Dr. John DiGiovanni: Preliminary Comments

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

In general, the literature review process is fairly well described and documented. One aspect that is difficult to assess is what information has been lost due to the exclusion of a large number of articles originally retrieved from the search criteria. In addition, a better description of the exclusion criteria might be helpful. It also seems that some more recent literature has been omitted likely due to the timeline for preparation of the document.

There are a couple of papers listed below that should be considered for citation that I did not find in the reference list:

1. Dose-response for B(a)P skin carcinogenesis in two different mouse strains
Reiners, J.J. et al Carcinogenesis, 3:301-307, 1984

2. Mapping of BPDE DNA adducts in the p53 gene of NHBE cells
Dessinenko, MF et al, Science 274:430-432, 1996

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 and animal 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 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?

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?

Collectively, the available human, animal and mechanistic data support the conclusion that B(a)P

is "carcinogenic to humans". In general, this section is well written and summarizes the available data from human epidemiology studies as well as relevant animal data and mechanistic data. The focus is on lung, bladder and skin cancers although other cancers have been linked to PAH such as B(a)P. Notably, there are no human epidemiologic studies where the exposure was to B(a)P alone. Environmental or occupational exposures to PAHs occur as mixtures of many PAHs, including B(a)P. Because there is no direct evidence in humans for B(a)P carcinogenesis this should be discussed and presented more adequately.

Note that this classification is consistent with IARC which also classifies B(a)P as carcinogenic to humans (Gropup 1). This classification by IARC is also based on the "strong and extensive experimental evidence for the carcinogenicity of B(a)P in many animal species, supported by the consistent and coherent mechanistic evidence from experimental and human studies that provide biological plausibility to support the overall classification of B(a)P as a human carcinogen".

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 $3x10^{-4}$ 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?

3b. **Inhalation reference concentration for effects other than cancer** (section 2.2). The draft assessment proposes an overall reference concentration of $2x10^{-6}$ mg/m^3 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?

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?

Two oral carcinogenesis studies, one conducted in male and female Wistar rats and one conducted in female B6C3F1 mice, were considered for the derivation of the oral slope factor for cancer (Table 2-7). The calculated oral slop factors from these two studies (for various cancer sites) ranged from 0.04 to 1 per mg/kg-d.

The oral slope factor for cancer (1 per m/kg-day) was ultimately derived from the dose-response study of alimentary tract tumors (forestomach, esophagus, tongue and larynx combined) in female B6C3F1 mice that received oral administration of B(a)P at different doses. The rational for selecting this slope factor is given as follows "As there are no data to support any one result as most relevant for extrapolating to humans, the most sensitive result was used to derive the oral slope factor". To base the oral slope factor for cancer on a single (albeit well designed and executed) study in only one sex seems somewhat problematic although the desire to err on the side of caution and select such a conservative value is understandable. Having data in both male and female B6C3F1 mice would be more desirable and would strengthen the determination of the oral slope using only mice. In addition, the justification for excluding the rat data and the derived slope factors from this data in the final calculation needs to be stronger.

It is clear from the oral carcinogenicity data in the studies by Kroese et al (male and female Wistar rats) and Beland and Culp (female B6C3F1 mice) as well as other data in the literature that the rodent forestomach is a very sensitive site for tumor development in both rats and mice. Because there is no human equivalent to the rodent forestomach this has been discussed in section 2.3.4 Uncertainties in the Derivation of the Oral Slope Factor and indicated in Table 2-8. It is not clear how concordance of this tumor site across mice and rats increases the relevance of the oral slope factor derivation to humans. Some additional discussion may be warranted here.

The Multistage Weibull model is used to derive the oral slope factor because it incorporates both the time at which death with tumor occurred as well as well as dose in the determination. While this seems to be appropriate, I think that there could be a little more detailed explanation for choosing this method over other possible methods that may have been considered. If other methods were compared what would be the oral slope factor for cancer? In the previous IRIS assessment several models were used to fit the data and a geometric mean of the estimates from 4 different models was recommended.

3d. **Inhalation unit risk for cancer** (section 2.4). The draft assessment proposes an inhalation unit risk of 0.5 per mg/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?

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?

The dermal slope factor (0.006 per µg/day) for skin tumors was calculated from the NIOSH study (Sivak et al, 1997) that examined B(a)P skin carcinogenesis in male C3H/HeJ mice which was then adjusted using a cross-species scaling approach similar to that used for derivation of the oral slope factor for cancer based on body weight. A number of other studies were considered but for various reasons they were excluded in the final determination.

There are significant differences in sensitivity of various mouse strains to skin carcinogenesis in addition to significant differences between species. The reliance of the derivation of the dermal slope factor on a single study using C3H/HeJ male mice leads to significant uncertainty in the calculated dermal slope factor. In addition, from the studies selected there is a suggestion of differences between male and female mice in terms of susceptibility to skin carcinogenesis by B(a)P. These uncertainties impact the derivation and accuracy of the dermal slope factor using only a single set of data from a single mouse strain and from a single sex (again in this case males). At the very least there should be some additional discussion of these two issues. Alternatively, a geometric or other type of mean could be considered by combining values from the different studies shown in Table 2.11.

As stated in the document, dermal exposure to PAH such as B(a)P in the environment likely occurs predominantly via soil contact and the available data on B(a)P carcinogenesis comes from studies where it was applied topically in a solvent. Thus, the use of a solvent may increase availablility of B(a)P in the skin. Skin carcinogenesis bioassays using a soil matrix are not available to address this question, however, there are studies that have been done to examine the availablility of PAH from soil and various particulates that might be considered in calculating the dermal slope factor. As with the other cancer slope factors derived in this document, B(a)P will be present in environmental samples as a mixture of many PAHs that may also impact is availability. This aspect should be considered more thoroughly in the document. Others have taken the availablility of B(a)P from soil into consideration in calculating a dermal slope factor for skin cancer (Knafla et al, 2011)

In addition, there are several assumptions that are made in calculating the dermal slope factor. First, the cross-species scaling used to calculate the dermal slope factor uses the same method of allometric scaling used for the oral slope factor for cancer i.e., ¾ power of body weight. This method of scaling may not be appropriate for the following reasons: i) skin carcinogenesis based on dermal exposure will be proportional to the area of skin exposed as well as the dose and not necessarily proportional to body weight. Thus, scaling methods based on a unit area of skin exposed and not body weight should be considered as discussed by Knafla et al, 2011; ii) there are significant differences between mouse skin and human skin, particularly in total skin thickness as well as dermal absorption characteristics. The assumption that toxicokinetic processes in the skin will scale similarly to interspecies differences in whole body toxicokinetics is not supported by the available data. These differences need to be taken into further consideration in the cross-species scaling. Again, this is discussed in the Knafla et al, 2011 paper where an adjustment is made for these differences in skin thickness in their calculation of a dermal slope factor for skin cancer.

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?

The available studies in both humans and animals support a mutagenic mode of action for B(a)P. The use of ADAFs for sensitive populations is appropriate based on a mutagenic mode of action for B(a)P.

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

Charge question on the public comments

2. 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