategy/Study Selection and Evaluation* section. Please comment on whether the literature search approach, screening, evaluation, and selection of studies for inclusion in the assessment are clearly described and supported. Please comment on whether EPA has clearly identified the criteria (e.g. study quality, risk of bias) used for selection of studies to review and for the selection of key studies to include in the assessment. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of benzo[a]pyrene.**

Overall, the literature search strategy and process were clearly described. With regard to the study selection, it was stated that based on a secondary keyword search followed by a preliminary "manual" screen of titles or abstracts was performed by a toxicologist, and that a more detailed "manual" review of titles, abstracts, and/or papers was then conducted. Were these "manual" searches and reviews performed independently by another investigator, with search results checked and disagreements resolved? This step would be important in ensuring reliability and without bias in the review of the studies.

**2. Hazard identification. In section 1, the draft assessment evaluates the available human, animal, and mechanistic studies to identify the types of toxicity that can be credibly associated with benzo[a]pyrene exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/backgrd.html>) to reach the following conclusions.**

2a. **Developmental toxicity** (sections 1.1.1, 1.2.1). The draft assessment concludes that developmental toxicity and developmental neurotoxicity are human hazards of benzo[a]pyrene exposure. Do the available human, animal and **mechanistic** studies support this conclusion?

Chen et al. (2012) was chosen as the basis for the proposed overall oral RfD. However, this study was conducted on neonate rats (post natal 6-11 days) to determine whether neurotoxic effects of postnatal BaP exposure on behavioral performance persist in juvenile and young adult stages. The draft assessment did not state whether studies on prenatal exposure assessment were considered.

Overall, the human and animal studies included were quite comprehensive. The comment in the summary stating the limitation in comparing specific endpoints across species is well-noted.

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**2c. Immunotoxicity (sections 1.1.3, 1.2.1). The draft assessment concludes that immunotoxicity is a potential human hazard of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?**

Evidence pertaining to the immune effects of BaP was based in animals (rats) (Table 1-8) studies. In particular, mainly on two studies - De Jong et al. 1999 and Kroese et al. 2001. Some considerations on generalizability:

- The results were based on males. What were the effects of females?
- The rats were 6 weeks of age. What would be the effects of mature rats? Hence, the age factor should be considered.

Urso et al. 1988 studied the effects of BaP on spleen function. BaP exacerbates the change of the leukocyte profile during pregnancy and preferentially affects the lymphocytes. However, no follow-up was performed on the offspring of the pregnant mice.

Rats and mice appeared to have been used interchangeably on the reporting of the results. What are the differences, biologically and mechanistically, between the two that need to be kept in mind when interpreting the findings? For example, spleen effects on rats and mice (1-38), reductions in IgA levels in male rats (De Jong et al., 1999), and non-significant reductions in IgG levels in female mice (Dean et al., 1983). The evidence in animals for the effects of BaP on the developing immune system is mainly based on the studies on mice (1-40). Postnatal exposure to BaP, however, was studied on rats. The differences between the animal subjects should again be noted in concluding that BaP may alter the developing immune response to infection or vaccination.

BaP effects on the immune functions in humans are mostly based on occupational studies, and that the effects studied were mostly with PAH mixtures (except for a small number of studies such as Wu et al., 2003b which measured BaP concentrations). This should be made aware when making interpretations. BaP is often used as an indicator chemical to measure PAH exposures.

In studying the immune suppression and sensitization (1-39), a statistically significant decrease in the splenic natural killer cell activity was observed in the De Jong et al., 1999 study while no decrease was found in the Munson et al., 1985 study. The report states that the magnitude of the dose and duration of the exposure may account for the discrepancy between these two studies. It should be noted, however, that male Wistar rats were studied in the former study, and B6C3F1 female mice were studied in the latter. The difference between the animals studied and the gender should also be noted.

The RfD for immunological effects was based on De Jong et al. (1999) where oral administration of B[a]p in male rats resulted not only in general toxicity, as indicated by the effects on body weight, but also in immunotoxicity, as indicated by the effects on bone marrow (decreased cell counts), (decrease weight in) thymus, spleen, and (decreased) lymph nodes. Red blood cells and white blood cells were significantly decreased. Most toxic effects were only observed in the highest-dose group (90 mg/kg), but compared to the general toxicity, some parameters indicating

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immunotoxic effects were also affected at lower doses (10 and 30 mg/kg), including thymus weight changed and spleen B-cell.

***2e. Other types of toxicity (section 1.1.4). The draft assessment concludes that the evidence does not support other types of noncancer toxicity as a potential human hazard. Are there other types of noncancer toxicity that can be credibly associated with benzo[a]pyrene exposure?***

The types of noncancer toxicity covered in the draft assessment appear to be quite comprehensive.

***3. Dose-response analysis. In section 2, the draft assessment uses the available human, animal, and mechanistic studies to derive candidate toxicity values for each hazard that is credibly associated with benzo[a]pyrene exposure in section 1, then proposes an overall toxicity value for each route of exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/backgrd.html/>) in the following analyses.***

**3e. Dermal slope factor for cancer** (section 2.5). The draft assessment proposes a dermal slope factor of 0.006 per ug/day based on skin tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and scaling from mice to humans? Does the method for cross-species scaling (section 2.5.4 and appendix E) reflect the appropriate scientific considerations?

2.5.1. The draft assessment based on skin tumors in mice given the availability of lifetime bioassays of dermal BaP exposure in mice.

On the choice of epidemiological studies in the analysis of carcinogenicity - limited studies are available. Occupational epidemiological studies included an extended case series of skin tumors among 606 tar-refinery workers who had been recorded as having had tar dermatitis between 1946 and 1996 (Letzel and Drexler, 1998). 4754 skin tumors had been identified and surgically removed up to the end of 1996, of which 90% (4280) had histological diagnoses. Most of the tumors occurred in areas that had been in contact with the tar or tar fumes, notably the facial area, forearms, and hands. It should be noted, however, that the size of the study population within which the 606 subjects with dermatitis were reported was not given. Given the large proportion of subjects with at least one malignant tumor, the high proportion of squamous-cell carcinomas and the occurrence on exposed areas suggest that occupational exposures at this tar refinery represented a risk factor for these tumors (IARC, 2010). Information on the length of the exposure and corresponding concentrations of these workers was not available.

Dermal exposure may occur from contact by using certain pharmaceutical products containing coal tar. However, epidemiological studies on the exposure to pharmaceutical coal tar are limited. One population-based case-control study with 404 subjects and 391 controls (Mitropoulos and Norman, 2005) studied self-reported use of coal tar/dandruff shampoo and the association with

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increased incidence of skin squamous cell carcinomas (SCC) (association between occupation and SCC was also studied). The study was limited in its design, exposure levels, and recall bias.

In addition, there are small scale studies with limitations in design and quality of the exposure data:

- BaP was detected in coal-tar-containing hair shampoos. van Schooten et al. (1994) studied the human exposure of PAH in coal-tar shampoos. Eleven healthy subjects applied the shampoo which contained high concentrations of 285 mg/kg pyrene and 56 mg/kg BaP. A dose of 20 g shampoo was used in the evening and the internal dose of PAH was assessed as urinary 1-hydroxypyrene. One day after exposure, the internal dose was 10 times higher than the background level, similar to that measured in coke-oven workers. On day 2, the mean increase was 5 times. It should be noted, however, that the characteristics of the subjects (occupation, smoking status) and the duration and extent of the shampoo application, the residual amount left on the scalp, and the coal tar absorption rate and amount were not considered in the study (Goldman, 1995).

- Bickers and Kappas (1978) on the induction of AHH by coal tar – 9 patients with psoriasis or atopic dermatitis applied coal tar solution to clinically unaffected skin in the lower lumbar region. A 2- to 5-fold increase in AHH activity was seen in the treated areas compared to the control sites.

- Černíková et al. (1983) on the absorption of a coal tar component through the skin on 28 patients

- Hukkelhoven et al. (1984) on the inducibility of AHH – coal tar was applied to a certain area of the scalp of each subject. It remained associated with the hair even after extensive washing. In addition, the effect of coal tar on AHH activity is restricted to the treated skin surface.

- Five subjects in Storer et al. (1984) applied a 2%-crude coal tar in petrolatum preparation to the trunk and extremities. Absorption of PAHs in crude coal tar occurred in a variable manner. PAH levels in blood ranged from undetectable amounts to 100 ppb.

- Van Cantfort et al. (1986) studied BP metabolism among 11 subjects who were treated with coal tar at 24-h intervals. A 2- to 8-fold increase in BP metabolism was reported.

- Merk et al. (1987) evaluated the effect of human exposure to a crude coal tar among 12 healthy subjects. The study found that human hair follicle enzymes are capable of converting BP 7,8-diol to tetrols.

- Jongeneelen et al. (1988) treated 5 female patients suffering from eczematous dermatitis on the arms and legs for several days with an ointment containing 10% coal tar. The concentration of 1-OP-H increased to about 100 to 1000 times the background level.

- Arnold et al. (1993) investigated the effects of topical application of isoquinoline (a component of coal tar) on human skin. The subjects included 18 volunteers with no history of skin diseases and 17 psoriasis patients who had received no therapy for 2 and/or 4 weeks prior to the study. Results showed that application of 0.2% isoquinoline or even crude coal tar did not have any significant influence on ODC (ornithine decarboxylase) induction.

- Hansen et al. (1993) studied the urinary excretion patterns of 1-OH-P and  $\alpha$ -naphthol in urine in 2 patients. Each subject was treated once a day with coal tar pitch covering

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>50% of the skin. After 1 week, the urinary concentration of 1-OH-P and 1-naphthol increased approximately 100 times. However, after 3 weeks, the urinary concentration decreased to approximately the pre-experiment levels, even though the treatment remained unchanged.

- VanRooij et al. (1993) applied a dose of 2.5 mg/cm<sup>2</sup> pharmaceutical grade coal tar ointment for three 6-h periods to either the volar forearm, hand, neck, trunk, or calf of 8 male subjects. There

were significant differences in the total excreted amount of 1-OH-P between individuals, but no significant differences in the extent of urinary 1-OH-P excretion after coal tar application between the various skin sites.

- Santella et al. (1994) where 57 psoriasis patients and 53 untreated subjects applied either an ointment or gel-based coal tar product, or both, to the entire body surface at least once a day. The estimated exposure was 20 to 100 g of tars/day. Urinary PAH metabolites were elevated in patients compared with untreated subjects.

- Viau and Vyskočil (1995) had one male volunteer suffering from psoriasis of the scalp undergo treatment with a coal tar shampoo. A single treatment with the coal tar shampoo resulted in at least a 10-fold increase in the excretion of 1-OH-P.

- It should, however, be noted that the use of 1-OH-P as a marker for PAH exposure has been criticized because its levels fluctuate and decline after initial exposure, thus preventing constant monitoring of PAH exposure from coal tar (Hansen 1993).

#### Charge question on the public comments

4. In August 2013, EPA asked for public comments on an earlier draft of this assessment. Appendix G summarizes the public comments and this assessment's responses to them. Please comment on EPA's responses to the scientific issues raised in the public comments. **Please consider in your review whether there are scientific issues that were raised by the public as described in Appendix G that may not have been adequately addressed by EPA.**

It appears that EPA has adequately addressed each of the comments/opinions/issues. With regard to the comment on inclusion of studies of patients therapeutically treated with coal tar, EPA's response on the limitation of the single population-based case-control study was well-stated. In addition, it should be noted that the generalizability of the results in this study is limited, as 97.7% of the subjects are Caucasians, and that male predominantly occupied the "high risk" professions.

#### Other comments:

1. Page 1-1, line 9: add as in the statement "...it is often used as an indicator chemical to measure exposure to PAH...."

## **Dr. Joanne English**

### **Charge Question 1: Literature search/study selection and Evaluation**

#### **Preliminary comments:**

The process used for the literature search is clearly described. Figure LS-1 is helpful in identifying the criteria used for study selection/exclusion. In reviewing the initial literature search strategy keywords (Appendix C) it is noted that search terms for several systems (developmental, reproductive, and immunologic) were included, but no queries were made that included the term "cardio" (i.e., cardiotoxicity; cardiovascular; cardiopulmonary), "vascular," "athero\*," etc. Given that the authors identified some evidence of cardiovascular system effects, omission of these search terms might have resulted in bias in the assessment of this endpoint. Please address.

Similarly in the literature search secondary refinement, it is noted that certain potential target organs are included in the search terms (e.g., thymus, spleen), but not others (e.g., liver, kidney). Again, it is unclear that the assessment of all potential targets identified in the hazard identification section (specifically section 1.1.4) was comprehensive and how bias was avoided. Please address if other search terms should be included.

The literature search and study selection strategy does not appear to include a review of the references in the primary literature, which is recommended as a means to surface potentially relevant articles not identified through the systematic searching and manual screening processes. Please indicate if pertinent references cited in the primary literature were reviewed, and consider including this step explicitly in the literature search and study selection strategy.

Where possible, were universal characters on a root word used to include word variations (e.g., teratog! To locate "teratogen," "teratogenic" and "teratogenicity" )? This approach may reduce the number of search terms needed.

Additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer health effects of benzo[a]pyrene are listed below:

Aboutabl ME, Zordoky BN , El-Kadi AO . (2009). 3-Methylcholanthrene and benzo( a )pyrene modulate cardiac cytochrome P450 gene expression and arachidonic acid metabolism in male Sprague Dawley rats . *Br J Pharmacol* , 158 , 1808 – 19 .

Aboutabl ME , Zordoky BN , Hammock BD , El-Kadi AO . (2011) . Inhibition of soluble epoxide hydrolase confers cardioprotection and prevents cardiac cytochrome P450 induction by benzo(a)pyrene. *J Cardiovasc Pharmacol*, 57, 273– 81.

Davila D, Romero D, Burchiel S . (1996). Human T cells are highly sensitive to suppression of mitogenesis by polycyclic aromatic hydrocarbons and this effect is differentially reversed by alphanaphthoflavone . *Toxicol Appl Pharmacol* , 139 , 333 – 41 .

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Jeng HA, Pan CH, Diawara N , Chang-Chien GP , Lin WY , Huang CT , et al . (2011) . Polycyclic aromatic hydrocarbon – induced oxidative stress and lipid peroxidation in relation to immunological alteration . *Occup Environ Med* , 68 , 653 – 8 .

Knaapen AM , Curfs DM , Pachen DM , Gottschalk RW , de Winther MP , Daemen MJ , Van Schooten FJ . (2007) . The environmental carcinogen benzo[ a ]pyrene induces expression of monocyte-chemoattractant protein-1 in vascular tissue: a possible role in atherogenesis . *Mutat Res* , 621 , 31 – 41 .

N ' Diaye M , Le Ferrec E , Kronenberg F , Dieplinger H , Le Vee M , Fardel O . (2009) . TNF  $\alpha$  - and NF-  $\kappa$  B-dependent induction of the chemokine CCL1 in human macrophages exposed to the atherogenic lipoprotein(a) . *Life Sci* , 84 , 451 – 7 .

Oesterling E , Toborek M , Hennig B . (2008) . Benzo[ a ]pyrene induces intercellular adhesion molecule-1 through a caveolae and aryl hydrocarbon receptor mediated pathway . *Toxicol Appl Pharmacol* , 232 , 309 – 16.

Yang H , Zhou L , Wang Z , Roberts LJ II , Lin X , Zhao Y , Guo Z . (2009) . Overexpression of antioxidant enzymes in ApoE-deficient mice suppresses benzo( a )pyrene-accelerated atherosclerosis. *Atherosclerosis* , 207 , 51 – 8.

Wester P , Muller J , Slob W , Mohn G , Dortant P , Kroese E . (2012). Carcinogenic activity of benzo[ a ]pyrene in a 2 year oral study in Wistar rats. *Food Chem Toxicol*, 50, 927– 35

## **Charge question 2: Hazard identification (Section 1)**

### **General comment:**

It is noted in question 2, that the draft assessment evaluates the available human, animal, and mechanistic studies to identify the types of toxicity that can be credibly associated with benzo[a]pyrene exposure. The tabulated summaries for human and animal studies are organized by target organ or system effect (e.g., kidney toxicity; nervous system effects). Tabulated summaries for mechanistic studies do not appear to be included, and it is unclear how this information was integrated into the assessment of certain hazards (see comments on cardiovascular system effects, charge question 2.e. “Other types of toxicity”).

For animal studies, tabulated summaries include helpful information on study design (species, strain, sex, number per group, dose levels, route of administration and dosing regimen/duration) and study results. Additional context regarding the overall study results is often needed to interpret the findings for a specific endpoint, including available toxicokinetic information for the relevant dose range, if organ weight changes were or were not accompanied by histopathological changes; and observations that inform the general health status of animals under study.

### **Charge question 2c: Immunotoxicity (Sections 1.1.3, 1.2.1)**

#### **Preliminary comment:**

Section 1.1.3 begins with the assertion that there are no human studies evaluating immune effects following exposure to benzo[a]pyrene alone for any route of exposure, and then discusses occupational studies. Consider including some additional human data here (Davila et al., 1996; Allan et al. 2006; Jeng et al. 2011) before animal studies are discussed.

Table 1-8 provides a clear summary of the evidence pertaining to immune effects of Benzo(a)pyrene in laboratory animals. Evidence of immunotoxicity is supported by data from multiple end-points (thymus, spleen, immunoglobulin alterations) of limited predictive capability, in combination with the mode of action analysis that suggests biological plausibility. Figure 1-5 "**Exposure-response array for immune effects following oral exposure**" nicely illustrates the NOAELs and LOAELs for repeated dose studies, showing NOAELs consistently in the 3 to 30 mg/kg-day range. This range is considerably higher than the ranges identified for developmental and reproductive toxicity endpoints. Toxicokinetic data might be helpful to inform the interpretation of the hazard data obtained in animal studies; e.g., at what dose levels is metabolic induction occurring; at what dose levels does clearance become saturated?

The authors concluded there was suggestive evidence that immunotoxicity is a potential human hazard of benzo[a]pyrene exposure. Please explain what are the criteria for the "suggestive evidence" conclusion? The preamble (Preamble 5.5) descriptor for characterizing the overall weight of evidence does not appear to be applicable to the results reviewed for immune system effects, viz.:

***"Suggestive of a causal relationship:*** At least one high-quality epidemiologic study shows an association but other studies are inconsistent."

Please provide the relevant categories of evidential weight for causality for immune system effects, and state how the evidence for benzo(a)pyrene fulfills the criteria for "suggestive evidence" and why other levels of evidence (e.g., clear evidence or equivocal evidence) were not chosen.

### **Charge question 2d. Other types of toxicity (Section 1.1.4)**

#### **Preliminary comment:**

The draft assessment evaluates the available human, animal, and mechanistic studies to identify the types of toxicity that can be credibly associated with benzo[a]pyrene exposure. Section 1.1.4 "Other Toxicity" begins with the statement that there is some evidence that benzo[a]pyrene can produce effects in the forestomach, liver, kidney, and cardiovascular system, as well as alter hematological parameters, but that there is less evidence for these effects compared to organ systems described earlier in Sections 1.1.1–1.1.3 (i.e., developmental toxicity, reproductive toxicity, and immunotoxicity). Overall, EPA concluded

that the available evidence does not support these noncancer effects as potential human hazards.

The potential hazards identified in the introductory paragraph; i.e., forestomach toxicity; hematological toxicity; liver toxicity; kidney toxicity; cardiovascular toxicity; as well as nervous system effects, are then discussed in the subsequent paragraphs of section 1.1.4. As noted in the response to charge question 1 - **Literature search/study selection and Evaluation**, it is unclear as to whether the search was sufficiently comprehensive to identify studies relevant to addressing the identification of all of these other hazards.

### Forestomach Toxicity

The discussion of forestomach toxicity may be out of place in this section of 1.1.4 and the introductory paragraph, as there appears to be considerable evidence that the forestomach is a target of benzo(a)pyrene. The authors indicate that forestomach effects observed in rodents support a human hazard, noting that humans do not have a forestomach but do have similar squamous epithelial tissue in their oral cavity. Therefore, human relevance is not a basis for excluding the credible evidence of forestomach toxicity associated with benzo(a)pyrene exposure. As a preneoplastic (i.e., nonneoplastic) lesion, it can be logically concluded that the evidence does indeed support this noncancer toxicity as a potential human hazard. This conclusion is at odds with the overall conclusion for this section that the available evidence does not support forestomach effects as representing a potential human hazard.

In section 1.2.1 **Weight of Evidence for Effects Other than Cancer**, the authors state:

“Forestomach hyperplasia was observed following oral and inhalation exposure; however, this endpoint most likely reflects early events in the neoplastic progression of forestomach tumors following benzo[a]pyrene exposure (see Section 1.1.4), and was not considered further for dose-response analysis and the derivation of reference values.”

The authors' decision to not consider forestomach toxicity further for dose-response analysis and the derivation of reference values should not be used as a justification for excluding forestomach toxicity as a hazard credibly associated with benzo(a)pyrene exposure. Forestomach toxicity may reflect a tumor promoting key event in the tumorigenic mode of action, and thus reflect part of a combination mode of action discussed by the authors in the section “other modes of action.”

For these reasons, forestomach toxicity is credibly associated with benzo(a)pyrene exposure, so it is reasonable to identify it as such in the hazard identification section of the document. Since humans lack a forestomach, consider clarifying that in humans, such toxicity might manifest as esophageal or other gastrointestinal tract toxicity.

### Hematological toxicity

The summary of hematological toxicity is well done. The data suggest that dose rate may influence blood cell parameters, but not in a reproducible fashion. Changes are minimal or statistically insignificant at all but the highest dose levels (repeated oral dosing of 90 or 100 mg/kg-day). The studies presented provide little evidence of appreciable hematotoxicity by benzo(a)pyrene. Noting the general comment to charge question 2, based on the authors' summary, I agree with the conclusion that the studies presented do not provide convincing evidence that hematological effects are a human hazard of benzo(a)pyrene exposure.

#### Liver toxicity

The studies described in this section reporting noncancer effects of benzo(a)pyrene to the liver can be summarized as identifying reproducible organ weight changes (all three studies) without associated histopathology in two studies. In the 3rd study, increased liver oval cell hyperplasia was reported only at the highest dose level (90 mg/kg-day) following 35-day gavage dosing (DeJong et al 1999). Clarify that histopathology evaluations of the liver were (or were not) performed by Knuckles et al. 2001. Noting the general comment to charge question 2, based on the authors' summary, I agree with the conclusion that these studies do not provide convincing evidence that noncancer liver effects are a human hazard of benzo(a)pyrene exposure. The results of Wester, et al. 2012, (not cited) should also be addressed which may provide added support for this conclusion.

#### Kidney toxicity

In the three studies discussed, there is no consistent finding indicative of kidney toxicity. Noting the general comment to charge question 2, based on the authors' summary, I agree with the conclusion that these studies do not provide convincing evidence that noncancer kidney effects are a human hazard of benzo(a)pyrene exposure. The results of Wester, et al. 2012, (not cited) should also be addressed which may provide added support for this conclusion.

#### Cardiovascular toxicity

The discussion does not convincingly lead to the conclusion that cardiovascular toxicity is not a human hazard of benzo(a)pyrene exposure. There are multiple modes of action by which chemicals may adversely impact the cardiovascular system, and it is unclear if different lines of evidence (i.e. mechanistic, animal and human) were integrated for hazard identification. Several studies showing an influence of benzo(a)pyrene on the severity and progression of atherosclerotic plaques in animal models (as cited by Oesterling et al., 2008 – not included in this section) are not addressed. Other studies to consider as part of the weight of evidence evaluation, but not cited in this section are Knappen et al (2007 and Yang et al. (2009) which address the induction of atherosclerosis by benzo(a)pyrene in rodents; and Aboutabl et al., 2009 and 2011, which examine cardiac hypertrophy and cardiac biomarkers after benzo(a)pyrene exposure. The induction of inflammatory cytokines by benzo(a)pyrene (e.g., N'Diaye et al. 2009 – not cited; and N'Diaye et al. 2006 – cited on p 1-77) should be included as part of the weight-of-evidence discussion of cardiotoxicity. Additionally, it is unclear as to

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whether the designs of the animal studies reviewed were suitable to identify adverse cardiovascular effects. Although limited, the two epidemiology studies cited (Burstyn et al. 2005; Friesen et al. 2010) lend credence to possible human relevance of this endpoint.

Since cardiovascular effects were identified in rats and mice effects following gestational exposures to benzo(a)pyrene, address whether such findings should be considered as part of the weight of evidence for the cardiovascular system as a potential adult target of benzo(a)pyrene exposure.

It is unclear at this time as to whether the search was sufficiently comprehensive to identify studies relevant to addressing the identification of cardiovascular system toxicity of benzo(a)pyrene exposure (see preliminary comments to charge question 1 – literature search/study selection and evaluation). Please address the references that are missing; if they were excluded, the basis for their exclusion. If not intentionally excluded, include the missing references as part of the weight of evidence evaluation, and be explicit as to the reasoning for concluding that the available evidence either does or does not support cardiovascular system toxicity as a potential human hazard.

#### Nervous system effects

This paragraph briefly describes 13 articles that address nervous system effects of benzo(a)pyrene in laboratory animals and concludes with a statement that “These data are consistent with the neurobehavioral effects observed following developmental exposure and suggest that benzo(a)pyrene exposure could be neurotoxic in adults.” However, only two of these studies were identified as informing the neurotoxic potential of benzo(a)pyrene exposure in adult animals following subchronic or chronic oral exposure and included in Table 1-9. Since hazard identification does not rely only on repeated subchronic or chronic exposure scenarios alone, it is not clear why the other studies discussed in this section were not also summarized in Table 1-9; thus Grova et al. 2007; *ibid* 2008; Saunders et al. 2001, *ibid* 2002; *ibid* 2006; Liu et al. 2002 ; Maciel et al. 2014; Chen 2011; Qiu et al. 2011; Xia et al. 2011; Bouayed et al. 2012) are all left out of the table. Considering the relatively low doses in laboratory animals at which behavioral alterations were reported to be observed, the reasoning for not considering the adult nervous system as a potential human target is unclear.

Since neurobehavioral effects were identified in rats and mice effects following gestational exposures to benzo(a)pyrene, address whether such findings should be considered as part of the weight of evidence for the nervous system as a potential adult target of benzo(a)pyrene exposure.

Decrements in short term memory were reported in two studies of workers exposed occupationally to PAH mixtures containing Benzo(a)pyrene (Niu et al. 2010; Qiu et al. 2013), lending possible credence of human relevance of this endpoint.

Be explicit as to the reasoning for concluding that the available evidence either does or does not support adult nervous system effects as a potential human hazard.

**Charge question 3a. Oral reference dose for effects other than cancer (section 2.1.3. Uncertainty factors)**

**Preliminary comments:**

For all endpoints, the intraspecies uncertainty factor (UF) chosen was 10x, and it is stated that insufficient information is available to derive a quantitative estimate of variability in human susceptibility. Addressing variation within the human population, if the critical effect was in a known sensitive population, a value of less than 10 may be used. It is asserted in the document that the developing fetus is the most susceptible human subpopulation to benzo(a)pyrene; so to the extent that the rodent developing fetus is a suitable model for humans, a value less than 10x could be considered for the intraspecies UF applied in Jules et al. 2012. Thus, the default 10x factor may not be warranted since the point of departure for developmental cardiovascular effects is based on exposure of a sensitive subpopulation. Since some uncertainty remains as to the variability in the susceptibility of the human developing fetus, infant and newborn to benzo(a)pyrene, a 3x UF is appropriate. A similar conclusion might be reached for the point of departure for early postnatal developmental neurobehavioral effects in Chen et al. 2012, as it is asserted under “**Susceptible Populations and Lifestages**” that the early postnatal period is also a period of heightened susceptibility to exposure to benzo(a)pyrene. However, it is as yet unclear whether the developing nervous system is more susceptible than the adult nervous system to benzo(a)pyrene exposure, based on the low dose effects in adult animals reported by Chengzhi et al. 2011 and Bouayed et al. 2009. Therefore the 10x intraspecies uncertainty factor is appropriate for the neurodevelopmental effects reported by Chen et al. 2012.

An interspecies uncertainty factor, UFA, of 3 ( $10^{1/2} = 3.16$ , rounded to 3) was applied, to all PODs except Chen et al. (2012), (neurobehavioral effects) because BW<sup>3/4</sup> scaling is being used to extrapolate oral doses from laboratory animals to humans. Justification provided was the absence of information on whether allometric (i.e., body weight) scaling holds when extrapolating doses from neonatal animals to adult humans due to presumed toxicokinetic and/or toxicodynamic differences between lifestages. Clarify why the required extrapolation is from neonatal animals to adult humans, and not from neonatal animals to neonatal humans.

Application of subchronic to chronic UF of 1x in the case of developmental endpoints is appropriate. An UF value of 10 was applied when the POD was based on studies that were 42–90 days in duration. A value of 10x is an appropriate default for studies that are subchronic (90-days) in duration, approximating 1/10 of the lifespan. For studies less than 90-days in duration, please provide justification for their use in deriving a chronic oral RfD.

Application of LOAEL to NOAEL UFs appear appropriate based on the information presented.

Selection of a database deficiency UF of 3x for all POD. The lack of a multigenerational reproduction study and lack of a neurodevelopmental toxicity study that includes exposure during gestation through lactation are identified as the data deficiencies. This is appropriate justification for the 3x UF for database sufficiency.

## Dr. Michael Foster

Responses to Assigned Questions: #3b, #3d, #4, and #5.

### 1. Question 3b.

Inhalation reference concentration for effects other than cancer (section 2.2). The draft assessment proposed an overall ref conc of  $2 \times 10^{-6}$  mg/m<sup>3</sup> based on decreased fetal survival during a critical window of development. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-analysis, calculating points of departure, and applying uncertainty factors?

Assignment: **2.2.1. Identification of studies and effects for dose-response analysis** – McIntyre, Foster, Walter.

Question 3b is focused on animal model (rodent) studies that support inhalation reference concentration for effects (developmental and reproductive toxicities) other than cancer. Reports in a female rat model were supportive of developmental (hippocampal downregulation in F-1 generation)(Wormley et al, 2004) and fetal survival (pregnant dams)(Archibong et al, 2002) effects following gestational inhalational exposures to B(a)P. A subsequent publication by Archibong et al, 2012, further supports reproductive effects (female ovarian function) of gestational exposures to B(a)P, and replicated the inhalational experimental design (dose response) of the earlier Archibong, et al, 2002 report. The Archibong et al, 2012 report (Reproductive Tox 34:635-43) was not included as a citation and reviewed for non-cancer effects and dose-response analysis (likely due to lateness of the report with respect to the timing of the searched publication data base prepared for the 2013 IRIS draft). Additional rodent model support of developmental effects of multiple doses and inhalational exposure of B(a)P is the report by Mackenzie and Angevine (1981) and summarized in Table 1-2 (pgs. 1-5 and 1-6) using an outbred female mouse model (CD-1). This same report additionally was expanded to include reproductive effects upon F-1 generation females and subsequent viability and litter size of F-2 generation.

With respect to section **2.2.2. Methods of analysis**, in this section, on pg. 2-18, reference is made to Table 2-4 that contains summary information on female and male rat models with respect to developmental and reproductive effects (non-cancer). Appears that for some of the results summarized for male rat models and fertility outcomes, that a report by Ramesh et al, 2008 (Exp Toxicol Path 60:269-80) is not identified as a source of some of the summary information listed in Table 2-4.

Question 3b continued: does the discussion of exposure scenarios (**section 2.2.5**) reflect the scientific considerations that are **inherent** for exposures during a critical window of development?

The rodent model studies (rats and mice) appear to be highly supportive of susceptibility of F-1 generation offspring to endure developmental and reproductive effects following gestational

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exposures and translational to associative developmental results observed for humans (Table 1-1, pg.1.4-1.5).

## 2. Question 3d.

Inhalation unit risk for cancer (section 2.4). The draft assessment proposes an inhalation unit risk of 0.6 per mg/m<sup>3</sup> based on a combination of several types of benign and malignant tumors in hamsters. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?

Assignment: **2.4.1. Analysis of Carcinogenicity Data (Choice of Studies)** – Foster, Moorthy, Schlesinger

Question 3d is focused on inhalation unit risk and the assignment for 2.4.1 is to critique choice of studies that support the inhalation unit risk. Recalling the overview slide (slide #16, in the presentation by Ms. Kathleen Newhouse overviewing the Draft Assessment) during our tele-conference of March 4, 2015, a principal study was identified [Thyssen et al, 1981; experimental design: adult hamster model (sex ?) with daily (3-4.5 hr/d) life time B(a)P submicronic aerosol exposures by nose-only inhalation, over average survival durations of 60 to 96 weeks and dose response readouts of body weight, and incidence and latency of tumors with segmental distributions, i.e., URT, trachea, lung, oro-pharynx, esophagus, and forestomach]. This report was relied upon by EPA due to the merits of seemingly being the “only inhalation route cancer bioassay available” (see Executive Summary, section on Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure, pg. xxxviii). Further support for reliance on the Thyssen et al, 1981, report, arises from a subsequent short communication by this same research group (see, J Pauluhn et al, 1985, Exp Path 28:31) and although limited in scope the survival results and presence of neoplastic alterations appeared replicatable with the experimental design in the hamster model for low B(a)P aerosol doses by nose-only inhalation.

Consistent with the incidence in a rodent model of URT and tracheal tumors reported by Thyssen et al, 1981, are numerous human epidemiologic studies that demonstrate/suggest associations between PAH related occupations and lung cancer (see Table 1-11, for summary of epidemiologic based reports of B(a)P in relation to lung cancer risk for Tier1 studies, pgs. 1-55 to 1-56) by Armstrong and Gibbs, 2009; Spinelli et al, 2006; and Xu et al, 1996, for aluminum smelter and iron-steel industry workers. For epidemiologic approaches, difficulties arise from exposure to source mixtures of PAHs and not just B(a)P and confounding by habituation and/or co-exposure to smoke products. A recent 2014 review (Gibbs and Labreche, JOEM, 56: S40-S48) of epidemiologic evidence associating increased risks of lung and bladder cancers with aluminum industry workers and occupational exposures to coal tar pitch volatiles, adds to the convincing association of lung cancer incidence in workers at differing locations world-wide.

Assignment: **2.5.5 Uncertainties in the Derivation of the Dermal Slope Factor** – All team members.

Derivation methods are not in my expertise and as well a significant familiarity with dermal exposure risk assessment. However background is clearly presented in the draft by EPA on the derivation of the dermal slope factor in section 2.5.5; and the Executive Summary (pg. xxxix) clearly identified the NIOSH report by Sivak and co-authors, 1997, for the data base used

by EPA to dose-response analysis and extrapolation to lifetime cancer risk following dermal exposure to B(a)P. A statement in the Executive Summary (same pg. xxxix) clearly acknowledges that the dermal slope factor "has been derived for a local effect, and it is not intended to estimate systemic risk of cancer following dermal absorption of B(a)P into the systemic circulation.

### 3. Question 4.

**Executive Summary.** Does the executive summary clearly and appropriately present the major conclusions of the assessment? – McIntyres, Foster, Gennings, Li, Lichtveld, Roberts

This is a fairly broad assignment; and my comments reflect upon the section of the Executive Summary focused on inhalation exposure with effects other than cancer (pg. xxxvi), and although concise, the conveyed information in the Executive Summary for this section is appropriate and centers on fetal survival, and neurodevelopmental effects and reproductive results for both sexes citing credible animal model studies as presented in Table 1-1 on Draft pgs.1.5 to 1.7.

With respect to the section on Confidence in the Overall Inhalation RfC (pg. xxxvii) it is not clear to me on how EPA may determine the degree of confidence (e.g., low-medium, in Table ES-2, pg. xxxvii) and thus would be helpful to perhaps provide a brief background on how confidence ranking is accomplished. The

Archibong et al, 2002, report cited in the section, has been to some degree replicated with a recent 2012 publication by this same group (Archibong et al, Reproductive Tox 34:635-43, perhaps a review of this recent citation would raise the ranking of the degree of confidence).

### 4. Question 5.

Charge question on the **public comments**. In August 2013, EPA asked for public comments on an earlier draft of this assessment. Appendix G summarizes the public comments and this assessment's responses to them. Please comment on EPA's responses to the scientific issues raised in the public comments. – Bartell, Baynes, Choi, DiGiovanni, Foster, Kissell, Poirer, Portier, Roberts, Schlesinger, Stayner, Stern, Vorhees

Section G is quite extensive (~12.5 pages, text dense, with additional 1.5 pgs of an example calculation). Overall the EPA Responses seem straight forward and direct in response to the public comments. Although the public comments are grouped by section (e.g., Additional Literature, Weight of Evidence, .....), it would seem to be helpful and perhaps easier to scan/read through, if the public comments were identified by numbering (e.g, 1,2, 3 .....).

Based on my own scientific background I focused on public comments in reference to Comments on the Inhalation Unit Risk (pgs. G-7 to G-9) and these comments largely dealt with the Thyssen et al, 1981 report of life time exposure of B(a)P in a hamster inhalation model. Public comments related to: a) respiratory particle overload, b) discrepancies in neoplastic incidence, c) differences in the numbers of animals at risk between the EPA analysis and as reported by Thyssen and co-authors, and d) to exposure dose variability during the course of the

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experimental design; for each of these comments, the responses by EPA seemed straightforward and responsive. In cases where appropriate, revisions were introduced by EPA (for example, Table D-13 of the Supplemental Information, on the incidence of benign vs malignant tumors, with respect to public comments from Arcadis and EPRI, pg. G-8).

## Dr. Helen Goeden

### 2. Literature Search/Study Selection and Evaluation.

*[All members – lead discussants Goeden & Li]*

The process for identifying and selecting pertinent studies for consideration in developing the assessment is detailed in the *Literature Search Strategy/Study Selection and Evaluation* section. Please comment on whether the literature search approach, screening, evaluation, and selection of studies for inclusion in the assessment are clearly described and supported. Please comment on whether EPA has clearly identified the criteria (e.g. study quality, risk of bias) used for selection of studies to review and for the selection of key studies to include in the assessment. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of benzo[a]pyrene

*Preliminary Response:*

*This section provides a concise high level, general description of the literature search strategy and study selection process conducted for benzo[a]pyrene (BaP). It should also be clear that there are additional steps beyond those portrayed in Figure LS-1. Once the chemical specific studies deemed relevant by EPA are identified additional literature to inform modes/mechanisms of action or specific areas of uncertainties may be searched for.*

*While Figure LS-1 provides general exclusion/inclusion criteria the reader has no efficient way to identify which specific references were excluded and why. It would be helpful if the HERO database search selection criteria could include a tag for 'not considered' along with an short explanation (e.g., duplicate). Likewise, the reader has no efficient way of understanding the utility of the retained/included references. For studies that EPA has chosen to provide study summaries in the Supplemental Information section a concise tabular summary that includes strengths/limitations and utility of the selected studies would greatly improve the clarity and transparency of the assessment.*

*It is noted within the document that studies with mixtures of chemicals were excluded. BaP, except in the laboratory, virtually never exists in isolation. Since this is the case risk assessments of BaP will virtually always require an assessment of BaP within a PAH mixture. At a minimum data from studies which examined both the effects of BaP alone and the effects of a PAH mixture containing BaP should be included in the current assessment as this information is type of information is essential for conducting risk characterization of BaP. EPA has undertaken an assessment of PAH mixtures and it is understandable that that effort should not be duplicated here. However, acknowledgement of what is generally known regarding synergistic, antagonistic, or additive relationships should be included in the current assessment.*

*This section does not describe how assessments by other national and international health organizations were identified or used within the current assessment.*

*Additional studies of potential interest include:*

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*Chepelev et al. Crit Rev Toxicol 2015, 45(1):44-52. Integrating toxicogenomics into human health risk assessment: Lessons learned from the benzo[a]pyrene case study.*

*Moffat et al. Crit Rev Toxicol 2015, 45(1):1-43. Review Article. Comparison of toxicogenomics and traditional approaches to inform mode of action and points of departure in human health risk assessment of benzo[ a ]pyrene in drinking water.*

*Zaccaria & McClure. Int J Toxicol 2013, Jul 32(4):236-50. Using immunotoxicity information to improve cancer risk assessment for polycyclic aromatic hydrocarbon mixtures.*

*Zhao et al. Food Chem Toxicol 2014, Jul 69:244-251. Exposure of mice to benzo(a)pyrene impairs endometrial receptivity and reduces the number of implantation sites during early pregnancy.*

*Health Canada has also released a draft document: "Benzo[a]pyrene in Drinking Water" as well, which can be found at: <http://www.hc-sc.gc.ca/ewh-semt/consult/2015/bap/draft-ebauche-eng.php>*

### **3. Hazard identification.**

In section 1, the draft assessment evaluates the available human, animal, and mechanistic studies to identify the types of toxicity that can be credibly associated with benzo[a]pyrene exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/backgrd.html/>) to reach the following conclusions.

#### *Preliminary Response:*

*Hazard identification is the process of identifying the type of hazard to human health (e.g., cancer, birth defects). Key aspects of hazard identification include identifying which health endpoints are of most concern (e.g., most sensitive - occurring at lower exposure doses than other endpoints) as well as toxicokinetics/toxicodynamics and potential MOAs as they relate to the health endpoints identified and susceptible populations (EPA 2014 Framework for Human Health Risk Assessment to Inform Decision Making).*

*The readability of this section would be greatly improved by the incorporation of:*

- 1) An introductory paragraph which outlines the purpose of the Hazard Identification section;*
- 2) Inclusion of an overview of toxicokinetics/toxicodynamics as they relate to health endpoints and potential susceptible populations; and*
- 3) A summary paragraph which addresses the strengths and limitations of the data, including areas for which data may be unavailable (data gaps), and describes how the results of the Hazard Identification are used in the subsequent Dose Response section.*

#### **2a. Developmental toxicity** (sections 1.1.1, 1.2.1).

*[Choi, McIntyres, Vorhees, Levin, Li, Poirier. Lead discussants: Levin, Vorhees]*

The draft assessment concludes that developmental toxicity and developmental neurotoxicity are human hazards of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?

*Preliminary Response:*

*Yes, the available human, animal, and mechanistic studies support the conclusion that BaP is a developmental hazard. The utility of the exposure-response array (Figure 1-2) could be improved by providing more context regarding dose comparisons. Currently the exposure-response arrays contain a mix of species and administration (e.g., gavage to pregnant animal, lactational exposure and direct dosing to neonatal animals) making true comparison across studies difficult. Chen et al 2012 directly dosed neonatal rats (PND5-11) whereas Bouayed et al 2009 exposed neonatal mice (PND1-14) via mother's milk and in McCallister et al 2008 fetal rats (GD14-17) were exposed in utero. This information is contained within Table 1-4 and at a minimum should be noted in Figure 1-2 to provide needed context. Calculation and presentation of Human Equivalent Doses (HEDs) should be also considered.*

*Toxicokinetic information regarding fetal exposures (e.g., Shendrikova and Aleksandrov, 1974. Comparative penetration of polycyclic hydrocarbons through the rat placenta into the fetus. Bull. Exp. Biol. Med., 77(2): 169–171) and lactational transfer should be included as they inform the comparative doses to developing organisms at different stages of development and exposed via different routes of administration. For example, it is likely that the neonatal animals directly dosed by Chen et al. received a higher dose than the developing organisms exposed to a comparable maternally administered dose in Bouayed et al and McCallister et al.*

**2b. Reproductive toxicity** (sections 1.1.2, 1.2.1).

*[McIntyres, Moorthy, Poirier, Walter. Lead discussants: McIntyres & Walter]*

The draft assessment concludes that male and female reproductive effects are a human hazard of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?

*Preliminary Response:*

*Yes, the available human, animal, and mechanistic studies support the conclusion that BaP is a reproductive hazard. The utility of the exposure-response arrays (and possibly the evidence tables as well) could be improved by presenting Human Equivalent Dose (HED) levels. This would greatly improve the cross study comparison. Currently the exposure-response arrays contain a mix of species making true comparison across studies difficult.*

**2c. Immunotoxicity** (sections 1.1.3, 1.2.1).

*[Burchiel, Choi, English. Lead discussants: Burchiel & Choi]*

The draft assessment concludes that immunotoxicity is a potential human hazard of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?

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*Preliminary Response:*

*Yes, the available human, animal, and mechanistic studies support the conclusion that BaP is a potential immunotoxicity hazard.*

**2d. Other types of toxicity** (section 1.1.4).

*[Burchiel, Choi, English, Li, Ramos, Moorthy, Vorhees. Lead discussants: English & Moorthy]*  
The draft assessment concludes that the evidence does not support other types of noncancer toxicity as a potential human hazard. Are there other types of noncancer toxicity that can be credibly associated with benzo[a]pyrene exposure?

*Preliminary Response:*

*The basis for arriving at this conclusion needs to be expanded for each of the health endpoints listed. The current text does not provide adequate rationale for why the evidence does not support hazard identification. Is the reason insufficient of data, inconsistent data, or sufficient data to conclude that the health endpoints is not a sensitive endpoint?*

*The information provided within Section 1.1.4 Forestomach Toxicity is not consistent with the conclusion drawn by EPA. There is clear evidence that BaP exposure causes forestomach hyperplasia and hyper keratosis. If it is EPA's policy that preneoplastic lesions cannot be used as the basis for deriving noncancer toxicity values or this effect is considered irrelevant to humans this should be clearly stated along with supporting rationale.*

*The evidence provided for hematological toxicity appears to be limited and suggests only a marginal effect on hematological parameters as the magnitude of the alterations may not be biologically significant.*

*The evidence provided for liver and kidney toxicity appears to be limited and suggests that while effects may be observed at higher exposure levels it does not appear to be a sensitive health endpoint.*

*The evidence provided for cardiovascular toxicity and adult neurotoxicity suggests potential toxicity at low dose levels, however, the data is too limited to utilize quantitatively. It is not clear why evidence pertaining to cardiovascular toxicity are not included in Table 1-9.*

*Relevant recently published articles include:*

*Gan et al. 2012. Biomed Environ Sci 25(5):549-56. Effects of benzo(a)pyrene on the contractile function of the thoracic aorta of Sprague-Dawley rats.*

*Jayasundara et al. 2015. Tox Sci 143(2):469-81. AHR2-Mediated Transcriptomic Responses Underlying the Synergistic Cardiac Developmental Toxicity of PAHs.*

*Liang et al. 2014. J Toxicol Sci 39(5):739-48. Adverse effect of sub-chronic exposure to benzo(a)pyrene and protective effect of butylated hydroxyanisole on learning and memory ability in male Sprague-Dawley rat.*

*Uno et al. 2014. Toxicology 316:34-42. Protective role of cytochrome P450 1A1 (CYP1A1) against benzo[a]pyrene-induced toxicity in mouse aorta.*

2e. **Cancer** (sections 1.1.5, 1.2.2).

*[Burchiel, DiGiovanni, Goeden, Moorthy, Poirier, Ramos, Stayner, Stern. Lead discussants: Poirier, Stayner]*

The draft assessment concludes that benzo[a]pyrene is “carcinogenic to humans” by all routes of exposure. Do the available human, animal, and mechanistic studies support this conclusion?

*Preliminary Response:*

*According to the EPA 2005 Cancer Guidelines the descriptor “carcinogenic to humans” is applied when there is strong evidence of human carcinogenicity. Combinations of the following evidence can be used to justify this classification:*

*Strong epidemiological evidence of an association between human exposure and either cancer or the key precursor event(s) of the mode of action but not enough for a causal association and there is extensive evidence of carcinogenicity in animals, and the mode(s) of carcinogenic action and associated key precursor events have been identified in animals, and 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.*

*The information provided in Section 1.2.2 and summarized in Table 1-18 does address each of the pieces of evidence necessary to identify BaP as “carcinogenic to humans”. This classification is consistent with IARC’s 2010 classification and Health Canada’s 2015 draft classification. While it is true that these assessments were prepared for different purposes, using different guidelines and methods it would be appropriate to include reference to these assessments in section 1.2.2.*

4. **Dose-response analysis.** In section 2, the draft assessment uses the available human, animal, and mechanistic studies to derive candidate toxicity values for each hazard that is credibly associated with benzo[a]pyrene exposure in section 1, then proposes an overall toxicity value for each route of exposure. The draft assessment uses EPA’s guidance documents (see <http://www.epa.gov/iris/backgrd.html/>) in the following analyses.

3a. **Oral reference dose for effects other than cancer** (section 2.1).

*[Bartell, Gennings, Levin, McIntyres, English, Hays, Roberts, Stern, Vorhees. Lead discussants: Stern & Bartell]*

The draft assessment proposes an overall reference dose of  $3 \times 10^{-4}$  mg/kg-d based on developmental toxicity during a critical window of development. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.1.5) reflect the

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scientific considerations that are inherent for exposures during a critical window of development?

*Preliminary Response:*

*The rationale provided by EPA for not selecting Bouayed et al 2009 for dose response analysis was because the doses were higher than Chen et al. However, Chen et al used direct dosing of 0.02, 0.2 and 2 mg/kg-d to neonatal rats (PND5-11) whereas lactating mice were dosed in Bouayed et al and exposure of neonatal mice PND1-14 was via milk. It is likely that the actual doses to the developing organisms in Bouayed et al were significantly lower than the maternal doses of 2 and 10 mg/kg-d. Toxicokinetic issues such as fetal and milk transfer vs direct dosing should be discussed.*

*Calculation of an HED was not done for Chen et al 2012 because doses were administered directly to neonatal animals. It is true that EPA 2011 recommends that allometric scaling not be done when extrapolating doses from neonatal animals to human adults. However, EPA 2011 acknowledges that there are instances where extrapolation from the young animal to a young human exposure may be desirable. When doing such an extrapolation key developmental processes need to be matched in a species-dependent manner, because the temporal pattern of development differs across species.*

*It is not clear why EPA did not consider extrapolating from neonatal animals to the corresponding life stage in humans. For example (for illustrative purposes only) if body weight data for the neonatal rats from PND5-11 and humans from birth to 2 years of age is used a DAF of approximately 0.2 is calculated. Rationale for why the standard default uncertainty factor rather than extrapolating from neonatal animals to the corresponding human life stage is preferable should be added, if that is actually the case.*

*The rationale provided for the UF selection is reasonable. The rationale provided for application of a full 10 subchronic-to-chronic UF should be expanded. Was the available data evaluated for information regarding increased severity or additional effects or decreasing PODs with increasing duration?*

**3b. Inhalation reference concentration for effects other than cancer** (section 2.2).

*[McIntyres, Foster, Goeden, Schlesinger, Walter. Lead discussants: McIntyres & Schlesinger]*

The draft assessment proposes an overall reference concentration of  $2 \times 10^{-6}$  mg/m<sup>3</sup> based on decreased fetal survival during a critical window of development. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.2.5) reflect the scientific considerations that are inherent for exposures during a critical window of development?

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*Preliminary Response:*

*Within the limitations of the available data the proposed overall reference concentration is scientifically supported. The exposure scenario discussion accurately reflects considerations regarding critical (and noncritical) windows of exposure.*

**3c. Oral slope factor for cancer** (section 2.3).

*[Bartell, DiGiovanni, Gennings, Portier, Roberts. Lead discussants: DiGiovanni & Portier]*

The draft assessment proposes an oral slope factor of 1 per mg/kg-d based on alimentary tract tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?

*Preliminary Response:*

*The proposed oral slope factor is scientifically supported. It would be relevant and appropriate to reference oral slope factors independently derived by other health organization. For example, oral slope factors recently derived by CalEPA OEHHA (2012 Public Health Goal for BaP) (1.7 per mg/kg-d) and Health Canada (2015 draft) (1.275 per mg/kg-d) are of similar magnitude.*

*It appears that data on mixtures from studies which evaluated both BaP alone and PAH mixtures containing BaP were excluded. BaP, except in the laboratory, virtually never exists in isolation. Since this is the case, risk assessments of BaP will virtually always require an assessment of BaP within a PAH mixture. Data from studies which examined both the effects of BaP alone and the effects of BaP within a PAH mixture (e.g., Culp et al 1998) should be included in the current assessment as this information is essential for conducting environmental risk characterization of BaP.*

*EPA has undertaken an assessment of PAH mixtures and it is understandable that that effort should not be duplicated here. However, acknowledgement of what is generally known regarding synergistic, antagonistic, or additive relationships should be included in the current assessment.*

**3d. Inhalation unit risk for cancer** (section 2.4).

*[Bartell, Foster, Gennings, Moorthy, Portier, Schlesinger. Lead discussants: Foster & Bartell]*

The draft assessment proposes an inhalation unit risk of 0.6 per mg/m<sup>3</sup> based on a combination of several types of benign and malignant tumors in hamsters. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?

**3e. Dermal slope factor for cancer** (section 2.5).

*[Bartell, Baynes, Bunge, Choi, DiGiovanni, Gennings, Hays, Kissel, Portier, Roberts, Stayner. Lead discussants: Baynes & Bartell]*

The draft assessment proposes a dermal slope factor of 0.006 per ug/day based on skin tumors in mice. Is this value scientifically supported, giving due consideration to the

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intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and scaling from mice to humans? Does the method for cross-species scaling (section 2.5.4 and appendix E) reflect the appropriate scientific considerations?

**3f. Age-dependent adjustment factors for cancer** (section 2.6).

[DiGiovanni, Goeden, Poirier, Ramos, Stern. Lead discussants: Ramos & Goeden]

The draft assessment proposes the application of age-dependent adjustment factors based on a determination that benzo[a]pyrene induces cancer through a mutagenic mode of action (see the mode-of-action analysis in section 1.1.5). Do the available mechanistic studies in humans and animals support a mutagenic mode of action for cancer induced by benzo[a]pyrene?

*Preliminary Response:*

*Yes, the available mechanistic studies support a mutagenic mode of action. Data demonstrating increased early life sensitivity to mutagenic mode of action carcinogens presented in the Supplemental Guidance (EPA 2005b) included data for BaP. Evaluations by several other health organizations have also identified mutagenicity as the primary mode of action for BaP.*

**5. Executive summary.**

[McIntyres, Foster, Gennings, Li, Lichtveld, Roberts. Lead discussants: Li & Roberts]

Does the executive summary clearly and appropriately present the major conclusions of the assessment?

**6. Charge question on the public comments**

[Bartell, Baynes, Choi, DiGiovanni, Foster, Kissell, Poirier, Portier, Roberts, Schlesinger, Stayner, Stern, Vorhees. Lead discussants: Roberts & Stern]

In August 2013, EPA asked for public comments on an earlier draft of this assessment. Appendix G summarizes the public comments and this assessment's responses to them. Please comment on EPA's responses to the scientific issues raised in the public comments. Please consider in your review whether there are scientific issues that were raised by the public as described in Appendix G that may not have been adequately addressed

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## Dr. Sean Hays

### Notes

#### RfD

- Jules et al – Is it appropriate to do D-R when data was reported for only top two doses.
- Bouayed et al. (2009a) and Mackenzie and Angevine (1981) – EPA did not use these, concluding that they used higher doses. The more important question is whether they produced consistent results.
- DAF:
  - BW<sup>3/4</sup> for adult dosing is appropriate.
  - BW scaling for early postnatal dosing? Not sure of approach. We have an opinion by time of meeting.
- Table 2-1: It would be good to insert a column showing the DAF for each calculation (row)
- UF\_D: Are there any PAH mixture studies that fill these data gaps?
- Table 2-2: It would be helpful to include the Confidence score (from Table 2-3) in Table 2-2. This helps the reader understand the conclusions in Table 2-3. It would be nice to include a column that has minor notes as to the justification for the Confidence score.

#### Dermal Slope Factor

##### 2.5.1

- Why cite ATSDR, IARC, IPCS for evidence for human carcinogenicity? Cite primary literature.
- 2-39, line 32; Instead of “shorter studies”, this should read “studies of shorter than lifetime duration”.

##### 2.5.2

- Time to tumor modeling & adjustments for time to tumor? Will have an opinion by time of meeting.

##### 2.5.4 Cross-species scaling of dosimetry

- No dose measure related to mg/cm<sup>2</sup>/day
  - mg/BW<sup>3/4</sup> – EPA's chosen approach, although, the report seems to indicate they used ug/d as the ultimate dose metric.
  - 1 to 1
  - mg/%skin
  - mg/kg
- Assumptions
  - Equal TK across species
  - Lifetime exposures
  - Linear below POD

##### 2.5.5 Uncertainties

## 2.6 ADAFs

- Dermal example (Table 2-15) uses a dose metric of ug/d. This example should use EPA's recommended approach ( $BW^{3/4}$ ). It's not really clear which dose metric EPA is actually advocating.

## Appendix E

- Less than Lifetime dose adjustment (pg E-75): "Equivalent lifetime doses were estimated by multiplying the relevant average daily doses by  $(L_e/104)^3$ , where  $L_e$  is the length of exposure, based on observations that tumor incidence tends to increase with age (Doll, 1971). Note that exposure periods <52 weeks would lead to a relatively large adjustment [i.e.,  $(52/104)^3 = 0.125$ , or an eightfold lower dose than administered], reflecting considerable uncertainty in lifetime equivalent dose estimates generated from relatively short studies.
- Table E-19.
  - It seems that the footnote 'c' should be applied to the animal in the middle dose group examined at day 663.
  - The low dose (0.014 ug/d) is a NOAEL
- E-112: Alternative Cross Species Scaling Factors
  - Approach 2:
    - Should be accounting for SA of dose of BaP applied. Text seems to indicate this is not the case. The description of the "Assumptions" does not seem correct.
    - They use 100 cm<sup>2</sup> as the SA for mice. Is this total body surface area or the area of skin painted in the D-R study? This seems to be the SA of an entire mouse. If so, this is not the correct approach.

## **Overall comments:**

### **RfD**

- Why not a dermal RfD?

### **Dermal Slope Factor**

- I do not agree with dose metric being used. It should be scaled to surface area of skin exposed in mouse painting studies. See Knafla et al. 2010 for a good start on how to do this.
- I'm afraid this slope factor is a valid slope factor for mice being painted for a lifetime to BaP alone and in a solvent (acetone) and is not valid for humans exposed to real world BaP/PAH exposures (in soil, etc.). I will have more to say at the meeting.
  - I would like to see more discussion of data on relative absorption of BaP into skin when applied in a solvent and when applied in relevant environmental matrix (soil, etc.).

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- This type of information should be provided to give guidance as to how the BaP dermal slope factor should be used for assessing risks from environmental studies.
- Could more studies be included in the D-R modeling using pooling amongst studies?
- Are there no PAH painting studies to base a dermal slope factor? I would like to see the potency of PAH mixture versus BaP alone when applied in the same solvent carrier. Again, this gets to whether the dermal slope factor derived by EPA has any utility beyond assessing risks to mice painted with BaP.
- I would like to see EPA do some calculations using their dermal slope factors to predict risks of skin cancer amongst some known human cohorts (e.g., psoriasis patients treated with relevant treatment regimens). A real world test like this would help determine whether this dermal slope factor is anywhere near valid. While a slope factor is not meant to be predictive, it would be nice to know it isn't overly conservative and/or is valid for more than just estimating risks to mice painted with BaP in solvent.

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**Dr. Ed Levin**

## **Literature Search Strategy/Study Selection and Evaluation**

Question #1

*Is the literature search strategy well documented?*

Yes, the authors of the review have done an excellent job describing their well-considered search strategy.

*Please identify additional peer-reviewed studies that might have been missed.*

Answer: None identified

**Hazard Identification Question #2a. Developmental Toxicity, Discussion Leaders:** Levin, Vorhees

***Hazard identification.** In section 1, the draft assessment evaluates the available human, animal, and mechanistic studies to identify the types of toxicity that can be credibly associated with benzo[a]pyrene exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/backgrd.html/>) to reach the following conclusions.*

***2a. Developmental toxicity** (sections 1.1.1, 1.2.1). The draft assessment concludes that developmental toxicity and developmental neurotoxicity are human hazards of benzo[a]pyrene exposure. Do the available human and animal studies support this conclusion?*

Answer: The evidence from the human and animal studies provides good evidence that benzo(a)pyrene exposure presents a risk for human developmental neurotoxicity. The epidemiological studies provide excellent correspondence to the more general public. Inherent in any epidemiological study there are limitations concerning the cause and effect relationship and parceling out the individual toxicants under study. The animal studies provide excellent determination of cause-and-effect relationships to individual chemicals. However the challenge of experimental animal studies is always how well do they relatedly to humans. Any study certainly has limitations but ignoring the weight of evidence the due to shortcomings of any particular study puts children at risk for neurodevelopmental disability. It is important to keep in mind that the way we use statistical analysis is to minimize the reports of false positives (alpha errors) but most studies do not minimize the reports of false negatives (beta errors). That being the case, a particular study failing to detect a significant effect must be interpreted as a failure to detect an effect not demonstration of no effect. Inasmuch as the goal is to protect the public from toxic risks, we need to be careful not to disregard studies that find significant effects just because another study fails to find a significant effect. In fact, given that most studies are statistically powered to minimize alpha but not beta errors we should expect beta errors to occur on a regular basis.

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### **Dose-Response Assessment Question #3a. Oral Reference Dose (RfD)**

*2.1.1. Identification of Studies and Effects for Dose-Response Analysis – Levin, Li, McIntyre, Vorhees*

*3. Dose-response analysis. In section 2, the draft assessment uses the available human, animal, and mechanistic studies to derive candidate toxicity values for each hazard that is credibly associated with benzo[a]pyrene exposure in section 1, then proposes an overall toxicity value for each route of exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/backgrd.html/>) in the following analyses.*

Answer: This section provides an excellent analysis, providing a well-considered integration of the extant literature.

*3a. Oral reference dose for effects other than cancer (section 2.1). The draft assessment proposes an overall reference dose of  $3 \times 10^{-4}$  mg/kg-d based on developmental toxicity during a critical window of development. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.1.5) reflect the scientific considerations that are implicit for exposures during a critical window of development?*

*2.1.4 – 2.1.6. All team members*

Answer: The authors have done a good job with the estimate of the oral reference dose for risks of non-cancer toxic consequences of BaP exposure.

### **Question #3b. Inhalation Reference Concentration (RfC)**

*3b. Inhalation reference concentration for effects other than cancer (section 2.2). The draft assessment proposes an overall reference concentration of  $2 \times 10^{-6}$  mg/m<sup>3</sup> based on decreased fetal survival during a critical window of development. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.2.5) reflect the scientific considerations that are implicit for exposures during a critical window of development?*

*2.2.4. – 2.2.8. All team members*

Answer: Yes, the authors of the report have done an excellent and well-considered job with this issue.

### **Question #3c. Oral Slope Factor**

*2.3.4. Uncertainties in the Derivation of the Oral Slope Factor – All team members.*

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Answer: There will always be uncertainties in the slope of the dose-effect function, particularly at the lower end of the dose range where there is a greater likelihood for variable responses. In addition non-linear dose response functions due to multiple mechanisms of effect (i.e. Ah receptors involved in different physiological processes may have differential effects in perturbing those processes).

*3c. Oral slope factor for cancer (section 2.3). The draft assessment proposes an oral slope factor of 1 per mg/kg-d based on alimentary tract tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?*

Answer: It is less than optimal to base this sort of calculation on only one line of evidence.

### **Question #3d. Inhalation Unit Risk**

*2.4.4. Uncertainties in the Derivation of the Inhalation Unit Risk – All team members*

*3d. Inhalation unit risk for cancer (section 2.4). The draft assessment proposes an inhalation unit risk of 0.5 per mg/m<sup>3</sup> based on a combination of several types of benign and malignant tumors in hamsters. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?*

Answer: Yes I think this is appropriate way to do this calculation based on the evidence available.

## Dr. Maureen Lichtveld

### Comments on executive summary

#### General comments:

- It is unclear who the target audience for the executive summary is. Specifically, will the same executive summary be used to inform the general public of the review findings, or will a separate literacy-competent version be developed?
- p xxxiv, lines 3-33: dark shaded text-: if the intent of that text is meant as a general overview, then the literacy level may need to be lowered. Some statements may have been oversimplified: for example, lines 14-17, the “magnitude of exposure” etc. also depends on the dose, route, and duration of course in addition to the other factors mentioned. In addition, from a community perspective there is sensitivity about always beginning this list with “lifestyle factors”, considered as a blaming strategy by some health disparate communities. While lifestyle factors certainly play an important role those could be listed as second or third.

#### Specific Comments

- p. xxxv line 3: *Effects other than cancer observed following oral exposure*: consider adding an example of an external measure of exposure
- p. xxxvi lines 1 and 9: *confidence in overall oral RfD*- define the qualitative term “medium”
- p. xxxviii: *quantitative estimate of carcinogenic risk from dermal exposure*- there were considerable comments from the panel members and those who presented during the public comment period of the March conference call requiring revision of this section. The details are best addressed after the upcoming in-person meeting

p. xxxix: *susceptible populations and life stages*- this section is limited in scope as presented. The *supplemental guidance for assessing early life exposure to carcinogens* has relevant information which can strengthen the current section. For example, on p. 34 of that guidance, factors influencing the analysis of susceptible life stages are outlined. A discussion of how those factors are applied in the context of B[a]P, including the derivation of the dermal slope factor, would elucidate the rationale and decision-making process regarding human health risk.

## Dr. Barry McIntyre

**Barry McIntyre's draft responses to assigned questions are in italics prefaced by "[BSM]". Note that question 2a has an editorial comment that I wanted to bring to EPA's attention. I do not think that this rises to the level of it needing to be circulated amongst the panel members.**

### 1. Literature search/study selection and Evaluation.

The process for identifying and selecting pertinent studies for consideration in developing the assessment is detailed in the *Literature Search Strategy/Study Selection and Evaluation* section. Please comment on whether the literature search approach, screening, evaluation, and selection of studies for inclusion in the assessment are clearly described and supported. Please comment on whether EPA has clearly identified the criteria (e.g. study quality, risk of bias) used for selection of studies to review and for the selection of key studies to include in the assessment. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of benzo[a]pyrene.

*[BSM]The literature search approach, screening, and selection of studies for inclusion are well documented, appropriately comprehensive, and transparent. However, it is less clear as to how animal studies were assessed for quality/risk of bias. In the Literature Search Strategy, it is simply stated that "All animal studies of benzo[a]pyrene involving repeated oral, inhalation, or dermal exposure that were considered to be of acceptable quality, whether yielding positive, negative, or null results, were considered in assessing the evidence for health effects associated with chronic exposure to benzo[a]pyrene." In addition, Section 6 of the preamble identifies various factors for defining study selection for deriving the toxicity values state "credible evidence of an association". However, it is stated that "Studies with adequate power to detect effects at lower exposures levels are preferred..." This may lead one to suspect that a poorly powered study that shows an adverse outcome would be accepted. Was any consideration given to appropriately powering the study for the endpoint of interest, or using a sufficient number of animals consistent with regulatory guideline studies? Was any consideration given purity of the test material or confirmation of dose formulations? If criteria were used, were they defined a priori?*

2. **Hazard identification.** In section 1, the draft assessment evaluates the available human, animal, and mechanistic studies to identify the types of toxicity that can be credibly associated with benzo[a]pyrene exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/backgrd.html/>) to reach the following conclusions.

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**2a. Developmental toxicity** (sections 1.1.1, 1.2.1). The draft assessment concludes that developmental toxicity and developmental neurotoxicity are human hazards of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?

*[BSM- editorial] P46-L 10- 15 (and others). One can't definitively state that there was an effect on fetal survival (although likely) since the authors did not stain the uteri of animals that did not litter (i.e. definitive evidence of post-implantation loss). This could be easily reworded (as appropriate) "number of litters/litter size on PND 1 was lower suggesting fetal/early perinatal loss..."*

*[BSM] The draft assessment includes a sufficient number of appropriately conducted animal studies that demonstrates an effect on the number of live litters/pups born (likely due to fetal death **and terata**- There are publications that indicate that B[a]P is a teratogen (see Shum et al Teratology 20(3)365 1979), growth retardation, effects on F1 fertility and fecundity when exposed in utero. These data are consistent with the limited information available from human B[a]P/PAH studies. Although there appears to be B[a]P-related changes in developmental neurological endpoints (indicative of neuro/developmental toxicity) , most of these studies utilized a small number of animals as compared to typical guideline studies (i.e. 15-20 animals/sex/group). Nevertheless, these appear to consistent with findings (e.g. cognitive ability) observed in developing humans exposed to B[a]P/PAH.*

*Taken together, there is a clear and compelling relationship between B[a]P exposure and developmental toxicity in rodents. In humans, there is a compelling relationship between B[a]P/PAH exposure and fetal loss and diminished cognitive ability, and is consistent with and supported by the rodent data.*

**2b. Reproductive toxicity** (sections 1.1.2, 1.2.1). The draft assessment concludes that male and female reproductive effects are a human hazard of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?

*[BSM] The draft assessment includes a sufficient number of appropriately conducted animal studies that demonstrate both a functional effect on reproductive endpoints indicative of B [a]p –related reproductive toxicity, as well as evidence for potential modes of action. The rodent data demonstrates clearly that B[a]P affects fertility and fecundity. These adverse functional effects in male rodents are associated with adverse changes in the testes and sperm. The observed changes in apical reproductive endpoints (e.g. sperm motility and T) are relevant and translatable biomarkers for assessing the association of B[a]P exposure and the potential for adverse effects in humans. In human males, changes in sperm quality and fertility have been observed in individuals exposed to PAH mixtures. Although not definitive evidence of causal relationship between B[a]p exposure and reproductive toxicity in humans, these findings are consistent with those observed in laboratory animals. Studies in female rodents that may explain the functional female effects are limited, and contradictory. The Xu (2010) study was a low-powered mixture study (n=6), rather than a typical toxicity study to designed to characterize dose-response relationships and target organ toxicity. This publication has other weaknesses including the use of pentobarbital (known to affect hormone secretion), small n for low weight tissues/hormone levels. For reference, guideline toxicity studies (and studies conducted by*

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*the National Toxicology Program) typically require ~10 rats/sex. Moreover, this effect on ovarian weight was not observed by Knuckles (20 rats/group) or Kroese (10 rats/group). This being said, the study by Mackenzie and Angevine provides compelling evidence that in utero exposure (sensitive window for ovary development) to B[a]P  $\geq$  10 mg/kg affects the developing rodent fetal ovary, resulting in infertility when the offspring are sexually mature (and in the absence of B[a]P). This finding in rodents is consistent with studies in humans examining the effects of in utero tobacco smoke and the effects on the future fertility of female offspring. Moreover, studies done by Neal et al with human tissues provides further support that the human ovary is also a target for B[a]P. The data reported by Wu et al, cannot be fully ascribed as to providing evidence that B[a]P is a human reproductive toxicant. Rather, these data are more consistent with developmental toxicity resulting in early embryonic death (which is also observed in rodents).*

*Taken together, there is a clear and compelling relationship between B[a]P exposure and effects on the rodent reproductive system, resulting in impaired fertility and fecundity. In humans, there is a strong relationship between B[a]P/PAH exposure and effects sperm quality and fertility, and targets the ovary, and is consistent with and supported by the rodent data.*

2c. **Immunotoxicity** (sections 1.1.3, 1.2.1). The draft assessment concludes that immunotoxicity is a potential human hazard of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?

2d. **Cancer** (sections 1.1.5, 1.2.2). The draft assessment concludes that benzo[a]pyrene is "carcinogenic to humans" by all routes of exposure. Do the available human, animal, and mechanistic studies support this conclusion?

2e. **Other types of toxicity** (section 1.1.4). The draft assessment concludes that the evidence does not support other types of noncancer toxicity as a potential human hazard. Are there other types of noncancer toxicity that can be credibly associated with benzo[a]pyrene exposure?

3. **Dose-response analysis.** In section 2, the draft assessment uses the available human, animal, and mechanistic studies to derive candidate toxicity values for each hazard that is credibly associated with benzo[a]pyrene exposure in section 1, then proposes an overall toxicity value for each route of exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/backgrd.html>) in the following analyses.

3a. **Oral reference dose for effects other than cancer** (section 2.1). The draft assessment proposes an overall reference dose of  $3 \times 10^{-4}$  mg/kg-d based on developmental toxicity during a critical window of development. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.1.5) reflect the scientific considerations that are inherent for exposures during a critical window of development?

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*[BSM] In principal, the selection of an overall reference dose based on developmental toxicity during a critical window of development is scientifically supported, but the selection of studies upon which it is based warrants further panel discussion. In the study by Chen (2012), one could argue that there are potential study design/conduct weaknesses that may decrease the confidence in the study findings. These include: rotating the pups amongst dams every few days, relatively small sample size (as compared to guideline studies), and potentially inappropriate statistical analyses. Specifically, rotation of pups amongst dams likely induces both pup and dam stress (very common for dams to reject their fostered young). Moreover, it does not "distribute" the maternal caretaking differences across the groups; it actually results in the loss of the ability to account or test for maternal/litter effects. This study utilized 10 animals/gender/group; the authors did test for gender effects, and polled if not significant (for an n of 20). For righting reflex (which exhibited an effect at the lowest dose) the effect of treatment x gender exhibited a p value of 0.10, whereas gender alone exhibited a p value of 0.06. Taken together, these appear to approach statistical significance, and may have attained the  $p < 0.05$  if more animals were used, and resulting data may be confounded by gender. These potential issues aside, the elevated plus maze is the most informative for dose-response analysis.*

*The study by Xu (2010) was a low-powered mixture study ( $n=6$ ), rather than a typical toxicity study designed to characterize dose-response relationships and target organ toxicity. This publication has other weaknesses including the use of pentobarbital (known to affect hormone secretion), small n for low weight tissue/hormone levels. Moreover, this effect on ovarian weight was not observed by Knuckles (20 rats/group) or Kroese (10 rats/group) at similar and higher dose levels. Therefore, the selection of this study for further dose-response analysis may not be appropriate. Obviously, study selection will impact (to some degree) the subsequent presentation and collective assessment. Conceptually, the assessment of candidate values, UFs and PODs is logical and appropriate.*

**3b. Inhalation reference concentration for effects other than cancer** (section 2.2). The draft assessment proposes an overall reference concentration of  $2 \times 10^{-6}$  mg/m<sup>3</sup> based on decreased fetal survival during a critical window of development. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.2.5) reflect the scientific considerations that are inherent for exposures during a critical window of development?

*[BSM] In principal, the selection of an overall reference concentration based on adverse effects during a critical window of development is scientifically supported, but the study selected appears to have deficiencies that warrant further panel discussion. The selected study by Archibong (2002) exhibits technical weakness that may impact overall study consideration. Blood samples were collected from the orbital plexus (a highly stressful technique); stress is known to elevate PRL levels (potential relationship between B[a]P and PRL is suspect), and based on the hormone data it appears that each dose (and*

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*corresponding control) was run in series (each dose group having its own control with discrepancies in control responses). These weaknesses aside, the apparent effects of B[a]P on fetal survival are compelling, and consistent with that observed in other studies. Obviously, study selection will impact (to some degree) the subsequent presentation and collective assessment. Conceptually, the assessment of candidate values, UFs and PODs is logical and appropriate.*

**3c. Oral slope factor for cancer** (section 2.3). The draft assessment proposes an oral slope factor of 1 per mg/kg-d based on alimentary tract tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?

**3d. Inhalation unit risk for cancer** (section 2.4). The draft assessment proposes an inhalation unit risk of 0.6 per mg/m<sup>3</sup> based on a combination of several types of benign and malignant tumors in hamsters. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?

**3e. Dermal slope factor for cancer** (section 2.5). The draft assessment proposes a dermal slope factor of 0.006 per ug/day based on skin tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and scaling from mice to humans? Does the method for cross-species scaling (section 2.5.4 and appendix E) reflect the appropriate scientific considerations?

**3f. Age-dependent adjustment factors for cancer** (section 2.6). The draft assessment proposes the application of age-dependent adjustment factors based on a determination that benzo[a]pyrene induces cancer through a mutagenic mode of action (see the mode-of-action analysis in section 1.1.5). Do the available mechanistic studies in humans and animals support a mutagenic mode of action for cancer induced by benzo[a]pyrene?

**4. Executive summary.** Does the executive summary clearly and appropriately present the major conclusions of the assessment?

*[BSM] The executive summary clearly presents the current major conclusions of the assessment (based on the selected studies that were used for calculation of the RfD, RfC, and cancer slope factors). The Summary also addresses the Key Issues and provides the context in which these were addressed.*

**5. Charge question on the public comments**

In August 2013, EPA asked for public comments on an earlier draft of this assessment. Appendix G summarizes the public comments and this assessment's responses to them. Please comment on EPA's responses to the scientific issues raised in the public comments. Please consider in your

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review whether there are scientific issues that were raised by the public as described in Appendix G that may not have been adequately addressed.

## **Dr. Bhagavatula Moorthy**

### Charge questions on the draft Toxicological Review

#### 6. Literature search/study selection and Evaluation.

The process for identifying and selecting pertinent studies for consideration in developing the assessment is detailed in the *Literature Search Strategy/Study Selection and Evaluation* section. Please comment on whether the literature search approach, screening, evaluation, and selection of studies for inclusion in the assessment are clearly described and supported. Please comment on whether EPA has clearly identified the criteria (e.g. study quality, risk of bias) used for selection of studies to review and for the selection of key studies to include in the assessment. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of benzo[a]pyrene

**Comments:** The overall strategy for literature search/study is appropriate. However, some references could be included in regard to the effect of maternal exposure to benzo[a]pyrene (BP) on fetal development. Recent epidemiological studies suggest an association between dietary BP intake and lower birth weight in children (Duarte-Salles et al., *Environment international* 60C, 217-223, 2013; Duarte-Salles et al., *Environment international* 45, 1-8, 2012; *Public health nutrition* 13, 2034-2043, 2010). These references could be included. Also, there is little emphasis on the effects of benzo[a]pyrene (BP) on non-cancer pulmonary toxicity. Our group recently published a paper in which we showed that maternal exposure of mice to BP leads to increased susceptibility of newborn mice to hyperoxic lung injury and chronic lung disease (CLD) (Couroucli et al., *Tox. Lett.*, 230: 322-332, 2014). Supplemental oxygen therapy is frequently encountered in premature infants and very low birth weight infants, and hyperoxia contributes to the development of bronchopulmonary dysplasia (BPD), also known as CLD, in these infants. Maternal smoking is one of the risk factors for preterm birth and for the development of bronchopulmonary dysplasia (BPD). Thus, I believe there should be some description on the effect of BP on pulmonary toxicity in infants as well as adults.

7. **Hazard identification.** In section 1, the draft assessment evaluates the available human, animal, and mechanistic studies to identify the types of toxicity that can be credibly associated with benzo[a]pyrene exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/backgrd.html/>) to reach the following conclusions.

2a. **Developmental toxicity** (sections 1.1.1, 1.2.1). The draft assessment concludes that developmental toxicity and developmental neurotoxicity are human hazards of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?

**Comments:** Yes, this is true. I will also add that additional emphasis could be given on the effect of BP on lung development because maternal exposure to BP through cigarette smoke or

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diet (e.g., charcoal broiled meats) could lead to abnormal lung development in the babies born to these mothers.

**2b. Reproductive toxicity** (sections 1.1.2, 1.2.1). The draft assessment concludes that male and female reproductive effects are a human hazard of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?

**Comments:** Yes, this is true. In a recent study ([PLoS One](#). 2014 Jan 29;9(1):e87439. doi: 10.1371/journal.pone.0087439), male transgenic lacI mice at different ages (7, 25 and 60 days old) were treated with BP at different doses (0, 50, 200 or 300 mg/kg body weight). Mutant frequency was then determined in a meiotic cell type (pachytene spermatocyte), a post-meiotic cell type (round spermatid) and epididymal spermatozoa after at least one cycle of spermatogenesis. The results show that (i) mice treated with BP at 7 or 25 days old, both being pre-adult ages, had significantly increased mutant frequencies in all spermatogenic cell types tested when they were 60 days old; (ii) spermatogenic cells from mice treated before puberty were more susceptible to BP-associated mutagenesis compared to adult mice; and (iii) unexpectedly, epididymal spermatozoa had the highest mutant frequency among the spermatogenic cell types tested. These data support the hypothesis that pre-adult exposure to BP increases the male germline mutant frequency in young adulthood. The data also suggest that exposure to environmental genotoxins at different life phases (e.g., pre-adult and adult) can have differential effects on reproductive health. This information could be included in the draft. In regard to females, Finuadi et al. ([Hum Reprod](#). 2014 Mar;29(3):548-54) showed that *in vivo* exposure to benzo(a)pyrene induces significant DNA damage in mouse oocytes and cumulus cells.

**2c. Immunotoxicity** (sections 1.1.3, 1.2.1). The draft assessment concludes that immunotoxicity is a potential human hazard of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?

**2d. Cancer** (sections 1.1.5, 1.2.2). The draft assessment concludes that benzo[a]pyrene is "carcinogenic to humans" by all routes of exposure. Do the available human, animal, and mechanistic studies support this conclusion?

**Comments:** Yes, the data do suggest that BP is a human carcinogen. The section is well organized and well written. While BP is understood to be a carcinogen by all routes of exposure, it needs to be emphasized that BP is not a liver carcinogen, and mainly causes cancer in organs such as lung, breast, skin and the route of exposure determines where the primary site of cancer formation is likely to occur. For example, dermal exposure leads to skin cancer, while inhalation exposure could lead to lung cancer, and chewing tobacco could lead to oral cancer. Also, while liver cytochrome P4501A/1B enzymes play a major role in the bioactivation of BP to form metabolites that can bind DNA, leading to DNA adducts, these hepatic adducts could not cause cancer in the liver. Actually some of the metabolites could be transported to lung or there is local metabolism in lung leading to adducts which cause tumorigenesis in the lung. We have a review article that is in press in *Toxicological Sciences* that in part discusses the latest aspects of PAH metabolism and lung cancer (Moorthy et al., Polycyclic Aromatic Hydrocarbons (PAHs): From Metabolism to Lung Cancer, *Toxicological Sciences*, in press, 2015).

**2e. Other types of toxicity** (section 1.1.4). The draft assessment concludes that the evidence does not support other types of noncancer toxicity as a potential human hazard. Are there other types of noncancer toxicity that can be credibly associated with benzo[a]pyrene exposure?

**Comments:** Yes, please see my comments in response to charge question 1.

**8. Dose-response analysis.** In section 2, the draft assessment uses the available human, animal, and mechanistic studies to derive candidate toxicity values for each hazard that is credibly associated with benzo[a]pyrene exposure in section 1, then proposes an overall toxicity value for each route of exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/backgrd.html/>) in the following analyses.

**3a. Oral reference dose for effects other than cancer** (section 2.1). The draft assessment proposes an overall reference dose of  $3 \times 10^{-4}$  mg/kg-d based on developmental toxicity during a critical window of development. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.1.5) reflect the scientific considerations that are inherent for exposures during a critical window of development?

**Comments:** Yes, this has been well presented. However, please see my comments in response to charge question 1 for the relationship between maternal BP and chronic lung disease in newborn mice.

**3b. Inhalation reference concentration for effects other than cancer** (section 2.2). The draft assessment proposes an overall reference concentration of  $2 \times 10^{-6}$  mg/m<sup>3</sup> based on decreased fetal survival during a critical window of development. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.2.5) reflect the scientific considerations that are inherent for exposures during a critical window of development?

**3c. Oral slope factor for cancer** (section 2.3). The draft assessment proposes an oral slope factor of 1 per mg/kg-d based on alimentary tract tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?

**3d. Inhalation unit risk for cancer** (section 2.4). The draft assessment proposes an inhalation unit risk of 0.6 per mg/m<sup>3</sup> based on a combination of several types of benign and malignant tumors in hamsters. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?

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**Comments:** While this may be appropriate, I believe additional studies in different species (e.g., rats, mice) must be done for extrapolation to humans.

**3e. Dermal slope factor for cancer** (section 2.5). The draft assessment proposes a dermal slope factor of 0.006 per ug/day based on skin tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and scaling from mice to humans? Does the method for cross-species scaling (section 2.5.4 and appendix E) reflect the appropriate scientific considerations?

**3f. Age-dependent adjustment factors for cancer** (section 2.6). The draft assessment proposes the application of age-dependent adjustment factors based on a determination that benzo[a]pyrene induces cancer through a mutagenic mode of action (see the mode-of-action analysis in section 1.1.5). Do the available mechanistic studies in humans and animals support a mutagenic mode of action for cancer induced by benzo[a]pyrene?

**9. Executive summary.** Does the executive summary clearly and appropriately present the major conclusions of the assessment?

**10. Charge question on the public comments**

In August 2013, EPA asked for public comments on an earlier draft of this assessment. Appendix G summarizes the public comments and this assessment's responses to them. Please comment on EPA's responses to the scientific issues raised in the public comments. Please consider in your review whether there are scientific issues that were raised by the public as described in Appendix G that may not have been adequately addressed

## **Miriam C. Poirier**

**Charge question on literature search/study selection and Evaluation** - The process for identifying and selecting pertinent studies for consideration in developing the assessment is detailed in the *Literature Search Strategy/Study Selection and Evaluation* section. Please comment on whether the literature search approach, screening, evaluation, and selection of studies for inclusion in the assessment are clearly described and supported. Please comment on whether EPA has clearly identified the criteria (e.g. study quality, risk of bias) used for selection of studies to review and for the selection of key studies to include in the assessment. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of benzo[a]pyrene.

**Response** - The approach to the literature search used for this evaluation has been described in the Preamble on page xvii, Section 3. The major standard databases (PubMed, ToxNet, NLM, Web of Science), as well as the EPA's HERO database were queried for items including benzo[a]pyrene, PAHs, and other relevant terms, with interest in animal data, human epidemiological data and other mechanistic and toxicokinetic studies. The original searches turned up 21,000 references, and those were pared down to about 700 references, which are currently cited in the Toxicological Review and fall into 7 different categories. All of these references are currently available in the HERO database.

Given the task at hand I believe that the EPA has done a fine job with this. However, given the incompleteness of modern databases and the variety of terms required to search this very complex topic, it is not surprising that there were occasional relevant papers missed. In addition there may be papers that have been left out intentionally due to evaluation criteria that might not be clear to those of us reviewing the document. Having a panel of experts review the literature chosen is an important aspect of this review, given the abovementioned difficulties in finding all the relevant papers.

**Conclusions** - I did find several papers that I believe to be relevant to this document that were both not listed in the document and not found in the HERO database. I have included these in the answers to my charge questions below, with accompanying descriptions of how I consider them relevant to this document.

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**Charge Question on Cancer** (sections 1.1.5 and 1.2.2) – “The draft assessment concludes that benzo[a]pyrene is ‘carcinogenic to humans’ by all routes of exposure. Do the available human, animal and mechanistic studies support this conclusion?”

**Response** – To answer this it is necessary to invoke the EPA guidelines for whether or not a compound is considered a human carcinogen. These are evaluated below with respect to the evidence presented in the “Toxicological Review of Benzo[a]pyrene”.

***The compound in question is “Carcinogenic to Humans” when there is convincing epidemiologic evidence of a causal association between human exposure and cancer.***

The epidemiologic data presented here in the “Toxicological Review” (p.1-83 and 1-84) strongly support the carcinogenicity of polycyclic aromatic hydrocarbon (PAH) exposures in humans. The Toxicological Review summarizes a large number of studies focused on lung, bladder and non-melanoma skin cancer, however, as this report states, when dealing with human exposures it is not possible to separate benzo[a]pyrene from the other PAHs that have been shown to increase tumor risk in humans. Therefore, from the epidemiologic studies there is no direct evidence that benzo[a]pyrene by itself (alone) is carcinogenic to humans. However, because there is the assumption that benzo[a]pyrene is likely a component of all the PAH mixtures that humans might be exposed to, the epidemiological data suggest that benzo[a]pyrene alone is likely to be a human carcinogen.

Evaluation of the data presented:

The Toxicological Review document focused only on the three cancers mentioned above (lung, bladder and skin), but there are other organs for which PAHs are carcinogenic, and additional data are presented in Supplemental information (p. D-28 to D-33). For example, the Toxicological Review does not include colon cancer risk, despite the fact that there is strong evidence for an association between PAH-exposure in heavily char-broiled meat (Rothman et al., HERO ID 84099) and colon cancer risk (Sinha, R. et al., HERO ID 1007703), as well as the correlations between PAH-DNA adduct formation, cooked meat ingestion and colon adenocarcinoma risk in the same population (Gunter et al., HERO ID1011897). An additional study, not in HERO, (Chen, S-Y et al., Int. J. Cancer 99:14-21, 2002) documented the risk of hepatocellular carcinoma and chemical class-specific PAH-DNA adducts in human liver samples, showing that smoking and hepatitis B status were both risk factors for liver cancer.

***The compound in question can be considered “Carcinogenic to Humans” when there is a lesser weight of epidemiological evidence but when all of the following conditions are met:***

a) *strong evidence of an association between human exposure and either cancer or the key precursor events of the agent’s mode of action but not enough for a causal association*

The Toxicological Review rightly concludes that benzo[a]pyrene is metabolized, damages DNA, and is carcinogenic through consequent mutagenic mechanisms, which occur prior to tumor formation. The document also states that in the environment benzo[a]pyrene is found in mixtures of carcinogenic PAHs, and it is therefore impossible to tell which PAH compounds contributed to the mutagenic burden in humans. The document fails to point out, however, that of

these steps (metabolism, DNA damage, mutagenesis) the only one that can be demonstrated specific to PAH exposure is the formation of PAH-DNA damage. Because many classes of carcinogen (in addition to PAHs) induce the formation of GC →TA and AT →TA transversions, not a single human mutation can be unequivocally traced back to a PAH exposure. Therefore, despite the indirect evidence presented in the Toxicological Review on p.1-84, the evaluation of mutation spectra alone cannot indicate the cause of those mutations.

In humans the presence of PAH-DNA adducts is a critical step in the continuum between exposure and tumor induction, however specific evidence for benzo[a]pyrene-induced DNA adducts is harder to find in PAH-exposed humans than in animal models exposed only to benzo[a]pyrene. In an excellent review (Boysen and Hecht, *"Analysis of DNA and protein adducts of benzo[a]pyrene in human tissues using structure-specific methods"* Mutation Research 543:17-30, 2003 – which is not in the HERO database), the authors document the use of structure-specific methods to quantify benzo[a]pyrene-induced DNA adducts in human tissues. They reported that in 39% of 705 human samples it was possible to detect the presence of the major stable DNA adduct associated with benzo[a]pyrene exposure and carcinogenesis, the r7, t8, t9-trihydroxy-c-10-(N<sup>2</sup>deoxyguanosyl)-7, 8, 9, 10-tetrahydro-benzo[a]pyrene (BPdG). In conclusion, evidence for BPdG formation in human tissues provides a direct link between PAH exposures and mutations considered likely to have come from benzo[a]pyrene exposures. There is a direct link between PAH exposures and PAH-DNA adducts, or benzo[a]pyrene exposure and the formation of BPdG, but there is no direct link between PAH-DNA or BPdG adduct formation and mutations leading to and/or found in human cancers.

Evaluation of the data presented:

The Supplemental information summarizes 6 *in vivo* studies (Table D-33), which evaluated benzo[a]pyrene-induced DNA adducts in humans. This table presents only a fraction of the studies available using chemical class-specific methods to measure PAH-DNA and BPdG adduct formation in human tissues.

In addition, there are a series of human studies where individuals with the highest PAH-DNA adducts (using chemical class-specific methods) also have the highest human cancer risk. This information would be useful if summarized in a table in the Supplemental information (see for example: Kyrtopoulos, S.A., Toxicology Letters 162:3-15, 2006 [not in HERO]; and Poirier, M.C., HERO ID 2558407).

Critical to our understanding of the published values for human benzo[a]pyrene-induced DNA adducts and PAH-DNA adducts, is knowledge of what is actually being measured by a specific assay. As mentioned (above), the gold standard is determination by structure-specific methods, and this includes mass-spectrometry based methods as well as the HPLC/fluorescence of Alexandrov and Rojas. Other assays can have compound specificity and can also be very useful. For example, the various immuno-methods (ELISA and immunohistochemistry) which employ monoclonal or polyclonal antibodies raised against benzo[a]pyrene-modified DNA (termed BPDE-DNA antisera), which are invariably specific for a family of carcinogenic PAHs (7-8 of these have been shown to cross-react) bound to DNA. These antisera are chemical class-specific indicators, and we use the term "PAH-DNA adducts" for measurements of DNA

damage in human tissues because it is not possible to say exactly what DNA adducts are being measured. The popular <sup>32</sup>P-postlabelling is not at all specific for PAH-DNA adducts because the method also detects adducts of many different chemical classes. In addition, synchronous fluorescence spectroscopy only shows benzo[a]pyrene when pre-purification steps are employed. Thus, choice of an assay will vastly impact the validity, reliability and conclusions of a particular study.

The Toxicological Review has no consistent discrimination between the various methods involved in human PAH-DNA or BPdG DNA analysis. This may be due to the lack of a consistent chemically-correct nomenclature for the methods on the part of the authors (of the different publications) themselves. These two documents (Toxicological Review and Supplemental information) would be improved if a table could be added to describe the characteristics of the methodologies in question.

Another problem with the document is the lack of a uniform terminology, not only for the assays used and what they measure, but also for the chemical nomenclature of terms like BPDE and BPdG, where the chemical specificity matters. With minimal effort the scholarship could be made more precise and the readability of this document substantially improved.

*b) extensive evidence of carcinogenicity in animals*

The document, on pages 1-62 to 1-69, and the summary on pages 1-85 and 1-86, provide a thorough documentation of many different studies all showing unequivocally that benzo[a]pyrene is a carcinogen in rodent models.

*c) the mode(s) of carcinogenic action and associated key precursor events have been identified in animals*

As demonstrated clearly in the Toxicological Review (and summarized in Supplemental Information Table D-33, p.D-98), in animal models exposed to benzo[a]pyrene there is extensive evidence of the formation of dose-related benzo[a]pyrene-induced DNA adducts, and other types of dose-related genotoxic events including: germline mutations, somatic mutations, micronuclei, sister-chromatid exchanges, chromosomal aberration, DNA strand breaks, and unscheduled DNA synthesis.

*d) 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*

The primary mechanistic evidence found in human tissues comprises PAH-DNA and BPdG adduct formation, which are key precursor events that provide a direct link between PAH exposures and mutations occurring as a result of benzo[a]pyrene exposure. There are other end points of interest, which also occur in a dose-related fashion in animals, and these include: DNA damage occurring through the radical cation and the o-Quinone pathways, chromosomal aberration, sister chromatid exchange, micronucleus formation, and GC→TA transversion mutations in oncogenes and tumor suppressor genes in benzo[a]pyrene-associated tumors

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(Supplemental Information page D-26). In addition, the mutations glycophorin A and *HPRT* can be found associated with exposure in humans. However, in humans, unlike in animals, only the PAH- or benzo[a]pyrene-specific DNA adducts can be shown to result directly from PAH exposures, as humans are not exposed to benzo[a]pyrene alone. The major stable DNA adduct of benzo[a]pyrene (BPdG) has been associated with mutagenesis in cell culture and whole animals, and tumor formation in animals, and because this adduct is also found in humans, it is no surprise that epidemiological case-control and other studies have found an association between PAH-DNA adduct formation and PAH-associated tumor risk.

**Conclusions** – The first step in the EPA analysis of whether or not a compound is a human carcinogen states ***“The compound in question is ‘Carcinogenic to Humans’ when there is convincing epidemiologic evidence of a causal association between human exposure and cancer”***. Whereas the available epidemiological data do show that PAHs are carcinogenic in humans, there is no data on human exposure to benzo[a]pyrene alone. The strong possibility that all PAH mixtures contain benzo[a]pyrene provides a likelihood that this is the case. However, considering only the epidemiological studies, benzo[a]pyrene cannot be considered a human carcinogen.

For the second step of the EPA requirements, ***“The compound in question can be considered ‘Carcinogenic to Humans’ when there is a lesser weight of epidemiological evidence but when all of the following conditions are met....”***, the data show that all four of the required conditions are met. Therefore, based on tumor studies in humans and animal models, and on mechanisms of action determined in both species, strong evidence of key precursor events related to benzo[a]pyrene exposure and found in humans indicates that benzo[a]pyrene can be considered a human carcinogen.

**Charge question 2a. Developmental toxicity** (sections 1.1.1, 1.2.1). The draft assessment concludes that developmental toxicity and developmental neurotoxicity are human hazards of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?

**Response** – As the Toxicological Review states on p. 1-1, section 1.1.1, there is evidence that in human pregnancies where the mother is exposed to ambient or dietary PAHs, PAH-DNA damage as an indicator of exposure is accompanied by: a reduction in fetal size, an increase in *in utero* fetal loss, a reduction in the size of head circumference (related to cognitive function), developmental delay in motor skills, increased anxiety/depression, and increased attention problems. With the exception of increased tendency to abort, and reduction in fetal size, all of the other end points are considered manifestations of neurotoxicity. Clearly these toxicities may have significant impact on the lives and future success of these children.

Many rodent studies support the human observations, though in a few areas the results diverge. In the rodent studies benzo[a]pyrene treatments during gestation were either by gavage or inhalation. In parallel to the human studies, there were significant increases in fetal loss, along with decreases in fetal weight and survival. In addition, examination of fertility in the offspring revealed changes in development of male and female reproductive organs, and decreased overall fertility which included fewer and smaller litters, compared to unexposed controls. Physical examination revealed organ weight decreases and abnormalities in testes and ovaries. Additionally, cardiovascular and neurological defects were observed, including increased blood pressure, altered learning and memory behaviors, and impaired neuromuscular and sensorimotor development. However in the rodents, unlike in the children, there was decreased anxiety-like behavior.

With the exception of anxiety-like behavior, all of the end points found in children have been reproduced in rodent models. Additional end points found in the rodents, for example the reproductive integrity and fertility-related issues, have yet to be documented in children, but are indicators of potential long-term consequences of PAH exposures in children.

**Conclusions** - This part of the Toxicological Review document is comprehensive and well-written, and I have no suggestions for additional studies or references. The data support the conclusions that developmental toxicity in both males and females are likely outcomes from transplacental benzo[a]pyrene exposure.

**Charge question 2b. Reproductive toxicity** (sections 1.1.2, 1.2.1). The draft assessment concludes that male and female reproductive effects are a human hazard of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?

**Response** – As the Toxicological Review states on p.1-22 in section 1.1.2, in humans there is evidence that environmental and occupational PAH exposures affect both male and female fertility. In two studies PAH-exposed males were shown to have low fertility, with their embryos having abnormally low implantation rates. Workers occupationally exposed to PAHs were more likely to have oligospermia and morphologically-abnormal sperm, than controls. In females, women who smoke are more likely to have ovulatory disorders and higher spontaneous abortion rates than women who do not. In addition, smoking during peri-menopause has been shown to accelerate the rate of menopause.

In rodent models, where benzo[a]pyrene exposure was largely by gavage or inhalation, the evidence is much more extensive. Changes in males were found in several studies, and these included decreases in sperm counts, decreases in sperm motility and altered sperm morphology. In addition, there were decreases in testicular weight, decreased epididymal tubule diameter, and decreases in testosterone levels. In benzo[a]pyrene exposed females, decreased fertility and fecundity, decreased ovary weight, decreases in the number of follicles, and a reduction in follicle stimulating hormone (FSH) were observed. In pregnant mice, benzo[a]pyrene exposure exposure has been shown to produce losses in progesterone, estradiol and prolactin. Also noted in females were altered estrus cyclicity and increased cervical epithelial inflammation.

Taken together the rodent studies support the observations of reduced fertility in human smokers and workers exposed to high levels of benzo[a]pyrene in PAH mixtures. The rodent studies add critical mechanistic insights that could not be obtained from the available human studies alone.

**Conclusions** - This part of the Toxicological Review document is comprehensive and well-written, and I have no suggestions for additional studies or references. The data support the conclusions that male and female reproductive effects are a human hazard of benzo[a]pyrene exposure.

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**Charge question 3f. age-dependent adjustment factors for cancer** (section 2.6). The draft assessment proposes the application of age-dependent adjustment factors based on a determination that benzo[a]pyrene induces cancer through a mutagenic mode of action (see the mode-of-action analysis in section 1.1.5). Do the available mechanistic studies in humans and animals support a mutagenic mode of action for cancer induced by benzo[a]pyrene?

**Response** - The document EPA/630/R-03/003F "Supplemental Guidance for Assessing Susceptibility from Early-Life Exposures to Carcinogens" lays out the rational approach of the EPA, to adjustment of tumor risk for exposures at different ages, for carcinogens with a mutagenic mode of action. The age-related adjustments are based on many animal experiments where exposures occurred at different ages, and tumor incidences were evaluated. Having read this document, and section 2.6 in the Toxicological Review of Benzo[a]pyrene, I agree that use of the proposed age-dependent adjustment factors (ADAFs) in evaluation of exposures in human infants and adolescents is science-based and reasonable. In addition the supporting references are complete, and there is nothing else I would add.

## Dr. Kenneth Portier

**Question #1: Literature Search Strategy/Study Selection and Evaluation:** The process for identifying and selecting pertinent studies for consideration in developing the assessment is detailed in the *Literature Search Strategy/Study Selection and Evaluation* section. Please comment on whether the literature search approach, screening, evaluation, and selection of studies for inclusion in the assessment are clearly described and supported. Please comment on whether EPA has clearly identified the criteria (e.g. study quality, risk of bias) used for selection of studies to review and for the selection of key studies to include in the assessment. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of benzo[a]pyrene

### *Preliminary Comments:*

- *Figure LS-1 references conditions that led to the exclusion of ~600 manuscripts in the manual screening of the ~1000 manuscripts considered for study inclusion. These criteria are not described or otherwise expanded upon in the Literature Search Strategy section of the BaP Tox Review document (the Report) or in the BaP Tox Supplemental Information (the Supplement). In particular, phrases such as “Inadequate basis” and “Inadequate reporting” sound subjective but it is assumed that some additional criteria were used to make this judgement (e.g. used no controls, inappropriate route of exposure, very low or no power to detect effects, etc.) While these issues may be discussed in A Review of the Reference Dose and Reference Concentration Processes (U.S. EPA, 2002) and Methods for Derivation of Inhalation Reference Concentrations and Application of Inhaled Dosimetry (U.S. EPA, 1994)., at a minimum, the BaP Tox Supplemental Information should be appended to include additional discussion of this issue. Without this additional information, the Study Selection cannot be duplicated. I agree that much of the detailed rationale for selected studies is provided in the Dose Response subsections of each section.*
- *No assessment is provided on the extent of information lost by not including animal in vivo and in vitro studies designed to identify potential therapeutic agents that would prevent the carcinogenicity or genotoxicity of benzo[a]pyrene. It is to be expected such studies might provide additional information on mode of action of benzo[a]pyrene. It may be that the extensive discussion in Appendix D covers everything that might be relevant from these therapeutic agent animal studies, but if this is the case EPA should so state.*

**Question #2: Hazard identification.** In section 1, the draft assessment evaluates the available human, animal, and mechanistic studies to identify the types of toxicity that can be credibly associated with benzo[a]pyrene exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/backgrd.html/>) to reach the following conclusions.

2a. **Developmental toxicity** (sections 1.1.1, 1.2.1). The draft assessment concludes that developmental toxicity and developmental neurotoxicity are human hazards of

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benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?

**2b. Reproductive toxicity** (sections 1.1.2, 1.2.1). The draft assessment concludes that male and female reproductive effects are a human hazard of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?

**2c. Immunotoxicity** (sections 1.1.3, 1.2.1). The draft assessment concludes that immunotoxicity is a potential human hazard of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this conclusion?

**2d. Cancer** (sections 1.1.5, 1.2.2). The draft assessment concludes that benzo[a]pyrene is "carcinogenic to humans" by all routes of exposure. Do the available human, animal, and mechanistic studies support this conclusion?

**2e. Other types of toxicity** (section 1.1.4). The draft assessment concludes that the evidence does not support other types of noncancer toxicity as a potential human hazard. Are there other types of noncancer toxicity that can be credibly associated with benzo[a]pyrene exposure?

## **Dose-Response Assessment (Section 2)**

**Question #3: Dose-response analysis.** In section 2, the draft assessment uses the available human, animal, and mechanistic studies to derive candidate toxicity values for each hazard that is credibly associated with benzo[a]pyrene exposure in section 1, then proposes an overall toxicity value for each route of exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/backgrd.html/>) in the following analyses.

**3a. Oral Reference Dose (RfD):** The draft assessment proposes an overall reference dose of  $3 \times 10^{-4}$  mg/kg-d based on developmental toxicity during a critical window of development. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.1.5) reflect the scientific considerations that are inherent for exposures during a critical window of development?

*Preliminary Comments:*

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- *The discussion around identification of studies and effects used in the dose-response analysis was clear and to the point. Analysis is consistent with EPA's Benchmark Dose Technical Guidance Document (U.S. EPA 2012c). Adequate information is provided in Appendix E of the Supplemental Information to assess model fit adequacy.*
- *A review of model fits found no situations where the chosen model looked inadequate.*
- *It seems appropriate to establish a P-value upper threshold of 0.01 to indicate inadequately fitting models allowing discarding of marginally adequate models and allowing the focus to remain on the best fitting models.*
- *The model selection protocol tends to favor PODs that are conservatively low. When multiple models produce BMDL estimates within 3x of each other, the model AIC criteria is used to select the value to be used, whereas when the BMDL estimates are not within 3x, the lowest BMDL is used. This (standard) selection protocol results in a value that could have very conservative properties.*

**3b. Inhalation Reference Concentration (RfC):** The draft assessment proposes an overall reference concentration of  $2 \times 10^{-6}$  mg/m<sup>3</sup> based on decreased fetal survival during a critical window of development (Section 2.2). Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.2.5) reflect the scientific considerations that are inherent for exposures during a critical window of development?

**3c: Oral slope factor for cancer** (section 2.3). The draft assessment proposes an oral slope factor of 1 per mg/kg-d based on alimentary tract tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?

*Preliminary Comments:*

- *This section presents the oral slope factor case in a straightforward discussion, providing adequate justification for decisions at each step.*
- *The assumption of equal cumulative exposure yielding equivalent outcomes (page 2-18, lines 18-21) - used to justify converting administered dose (5days/week) to equivalent continuous dose (7days.week) – is not discussed in either the report or in the Supplement. Is this a generally accepted assumption? Is this the only method of converting administered dose to equivalent continuous dose? Originally I wondered whether the PBPK animal models (Appendix D, section D.2) might offer additional approaches, but the models have high uncertainties and model a limited number of the important pathways and or sources/sinks that limit their utility.*
- *Uncertainties in the Derivation of the Oral Slope Factor - Use of Multistage Weibull model is well supported – Desire to incorporate time of death in the modeling is*

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*appropriate and would be expected to improve model fits. The analysis presented seems to represent a due-diligence effort that is supported by reviews of this model and approach by previous expert panels. This does not mean that multistage Weibull is the perfect model or that some other model incorporating time of death could be found to fit better. But, there do not seem to be other obvious models that could have been fit that would incorporate the available data.*

- *Statistical uncertainty is appropriately accounted for and use of BMDL justified.*
- *Previous IRIS Assessment Oral Slope Factor - The major difference with the previous IRIS assessment seems to be due in a large part to the use of  $BW^{2/3}$  scaling instead of the  $BW^{3/4}$  scaling used in this assessment.*

**3d: Inhalation Unit Risk:** The draft assessment proposes an inhalation unit risk of 0.6 per  $\text{mg}/\text{m}^3$  based on a combination of several types of benign and malignant tumors in hamsters. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of departure?

*Preliminary Comments:*

- *This section presents the approach to estimation of the inhalation unit risk case in a straightforward way, providing adequate justification for decisions at each step. The approach used (accommodating time-to-tumor data in a Multistage Weibull model) is similar to that used to estimate the oral slope factor. Model fits look adequate for obtaining reasonable estimates.*
- *The analysis proceeds by considering tumors to be either all fatals or all incidentals. This seems reasonable given the absence of investigator-determined cause of death and the likelihood that all tumors are unlikely to be fatal.*
- *Not discussed in Table 2-10 (uncertainties).*
  - *The impact on estimates of assuming something other than equal risk for all species is associated with equal concentrations in air (page 2-35, lines 4-5).*
  - *The impact on estimates of assuming something other than equal cumulative exposure yields equivalent outcomes (page 2-18, lines 18-21- used to justify converting administered dose (5days/week) to equivalent continuous dose (7days.week)) This is not discussed in either the report or in the Supplement. Is this a generally accepted assumption? Is this the only method of converting administered dose to equivalent continuous dose? Originally I wondered whether the PBPK animal models (Appendix D, section D.2) might offer additional approaches, but the models have high uncertainties and model a limited number of the important pathways and or sources/sinks that limit their utility.*
  - *The impact on estimates of assuming that the latency time,  $t_0$  (the time between a tumor first becoming observable and causing death) is different from zero (page E-66, line 23-24).*
  - *The impact on estimates of eliminating from the analysis all animals without confirmation of one or more of the pharynx or respiratory tract tissues being*

*examined, unless a tumor was diagnosed in those that were examined (page E-66, lines 25-27). This decision impacts the denominator of the cases fraction and hence has the potential to impact significantly the model results. On the other hand, sample sizes are quite good and if not a lot of cases are excluded the impact could be small. Note: In Appendix G (page G-8, lines 43-46) we are informed that 5 low-exposure animals are omitted in the dose response modeling. Including this information directly into the Report or even into the body of the Supplement and discussing it briefly would resolve this comment.*

**3e: Dermal Slope Factor for cancer:** The draft assessment proposes a dermal slope factor of 0.006 per ug/day based on skin tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and scaling from mice to humans? Does the method for cross-species scaling (section 2.5.4 and appendix E) reflect the appropriate scientific considerations?

*Preliminary Comments:*

- *The approach to estimation of the dermal slope factor is presented in a straightforward manner*
- *There is more dermal studies, with more dose levels within studies and more than adequate animals/dose. This resulted in EPA having to fit multiple models for each dataset and then select among multiple model forms for those best fitting. As a result the modeling section in Appendix E is larger and harder to follow. The modeling results presented in Appendix E do allow following and duplicating the EPA analysis feasible.*
- *Cannot comment on the method used for interspecies scaling of the dermal slope factor, but this is an important question because it contributes quite significantly to the uncertainty in the final estimate (see also comment and answers on page G-12, lines 5-7).*

**3f: Age-dependent adjustment factors for cancer** (section 2.6). The draft assessment proposes the application of age-dependent adjustment factors based on a determination that benzo[a]pyrene induces cancer through a mutagenic mode of action (see the mode-of-action analysis in section 1.1.5). Do the available mechanistic studies in humans and animals support a mutagenic mode of action for cancer induced by benzo[a]pyrene?

**Questions #4: Executive summary.** Does the executive summary clearly and appropriately present the major conclusions of the assessment?

*Preliminary Comments:*

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- *The Executive Summary is 6 and ½ pages in length – long for a summary. A lot of the text is duplicated from the body of the Report. Including only the table information (combined into one long table including cancer slope factors and estimates using ADAFs – add uncertainty factors where utilized) followed by a list of notes – one per table row in bullet format, I estimate the summary could be just as understandable in about 3 pages. Additional bullet notes could be added to summarize estimation method issues (eg. Use of Multistage Weibull and Multistage Cancer models to accommodate time-to-tumor data in estimating cancer slope factors.) A reduced Executive Summary would further meet the spirit of NRC recommendation 1.*
- *An Executive Summary should be able to stand-alone and not make references back to the body of the Report or to the Supplement (see page xxxvi, line 19).*

**Question #5: Charge Question on Public Comments:** In August 2013, EPA asked for public comments on an earlier draft of this assessment. Appendix G summarizes the public comments and this assessment's responses to them. Please comment on EPA's responses to the scientific issues raised in the public comments. Please consider in your review whether there are scientific issues that were raised by the public as described in Appendix G that may not have been adequately addressed

*Preliminary Comments:*

- *The EPA response to the comment on “Apparent threshold in animal cancer bioassays” (page G-7, line 7-25) is in line with good statistical practice/thinking. The argument for or against an exposure threshold below which cancer effects might not occur must be answered through increased biological processes understanding because there are simply not enough test animals available to answer the question experimentally and through empirical models.*
- *The EPA response to the comment on “Exposure variability in the study used to derive inhalation unit risk” (page G-9, lines 1-14) provides an answer that essentially “begs the question”. Agreed, the way EPA “eliminates” this issue is by assuming that cancer risk is proportional to cumulative exposure, but still the impact of exposure variability should be better addressed in the body of the Supplement.*

*Other Comments that do not necessarily fit in any one section:*

- *Page 2-13, line 16-17 states: “...and the study used to derive a candidate value based on decreased testosterone (Zheng et al., 2010) did not observe a dose-response relationship (a 15% decrease in testosterone was seen at the low and high doses, with statistical significance at the high dose).” The study did not observe a monotonic dose-response pattern as might be expected for a toxin, but the inverse U shaped response pattern is not discussed further. My limited understanding of such patterns is that they may be suggestive of endocrine disruption. This issue is not discussed. I did note that the*

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*literature review key words did include endocrine disruption but none of the papers referenced in the report or the supplement discuss endocrine disruption.*

- *Good point made on page 2-14, lines 25-28 regarding exposure level at critical development windows. Need more be said? The discussion on ADAF in, section 2.6 does not come back to this critical developmental life stage discussion.*
- *In a few places in the Report and/or the Summary, reference was made to historical incidence rates of outcomes. All the multistage models assume a background factor in estimating the response likelihood. The key data allowing estimation of this parameter is the control animals from each study. For many of the test species, background incidence rates for tumors can also be estimated from historical control datasets. Incorporating historical control information into the modeling process conceptually can be done and would reduce the uncertainty of the response at the 0 dose and could as a result reduce the overall uncertainty in the model and the BMDL specifically. I have not seen this done anywhere but wondered if this was attempted for any of the exposure scenarios presented. The Report refers to the potential for using historical controls (see page xx, lines 30-41) but I can't find where this was actually done in this Report.*

## **Dr. Steve Roberts**

### **2.1.2 Methods of Analysis, Dosimetric Adjustment Factor**

PODs estimated based on effects in adult animals were converted to human equivalent doses (HEDs) employing a Dosimetric Adjustment Factor consistent with current EPA guidance.  $BW^{3/4}$  scaling was not applied to calculate HEDs from studies in which doses were administered to early postnatal animals, which I think is also consistent with EPA guidance [will confirm]. I have no comments or suggestions for improvement.

### **2.3.1 Analysis of carcinogenicity data (Choice of studies)**

Available studies are identified and the rationale for selection of Kroese et al. (2001) and Beland and Culp (1998) as the best studies is presented clearly and concisely. I agree with the selection of these studies as the most appropriate for conducting dose-response analysis.

### **2.5.1 Analysis of carcinogenicity data (Choice of studies)**

Available studies are identified and described succinctly, with details presented in the Appendix. The rationale for selection of the NIOSH study as the best for dose-response analysis is presented clearly and concisely. I agree with the selection of this study.

### **2.5.2 Dose-response analysis**

It is clear from the discussion in this section and in Appendix E that the appropriate dose metric has not been established for skin tumorigenicity from benzo(a)pyrene. Appendix E discusses some options for dose-metrics, but this discussion is in the context of how to extrapolate observations in mice to humans and seems to ignore the basic question of what is the appropriate metric in *any* species (mass; mass per unit area; something else?). This is not just an issue for extrapolation among species, but also for the fundamental form that dermal cancer potency factor should take. This question could be addressed experimentally, but to my knowledge has not to date. Until this issue is resolved, developing a dermal slope factor is premature, in my opinion.

## **Executive Summary**

In general, the Executive Summary clearly presents the major decisions and conclusions of the assessment. The extent to which the committee agrees with those decisions and conclusions will be determined during the face-to-face meeting. There will be some suggestions for improvement; for example, under "Key Issues Addressed in Assessment" the issue of applicability of the overall RfD and RfC values to risk assessment for the general population is raised, which is an important one. Unfortunately, the Executive Summary says nothing about it other than to refer the reader to two sections in the main body of the report. The Executive Summary should bring forward at least the main ideas regarding this topic.

Parenthetically, the Executive Summary makes statements such as, "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." The statement is accurate, but the reference values as presented are arguably unsuitable for cumulative risk assessment without some modification. [The issue is that the  $UF_D$  one would apply to produce a candidate reference value that might be selected as the overall reference value, as in this report, could be different from the  $UF_D$  one would apply to create a value that applies to one specific type of toxicity, e.g.,

hepatotoxicity, in a cumulative risk assessment). An explanation of this issue may not be appropriate for the toxicological review, but should be articulated by EPA *somewhere*. The current draft TMB review suggests that EPA provide some discussion and/or guidance on this subject. This committee may want to decide whether to bother reiterating that suggestion.

### **Summary of External Peer Review**

It will be important to make clear in our response to this charge question that we are considering only public comment summaries presented in Appendix G. Without seeing the original public comments, we have no way of knowing how accurately the comments were summarized and whether the points made by the public commenters were adequately captured.

EPA accepted some public comments and made suggested changes while rejecting others. When the EPA disagreed with a comment, a clear explanation of the basis for disagreement was provided. Our charge includes determining "whether there are scientific issues that were raised by the public as described in Appendix G that may not have been adequately addressed."

Presumably this means situations where the committee either disagrees with the EPA response or thinks that they missed the point. Some (hopefully most) of these situations will become clear as the earlier charge questions are discussed by the committee. Points on which there is disagreement between the EPA and public commenters that are not addressed previously by the committee will need to be discussed under this charge question. Points potentially include: [my initial impressions in brackets]

- Low confidence placed by the EPA in skin cancer studies using mice with human skin grafts [EPA makes some good points on limitations of these studies]
- Disagreement by EPA with public comments indicating that coal tar studies in humans demonstrate that benzo(a)pyrene does not cause skin cancer in humans [EPA makes some good points on limitations of these studies]
- Contention that EPA has mischaracterized the evidence supporting an association between benzo(a)pyrene exposure and lung and skin cancer in humans [lung cancer association looks solid to me, but the association with skin cancer might be overstated]
- Why is "decreased anxiety" a critical effect? [who wouldn't want decreased anxiety? ... but it does comport with EPA guidance]
- Disagreement by EPA with public comments that dose-response modeling of data from cancer bioassays from oral, inhalation, and dermal routes show thresholds. [Agree with EPA that this type of modeling cannot identify thresholds for carcinogenesis.]
- Disagreement by EPA with public comments indicating that the Thyssen et al. (1981) inhalation study was unsuitable for development of an inhalation unit risk because the maximum tolerated dose was exceeded and exposures were highly variable over time. [Still thinking about this one.]
- Disagreement by EPA with public comments indicating that studies show non-linear dose-response relationships for skin cancer and benzo(a)pyrene and there is a MOA based upon inflammation, cell killing, and cell replication, consistent with non-linearity. [I concur with EPA's response on this one]
- Disagreement by EPA with public comments indicating that PAHs are not casually related to human skin cancers because PAH-induced tumors in mouse skin have a different genetic signature than human skin tumors. [Generally concur with the EPA response]

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- Disagreement by EPA with comments that the study by Sivak et al. (1997) should not be used to develop a dermal slope factor because the maximally tolerated dose was exceeded. [EPA response seems adequate.]
- Disagreement regarding EPA's approach for extrapolating the dermal slope factor from mouse to human skin and with the expression of the slope factor in  $\mu\text{g}/\text{d}$  [I also disagree with EPA's approach. This will no doubt be discussed in the context of other charge questions.]
- Disagreement over the risks that would be estimated for the general population based upon the proposed dermal slope factor and typical PAH exposures. [I have not yet gone through the math.]

## **Dr. Richard Schlesinger**

### **Charge Question 3b:**

Based upon the available database, the appropriate critical toxicological effects for estimation of an RfC are developmental and reproductive parameters. This choice of endpoints is supported by consistent qualitative effects from exposure across species. Unfortunately, there are very limited datasets for evaluation of chronic effects in this regard from inhalation exposure. The specific key study used in the Assessment for derivation of the RfC, namely that of Archibong et al (2002), employed three concentrations of B(a)P and examined decreased fetal survival; since the lowest level used still resulted in toxic effects, this was considered as the LOAEL for the POD for dose-response analysis.

**p. 2-18. L. 1-18.** The rationale for use of a value of 860 mL for tidal volume (TV) and 50 mL for volume of the upper respiratory tract (URT) is not clear. On the average, TV ranges from 7-9 mL/kg BW, so for a 70 kg person (the default body wt for human), the value should range from 490-630 mL. Using the value of 16bpm noted in the B(a)P Document, the minute volume would range from 7.8-10.1 L/min. Regarding FRC, which is RV + ERV, the average value in males is 2400 mL, so the value in the Document of 3,300 mL seems high.

**p. 2-19. Section 2.2.3.** This section discusses the rationale for the various UFs used. The UF for intraspecies and interspecies differences are appropriate and consistent with established guidelines (Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry, USEPA, 1994; A Review of the Reference Dose and Reference Concentration Processes, USEPA, 2002). Similarly, the UF used for LOAEL when NOAEL is not available is also appropriate. However, what is not clear is the rationale for the UF used for subchronic to chronic extrapolation, especially since the key study for derivation of RfC was subacute. The B(a)P Document indicates a UF of 1; the default in this case is 10, and this may have been a better value to use since the inherent extrapolation in the Assessment involves subacute to chronic. However, the Methods for Derivation of RfC as noted in the 2002 Document cited above is that the UF total should be less than or equal to 3000, and a full value of 10 should not be used in 4 or more areas of extrapolation. The current B(a)P Assessment fits this criterion using 1 for the subchronic to chronic, but would not if using the more appropriate value of 10.

**p.2-20, L. 9.** What is meant by, "...these studies observed a high magnitude of response"?

**p.2-20, L. 30-32.** This statement seems to contradict the selection of the POD for the RfC.

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**p.20-23, Section 2.2.6.** Confidence in the derived value for RfC is noted as low to medium. Confidence in the database is indicated as low for a number of reasons, while confidence in the key study is noted as medium. The rationale for the range of confidence up to medium for the derived RfC value is noted as due to "...consistent systemic effects observed by the oral route...and similar effects observed in human populations exposed to PAH mixtures." However, while there is consistency in qualitative effects between oral and inhalation routes of exposure, there may not be consistency in the dose-response relationship between different routes of exposure. Furthermore, effects of B(a)P do occur at site of entry, so some effects will differ between oral and inhalation exposure. Thus, using the rationale above to increase confidence of the RfC value to medium is questionable.

On page 2-25, lines 1-5, it is noted that the study selected as the basis for the RfC "...provided limited information regarding the inhalation exposures of the animals..." in that it was not clear whether the concentrations noted in the paper were target values or analytical concentrations nor was the "...method used to quantify benzo(a)pyrene in the generated aerosols..." reported. This, together with the comments above and the magnitude of the UFs used, indicate that the overall RfC confidence should be low, rather than low to medium. In fact, the Methods for Derivation of RfC document notes, "Low confidence in an RfC is usually applied to a derivation that is based on several extrapolations and indicates an estimate that may be especially vulnerable to change if additional data become available. For some chemicals, the data base is so weak that the derivation of a low confidence RfC is not possible." I think the current case is the former rather than the latter.

The preference for a POD for the RfC is a NOAEL, but the study used for the B(a)P value is a "default" LOAEL based upon the lowest of a number of discrete concentrations used in the key study rather than an extensive dose-response relationship. Thus, the actual "true" LOAEL is not clear, and necessitated use of UFs to compensate.

Finally, in the Methods for Derivation of RfC, the availability of only one inhalation bioassay is noted as the minimum database for estimation of an RfC and the confidence will then be low. Furthermore, the derivation procedure notes that in this case a chronic study is preferred, but a subchronic is acceptable. In the B(a)P RfC derivation, it is a subacute study (Archibong et al. 2002) that is being used for POD. Thus, all of the above strongly suggests a confidence level of low for the derived RfC, and not low to medium.

**Charge Question 3d:**

**Page 2-35, L. 26-28.** What is the basis for this statement and how useful is the derived unit risk in this case.

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**Page 2-36, Section 2.4.4.** A number of uncertainties are discussed, but what is the overall uncertainty or level of confidence for the number derived.

## **Dr. Leslie Stayner**

1. **Literature search/study selection.** Is the literature search strategy well documented? Please identify additional peer-reviewed studies that might have been missed.

The literature search strategy was thorough and very well documented in the toxicological review. Nonetheless, it appears that the epidemiologic literature presented in the toxicological review and supplemental information regarding the carcinogenicity of BAP was incomplete and somewhat out of date.

The toxicological review emphasized studies that met their criteria for high quality (i.e. Tier 1). Although I agree with most of the criteria they chose for identifying high quality studies, I believe that requiring a detailed exposure assessment for BAP is unnecessarily restrictive. Studies with a detailed exposure assessment would be most relevant for an exposure-response assessment, but are not necessary for hazard identification. The review only considered that three studies met their criteria for Tier 1 for lung cancer (Armstrong and Gibbs 2009, Spinelli et al 2006, Xu et al. 1996) and bladder cancer (Gibbs and Sevigny 2007a and 2007b, Spinelli et al 2006, Burstyn et al 2007). The Tier 1 studies only included studies of the aluminum and iron and steel manufacturing. It did not include any studies of workers from the coke ovens, roofing or asphalt industries which would have very high exposures to BAP and thus should be relevant for determining causality even though they may not have had detailed exposure assessments for BAP. Tier 2 studies are presented in a table in the report. However, there are many studies missing from these tables (e.g. Romunstadt et al. 2000, Ronneberg 1999, that have been included in prior reviews (i.e. see Table 1 in Bosetti et al. 2007, and Rota et al. 2014).

There is a disconnect between the review presented in the toxicological review and the supplemental information section. Normally I would expect a supplement to provide additional information then what is presented in the main body of the report. However, the supplemental information section did not follow the same logic of reviewing Tier 1 and Tier 2 studies, and did not provide a more detailed review of the studies then the main report.

The review presented in the supplemental information section relied heavily on a systematic review and meta-analysis reported by Bosetti et al. in 2007, and by Armstrong et al. in 2004. It seems inappropriate for EPA to rely solely on a review articles rather than a review of the primary literature. There is also a more recent meta-analysis that they did not include in their review (Rota et al. 2014). Many of the epidemiologic studies cited in Bosetti and Rota are not discussed in the EPA supplemental document. For aluminum production workers the EPA only discusses the studies by Spinelli et al. (1991, 2006), Romundstad et al. (2000a and 2000b) and Xu et al. (1996). There are 10 other studies of aluminum production workers cited in the Bosetti review (see Table 1 of Bosetti), and five additional studies cited in the Rota review article (see Table 1 of Rota). It is unclear why the EPA only included the few studies that they did review in their report.

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The EPA supplemental review doesn't discuss any of the studies of asphalt workers and roofers or coke oven workers. For asphalt and roofers they refer the readers to the Bosetti et al (2007) review, which as mentioned above, was updated by Rota et al. (2014). They cite five papers as providing evidence of an excess risk of lung cancer and weak evidence for bladder cancer among asphalt workers and roofers (Burstyn 2007, Partanen 1994, Chiazze 1991, Hansen 1991, 1989, and Hammond 1976). They seemed to have overlooked studies cited in Bosetti (see Table 1) of roofers by Swaen et al., and of asphalt workers cited in Rota (see Table 1) by Behrens et al. (2009) and Zanardi et al. (2013). In addition given the differences in the nature of exposure, I think that it would be appropriate to separately discuss the findings for asphalt workers and roofers.

For coke oven workers, coal gasification and iron and steel foundry workers the supplemental report relies entirely on the reviews by Boffetta et al. (1997), Bosetti et al. (2007) and Armstrong et al. (2004). The more recent review by Rota (2014) identified two new studies of iron and steel workers (see Table 1) that were not considered in the earlier reviews. I was able to identify one additional study of coke oven workers by Miller et al. (2013), which was not included in the Rota or EPA reviews.

Finally, it is not clear why some of the studies of coal tar that were identified in the comments from the American Coke and Coal industry were not included in the EPA review. In particular the studies by Bhate et al (1993), Hannuksela-Svahn et al (2000), Jemec, G.B.E. and A. Østerlind (1994), Jones S.K. et al (1985), Menter A. and D.L. Cram (1983), and Muller and Kierland (1964) seem relevant.

2e. **Cancer** (sections 1.1.5, 1.2.2). The draft assessment concludes that benzo[a]pyrene is "carcinogenic to humans" by all routes of exposure. Do the available human, animal, and mechanistic studies support this conclusion?

The epidemiologic data alone is not sufficient to conclude that BAP is carcinogenic to humans. There is strong evidence that workers in industries with high exposures to BAP are at increased risk of lung and to a lesser extent for bladder cancer. However, workers in these industries are all exposed to other PAHs and it was impossible to single out BAP in the analyses of these studies. However, the epidemiologic data combined with the animal and mechanistic data do provide strong support for the conclusion that was reached by the EPA that BAP is carcinogenic to humans.

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## **Dr. Alan Stern**

### **Comments on Charge Question 2d**

*Cancer (sections 1.1.5, 1.2.2). The draft assessment concludes that benzo[a]pyrene is "carcinogenic to humans" by all routes of exposure. Do the available human, animal, and mechanistic studies support this conclusion?*

The evidence for BaP carcinogenicity by all routes of exposure in animal models is strong, and EPA has done a good job of presenting these animal data. Given the global potential for BaP to cause cancer in animals, it is also highly likely that BaP can also cause cancer in humans by all routes of exposure. However, at least two requirements for assignment of the category of "carcinogenic to humans" in EPA's 2005 Guidelines for Risk Assessment present logical problems relative to making this assignment for BaP based on the arguments presented in the draft IRIS document.

The first, is "(a) there is strong evidence of an association between human exposure and either cancer or the key precursor events of the agent's mode of action but not enough for a causal association." With respect to this requirement, there does not appear to be any epidemiologic evidence that addresses exposure to BaP in isolation from other PAHs, at least some of which are also known animal carcinogens. EPA presents an argument on pg. 1-83, lines 26-29 that "...the exposure-response patterns seen with the BaP measures make it unlikely that these results represent confounding by other exposures." However, the specific evidence behind this statement is not clear. With respect to dermal carcinogenicity in humans, there is likewise no evidence from BaP-specific exposures. In this case, EPA's argument (pg. 1-84, lines 3-7) is based on the relationship between benzo[a]pyrene diol epoxide-DNA (BDE-DNA) adducts as both a marker of BaP exposure and a causal step in skin tumor production. However, while it seems clear that BDE-DNA adducts are a marker of BaP exposure, the role of BDE-DNA adducts as a necessary step in BaP carcinogenicity is less clear. For example, on pg. 1-74, lines 30-38, EPA in its review of Culp et al. (1996) notes that in mice exposed to BaP in the diet had a sharp increase in tumor response between the lowest and next highest dose while the BDE-adduct concentration increased linearly. EPA's argument that BaP meets this first criterion for assignment of the "carcinogenic to humans" category may hinge on the "...but not enough for a causal association" portion of this criterion (although EPA does not explicitly say so, this requirement appears to function when the first, stand-alone requirement for the assignment of "carcinogenic to humans": "... *convincing epidemiologic evidence of a causal association between human exposure and cancer*" is not met). Clearly, this requires interpretation of specificity of this requirement, but I think that EPA could do a better job of making this case.

The second logical problem relates to the linked requirement in the 2005 guidelines that "(d) 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." In the IRIS document's discussion of carcinogenic modes of action, EPA presents three possible (and non-mutually exclusive) modes of action (pg. 1-69), BDE-DNA adducts,

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radical cation, and *o*-quinone-ROS. As discussed above, notwithstanding the qualitative carcinogenic potential of BDE, the quantitative relationship between BDE-DNA and tumor production does not appear to be clear and if possible, EPA should present a synthesis of evidence in animals and/or humans that supports a dose-response relationship for BDE-DNA and tumors. As for the other two possible modes of action, it appears that the evidence supporting these modes of action is from animal and/or *in vitro* studies. It may be sufficient for EPA to make the case that these are both key-precursor events and highly likely to function in humans given the basic underlying biochemistry and molecular biology in mammals. However, the current document does not explicitly make this case.

The above notwithstanding, I want to make it clear that these considerations relate only to evidence requirements for the assignment of the specific category of "carcinogenic to humans" and not to the highly likely carcinogenic potential for BaP to humans.

### **Comments on Charge Question 3a (section 2.1.3 – Uncertainty Factors – RfD)**

EPA's discussion of uncertainty factors (UFs) begins with the  $UF_H$ . Given that the starting point for this process is the animal-based POD, the  $UF_H$  is not a logical place to begin this discussion. Rather, the discussion should begin with the uncertainty factor for LOAEL-NOAEL conversion ( $UF_L$ ), followed by the UF for subchronic-chronic conversion ( $UF_S$ ), then the UF for animal-human ( $UF_A$ ) and *then* proceed to the  $UF_H$ .

On pg. 2-9, lines 5-10, EPA provides the rationale for applying a UF of 3 (as opposed to the full standard UF of 10) for accounting for potential differences between animals and humans. The reduction of the UF to 3 by the prior application of  $bw^{3/4}$  allometric scaling is consistent with EPA's risk assessment guidance. However, the rationale presented in the document at this point, that the  $bw^{3/4}$  accounts for aspects of toxicodynamic as well as toxicokinetic differences between animals and humans, does not appear to be consistent with EPA guidance, nor does it make toxicological sense to me. What is the basis for stating that allometric scaling necessarily addresses interspecies toxicodynamic differences? Toxicodynamic differences would conceptually appear to arise from differences in genetics and biochemistry and these factors should not necessarily scale as a function of body weight. If EPA believes otherwise, the document should either cite prior EPA guidance to this effect, or provide a more detailed basis for this statement. The issue here is not the value *per se* of this UF (with which I agree), but with its explanation.

The application and justification of the other UF for non-cancer oral dose endpoints appears reasonable and consistent with EPA guidance.

### **Comments on Charge Question 3b (section 2.2.3 – Uncertainty Factors – RfC)**

As per my comments on the presentation of UFs for the RfD, EPA's discussion of uncertainty factors for the RfC begins with the  $UF_H$ . Given that the starting point for this process is the animal-based POD, the  $UF_H$  is not a logical place to begin this discussion. Rather, the discussion should begin with the uncertainty factor for LOAEL-NOAEL conversion ( $UF_L$ ), followed by the UF for subchronic-chronic conversion ( $UF_S$ ), then the UF for animal-human ( $UF_A$ ) and *then* proceed to the  $UF_H$ .

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A  $UF_A$  of 3 was chosen to account for uncertainty in extrapolation from animals (rats) to humans. The rationale for not applying the full UF of 10 was the application of the regional deposition dose ratio (RDDR) based on EPA's 1994 guidance. The text states that the application of a dose adjustment factor (DAF) derived using this methodology accounts for interspecies toxicokinetic differences and therefore, only the residual uncertainty in the potential interspecies toxicodynamic differences remain to be addressed by the application of a UF. However, the RDDR only addresses interspecies differences in particle deposition. Since the developmental and reproductive PODs to which this UF are applied are systemic in nature (i.e., result from effects occurring outside the respiratory tract subsequent to absorption from the respiratory tract), interspecies differences in particle deposition do not account for interspecies differences in toxicokinetics subsequent to respiratory deposition. Unlike the estimation of the human equivalent dose (HED) in the derivation of the RfD, where toxicokinetic differences are addressed by a  $bw^{3/4}$  adjustment, the use of only the RDDR implicitly assumes a body weight adjustment on a linear basis and the text does not appear to address any other body weight-based adjustments. This would imply that some interspecies toxicodynamic uncertainty remains. It is not clear that this necessarily requires the application of the full  $UF_A$  of 10, however, this residual toxicokinetic uncertainty should be addressed.

The application of the remaining UFs appears to be consistent with EPA guidance.

### **Comments on Charge Question 3f (section 2.6 – Age Dependent Adjustment Factor)**

The discussion in section 1.1.5 presents three strong lines of evidence for a mutagenic mode of action (or several mutagenic modes of action) for BaP: The observation that BaP is a complete carcinogen in skin painting studies; the production of DNA base transversions resulting from benzo[a]pyrene diol epoxide (BDE) adducts, and the consistently positive results in bacterial mutagenicity models (with metabolic activation). Two other carcinogenic mechanisms, radical cation production and *o*-quinone/ROS production, although consistent with mutagenicity, provide weaker evidence for a mutagenic mode of action, as they are also consistent with non-mutagenic modes of action. I believe that this evidence makes a plausible case that BaP can cause cancer through a mutagenic mode of action and hence, justifies the application of the Age Dependent Adjustment Factor (ADAF). However, this case would be strengthened if EPA could provide evidence as to the relative contribution of each of these mechanisms to overall cancer risk showing that those mechanisms resulting in mutagenicity predominate or at least can be assumed to account for a large portion of the modeled tumorigenicity. Such evidence could take the form of (e.g.) the relative production and potency of BDE adducts compared to radical cation production and *o*-quinone/ROS production at relevant doses of BaP, and/or the relative kinetics and half-lives in the nucleus of these proximate carcinogenic agents.

## Dr. Charles Vorhees

### 1. **Literature search/study selection.** Is the literature search strategy well documented? Please identify additional peer-reviewed studies that might have been missed.

The literature search strategy is thorough, well-documented and comprehensive. In my own searches I identified several additional articles that may provide some additional relevant information. These are:

Perera et al. (2014) on PAH exposure and ADHD in children (Perera et al., 2014) and two experiments in animals. One by Patri et al. on BaP in developing rats on learning and the role of NA as a potential protective factor (Patri et al., 2013) and one on BaP in adult rats on motor and cognitive behavior (Maciel et al., 2014), although the latter is less relevant to developmental neurotoxicity.

2. **Hazard identification.** In section 1, the draft assessment evaluates the available human, animal, and mechanistic studies to identify the types of toxicity that can be credibly associated with benzo[a]pyrene exposure. The draft assessment uses EPA's guidance documents (see <http://www.epa.gov/iris/backgrd.html/>) to reach the following conclusions.

Hazard identification will be discussed later.

2a. **Developmental toxicity** (sections 1.1.1, 1.2.1). The draft assessment concludes that developmental toxicity and developmental neurotoxicity are human hazards of benzo[a]pyrene exposure. Do the available human and animal studies support this conclusion?

The focus of my preliminary comments will be on the developmental neurotoxicity animal data. There are a series of relevant human epidemiological studies which will also be considered, including, but not limited to: (Perera et al., 2012b; Perera et al., 2011; Tang et al., 2006; Perera et al., 2005; Perera et al., 2004; Tang et al., 2008; Perera et al., 2012a; Perera et al., 2009)

Descriptions of the key animal experiment about BaP are summarized in the EPA Toxicological Report and will not be repeated here. Here the focus will be on the strengths and weaknesses of these studies.

Tang et al. (Tang et al., 2011) treated Wistar rats starting at weaning for 14 weeks with 1, 2.5, or 6.25 mg/kg BaP i.p. from approximately P21-218 and assessed the animals in the Morris water maze (MWM) to a hidden platform as a test of spatial learning starting one day after the end of treatment. In this procedure rats were tested in a circular pool 180 cm in diameter and apparently given 1 trial/day although the authors do not specify this parameter and it may have been several

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trials per day. They found significant increases in maze latency on all 5 days of testing in the 2.5 and 6.25 mg/kg BaP doses but only on day-3 in the 1 mg/kg dose group. They gave a reference memory (probe) trial after the last learning trial on day-5. On this trial, they found effects of BaP at all doses on platform site crossovers and reductions target quadrant bias in the 2.5 and 6.25 mg/kg BaP dose group. Strengths: The tested multiple doses, groups sizes (9/group) were minimally adequate, the maze was appropriately sized for rats, reasonable learning curves were obtained, and the data appropriately analyzed. Weaknesses: Latency is a potentially confounded index of learning if performance, such as swim speed, is affected by the independent variable, an issue the authors fail to address. Also, the probe trial was given shortly after the last learning trial therefore it cannot be determined if the effects were on working or reference memory (the probe trial should have been given 24 h later). Also the probe trial was too long at 120 s; it is known that spatial bias begins to deteriorate after 30 s. This is mitigated by the fact that the effects of BaP were still significant even with a long probe trial. More importantly, while treatment began on approximately P21, this was not an early but rather a late developmental exposure period that extended well into adulthood. Moreover, it is not clear that the effects were irreversible since testing began shortly after the last treatment rather than allowing for the compound to be cleared in order to determine the permanence of any learning effects.

Qiu et al. (Qiu et al., 2011) similarly to Tang et al. (2011) above gave Sprague-Dawley male rats 6.25 mg/kg BaP i.p. but they used P28 rats and treated them for 14 weeks and tested them an unspecified number of days after the last treatment in a smaller 130 cm diameter MWM with a 9 cm hidden platform. They gave 4 trials/day from different start locations for 5 days following a habituation day in the pool with no platform present as acclimation. Apparently the probe trial was given after the last day of learning trials. They found a significant increase in latency to find the platform across all 5 days of testing and a reduction in the number of platform site crossings and time in the target quadrant on the probe trial. Strengths: They used 8 rats/group, a minimally sufficient sample size, the data were appropriately analyzed, and the MWM procedures were generally appropriate with some caveats. Weaknesses: A 130 cm maze for adult male SD rats is too small to provide a good test of spatial navigation. Adult rats should be assessed in mazes no less than ~183 cm (6 ft.) in diameter. Probe trials should be given 24 h or more after the last learning trial, and latency is a potentially confounded index of learning and should be cross-checked against swim speed and analysis of path length data, neither of which were apparently measured in this experiment. But the greatest concern about this experiment is that the BaP and Control groups differed significantly on Day-1 of MWM testing. This raises the concern that the BaP animals started out different. It is a fundamental concept in learning and memory that if groups start out different they are likely to be different in some performance parameter unrelated to learning. This can be resolved by examining the trials on day-1 individually. Ideally, both groups start out the same on trial-1 when animals in neither group know where to go to find the platform. If the groups then begin to diverge on subsequent trials it suggests that the treatment animals are less able to find and/or remember where the platform is after having found it at least once. Unfortunately, these authors did not address this issue leaving it unresolved. This experiment is also not a test of early but rather late developmental effects.

Xia et al. (Xia et al., 2011) like Qiu et al. (2011) above used male SD rats and started treatment at P28 and treated them for 13 rather than 14 weeks. They used 8 rats/group and the dose groups

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were Control, 1, 2.5, and 6.25 mg/kg BaP given daily by i.p. injection in DMSO diluted with corn oil. In this experiment, rats were tested in the MWM before BaP treatment (where no group differences were found, as expected) and after the end of treatment. This maze tank was also 130 cm in diameter but platform size was unspecified. For the post-treatment MWM assessment, the rats were given 4 trials per day for 5 days with a probe trial given shortly after the last learning trial on day-5. Significant increases in escape latencies were found in the 2.5 and 6.25 mg/kg BaP groups and as in the Qiu et al. study, the effects were uniform on all days including day-1, again raising a concern about swim speed or other interfering performance effects of the compound such that the animals in the treated groups may not have started the test equally capable for performing it. On the probe trial, an effect of 6.25 mg/kg BaP was found on platform site crossovers and on time in the target quadrant. Standard control methods for such issues are to conduct separate cued trials with a visible platform and curtains closed around the maze to prevent use of distal cues, track swim speed during learning trials, or to report path length which is largely immune to speed differences. Strengths: The study has minimally sufficient sample sizes, it included 3 BaP doses levels and two controls groups (vehicle and what they refer to as 0 mg/kg), the data were appropriately analyzed, and the effects at the two higher doses clear-cut. Weaknesses: As in several of the above studies, concerns exist about the small size of the maze for adult male rats, the reliance exclusively on latency without convergent measures less prone to confounding, the differences on day-1 of the test with no analysis of day-1 data trial-by-trial, and the fact that the probe trial was not given 24 h or more after the last learning trial.

Chen et al. (Chen et al., 2012) appears to be one of the strongest studies on BaP during early development. They mated SD rats in-house and culled litters to 8 (4 M and 4 F), randomized pups several times among dams with the goal of randomizing litter effects, used 40 litters in the experiment with 10 males and 10 females. Progeny were treated with 0.02, 0.2, or 2 mg/kg BaP by gavage on P5-11 and the offspring tested for landmark development on P12, 14, 16, and 18, and at later specific ages in an open-field, elevated plus maze (EPM), and MWM, the later 2 at ~P35 and again at ~P70. Most of the behavioral tests were standard but the MWM requires examination because details matter greatly on this test. The pool was 130 cm in diameter with a 9 cm hidden platform. On day-1 rats were given a 60 s trial with no platform as habituation as the authors call it. Spatial learning occurred on 4 days with 4 trials/day with an ITI of 5 min. On day-5 rats received a 60 s probe trial with the platform removed. Significant but modest body weight reductions were seen on P36 and 71 in the 2 mg/kg group but none of the physical landmarks of development were affected. There were delays in surface righting in the low, mid and high dose groups but on different days, and delays in the sloped board right test (incorrectly call negative geotaxis by the authors) at all doses on P12 and only in the high dose on P14. In the open-field there were increases in activity and rearing at P34 and 69 but not at P18 or P20, and these effects were mostly in the high dose group with one effect at P69 in the mid dose group on activity but not rearing. There were also effects in the EPM at P70 with increased time in open, reduced latency to first open entry, increased number of open arm entries, and decreased entries into closed arms. The effects were far more prominent in the high dose group than any other but effects were seen on some measures in the mid and even low dose groups. But by far the most striking findings in this study were in the MWM. In both males and females, and at both P36-39 and P71-74, escape latencies to find the hidden platform were markedly longer in the high dose group than in Controls or the low dose group. At the adult age, there were also significant latency increased found in the mid dose group. On the probe trial, in both males and

females at P40 and P75 time in the target and number of site crossovers were significantly reduced in the high dose group, and in the adults also in the mid dose group. Strengths: This study has a number of strengths; first was the care to use in-house breeding, standardizing litter size, balancing for sex, testing multiple dose levels of BaP, administered BaP by gavage rather than i.p injection, efforts to neutralize litter effects, use of multiple behavioral tests, appropriate statistical analyses of the data (but see one caveat), and use of generally good if not optimal MWM procedures. Weaknesses: Despite these strengths the study has weaknesses many of which are described above. The size of the MWM while appropriate for the P36-39 animals was undersized for adult rats, rendering the test less sensitive. Mitigating this is the experimental effects that were seen despite the small size of the maze. Another concern is the reliance on latency as the sole index of performance as a presumptive reflection of learning, an issue elevated by the fact that in all cases the affected BaP groups showed marked latency differences even on day-1 of testing. No sub-analysis of each trial on day-1 was performed, no cued trials were given, no measurements of path length or swim speed were recorded, the probe trial was given immediately after the last learning trial thereby limiting its interpretive value, and for this and the above studies no reversal learning was assessed.

Li et al. (Li et al., 2012) conducted an experiment using an inbred mouse strain with a Loss of Function (LOF) mutation in the Cpr gene which encodes for the P450 enzyme oxidoreductase involved in BaB metabolism. This is a specialized experiment to test a specific hypothesis about BaP. It is of interest because the KO and WT mice were given BaP on E14-17 by inhalation at a dose of 100 g/m<sup>3</sup>. Of particular interest in terms of developmental neurotoxicity was that among other parameters assessed in the offspring, mice were tested on an object discrimination task which was simply a modified Novel Object Recognition test (NOR). Setting the details aside at present, the upshot was that the BaP exposed KO mice but not the BaP WT mice showed a marked reduction in novel object preference suggesting a hippocampally-mediated non-spatial learning deficit. Because the effect occurred only in the KO mice that were deficient in metabolizing BaP, the data suggest that BaP is more toxic in those with reduced oxidoreductase capacity via genetic variation as in a copy number variant or SNP polymorphism. Unfortunately, only 4-5 mice were testing per group and this is a test that is known for its variability, reducing the confidence that the effect is replicable.

Bouayed et al. (Bouayed et al., 2009) also used mice. In this experiment adult Swiss albino mice were treated with 0, 2 or 20 mg/kg BaP by gavage on P0-14 and behaviors assessed, including physical development, maternal behavior (nest building and pup retrieval), surface righting, sloped board test (a.k.a. negative geotaxis), forelimb grip; open-field on P15, water escape pole climbing test on P20, EPM on P32, and spontaneous alternation on P40. No effects of BaP were found on physical development or maternal behavior. Delays in surface righting were found in both BaP groups on P3 and 5, on sloped board in the high dose group on P5, 7, and 9, on the wire suspension test on P9 and 11, no effects in the open-field, male delays in the high dose group on the water escape test, increased time in open and several other measures in the EPM. One low dose effects was also seen in terms of increased alternation frequency in the Y-maze, an effect not seen at the dose 10 times higher. Strengths: This is one of the few developmental neurotoxicity experiments in mice and therefore offers a slightly different species perspective.

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**Strengths:** The study has a number of strengths, including testing more than one dose of BaP, including multiple behavioral tests, and appropriate statistical analyses. **Weaknesses:** Only 5 litters were used in each group and there is no evidence that litter effects were accounted for. Many of the tests, while affected, are of limited interpretative value, and the doses of BaP are high.

In a study not included in the EPA review is by Maciel et al. (Maciel et al., 2014) but also they assessed motor and cognitive effects in this study done in Wistar rats, its relevance to the current assessment is marginal since the exposure and assessment were both to adult rats, therefore, the details will not be reviewed.

More relevant is a study not included in the EPA review by Patri et al. (Patri et al., 2013). In this unusual design, P5 Wistar rats were given a single, intracisternal injection of 0.1  $\mu$ M of BaP, raised and testing in the MWM before 6 weeks of age. Starting at P28, rats were tested in a 143 cm diameter maze for 8 days, 4 trials/day with a probe trial given 24 h after the last learning trial. The BaP group had significantly longer escape latencies than untreated or vehicle treated controls on days 3-8. Significantly, not only were their latencies longer, they had much longer path lengths than controls. Furthermore, swim speed was assessed and no differences found. On the probe trial the BaP groups has fewer site crossovers and reduced time in the target quadrant. **Strengths:** This experiment conducted the MWM better than in any of the above mentioned studies because they appropriately accounts for and eliminated concerns over potential swim speed differences by directly measuring swim speed and analyzing path length. They also conducted their probe trial 24 h after the last learning trial, making a reference memory deficit apparent without confounding with a possible working memory effects. **Weaknesses:** The intracisternal route of BaP administration makes this study more difficult to utilize in terms of risk assessment, and the groups sizes were marginal: N = 4 in the untreated group, N = 7 in the DMSO vehicle group, and N = 8 in the BaP group. In addition, it is not stated how many litters these male rats came from leaving open some concern that they may have been drawn from a small number of litter without proper attention to proper litter sampling.

**Preliminary Synthesis:** The above developmental neurobehavioral studies on BaP provide reasonable evidence that this compound induces developmental neurotoxicity in animal model systems. Several of the studies are fairly well done and provide reasonably compelling data. Nevertheless, each of the studies reviewed has limitation and some of these are of significant concern. This applies especially to some tests with known experiment to experiment variability and questionable replicability, such as the EPM and NOR. Studies using these methods should be replicated by the lab in a separate set of experiments or another lab with the same findings before significant weight should be placed on these effects. There are countless examples in the literature where findings with these methods cannot be replicated. Tests such as the open-field test of locomotor activity tend to be more reliable provided the test is properly done. This includes using an automated method, testing for a sufficient length of time (30-60 min, rather than 5 min to sample enough behavior) and proper environmental controls. The MWM has been heavily represented in the above reviewed experiments, largely in the absence of other tests of learning and memory. While the MWM is a superb test when properly conducted to assess spatial learning and reference memory, a strongly hippocampally-dependent form of cognition, it

is unfortunate that the above dataset does not derive the benefit of convergence by having other learning and memory tests utilized. On the other side of the issue, the fact that there are multiple experiments using this test, increases the confidence that developmental BaP has effects on spatial learning and memory, and this is a definite strength of these data. More significantly, however, are the deficiencies in the MWM methods in virtually every experiment reviewed. This raises concerns about how much weight to place on these data. The caveats are not trivial. Failure to include proper maze scaling, and most importantly proper control for potential confounding non-cognitive performance factors casts much of the data in doubt. Why these experiments have these concerns is unclear but as they stand, considerable doubt cannot be avoided in interpreting their meaning.

**2b. Reproductive toxicity** (sections 1.1.2, 1.2.1). The draft assessment concludes that male and female reproductive effects are a human hazard of benzo[a]pyrene exposure. Do the available human and animal studies support this conclusion?

**2c. Immunotoxicity** (sections 1.1.3, 1.2.1). The draft assessment concludes that immunotoxicity is a potential human hazard of benzo[a]pyrene exposure. Do the available human and animal studies support this conclusion?

**2d. Other types of toxicity** (section 1.1.4). The draft assessment concludes that the evidence does not support other types of noncancer toxicity as a potential human hazard. Are there other types of noncancer toxicity that can be credibly associated with benzo[a]pyrene exposure?

**3. Dose-response analysis.** In section 2, the draft assessment uses the available human, animal, and mechanistic studies to derive candidate toxicity values for each hazard that is credibly associated with benzo[a]pyrene exposure in section 1, then proposes an overall toxicity value for each route of exposure.

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**Dr. Christi Walter**

## **Response to Charge Question 1. Literature Search/Study Selection and Evaluation**

The processes for searching, selecting and evaluating literature were clearly described. The most recent references are around 2011. Additional references are provided in comments on specific sections. However, EPA should continue to compile references from 2011 to update the reference list. It is understandable that the document had to be submitted and stop evolving until it was evaluated, but the collection of relevant references should be an ongoing process. References for reproductive toxicology have been provided in the comments for that section.

## **Response to Charge Question 2b. Reproductive Toxicity**

### **Section 1.1.2**

The current document conveys a summary of traditional toxicological outcomes of BaP on reproduction. Because spermatogenesis is an ongoing process that renews itself, it is important to distinguish between an immediate effect and a lasting effect on male germ cells. In contrast, since oocytes develop in utero and are not continuously renewed, it is likely to be a major difference between male and female germlines. The distinction between immediate and long lasting effects on reproduction is rarely made in the literature so it is not surprising that it is not covered in the current document. There are many instances when an agent disrupts spermatogenesis, but the short term effects can be lost and long term normal spermatogenesis restored. This is an important aspect of male reproductive biology. If the intent for this review is to advise only about the immediate effects of BaP exposure on male reproduction, then the document is largely adequate. However, if the full ramifications of BaP exposure on reproduction are intended, there should be some discussion of the timeframe between treatment and observations and whether or not there was time for an additional wave or more of spermatogenesis before the outcomes were measured. Have the measurements been performed before spermatogenesis is restored, or after? The reversal of short term effects can involve testis size and weight, since this is a direct reflection of the amount of spermatogenesis, number and shape of sperm in the epididymis, count of ejaculated sperm, and histological appearance of the testis. However, it is also true that high concentrations of toxic agents may kill spermatogonial stem cells and have more permanent effects of spermatogenesis, testis, size and weight, and etc. Distinguishing between the different outcomes is important if we seek to inform that there can be short term consequences, but with enough time, many/most will go away.

An aspect of the document that would benefit from additional consideration is life stage and cell type. Because these cells will direct development of the next generation, successful reproduction may be compromised if germ cell mutagenesis is increased. *De novo* germ line mutations can result in genetic disease, miscarriage, infertility, etc. Life stage at exposure is of critical importance. Pre-spermatogonial stem cells proliferate extensively while migrating to and colonizing the embryonic gonad and after birth. This is a window of susceptibility to mutagenesis

that could result in lifelong increased mutant frequency in mature germ cells that would subsequently affect reproductive outcome (Xu et al., 2014). Further, stem cells are on the unprotected side of the testis blood barrier and more likely to be exposed to higher amounts of genotoxins. Because the stem cells are the cells that will continue to give rise to sperm, the impact of mutagenesis on stem cells can affect reproduction. There are no direct studies of the effects of BaP on spermatogonial stem cell mutagenesis, but there is a reference that implicates stem cell mutagenesis (Olsen et al., 2010). There are additional papers on the effects of BaP on adduct formation, mutagenesis, and gene expression (Verhofstad et al., 2010a; Verhofstad et al., 2010b; Verhofstad et al., 2011). To the best of my knowledge no studies on the mutagenic effects on oocytes has been performed and is likely due to the difficulty in obtaining adequate numbers of cells.

There are few studies on BaP effects on ovary, oocytes, etc. I am providing 4 I did not find in the HERO database, to help shore up the interpretations. (Einaudi et al., 2014; Kummer et al., 2013; Sadeu and Foster, 2011; Sadeu and Foster, 2013)

The tables figures to be particularly helpful. However, the document contains far too many abbreviations if the intention is to make the document understood by non-experts. While many who will read the report will be familiar with the abbreviations, it is dismaying if you aren't familiar with them. What is the target audience for the final document?

Available studies do support the conclusion that BaP exerts hazardous effects on human reproduction.

#### Recommended additional references for reproductive toxicology:

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## **Response to Charge Question 3b. Inhalation Reference Concentration**

### **Section 2.3.1-5**

Inhalation is a common route of exposure, thus it is important to have an RfC since BaP is a near-ubiquitous pollutant. While the RfC for inhalation of BaP is important, all cited studies fall short of the mark in providing the needed data. There are many assumptions, manipulation through conversion factors and extensive extrapolation of the limited data. Unfortunately, the limited number of studies and inadequacies of published studies to address the criteria needed for establishing a robust RfC, result in a very low confidence in the RfC and lead to questions as to whether it is worth publishing. Additional comments are provided below in case the group decides to go forward and publish this section of the review.

Page 2-16 lines 10-11. Human inhalation data, which had been discounted as useful for determining the RfC on page 2-15 is cited as supporting the animal data. Discounted data cannot be used to support animal data. Rather, they are consistent with the animal data, which is also inadequate for determining an RfC.

Page 2-16 Developmental and Reproductive Toxicity. Traditional toxicological endpoints are discussed, but the effects of BaP on development and reproduction could also stem from the effects on the genome and epigenome and these are not discussed. If the inhalation RfC is included in final review, information on the genetic and epigenetic changes identified in development and reproduction should be included. Mutations and epi-mutations during development can result in developmental outcomes that do not necessarily involve fetal death. Thus, it is suggested that information on toxicogenomics be included in these two sections.

With regard to reproductive toxicity, the toxicogenomics are also highly relevant and missing. Further, few studies examine whether spermatogenesis recovers after delays in exposure. This may be less important if one is considering continuous lifetime exposure, but it is important to know if spermatogenesis is impaired and whether BaP is mutagenic in spermatogenic cells, at the RfC.

Not clear how results from a different route of exposure can be considered to bolster the inhalation effects when routes are considered separately? Page 2-23 lines 33-34.