

**Clarifications/Comments for the SAB Draft Peer Review Report on Benzo[a]pyrene:
Public Teleconference**

August 21, 2015

EPA would like to clarify some areas in the draft benzo[a]pyrene (BaP) assessment that have led to unintended interpretations, and to ask for further clarification of some recommendations in the SAB's draft report. For each topic, text from the draft SAB report is repeated below, followed by EPA's bulleted clarifications/questions.

Regarding forestomach toxicity as a hazard, the panel report states the following:

p. 3, lines 16- 21. The potential hazards from BaP exposure identified and discussed in Section 1.1.4 include forestomach toxicity, hematological toxicity, liver toxicity, kidney toxicity, cardiovascular toxicity, and adult nervous system effects. Overall, the EPA concluded that the available evidence does not support these noncancer effects as potential human hazards. The SAB recommends that EPA's basis for arriving at this conclusion be expanded for these health endpoints. In addition, the SAB finds that the evidence presented in the assessment does not support EPA's conclusion that forestomach toxicity in rodents, cardiovascular toxicity, and adult nervous system toxicity are not potential human hazards.

p. 23, lines 43-44. The available evidence presented does not support EPA's conclusion that forestomach toxicity in rodents is not a potential human health hazard.

p. 24, lines 1-16. The document should be internally consistent regarding the human health hazard of forestomach toxicity. The EPA did not consider human relevance to be an appropriate basis for excluding the credible evidence of forestomach toxicity associated with BaP exposure, noting that humans do not have a forestomach but do have similar squamous epithelial tissue in their oral cavity. 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.

The decision to not consider forestomach toxicity further for dose-response analysis and the derivation of reference values, as explained in section 1.2.1 "Weight of Evidence for Effects Other than Cancer," should not be used as a justification for excluding forestomach toxicity as a hazard credibly associated with BaP 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 EPA in the section "other modes of action."

For these reasons, forestomach toxicity is credibly associated with BaP exposure, so it is reasonable to identify it as such in the hazard identification section of the document.

- EPA would like to clarify that the Agency considered forestomach hyperplasia to be a hazard but did not consider this endpoint further for dose-response analysis and subsequent derivation of noncancer reference values due to the conclusion that forestomach hyperplasia "most likely reflects early events in the neoplastic progression of forestomach tumors following benzo[a]pyrene exposure (see Section 1.1.4)" (see p. 1-82 of the draft assessment).

The organization of forestomach effects into section 1.1.4 ("Other Toxicities") introduced confusion due to the overarching statement in the introduction of Section 1.1.4.: "Overall, EPA concluded that the available evidence does not support these noncancer effects as potential

human hazards.” This statement was not meant to apply to forestomach toxicity. EPA will clarify this point in the next version of the assessment.

Regarding cervical hyperplasia and inflammation as critical endpoints for the RfD, the panel report states the following:

Letter to the Administrator, p. 1, lines 35-40. For derivation of the oral reference dose (RfD), the EPA has not made a sufficiently strong case that the available developmental endpoints are the most appropriate non-cancer endpoints for deriving an RfD, or that among the available neurodevelopmental endpoints, the most appropriate results have been used. The SAB suggests that the agency give more consideration to the available data on reproductive outcomes including cervical hyperplasia and cervical inflammation, and provide a firmer justification for not selecting these as critical endpoints.

p. 3, lines 36-39. With respect to developmental toxicity as the most appropriate category of non-cancer effects, the SAB suggests that EPA give more consideration to the available reproductive outcomes including cervical hyperplasia and cervical inflammation in Gao et al. (2011), and at least provide a firmer justification for not selecting these as critical endpoints.

p. 28, lines 27-30. With respect to developmental toxicity as the most appropriate category of non-cancer effects, the SAB suggests that EPA give more consideration to the available reproductive outcomes, including cervical hyperplasia and cervical inflammation in Gao et al. (2011), and at least provide a firmer justification for not selecting these as critical endpoints.

- Could the SAB clarify whether they are suggesting that the cervical hyperplasia and inflammation observed in the single study by Gao et al. (2011) are more appropriate endpoints for the basis of a reproductive RfD (instead of decreased ovary weight) or the overall RfD (instead of neurodevelopmental effects)?
- Could the SAB expand on whether they consider cervical hyperplasia or inflammation to be directly related to impaired reproductive function?

Regarding the database uncertainty factor for the RfD, the panel report states the following:

Letter to the Administrator p. 1, lines 44-47, p. 2, line 1. In addition, EPA should further justify the application of a database uncertainty factor of 3 that is based, in part, on the absence of a multi-generational study or extended one generation study, and the lack of a study examining functional neurological endpoints following exposure from gestation through lactation.

p. 4, lines 3-8. In addition, the SAB recommends that EPA further justify the application of a database uncertainty factor of 3 that is based, in part, on the absence of a multi-generational study or extended one generation study, and the lack of a study examining functional neurological endpoints following exposure from gestation through lactation. The EPA might consider whether an EPA developmental neurotoxicity guideline study and/or extended 1-gen study with a DNT cohort is likely to result in a NOAEL below that of Chen et al. (2012).

p. 29, lines 34-46 and p. 30 lines 1-7. With respect to the application of uncertainty factors in derivation of the RfD. The SAB suggests that the EPA further justify the application of an UF of 3 for database deficiency that is based, in part, on the absence of a multi-generational study or extended one generation study (OECD 443 – which is considered a replacement for the multigenerational study). The SAB suggests that the current data base could be considered sufficient as multigenerational studies were conducted and adverse outcomes were demonstrated that are supported by mode of action

studies. With the advent of the OECD 443, F1 animals, which have been continually dosed, are only assessed for reproductive effects if triggered (Parental generation are only required to be dosed for 2-weeks prior to mating). Therefore, it is questionable that the OECD 443 will provide any additionally useful reproductive information.

The lack of a study examining functional neurological endpoints following exposure from gestation through lactation was also used as justification for the UF of 3. However, there were 2 oral studies exposing dams at GD 14-17 (McCallister et al. 2008; Sheng et al. 2010), and 2 oral studies exposing dams or pups directly postnatally (Bouayed et al. 2009; Chen et al. 2012) that evaluated functional endpoints. There were additional gestational exposure studies evaluating receptor gene expression, although there were no studies that examined both gestational and lactational exposure. The EPA should address the question of whether the absence of such a study warrants an additional UF of 3 given that the UF of 10 for inter-individual differences is already included. As part of this deliberation, EPA might also consider whether an EPA developmental neurotoxicity testing (DNT) guideline study and/or extended 1-gen study with a DNT cohort is likely to result in a NOAEL below that of Chen et al. (2012).

- [For context] EPA states in the assessment (p. 2-9, starting on line 37) that “[a] database uncertainty factor, UF_D , of 3 was applied to account for database deficiencies, including the lack of a standard multigenerational study or extended 1-generation study that includes exposure from pre-mating through lactation, considering that benzo[a]pyrene has been shown to affect fertility in adult male and female animals by multiple routes of exposure (see Section 1.1.2). Considering that decreased fertility in adult male and female mice is observed following gestational exposure, it is assumed that exposure occurring over this more comprehensive period of development could result in a lower POD. Also, the lack of a study examining functional neurological endpoints following a more comprehensive period of developmental exposure (i.e., gestation through lactation) is a data gap, considering human and animal evidence indicating altered neurological development (see Section 1.1.1)
- EPA would like to clarify that there was no standard multigenerational or extended one-generation study available. Note that one statement in the draft assessment inadvertently describes two developmental studies as multigenerational studies (p. 1-36, line 32). While several studies cover different portions of the developmental period, they do not address cumulative exposure throughout the developmental period. More specifically, the study used for the derivation of the developmental (and overall) RfD, Chen et al. (2012), treated animals from PNDs 5-11. Additional studies in the oral database support that neurodevelopmental effects may occur after exposure to BaP from GDs 14-17 (McCallister et al. 2008; Sheng et al. 2010) or following lactational exposure from PNDs 0-14 (Bouayed et al. 2009). However, these supporting studies do not cover the low-dose region as thoroughly as Chen et al. (2012) (e.g., these studies observed effects at the lowest doses tested; doses which are higher than the POD identified for Chen et al. [2012]).
- Can the SAB elaborate on whether they believe the full period of susceptibility (in terms of the observed neurodevelopmental effects) has been covered by the available studies and whether a neurodevelopmental study which treats animals from gestation to lactation would be unlikely to result in a higher magnitude of effects or effects at lower doses levels?

Regarding recommendations for the reproductive toxicity section, the panel report states the following:

p. 15, lines 16-17. The SAB recommends that the EPA consider additional endpoints (i.e., ovarian and testicular effects) be considered for point of departure/BMD analyses and RfD derivation.

p. 15, lines 19-20. The SAB recommends that the EPA provide additional clarity as to why certain studies, or parts of studies, are brought forward while others are not.

- EPA assumes that these two recommendations are directed at reproductive studies/endpoints brought forward for dose-response and discussed in Chapter 2 of the draft assessment. EPA notes that the consideration of reproductive toxicity studies and endpoints for POD/BMD analyses and RfD derivation are discussed on pp. 2-4 and 2-5 of the draft assessment. Can the SAB clarify which additional ovarian and testicular effects/studies should be considered? Also, could the SAB expand on the recommendation regarding the studies or parts of studies brought forward for dose-response analysis?

Regarding confusion with respect to charge question 3a, the panel report states the following:

p.30, lines 19-32. The SAB found the last portion of charge question 3a, (*Does the discussion of exposure scenarios (section 2.1.5) reflect the scientific considerations that are inherent for exposures during a critical 20 window of development?*) somewhat vague. In section 2.1.5, the assessment notes that the most sensitive endpoint for RfD development is based on “neurobehavioral changes in rats exposed to benzo[a]pyrene during a susceptible lifestage,” i.e., rats exposed *in utero*. Thus, this endpoint is a neurodevelopmental endpoint. The assessment notes in section 2.1.5 that while the RfD derived from this endpoint should be applied to the general population, averaging of exposures over a lifetime should take into account that the critical window of exposure for this developmental endpoint can be much shorter than a lifetime exposure. The SAB interprets this portion of the charge question as asking whether the explanation in section 2.1.5 of the applicability of the concept of a critical window of exposure to the RfD (which is intended to be without significant risk during a *lifetime* of exposure) is appropriate and appropriately conveys the relationship of the critical window of exposure to the lifetime exposure framework of the RfD. Given this interpretation, the SAB agrees that section 2.1.5 is appropriate and appropriately conveys this concept.

- EPA would like to clarify that the panel has correctly interpreted this charge question, and would appreciate any wording suggestions to help make this type of charge question more clear in the future.

Regarding allometric scaling for larynx, esophagus and forestomach tumors, the panel report states the following:

Letter to the Administrator p. 2, lines 20-23. The SAB also questions the use of default cross-species scaling applied to all of the tumor sites identified in the two studies. The SAB commented that allometric scaling for alimentary tract sites (larynx, esophagus, forestomach) which can be considered portal-of-entry tumor sites may not be needed.

p. 4, lines 31-37. The SAB also has questions regarding the choice of cross-species scaling factors. Using this approach, time-weighted daily average doses are converted to human equivalent doses (HEDs) on the basis of $BW^{3/4}$ scaling. This allometric scaling is based on current EPA guidelines. However, there is uncertainty as to whether this scaling should be applied to all of the tumor sites identified in the two

studies. In particular, alimentary tract sites (larynx, esophagus, forestomach) can be considered portal-of-entry tumor sites, and allometric scaling may not be appropriate for these sites.

p. 33, lines 28-32, p. 34 lines 1-2. The adjustments for approximating human equivalent slope factors use the EPA cross-species scaling methodology. Using this approach, time-weighted daily average doses are converted to HEDs on the basis of $BW^{3/4}$ scaling. This allometric scaling is based on current EPA Guidelines (USEPA, 2005a). However, there is uncertainty as to whether this scaling should be applied to all of the tumor sites identified in the two studies. In particular, alimentary tract sites (larynx, esophagus, forestomach) can be considered portal-of-entry tumor sites, and may not require the application of allometric scaling for these sites.

- Could the SAB elaborate on this recommendation and provide relevant references for consideration?

Regarding additional studies for quantitative consideration for the dermal slope factor, the panel report states the following:

Letter to the Administrator p. 2, lines 33-34. The SAB recommends that the EPA include two additional studies for review and consider combining results from the mouse skin tumor bioassays to strengthen the derived DSF.

p. 37, lines 4-6. EPA should consider adding Nesnow et al. (1983) and Levin et al. (1977) studies to Table 2-11 and should consider combining results from the different studies shown in Table 2-11. This would strengthen the derived DSF. (also see p. 5, lines 9-11)

- EPA notes that the intent of table 2-11 was to capture the studies considered most appropriate for dose-response analysis and derivation of a cancer slope factor for lifetime dermal exposure. Based on several criteria (see p. 2-39 of the draft assessment), ten studies were incorporated into table 2-11 and several others were excluded. One of the major criterion was duration of exposure, in which the Agency focused on studies of approximately 2-years duration (i.e., lifetime). As noted in the draft assessment, less-than-lifetime studies “would tend to underestimate lifetime risk by overlooking the potential for the development of tumors in later life” (p. 2-39, lines 34-35). The studies noted by the SAB (Nesnow et al. [1983] and Levin et al. [1977]) treated mice for less than 2 years. Could the SAB elaborate on whether the relatively shorter durations of these two studies would decrease confidence in their utility for the derivation of a lifetime cancer slope factor? The assessment listed other less-than-lifetime studies; could the SAB clarify why these two studies were recommended and not the others?