UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C. 20460

OFFICE OF THE ADMINISTRATOR
SCIENCE ADVISORY BOARD

April 5, 2016

EPA-SAB-16-003

The Honorable Gina McCarthy
Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, D.C. 20460

Subject: Review of EPA's Draft Assessment entitled *Toxicological Review of Benzo[a]pyrene (September 2014)*

Dear Administrator McCarthy:

The EPA's National Center for Environmental Assessment (NCEA) requested that the Science Advisory Board (SAB) review the draft assessment, entitled *Draft Toxicological Review of Benzo[a]pyrene.* The assessment consists of a review of publicly available scientific literature on the toxicity of benzo[a]pyrene (BaP). The SAB was asked to comment on the scientific soundness of the hazard and dose-response assessment of BaP-induced cancer and non-cancer health effects. In response to the EPA's request, the SAB convened a panel consisting of members of the SAB Chemical Assessment Advisory Committee (CAAC) augmented with subject matter experts to conduct the review. The enclosed report provides the SAB's consensus advice and recommendations. This letter briefly conveys the major findings.

With regard to hazard identification, the SAB agrees that the available human, animal, and mechanistic studies support the EPA's conclusions that developmental neurotoxicity, developmental toxicity, male and female reproductive effects, and immunotoxicity are human hazards of BaP exposure. In addition, the SAB agrees with the classification of BaP as *carcinogenic to humans* by all routes of exposure in accordance with EPA's *Guidelines for Carcinogen Risk Assessment*. Furthermore, the SAB agrees that BaP-induced tumors arise primarily through a mutagenic mode of action resulting from BaP-induced DNA damage. However, the evidence presented in the assessment does not support EPA's conclusion that forestomach toxicity in rodents is not supportive of potential human hazard, and that cardiovascular toxicity and adult nervous system toxicity are not potential human hazards. Further evaluation and explanation should be provided for these conclusions.

For derivation of the oral reference dose (RfD), the SAB agrees that developmental endpoints, and in particular neurodevelopmental endpoints, are the appropriate basis for deriving an RfD for BaP. However, the EPA has not sufficiently justified that the developmental effects presented in the assessment 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 recommends that the EPA consider the overall picture of neurodevelopmental effects from a broader set

of the neurodevelopmental endpoints to justify and support the choice of the critical endpoint. The SAB suggests that the EPA 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.

With respect to the application of uncertainty factors, the SAB supports the application of a factor of 10 for intra-human variability. The SAB also recommends that the EPA consider applying a body weight $^{3/4}$ ($BW^{3/4}$) adjustment factor for interspecies extrapolation from neonatal animal to neonatal human. In addition, the EPA should provide further justification for 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.

For derivation of the inhalation reference concentration (RfC), the SAB found that the RfC value provided in the assessment is not scientifically supported. While the endpoint (decreased fetal survival) and key study selected are appropriate, the RfC is based only upon this one study that has some technical deficiencies that decrease the confidence in the RfC. Furthermore, the rationale for not employing a benchmark dose (BMD) approach to derive the point of departure is unclear. Regarding UFs, the EPA application of a UF of 3 to address residual uncertainty for interspecies extrapolation may be too low, since the regional deposited dose ratio (RDDR) adjustment used with the key study may not completely account for systemic toxicokinetics following an inhalation exposure. Additionally, because the effect was found at all exposure levels, the lowest-observed-adverse-effect level (LOAEL) from this study provides a weaker basis than a no-observed-adverse-effect level (NOAEL) for derivation of the RfC. The SAB recommends two studies that should be considered by the EPA to develop a more comprehensive dose-response relationship for BaP.

For derivation of the oral slope factor for cancer, the SAB finds that appropriate studies and models were selected for dose-response analysis. However, insufficient justification was provided for the derivation of the final slope factor solely based on a single-sex mouse study that produced the largest cancer slope factor. The SAB suggests that data from all studies be incorporated in the derivation of the oral cancer slope factor. The SAB also questions the use of a default cross-species scaling factor applied to all of the tumor sites identified in the two studies. The SAB recommends that the EPA provide a brief explanation of the rationale for its use of the allometric scaling factor when deriving the BaP oral slope factor, given what is known about the BaP mode of action for carcinogenicity, reaction rates, toxicokinetics, and the portal of entry effect for alimentary tract tumors.

For the derivation of the inhalation unit risk (IUR) for cancer, the SAB finds that the EPA has selected an appropriate study for dose-response analysis, and that appropriate models were used. The SAB recommends additional discussion of key assumptions, conducting sensitivity analyses, and encourages the EPA to reconsider the decision not to use epidemiological data to support the derivation of the IUR.

The SAB commends the EPA's efforts in deriving the IRIS Program's first dermal slope factor (DSF). However, the proposed DSF is not sufficiently supported scientifically. The SAB agrees that studies of skin tumors in mice are relevant to humans based on evidence of a similar mode of action and can be used to derive a DSF. However, 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. The SAB also recommends that the EPA more thoroughly review the evidence of skin cancer in studies of coke, steel and iron, coal gasification and aluminum workers given their relevance for

evaluating the appropriateness of using the mouse-based risk assessment model for predicting skin cancer risk in humans.

The assessment used mass rather than mass/area as the dose metric for cancer risk at "low dose" exposure to BaP. The SAB does not have a specific recommendation as to the dose metric, but strongly recommends that in the absence of empirical data, the decision be based upon a clearly articulated, logical, scientific structure that includes what is known about the dermal absorption of BaP under both conditions of the bioassays and anticipated human exposure, as well as the mechanism of skin carcinogenesis of BaP. The SAB also recommends that cancer risk calculated from the derived DSF should use the absorbed dose, and not the applied dose. Moreover, the SAB recommends that the EPA describe what constitutes a "low dose" exposure when using the mass of BaP as the dose metric.

The SAB believes the cross-species scaling approach used in the assessment should be supported by a coherent logical structure. In addition, differences between mouse and human skin should be considered, such as thickness of and metabolic rates in the target tissue (i.e., the viable epidermis layer).

Finally, the SAB concludes that the available mechanistic studies in humans and animals support a mutagenic mode of action for BaP-induced cancers, and the proposed use of age-dependent adjustment factors is justified.

The SAB appreciates this opportunity to review EPA's *Draft Toxicological Review of Benzo[a]pyrene* and looks forward to the EPA's response to these recommendations.

Sincerely,

/Signed/

Dr. Peter S. Thorne
Chair
EPA Science Advisory Board

/Signed/

Dr. Elaine M. Faustman
Chair
SAB Chemical Assessment Advisory Committee
Augmented for the Review of the Draft IRIS
Benzo[a]pyrene Assessment

Enclosure

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TABLE OF CONTENTS

Abbreviations and Acronyms

AhR	aryl hydrocarbon receptor
AIC	Akaike Information Criteria
ADAF	age-dependent adjustment factor
ADHD	attention deficit hyperactivity disorder
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate
ANOVA	analysis of variance
ATSDR	Agency for Toxic Substances and Disease Registry
BMC	benchmark concentration
BMCL	lower 95% confidence limit of the benchmark concentration
BMD	benchmark dose
BMDL	lower 95% confidence limit of the benchmark dose
BMR	benchmark response
BW	body weight
CAAC	Chemical Assessment Advisory Committee
CI	confidence interval
DSF	dermal slope factor
EPA	Environmental Protection Agency
ET	extrathoracic respiratory tract region
HED	human equivalent dose
HERO	Health and Environmental Research Online
HPBMC	human peripheral blood mononuclear cell
5-HT	5-hydroxytrytamine
IARC	International Agency for Research on Cancer
Ig	immunoglobulin
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
LOAEL	Lowest-Observed-Adverse-Effect Level
MOA	mode of action
NAS	National Academy of Sciences
NCI	National Cancer Institute
NIOSH	National Institute for Occupational Safety and Health
NMDA	N-methyl-D-aspartate
NOAEL	No-Observed-Adverse-Effect Level
NRC	National Research Council
NTP	National Toxicology Program
OECD	Organization for Economic Co-operation and Development
OR	odds ratio
ORD	Office of Research and Development
PAH	polycyclic aromatic hydrocarbons
PBMC	peripheral blood mononuclear cell
PFC	plaque forming cell
PHA	phytohemagglutinin
POD	point of departure
PU	pulmonary respiratory tract region
RfC	reference concentration

RfD	reference dose
RDDR	regional deposited dose ratio
ROS	reactive oxygen species
RR	relative risk
TDAR	T-dependent antibody response
UCL	Upper Confidence Limit
UF	uncertainty factor
UF_D	Database uncertainty factor
UF_H	Human inter-individual variability uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WHO	World Health Organization

1. EXECUTIVE SUMMARY

The Science Advisory Board (SAB) was asked by the EPA Integrated Risk Information System (IRIS) program to review the EPA's *Draft IRIS Toxicological Review of Benzo[a]pyrene (September 2014)* (hereafter referred to as the draft assessment). EPA's IRIS is a human health assessment program that evaluates information on health effects that may result from exposure to environmental contaminants. The assessment consists of a review of publicly available scientific literature on benzo[a]pyrene (BaP). The assessment was revised in September 2014 and a summary of EPA's disposition of the public comments received on an earlier draft of the assessment was added in Appendix G of the Supplemental Information to the Toxicological Review.

EPA asked the SAB to conduct a review of the scientific soundness of the conclusions presented in the draft BaP assessment. The SAB panel charged with conducting the review included members of the SAB Chemical Assessment Advisory Committee augmented with additional subject matter experts. An overview of the SAB's recommendations and advice on how to improve the clarity and strengthen the scientific basis of the assessment are presented below and discussed in greater depth in the body of the report.

Literature Search Strategy, Study Selection and Evaluation

In general, the literature search process is well described and documented. While the EPA did a thorough job documenting search terms used to identify studies for evaluation, the SAB notes that search terms for certain potential target organs are included but not others. The SAB recommends that the EPA review the references in the primary and secondary literature to identify potentially relevant articles not identified through the systematic searching and manual screening processes. In addition, secondary literature searches should be conducted whenever evidence for additional effects (e.g., cardiovascular effects) and specific data gaps emerge.

The SAB appreciates that the EPA is developing a handbook for the IRIS program which will outline the tools and processes to address study quality and risk of bias. In the interim, the EPA should provide sufficiently detailed criteria for each step of the process leading to the selection of key studies for the establishment of a point of departure. This will ensure not only that the rationale for initial study inclusion or exclusion is understood, but also that the strengths and weakness of the evaluated studies will be fully transparent. The SAB also requests clarification of how in vitro and mechanistic studies were included or excluded.

The SAB found that requiring a direct measure of BaP exposure is unnecessarily restrictive, especially in regards to epidemiology studies, as these studies could be relevant as supplemental information for hazard identification. Epidemiological studies of coke oven workers and other occupational groups with known exposures to BaP should at least be reviewed in the tables if not the text. The review of the epidemiology studies presented in the supplemental information relied heavily on the systematic review and meta-analysis reported by Bosetti et al. (2007) and Armstrong et al. (2004), respectively. It seems inappropriate for the EPA to rely solely on review articles rather than a review of the primary literature. In addition, the draft Supplemental Information document does not discuss any of the studies of asphalt workers and roofers or coke oven workers. The SAB has identified some epidemiology studies that EPA may consider for evaluation, including some of the studies of coal tar that were identified in the public comments.

The SAB has also provided a list of peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects of BaP.

Hazard Identification

Developmental Neurotoxicity and Developmental Toxicity

The SAB concurs with the EPA that BaP is a developmental neurotoxic agent in animals, with supporting evidence in humans. Prenatal airborne polycyclic aromatic hydrocarbon (PAH) exposures have been found to affect children's IQ adversely and may also contribute to attention deficit hyperactivity disorder (ADHD). In addition, there were plausible mechanistic studies that implicate N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) glutamate receptors, 5-hydroxytrytamine (5-HT) receptors, as well as oxidative DNA damage, as potentially mediating the observed neurobehavioral effects. Thus, there are sufficient studies, when considering the human, animal and mechanistic studies, to provide evidence of developmental neurotoxicity and effects on brain development and behavior. While each study has limitations, the weight of the evidence supports the conclusion that BaP can act as a developmental neurotoxicant.

The SAB concurs with the EPA that the available human studies support a contribution of BaP to human developmental toxicity. Studies with PAH mixtures have shown a correlation between PAH exposure and lower birth weights, increased risk of fetal death, and BaP DNA adducts. BaP exposure *in utero* has been demonstrated to cause fetal death, lower fetal/offspring weights and affect fetal germ cells. Additional studies showing BaP-related effects on fetal lung growth and function, and teratogenicity should be considered for inclusion.

Reproductive Toxicity

The SAB agrees that the data support the conclusion that BaP is a male and female reproductive toxicant through the oral and inhalation routes of exposure. The rodent data demonstrate convincingly that BaP affects fertility and fecundity. The functional effects in male rodents include adverse changes in testes and sperm and hormonal changes. Similar changes in sperm quality and fertility have been detected in humans exposed to PAH mixtures. The SAB recommends that the EPA give greater consideration to the genotoxic effects of BaP on male germ cells as a possible mode of action. BaP is mutagenic and mutagenesis in the germline can be detrimental to reproductive health.

BaP has a direct effect on adult rodent ovarian follicles. A recent study showed that *in vivo* exposure to BaP induces significant DNA damage in mouse oocytes and cumulus cells. *In utero* exposure of developing females to BaP provides compelling evidence that there is a sensitive window for exposure to BaP for the developing ovary.

Immunotoxicity

The SAB finds that the available immunotoxicity data based on animal models of pure BaP and complex PAH mixture exposures to humans (coke oven workers) support the claim that BaP is a human hazard for the immune system. The evidence for immunotoxicity in humans is based upon complex PAH mixture exposures. BaP as a pure chemical can cause suppression of human peripheral blood mononuclear cell responses at low concentrations (10-100 nM) *in vitro*. Immunotoxicity is caused by a combination of genotoxic (i.e. DNA adducts and p53-induced cell death) and non-genotoxic mechanisms (i.e. signaling due to AhR activation and oxidative stress). Animal studies provide strong evidence that BaP suppresses immune function leading to adverse consequences for host resistance to

infections and perhaps cancer. In addition to the evidence that BaP alters T cell development *in utero* and in adults, there is evidence that BaP alters B cell development in the bone marrow of adults. It is likely that the developing immune system is more sensitive to BaP exposures than adult exposures.

Cancer

The SAB finds that, in accordance with EPA's Cancer Guidelines, the EPA has demonstrated that BaP is a human carcinogen. This conclusion was based primarily on: (1) extensive evidence of carcinogenicity in animal studies, (2) the mode of carcinogenic action – mutagenic, and associated key precursor events have been identified in animals, (3) strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, and (4) strong support from an excess of lung cancer in humans who were exposed to PAHs, although not to BaP alone. This conclusion is consistent with the evaluations by other agencies, including the World Health Organization, International Agency for Research on Cancer and Health Canada.

Other Toxicity

Other potential hazards from BaP exposure are identified and discussed in Section 1.1.4 of the draft assessment; these 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 non-cancer effects as potential human hazards. The SAB recommends that the EPA clarify whether this conclusion is due to insufficient data, inconsistent data, or sufficient data to conclude that these health endpoints are not potential human hazards. In addition, the SAB finds that the evidence presented in the draft assessment does not support EPA's conclusion that squamous epithelium in the oral cavity (as implied by forestomach toxicity in rodents), cardiovascular toxicity, and adult nervous system toxicity are not potential human hazards from BaP exposure. The SAB also notes that the literature search was not sufficiently comprehensive to identify studies relevant to the characterization of cardiovascular system toxicity due to BaP exposure. Furthermore, the SAB identifies adult and developmental pulmonary toxicity as non-cancer endpoints that can be credibly associated with BaP exposure, but were not identified in the draft assessment.

Dose-Response Analysis

Oral Reference Dose for Effects Other Than Cancer

The SAB agrees that developmental endpoints, and neurodevelopmental endpoints in particular, are the appropriate basis for deriving an RfD for BaP. However, the SAB does not find that EPA has made a sufficiently strong case that the available developmental endpoints are the most appropriate non-cancer endpoints for setting an RfD, or that among the available neurodevelopmental endpoints, the observed results from the elevated plus maze test in Chen et al. (2012) are the most appropriate results.

With respect to developmental toxicity as the most appropriate category of non-cancer effects, the SAB suggests that the 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.

With respect to the choice of specific neurodevelopmental endpoints, the SAB recommends that the EPA consider the overall picture of neurodevelopmental impact from all of the neurodevelopmental endpoints in Chen et al. (2012)—including plus maze, reflex, locomotor activity and water maze—to justify and support the choice of the critical endpoint. In particular, the SAB suggests that the EPA reconsider or provide stronger justification for not using escape latency from the Morris water maze.

With respect to the application of uncertainty factors, the SAB supports the application of a UF of 10 for intrahuman variability. For interspecies extrapolation, the SAB recommends that the EPA consider application of a $BW^{3/4}$ adjustment as per the EPA's 2011 allometric scaling guidance for extrapolation from neonatal animal to neonatal human. In addition, the SAB recommends that the 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, and the lack of a study examining functional neurological endpoints following exposure from gestation through lactation.

Inhalation Reference Concentration for Effects other than Cancer

The RfC value as provided in the draft assessment is not scientifically supported due to: (1) the use of only one study (Archibong et al. 2002) for determining the point of departure (POD), (2) some technical limitations and specific deficiencies with this study, and (3) issues involving UF values. The rationale for not employing a benchmark dose (BMD) approach is unclear. Regarding uncertainty factors, since the regional deposited dose ratio (RDDR) adjustment used with the key study may not completely account for systemic toxicokinetics following particle deposition in the respiratory tract leading to extrarespiratory systemic effects, the EPA application of a UF of 3 to address residual uncertainty for interspecies extrapolation may be too low. Moreover, the Archibong et al. (2002) study found effects at all exposure levels. Thus, the use of the LOAEL for decreased fetal survival from this study for derivation of the RfC provides a weaker basis than a NOAEL. The SAB recommends that the EPA consider studies by Wu et al. (2003) and Archibong et al. (2012). While these two studies are not replicates of the key study, they may be useful in developing a more comprehensive dose-response relationship for BaP and, thus, may increase confidence in the LOAEL value used or further support use of BMD based approach.

Oral Slope Factor for Cancer

The SAB finds that appropriate studies and models were selected for dose-response analysis. However, an insufficient justification was provided for the selection of the final slope factor solely from the Beland and Culp (1998) mouse study, instead of the slope factor from the Kroese et al. (2001) rat study, or an average of the two, i.e., the EPA's choice of the single-sex mouse study that produces the largest cancer slope factor instead of a slope factor that incorporates data from all studies. 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 and is surrounded by considerable uncertainty. The SAB recommends that the EPA provide a brief explanation of the rationale for selecting an allometric scaling factor for the BaP oral cancer slope factor given what is known about the BaP mode of action for carcinogenicity, reaction rates, and toxicokinetics, and specifically, how the selection of the allometric scaling factor applies when there is a portal of entry effect for alimentary tract tumors.

Inhalation Unit Risk for Cancer

The SAB concludes that the EPA has selected an appropriate study (Thyssen et al. 1981) for dose-response analysis and that appropriate models were used to derive the inhalation unit risk (IUR). Although the IUR value is scientifically supported, the SAB recommends additional discussion of the key assumptions, conducting several sensitivity analyses, and reconsidering the use of epidemiological data for the derivation of inhalation unit risk values. The SAB also suggests the inclusion of an explicit conclusion statement regarding overall uncertainty of the unit risk value, and a brief discussion of the applicability of this value to typical environmental exposures (especially for sensitive subpopulations).

Dermal Slope Factor for Cancer

The SAB found the proposed dermal slope factor (DSF) and the proposed method for cross-species scaling to be not sufficiently scientifically supported. The key findings and recommendations of the SAB are summarized below:

- Choice of Studies:

  The SAB agrees that studies of mouse skin tumors are relevant to humans based on evidence for a similar mode of action. The draft assessment reviewed 10 complete carcinogenicity mouse skin tumor bioassays and Sivak et al. (1997) was chosen as the principal study. The SAB recommends that the EPA consider adding Nesnow et al. (1983) and Levin et al. (1997) for review and consider combining results from the different studies to strengthen the derived DSF. The SAB found the EPA's review of the epidemiological evidence of skin cancer in humans was not adequate. The SAB recommends that the EPA more thoroughly review the evidence for skin cancer in studies of coke, steel and iron, coal gasification and aluminum workers given their relevance for evaluating the appropriateness of using the mouse-based risk assessment model for predicting skin cancer risk in humans. The SAB agrees with the EPA that epidemiologic studies of therapeutic use of coal tar preparations do not provide an adequate basis for either hazard identification or the derivation of a dermal slope factor.

- Dose-Response Analysis:

  In evaluating the mouse (dermal) data, the EPA makes an adjustment if the dosing regimen is less than the expected life span. Doses in studies known or assumed to be shorter than 104 weeks are adjusted by a factor of $(Le/104)^3$, where Le is exposure duration in weeks and 104 weeks is the life expectancy of a mouse. The EPA should explain how a coefficient of 3 was chosen and how well it describes temporal dependence of the time-to-tumor data from the Sivak et al. (1997) study.

  The draft assessment used mass rather than mass/skin area as the dose metric for cancer risk at "low doses" of BaP. Published dermal slope factors for BaP skin carcinogenesis have used mass and mass/skin area as dose metrics and there do not appear to be any empirical data available to inform a choice between these two dose metrics or another metric. The SAB does not have a specific recommendation as to BaP dose metric, but strongly recommends that in the absence of empirical data the decision be based upon a clearly articulated, logical, scientific structure that includes what is known about the dermal absorption of BaP under both conditions of the bioassays and anticipated human exposures, as well as the mechanism of skin carcinogenesis of BaP. The SAB recommends that cancer risk calculated from the derived DSF should use absorbed dose, and not applied dose. The SAB also recommends that the EPA describe what constitutes a "low dose" if the assumption that mass of BaP is the appropriate dose metric for calculating the DSF from the skin cancer bioassay and for estimating cancer risk in humans.

- Dermal Slope Factor Cross-Species Scaling:

  Experimental cancer risk information for scaling from mouse to human skin cancer resulting from dermal exposure is not available. The science for selecting the allometric scaling approach employed by the EPA using body weight to the ¾ power is uncertain. However, the chosen cross-species scaling approach should be supported by a coherent logical structure. In addition, differences between mouse and human skin should be considered, such as thickness of and metabolic rates in the target tissue (i.e., the viable epidermis layer).

  The SAB has made other recommendations for describing the cancer risk calculated with the DSF. The recommendations include the need to state clearly how the absorbed dose is estimated from the exposed dose. In actual BaP exposures (from soil and other environmental media), the absorbed dose should be estimated from the exposed dose and the exposure scenario.

Age-dependent Adjustment Factors for Cancer
The SAB finds that the available mechanistic studies in humans and animals support a mutagenic mode of action for BaP-induced cancers. Given that the EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposures to Carcinogens* establishes a rational approach for the adjustment of tumor risk for exposures at different ages for carcinogens with a mutagenic mode of action, the SAB concludes that the proposed use of age-dependent adjustment factors (ADAFs) is justified.

Executive Summary
The SAB found that the major conclusions of the draft assessment for BaP were clearly and appropriately presented in the Executive Summary. Changes made to the body of the assessment in response to the SAB recommendations regarding the derivation of the chronic RfD/RfC, or cancer slope factors, should be incorporated into the Executive Summary. In addition, the SAB provides a number of suggestions for improvement of the Executive Summary.

Disposition of Public Comments
The SAB found that most of the scientific issues raised by the public, as described in Appendix G, were adequately addressed by the EPA. However, there were some issues on which the SAB differs from the EPA responses or provides additional comments on the topic. These issues were identified and referenced to relevant sections of the SAB report. The SAB encouraged EPA to provide additional transparency and were supportive of a draft response summary table that was prepared in real time for the SAB to review. The SAB thanks the public for these comments.

2. INTRODUCTION

The Science Advisory Board (SAB) was asked by the EPA Integrated Risk Information System (IRIS) program to review the EPA's *Draft IRIS Toxicological Review of Benzo[a]pyrene* (hereafter referred to as the assessment). EPA's IRIS is a human health assessment program that evaluates information on health effects that may result from exposure to environmental contaminants. The assessment consists of a review of publicly available scientific literature on benzo[a]pyrene (BaP). The assessment was revised in September 2014 and a summary of EPA's disposition of the public comments received on an earlier draft of the assessment was added in Appendix G of the Supplemental Information to the Toxicological Review.

In response to the EPA's request, the SAB convened an expert panel consisting of members of the Chemical Assessment Advisory Committee augmented with subject matter experts to conduct the review. The SAB panel held a teleconference on March 4, 2015, to discuss EPA's charge questions (see Appendix A), and a face-to-face meeting on April 15-17, 2015, to discuss responses to charge questions and consider public comments. The SAB panel also held teleconferences to discuss their draft reports on August 21, 2015, and September 2, 2015. Oral and written public comments have been considered throughout the advisory process.

This report is organized to follow the order of the charge questions. The full charge to the SAB is provided as Appendix A. The SAB also identified additional references to be considered by the EPA in their report (Appendix B). Appendix C provides suggestions on the format of the charge questions and organization of review.

3. RESPONSES TO EPA'S CHARGE QUESTIONS

3.1. Literature Search, Study Selection and Evaluation

Charge Question 1. 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 literature review process is well described and documented. The EPA did a thorough job documenting search terms used to identify studies in the main and supplementary report. In reviewing the initial literature search strategy keywords (Table LS-1 and Appendix C), the SAB noted that search terms for certain potential target organs are included but not others. To ensure that the literature search was comprehensive and bias was avoided, the SAB recommends that the EPA specify whether the search strategy included: (1) a review of the references in the primary and secondary literature as a means to identify potentially relevant articles not identified through the systematic searching and manual screening processes, and (2) conducting secondary literature searches as evidence for additional effects (e.g., cardiovascular) or specific data gaps (e.g., mechanistic, *in vitro* studies) that emerged. These steps should be included explicitly in the literature search and study selection strategy.

Figure LS-1 is helpful in identifying the general criteria used for study selection or exclusion. However, it is difficult to assess what information has been lost due to the exclusion of ~600 articles originally retrieved using the search criteria (3rd dotted line box) and why. It is appropriate to exclude papers that are "not relevant to BaP toxicity in mammals" or have "inadequate reporting of study methods or results" or "inadequate basis to infer exposure." The SAB appreciates that the EPA is developing a handbook for the IRIS program which will outline the tools and processes to address study quality and risk of bias. In the interim the EPA should provide sufficiently detailed criteria for each step of the process leading to the selection of key studies for the point of departure (POD) assessment. This will ensure that not only the rationale for initial study inclusion or exclusion is clearly understood, but also that the strengths and weaknesses of studies selected (as well as those that are not) for POD assessment are fully transparent. The EPA should consider identifying these criteria in one location within the Literature Search and Study Selection section, rather than directing the reader to other sections of the draft assessment or EPA references.

To increase transparency regarding excluded studies, a table containing the list of excluded references, grouped by the applicable exclusion criteria, should have been included in the supplementary information. For the draft assessment this will provide needed clarity regarding which epidemiological studies and animal studies were eliminated due to inadequate basis to infer exposure, inadequate reporting of study methods/results, and studies with mixtures.

The draft assessment separated the identified epidemiologic studies into tiers according to the extent and quality of the exposure analysis and other study design features. Tier 1 studies have detailed exposure assessment, large sample size, and adequate follow-up period. Tier 2 studies did not meet the criteria for

Tier 1 regarding exposure assessment, sample size, or follow-up period. The SAB finds requiring a direct measure of BaP exposure unnecessarily restrictive, especially for epidemiology studies, as these studies could be relevant as supplemental information for hazard identification. Epidemiological studies of coke oven workers and other occupational groups with known exposures to BaP are valuable sources of information for determining causality even if they do not include quantification of BaP exposures. These studies should at least be reviewed in the tables, if not the text. The draft assessment only considered that three epidemiology studies met this criterion for Tier 1 for lung cancer (Xu et al. 1996; Spinelli et al. 2006; Armstrong and Gibbs 2009) and four studies for bladder cancer (Spinelli et al. 2006; Burstyn et al. 2007; Gibbs and Sevigny 2007a, 2007b). 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, and 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 draft assessment. However, there are many studies missing from these tables (e.g., Ronneberg 1999; Romunstadt et al. 2000), that were included in prior assessments (i.e., see Table 1 in Bosetti et al. 2007 and Rota et al. 2014).

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

For asphalt workers and roofers, the Supplemental Information document refers the reader to the Bosetti et al. (2007) review. Six papers were cited to provide evidence of an excess risk of lung cancer and weak evidence for bladder cancer among asphalt workers and roofers (Hammond et al. 1976; Hansen 1989, 1991; Chiazze et al. 1991; Partanen and Bofetta 1994; Burstyn 2007). Studies cited in Bosetti (see Table 1) of roofers by Swaen et al. (1991) and of asphalt workers cited in Rota (see Table 1) by Behrens et al. (2009) and Zanardi et al. (2013) seem to have been overlooked. For coke oven workers, coal gasification, and iron and steel foundry workers the supplemental document relies entirely on the reviews by Boffetta et al. (1997), Armstrong et al. (2004), and Bosetti et al. (2007). The more recent review by Rota et al. (2014) identified two new studies of iron and steel workers (see Table 1) that were not considered in the earlier 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 Chemicals Institute were not included in the EPA assessment. In particular, the studies by Muller and Kierland (1964), Menter and Cram (1983), Jones et al. (1985), Bhate et al. (1993), Jemec and Østerlind (1994), and Hannuksela-Svahn et al. (2000) seem to meet the criteria for review, although the SAB noted that limitations in these studies make them of limited value for the assessment.

It also appears that *in vitro* studies (other than genotoxicity studies) and animal *in vivo* studies designed to identify potential therapeutic agents that would prevent the carcinogenicity or genotoxicity of BaP were not included. It would be expected that such studies might provide valuable additional information on mode of action of BaP.

In Appendix B, the SAB recommends a number of additional peer-reviewed studies from the primary literature, including some that are in HERO but were not used in the draft assessment, which the agency should consider in the assessment of noncancer and cancer health effects of BaP.

***Recommendation*s**

- The EPA should specify whether the literature search strategy included: (1) a review of the references in the primary and secondary literature as a means to identify potentially relevant articles not identified through the systematic searching and manual screening processes, and (2) conducting secondary literature searches as evidence for additional effects (e.g., cardio) or specific data gaps (e.g., mechanistic, *in vitro* studies) that emerged.
- The EPA should provide sufficiently detailed criteria for each step of the process leading to the selection of key studies for the point of departure (POD) assessment while the handbook which will outline the tools and processes is being developed.

3.2. Hazard Identification

In section 1 of the draft assessment, the EPA evaluates the available human, animal, and mechanistic studies to identify the types of toxicity that can be credibly associated with BaP exposure. The draft assessment uses EPA's guidance documents to reach conclusions about developmental toxicity, reproductive toxicity, immunotoxicity, carcinogenicity and other types of toxicity associated with BaP exposure. The SAB discusses the strength of the scientific evidence for each of these types of toxicity in the sections that follow.

3.2.1. Developmental Toxicity

Charge Question 2a. 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 SAB subdivided this Charge Question into two parts: developmental neurotoxicity; and developmental toxicity other than neurodevelopment.

Developmental Neurotoxicity

The SAB found the draft assessment to be thorough with regard to identifying studies pertaining to developmental neurotoxicity and found no additional literature. The SAB concurs with the EPA that the available human studies support the conclusion that BaP exposure contributes to human developmental neurotoxicity. There are relevant human epidemiological studies on effects on neurodevelopment resulting from exposure to BaP-PAH mixtures (Perera et al. 2004, 2005, 2006, 2009, 2011, 2012a, 2012b; Tang et al. 2006, 2008). For example, in a prospective cohort study in New York City, prenatal exposure to airborne PAH was found to affect children's IQ adversely (Perera et al. 2009). When the cohort was followed to the age of 9 years, the investigators concluded that early life exposure to environmental PAH may also contribute to attention deficit hyperactivity disorder (ADHD) behavior

problems in children (Perera et al. 2014). The draft assessment appropriately notes that in human studies the exposures are to PAH mixtures, and, therefore, the effects of BaP alone on child neurodevelopment cannot be isolated and determined to be exclusively attributable to BaP rather than to the sum, interaction, or antagonist effect of multiple PAHs acting in concert. However, the human prospective cohort studies have many strengths. These include the fact that (1) they are conducted in the target species (human), (2) they are prospective, and (3) they are from two separate populations with one cohort followed from before birth to the age of 9 years. An important aspect of the human studies that adds additional weight to their validity is that they measured BaP-specific DNA adducts in maternal and umbilical cord blood plasma and also used individually-worn air samplers on the mothers and found general agreement between the air sampling and internal dose metrics (Perera et al. 2012b). Of importance is that the method used for the BaP DNA adduct determinations in most of these studies was specific for BaP adducts and not generic for other PAH DNA adducts. The fact that the New York City Children's Study (Perera et al. 2006, 2012b, 2014) used an assay for a specific BaP-DNA adduct (Alexandrov et al. 1992) is a significant strength of these data.

The SAB also concurs with the draft assessment that the animal data support the view that BaP is developmentally neurotoxic in rodents. The SAB concludes that the draft assessment correctly identified the key studies, but did not consistently address the quality of the studies. Of these, the Chen et al. (2012) study was viewed as providing the best evidence despite some deficiencies. This study had a number of strengths; these included (1) using in-house breeding (to avoid maternal stress by shipping pregnant animals), (2) using 40 litters, (3) standardizing litter size, (4) blind observations of observer-rated behaviors, (5) balancing the time of testing across dose group, (6) testing multiple dose levels of BaP, (7) administering BaP by gavage, (8) efforts to neutralize litter effects, (9) use of multiple behavioral tests, (10) appropriate ANOVA methods as the main way of analyzing the data (see caveat below on post hoc testing), and (11) use of the Morris water maze (MWM). The study used a split-litter design which has both strength and weakness (discussed at the end of the next paragraph).

The SAB has also identified weaknesses in Chen et al. (2012). The MWM was undersized for adult rats, and the reliance on latency as the sole index of performance on learning trials may be insufficient without swim speed data; however, they report no swim speed differences on the probe trials. The use of the Least Significant Difference (LSD) test is a concern as it over-emphasizes differences as significant that may not be. The draft assessment correctly notes the importance of the parallelism of the learning curves. Learning rate was not shown to differ between groups. Rather the significant differences in latency between treatment groups seen throughout testing was likely due to some other long-lasting behavioral effect caused by developmental BaP exposure. The EPA also expressed concern about the interpretative value of the probe trial data in light of the fact that the affected BaP groups never reached the same level of proficiency on the learning trials as controls prior to being tested for memory and this concern remains. The pup randomization and litter rotation among dams used in the study is an unproven method of trying to prevent litter effects. It may work as intended or it may introduce unknown effects. While effects, if any, would be expected to be randomly distributed across litters, there exists the potential for interactions between groups created by this method of transferring pups between dams. Concern was raised about having all dose groups within litters. This could cause cross-contamination of BaP from higher dose groups to lower dose or control groups. Further, it is unknown if the dams could distinguish differences among the differently dosed pups and thereby differentially care for their offspring.

Despite these concerns and despite issues concerning whether the data reflect a spatial learning deficit or not, the MWM data show a BaP dose-dependent effect. Compared to the Elevated Plus Maze (EPM) data, the increased escape latency in the MWM appears to be a more stable behavioral change that was repeated over 4 days for two separate groups (cohorts) of animals. Rather than placing reliance only on the EPM data and dismissing the MWM data, the SAB recommends taking into account all the data in this study collectively and viewing them in their totality as evidence of a developmental neurobehavioral effect of neonatal BaP exposure with long-term adverse central nervous system effects.

With regard to neurobehavioral assessment, it is important to focus on the mutually supportive effects across behavioral domains in determining the reliability and pervasiveness of the low dose neurodevelopmental BaP effects. With regard to the elevated plus maze specifically as a test of anxiety, the significant effects of neurodevelopmental BaP exposure were found on all four measures used with this test and showed increased movement of the BaP exposed groups into the open arms of the maze relative to unexposed controls. This could be interpreted as decreased anxiety or increased risk taking of the animals. However, with tests such as this, the anthropomorphic judgment of its meaning in human terms is less important than the fact that it represents a persistent behavioral change caused by developmental BaP exposure that is significantly different from control behavior and as such may be regarded as an abnormal response. Given that BaP induced behavioral changes in other behavioral tests ranging from reflex development to Morris water maze performance, the results of this study provide converging evidence that shows a consistent pattern of alterations caused by developmental BaP exposure that can be seen from early development to adulthood that may be irreversible.

The SAB understands the EPA's desire to use the Chen et al. (2012) data to generate an RfD. Given the uncertainties identified, however, the draft assessment should consider if the resultant RfD emphasizing the EPM effects is the most appropriate outcome, or if using other end points, including the MWM results, may be more stable and reliable.

The SAB further notes that the Chen et al. (2012) data are supported by other studies. Bouayed et al. (2009) used mice treated with 0, 2 or 20 mg/kg BaP by gavage on postnatal day 0-14, that were assessed at different ages, and appropriate statistical analyses were used. This is a low-quality study with inadequate (small) sample size of five litters/dose, oversampling of four pups/litter without including litter as a factor in the statistical analyses, and no mention of whether the observations were conducted blind to treatment level and the order of testing counterbalanced across treatment level. Nevertheless, many of the tests were affected and the data were generally in alignment with those of Chen et al. (2012).

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 postnatal day 21 onward. Although the route of exposure is not directly relevant to humans, they too found increases in MWM latency as their measure of learning and on the probe trial to test for reference memory. They found effects at all doses of BaP. The study had reasonable group sizes (9/group), reasonable learning curves, and the data were appropriately analyzed. These researchers also relied on latency as their index of learning but their findings are in general agreement with those of Chen et al. (2012).

Relevant to the derivation of the inhalation RfC, Wormley et al. (2004) is an inhalation developmental neurotoxicity rat study in which exposure to BaP was on gestational days 11-21. The adult BaP-exposed offspring showed reduced perforant pathway long-term potentiation and reduced hippocampal NMDA-

NR1 receptor expression. The exposure system used restraint and dams were also exposed to isoflurane and minor surgery on gestational day 8 for which controls for these procedures were not included. However, the sample size was adequate and the study supports the developmental neurotoxicity of BaP.

The SAB concurs with the EPA that there are plausible mechanistic studies identified for how BaP may affect neurobehavioral development. Brown et al. (2007) and McCallister et al. (2008) treated rats with BaP by gavage on gestational days 14-17 and found metabolites in higher concentrations in brain than liver of the offspring. In addition, *in utero* BaP exposure reduced mRNA expression of glutamate receptor subunits, NMDA-NR2A and NR2B, and AMPA receptor expression and protein concentrations in hippocampus and inhibited NMDA-dependent cortical barrel field post-stimulation spikes by 50 percent. Bouayed et al. (2009) gave Swiss mice BaP on PND 0-14 and found effects on surface righting, forelimb grip strength, and EPM similar to that found by Chen et al., along with reduced spontaneous alternation and brain mRNA expression of 5-HT1A receptors. These findings implicate NMDA and AMPA glutamate receptors, as well as 5-HT receptors as potentially mediating the neurobehavioral effects seen by Chen et al. (2012) and others. They also support the view that developmental exposure to BaP adversely effects brain development and behavior. There is also data that prenatal BaP treatment in mice induces reactive oxygen species (ROS) (Winn and Wells 1997; Wells et al. 2010). The most salient evidence for ROS-induced injury is BaP-induced increased generation of 8-oxoguanine that causes GC-to-TA mutation in exposed embryos as another potential mechanism of BaP-induced developmental neurotoxicity.

The SAB concluded that the EPA correctly identified BaP as a developmental neurotoxic agent in animals with supporting evidence in humans. When reading across the human, animal, and mechanistic data, there are sufficient studies that provide evidence of developmental neurotoxicity and the data are convergent in showing BaP effects on brain development and behavior. While each study has limitations, the weight of evidence supports BaP as developmentally neurotoxic.

Looking across all developmental neurotoxicity studies, the SAB made two additional observations about the existing data. First, the existing studies have significant exposure gaps in brain development. Among the prenatal studies, there are exposures from GD14-17 (Brown et al. 2007; McCallister et al. 2008) but earlier and later exposure period BaP exposure studies could not be found. Among postnatal studies, there are exposures from PND 5-11 (Chen et al. 2012) and PND 0-14 (Bouayed 2009) but later exposure period BaP studies could not be found. This leaves major gaps in exposure periods from implantation (GD 6) to GD 14 and from GD 18-22. Similarly, for postnatal brain development there is a gap from PND 14-21. In the absence of studies with exposures spanning these missing stages of brain development it is not possible to rule out the possibility of other, yet unknown, developmental neurotoxic effects. Second, no studies were identified that assessed the effect of continuous exposure from implantation through parturition and lactation up to the age of weaning. The SAB notes that in the absence of data with chronic developmental gestational and lactational exposure, it is not possible to rule out the possibility that other developmental neurotoxic effects may occur. These gaps should be considered by the EPA in the overall evaluation of BaP developmental neurotoxicity. The significance of the gaps in terms of identifying effect levels lower than that reported by Chen et al. 2012 (0.02 and 0.2 mg/kg/day) is unknown.

Recommendations

- Rather than relying only on the EPM data and dismissing the MWM data, the EPA should take into account all the data in Chen et al. (2012) collectively, and view them in their totality as evidence of a developmental neurobehavioral effect of neonatal BaP exposure.

- EPA should consider the significant exposure gaps in brain development in existing studies in the overall evaluation of BaP developmental neurotoxicity.

Developmental Toxicity

The SAB concurs with the EPA that the available human studies also support a contribution of BaP to human developmental toxicity. Studies with PAH mixtures have shown a relationship amongst PAH exposure, lower birth weights, increased risk of fetal death, and BaP DNA adduct formation (see also Dejmek et al. 2000).

The SAB also concurs with the EPA that the animal studies presented support the conclusion that BaP is a developmental toxicant in animals. BaP exposure *in utero* has been demonstrated to cause fetal death, lower fetal/offspring weights and to affect fetal germ cells. The duration of oral BaP exposure included the time of implantation through major organogenesis in the mouse (GD 7-16; Mackenzie and Angevine 1981). Duration of inhalation BaP exposure included the latter part of organogenesis and histogenesis (GD 11- 20; Archibong et al. 2002). Additional studies that should be considered include reports on BaP-related effects on fetal lung growth/function (Thakur et al. 2014) and teratogenicity (Rigdon and Rennels 1964; Nebert et al. 1977; Shum et al. 1979). The SAB further recommends that the EPA's literature search include consideration of the relevant windows of prenatal development, recognizing that appropriately powered, conducted, and reported teratology studies may have been conducted prior to changes in testing guidelines that extended the dosing period to include the day prior to parturition. Based on these literature searches, the EPA should include justification as to the appropriateness and adequacy of the respective dosing paradigm, and the subsequent effects.

A brief survey of the literature indicates that there are additional reports that provide perspective on the likely mode/mechanism of action leading to BaP-related developmental toxicity that are not mentioned in the draft assessment. For example, there are studies on the formation of BaP adducts in rapidly dividing cells, including fetal tissues (Lu et al. 1986), the severity of developmental toxicity associated with Ah receptor status (Nebert et al. 1977), and the role of oxidative stress (Wells et al. 1997; Nakamura et al. 2012; Thakur et al. 2014). Therefore, the SAB suggests that the EPA consider including additional examples, as warranted, of mechanistic studies.

Toxicokinetic information regarding fetal exposures (Schlede and Merker 1972; Shendrikova and Aleksandrov 1974) and lactational transfer should also be included as they inform the comparative doses to developing organisms at different stages of development and exposed via different routes of administration.

Regarding other windows of susceptibility and the potential for adverse developmental outcomes, the SAB agrees that the postnatal development of other organ/systems may be impacted by BaP exposure; specifically, the immune system (see Section 3.2.3, SAB Response for Charge Question 2c), lung maturation/function, and cardiovascular changes (as identified in the EPA assessment). The SAB encourages the EPA to further review the literature to identify potential additional studies that may be useful in characterizing BaP-mediated developmental toxicity and dose-response relationships.

Recommendations

- The EPA should conduct a more complete literature search on developmental toxicity of BaP to characterize BaP-mediated developmental toxicity. Adverse outcomes resulting from BaP

exposure should take into context the susceptible window of exposure [i.e. whether exposure occurs in early gestation, late gestation (GD 6-12/15), or postnatal exposure].
- The EPA should consider including mechanistic studies that provide perspectives on the likely mode of action leading to BaP-related developmental toxicity.
- Toxicokinetic information regarding fetal exposures and lactational transfer should be included.

3.2.2. Reproductive Toxicity

Charge Question 2b. 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?

The SAB agrees that the data support the conclusion that BaP is a male and female reproductive toxicant through oral and inhalation routes of exposure. A sufficient number of appropriately conducted animal studies are included that demonstrate a functional effect on reproductive endpoints indicative of BaP-related reproductive toxicity and evidence for potential modes of action. The rodent data demonstrate convincingly that BaP affects fertility and fecundity.

Male Reproductive Hazards

The functional effects in male rodents include adverse changes in testes and sperm and hormonal changes. Changes in apical reproductive endpoints (e.g., sperm motility) (Mohamed et al. 2010; Chen et al. 2011; Chung et al. 2011; Archibong et al. 2008; Ramesh et al. 2008) are relevant and useful biomarkers that can be translated for assessing the association of BaP exposure and the potential for adverse effects in humans. Similar changes in sperm quality and fertility have been detected in humans exposed to PAH mixtures (Soares and Melo 2008; Hsu et al. 2006). The exposure to PAH mixtures prevents establishing a causal link between BaP exposure and reproductive toxicity in humans, but the findings are sufficiently consistent with the effects of BaP in rodents to deduce that BaP is a reproductive toxicant in humans.

The SAB recommends that the EPA consider the timing between the treatment with BaP and the measurement of endpoints. Because it is a proliferative tissue, the testis has the potential to recover from exposure to an insult after it is ended. Recovery can include but is not limited to restoration of normal weight based on restoration of spermatogenesis and production of sperm with normal morphology with subsequent waves of spermatogenesis. For sub-chronic studies, it could be informative to determine if the testes had time to recover in the absence of continued exposure. There is the possibility of an immediate effect from BaP or a PAH mixture that resolves with recovery time, could be dose-dependent and therefore could be missed depending on the timing of examination. The SAB requests that the EPA consider these factors when assessing the potential for male reproductive toxicity.

The SAB recommends that the EPA consider other hazard endpoints in addition to the classical reproductive hazard endpoints included in the draft assessment. For example, BaP is mutagenic and mutagenesis in the germline can be detrimental to reproductive health. Therefore, the SAB recommends that the EPA give greater consideration to genotoxic effects on male germ cells as a possible mode of action. The SAB recommends that the EPA consider inclusion of additional studies demonstrating that exposure at different life stages (e.g., pre-adult vs. adult), can have differential effects on reproductive health. References such as Liang et al. (2012) and Xu et al. (2014) could be used for this purpose.

Female Reproductive Hazards

As noted by the EPA, studies in female rodents that may explain the functional effects of BaP are limited and inconsistent. BaP has a direct effect on adult rodent ovarian follicles (Mattison1980; Mattison et al. 1980; Swartz and Mattison 1985; Borman et al. 2000), as well as data presented in Xu et al. (2010). Moreover, a recent study by Einaudi et al. (2014) showed that *in vivo* exposure to BaP induces significant DNA damage in mouse oocytes and cumulus cells. Collectively these aforementioned studies provide insight on the mode of action for BaP-related decreases in fertility and fecundity. The Xu et al. (2010) study was a low-powered (n=6) mixture study, rather than a typical toxicity study designed to characterize dose-response relationships and target organ toxicity. Other weaknesses found in this publication include the use of pentobarbital, which is known to affect hormone secretion, and a small number of experimental animals to assess low weight tissues to hormone levels. Guidelines for toxicity studies, including those conducted by the National Toxicology Program, require approximately 10 rats for each gender. The sub-chronic studies by Knuckles et al. (2001; 20 rats/group) and Kroese et al. (2001; 10 rats/group) did not detect changes in ovarian weight, revealing the inconsistent outcomes observed in different studies.

In utero exposure of developing females to BaP provides compelling evidence that there is a sensitive window for exposure to BaP for the developing ovary (Mackenzie and Angevine 1981). BaP ≥ 10mg/kg affects the developing fetal ovary, resulting in subsequent adult infertility (even in the absence of additional BaP exposure). Because fetal oocyte numbers are fixed prior to birth, as compared with the continual replenishment of sperm after puberty in males, BaP-related loss in oocytes indicates a permanent adverse effect. In humans, tobacco smoke during *in utero* development produces similar effects as BaP, including effects on subsequent adult fertility. Additional studies cited by the EPA demonstrate that the human ovary is a target for BaP. The results reported from Wu et al. (2010) could be considered relevant to developmental toxicity as well as reproductive toxicity due to early embryonic death, an endpoint also observed in rodent experiments.

General Comments

Germ cells are unique in that they will direct the development of the next generation. The success of the developmental process in producing normal offspring is dependent on the quality of the germ cells and the integrity of their DNA. The genotoxic effects of BaP have not been discussed in the draft assessment with regard to reproductive toxicity. These genotoxic effects have the potential to result in miscarriages, birth defects and genetic disease – all reproductive hazards. 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). Some papers discuss the mutagenic potential of BaP in somatic cells, but the mechanism is likely the same in germ cells (Young et al. 2014). There are additional references on the effects of BaP on adduct formation, mutagenesis, and gene expression in spermatogenic cells (Verhofstad et al. 2010a, 2010b, 2011). Other papers discuss the processing of BaP adducts during DNA replication and how different polymerases process the damage differently (Starostenko et al. 2014); such differences could contribute to the genotoxic effects in reproductive cells and during development. The Einaudi et al. (2014) study describes DNA damage in oocytes emanating from BaP exposure. The implication of increased DNA damage and mutagenesis in germ cells causes an increased risk of embryo-fetal death, birth defects and genetic disease among offspring. The EPA should consider these points as they discuss the potential for female reproductive impacts.

Recommendations

- The SAB recommends that genotoxic and mutagenic aspects of reproductive hazard be addressed, especially as they provide perspective on likely mode of action, or a clear explanation be provided as to why they are not addressed.

- The SAB recommends that the EPA consider additional endpoints (i.e., ovarian and testicular effects) for point of departure/BMD analyses and RfD derivation. The SAB suggests that follicular counts be considered for females. For male studies, the SAB recommends considering the recovery time after treatment prior to whatever endpoint is measured since the testis is proliferative and new rounds of spermatogenesis could change the outcome. The SAB also recommends that the EPA consider adding the biologically relevant endpoint of germline mutagenesis, since BaP is a mutagen. The SAB recommends considering that the life stage at which the animals are exposed to BaP and the life stage at which endpoints are measured be added since the testis matures after birth. The abundance of BaP lesions incurred by germ cells is another relevant measure for male and female studies that could be considered.

- 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; e.g., uterine hyperplasia/inflammation observed in the Gao et al. (2011) study was not included. The draft assessment does mention effects on the ovary but little attention is paid to the actual mode of action (decreases in the follicle pool) and there is no connection to the calculation of a point of departure. The SAB recommends that the EPA either include these endpoints, or provide appropriate justification as to why that they are not suitable for RfD determination (e.g., they support the mode of action but given limitations in experimental design - such as appropriateness of the route of administration and the short exposure duration— they are not suitable for generation of an RfD).

- The EPA should provide context as to the likely applicability of the inflammatory cervical response described in the Gao et al. (2011) study for BMD/RfD generation. The EPA may also want to consider if this finding should be categorized under "reproductive effect" or "other toxicity."

- The following reference could be added to sperm effects: Jeng et al. (2015).

- The following references could be added to ovarian effects: Kummer et al. (2013); Mattison (1980); Mattison et al. (1980); Sadeu and Foster (2011).

- The following reference could be added to mode of action-female reproductive effects: Sadeu and Foster (2013); Young et al. (2014).

3.2.3. Immunotoxicity

Charge Question 2c. 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?

The SAB believes that the available immunotoxicity data based on animal models of pure BaP and complex mixture exposures to humans (coke oven workers) support the claim that BaP is a human hazard for the immune system.

The evidence for immunotoxicity in humans is based upon complex mixture exposures. There is no doubt that BaP as a pure chemical can cause suppression of human peripheral blood mononuclear cell (HPBMC) responses at low concentrations *in vitro* (10-100 nM, Davila et al. 1996). It is unclear whether the levels of exposure demonstrated to have effects *in vitro* can be achieved from *in vivo* environmental inhalation exposures or ingestion of cooked foods. Immunotoxicity can be caused by a combination of genotoxic (DNA adducts and p53–induced cell death) and non-genotoxic mechanisms (signaling due to AhR activation and oxidative stress, Burchiel and Luster 2001). Some of these mechanisms are similar to cancer initiation and promotion, and there may, in fact, be a relationship between the carcinogenicity of certain PAHs, such as BaP, and their immunotoxicity.

The effects of BaP can vary by dose and time and sometimes lead to complicated non-linear dose-responses resulting in either increased or decreased immune parameters (Burchiel and Luster 2001). BaP and other similar PAHs have specific structure-activity relationships that are associated with AhR activation and increased P450 CYP1A1, CYP1A2, and CYP1B1 activities. BaP metabolites are likely responsible for the immunotoxicity seen *in vivo*. Thus, complicated dose-response relationships can be seen, which result from the actions of different metabolites of BaP (e.g., BP-diol-epoxides vs. BP-quinones).

Human Studies

The EPA has captured the key evidence, all of which is based upon exposure to mixtures, which makes a strong case for the immunotoxicity of BaP in humans.

Szczeklik et al. (1994) reported decreased serum immunoglobulins (Igs) in coke workers with inhalation exposures. Zhang et al. (2012) studied 129 coke oven workers (compared to 37 warehouse controls) for early and late apoptosis (Annexin V/PI) in HPBMC. The concentrations of BaP were 10-1,600 ng/m^3 in the working environment; 2.78-3.66 ng 1-hydroxypyrene (1-OHP) were measured in urine. Karakaya et al. (1999) found an increase in serum Ig, which is not consistent with Szczeklik et al. (1994), and may be associated with a difference in exposure dose and/or duration.

Winker et al. (1997) conducted an immune function and phenotype study of HPBMC comparing old and new coke facilities. These studies show depression of T cell activation in exposed workers, and the results are very compelling. Karakaya et al. (2004) also showed decreased T cell proliferative responses in asphalt and coke workers.

Because BaP is present in cigarette smoke, cigarette smoke studies are relevant for consideration. Numerous cigarette smoking studies have demonstrated immune suppression, but the interpretation of these effects is complicated by the strong action of nicotine, which in itself is immunosuppressive. Therefore, the SAB agrees that inclusion of cigarette smoking studies is not recommended for this IRIS

review. Cigarette smoking can also be an important confounder for other environmental cohort studies, and must be examined as an independent variable (Karakaya et al. 2004).

Animal Studies

The EPA focuses on De Jong et al. (1999) and Kroese et al. (2001) studies in rats with the toxic endpoint being thymic atrophy at 90 mg/kg to establish its RfD. However, these studies did not employ immune function studies that are known to be more sensitive. The EPA acknowledges that thymic atrophy may not be a reliable indicator of immunotoxicity (page 2-5, line 19, of the draft assessment).

Most immunotoxicity animal studies utilize mouse models (not rat) and they rely upon sensitive functional assays, such as the T-dependent antibody response (TDAR). In the draft assessment, the EPA has acknowledged the mouse immune function studies (page 1-38, lines 20-28), but they have not been included in the RfD calculation, presumably because these studies employed parenteral routes of administration and did not utilize adequate numbers of animals per group and a sufficient number of doses for evaluation. This is a common limitation of studies designed for assessing mechanism of action rather than regulatory needs.

The dose required to produce thymic atrophy is known to be quite high in mice and rats compared to that required to alter immune function (Luster et al. 1992). There is an overall consistency of findings for BaP immunotoxicity in mice and some rat strains. Temple et al. (1993) showed decreased IgM response and plaque forming cells (PFC) in mouse spleen at 5, 20, and 40 mg/kg and in Fischer 344 rats treated at 10 and 40 mg/kg for 14 days with subcutaneous injection, but the use of the rat model is limited by the lack of a substantial immunotoxicity database.

Important structure-activity relationships established early on by Dean et al. (1983) showed suppression of phytohemagglutinin (PHA)-induced T cell proliferation response of mouse spleen cells following exposure of mice to 50 mg/kg BaP, but not by benzo(e)pyrene (BeP), a non-carcinogenic congener. In mice, Ladics et al. (1992) have shown that BaP metabolites are responsible for suppression of the TDAR in mouse spleen.

Immune function tests indicate that BaP is suppressive and might result in increased risk of infections and perhaps cancer. This is evidenced by Munson et al. (1985) who showed a decreased resistance to Strep, Herpes, and B16 melanoma by BaP but not by BeP. Influenza infectivity was not affected by BaP and Listeria resistance was increased, thus demonstrating the complicated dose responses discussed above. Kong et al. (1994) also demonstrated decreased lung resistance to tumor cell challenge in Fischer 344 rats following intratracheal administration of BaP.

Collectively, these animal studies provide strong evidence that BaP suppresses immune function leading to adverse consequences for host resistance to infections. The limitation of most of these studies is that adequate exposure dose ranges were not explored that would assist the EPA in establishing an RfD based on immune function tests.

Developmental Immunotoxicity

Developmental immunotoxicity is not well-addressed in the draft assessment. There is no recommendation for calculation of an RfD based upon developmental immune exposures. Although BaP was found to produce alterations in T cell development by several investigators (Urso and Gengozian 1982, 1984; Urso and Johnson 1987; Rodriguez et al. 1999), these studies were limited by the use of a

single high dose (150 mg/kg) of BaP. Holliday and Smith (1994) found that 50 mg/kg total cumulative doses were able to decrease thymus cellularity and inhibit T cell development in the thymus of mice exposed gestationally. A decreased number of spleen cells was also seen by these investigators (Holladay and Smith 1995).

In addition to the evidence that BaP alters T cell development *in utero* and in adults, there is also evidence that BaP alters B cell development in the bone marrow of adults (Hardin et al. 1992). These effects may be dependent on the expression and activity of the aryl hydrocarbon receptor (AhR).

It is likely that the developing immune system is more sensitive to BaP exposures than adult exposures (Dietert et al. 2000, 2006; Luebke et al. 2006; WHO 2012). It is unclear whether the application of uncertainty factors can address these concerns regarding the inadequacy of the database. It is generally well known that developmental immunotoxicity is produced at much lower doses than those required to produce immunotoxicity in adults. However, this may not be well documented for BaP in the present literature used for the draft assessment.

Recommendations

This report could be improved by a well-defined, unified approach for immunotoxicity risk assessment (e.g., through a guidance document) that identifies sensitive biomarkers of exposure and effect for the immune system of animals and humans.

- There are concerns that sensitive immune function endpoints are not available to permit proper evaluation of BaP immunotoxicity in animal models, including adult, developing and juvenile animals, as well as assessing potential gender differences. These identified data gaps should be acknowledged in the draft assessment.

- The EPA should discuss how the point of departure and uncertainty factors used in the oral RfD derivation have addressed the potential for developmental immunotoxicity.

- The EPA should consider developing guidelines for immunotoxicity risk assessment, as has been done by the WHO (2012).

- *In vitro* human PBMC studies should be included that support an understanding of mechanisms of action that can guide the draft assessment.

- Associations between immunologically relevant endpoints and BaP adducts have been found in some human birth cohort studies (Jedrychowski et al. 2011; Tang et al. 2012; Jung et al. 2015). These studies are discussed elsewhere in this draft assessment in regard to neurodevelopment in Section 3.2.1 and should be linked with this discussion of developmental immunotoxicity.

3.2.4. Cancer

Charge Question 2d. 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 SAB finds that the EPA has demonstrated that BaP is a human carcinogen in accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA 2005a). This conclusion was based primarily on animal studies and mechanistic data, with strong support from an excess of lung cancer in humans who

are exposed to PAHs, but not to BaP alone. This conclusion is consistent with the evaluations by other agencies, including the World Health Organization's International Agency for Research on Cancer (IARC 2010) and Health Canada (2015). Detailed consideration of the EPA criteria for whether or not a compound is considered a human carcinogen, as applied to BaP, follows.

EPA Criterion 1 - The compound in question is "Carcinogenic to Humans" when there is convincing epidemiologic evidence of a causal association between human exposure and cancer.

The SAB agrees that occupational studies strongly indicate that PAH mixtures are carcinogenic to humans. Relevant occupations include, but are not limited to, chimney sweeps and workers in coke oven, iron, steel, and aluminum production. Other sources of significant human PAH exposure associated with cancer include chronic ingestion of PAH-contaminated food, and chronic inhalation of fumes from both cooking food and indoor heating with particular kinds of coal. However, as the draft assessment states, in the arena of human exposure, it is not possible to separate BaP from other carcinogenic PAHs. Therefore, from the epidemiologic studies there is no direct evidence that BaP alone is carcinogenic. Because there is the assumption that BaP is always a component of the PAH mixtures that humans are exposed to, one conclusion is that BaP alone is likely to be a human carcinogen based on the epidemiologic evidence. However, this assumption alone is likely not sufficient to satisfy the first EPA criterion.

The draft assessment focused on lung, bladder and skin cancers, but these are not the only organs for which PAHs are carcinogenic. There is strong evidence for an association between PAH-exposure in heavily char-broiled meat (Rothman et al. 1993) and colon adenoma risk (Sinha et al. 2005). In addition, there are strong associations between PAH-DNA adduct formation, cooked meat ingestion and colon adenoma risk in the same population (Gunter et al. 2007).

The SAB suggests that the EPA reconsider the requirement for individual monitoring data (Tier 1 studies) in choosing to present epidemiological studies because some important papers have been overlooked (see Appendix B). The Supplemental Information document summarizes six human studies (Table D-33) which evaluated BaP-induced DNA adducts in humans. This is a small fraction of the available studies that employ chemical class-specific methods to measure PAH-DNA or the major stable DNA adduct of BaP, the r7,t8 ,t9-trihydroxy-c-10-(N^2-deoxyguanosyl)-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPdG), in human tissues. It is possible that some epidemiological studies have been omitted by the EPA for lack of individual personal monitoring data. One could argue that for biomarker association studies, and for establishing or supporting hazard identification in a workplace known to be polluted, personal monitoring is not necessary. The presence of high ambient levels of BaP and/or PAHs, high levels of urinary 8-hydroxy-pyrene, and/or high levels of BPdG are all strong indicators of exposure.

There are a series of human epidemiological studies, involving cohorts of individuals, where subjects have been stratified into quartiles or quintiles for their PAH-DNA adduct level (using chemical class-specific methods). These studies have reported significant increases in cancer risk in individuals having the highest PAH-DNA adduct levels, compared to those having the lowest levels. Compiling this data into a table in the Supplemental Information would be very useful (see: Kyrtopoulos 2006; Poirier 2012).

The issue of the lack of an excess of skin tumors observed in most studies of therapeutic coal tar use (Muller and Kierland 1964; Jones et al. 1985) was discussed by the SAB, and there appear to be two major components to the overall consideration: (1) the hallmark characteristic of psoriatic skin is hyperkeratosis caused by abnormally rapid proliferation; and (2) the clinical studies involving the use of coal tar are incomplete. First, the skin of psoriasis patients who receive these treatments is not normal skin, and therefore psoriasis patients are unlikely to experience the same risk from coal tar exposure as the general population. In addition, psoriasis patients are known to shed skin cells at greatly increased rates (Weinstein and McCullough 1973). Desquamation can reduce penetration of compounds past the stratum corneum, so lipophilic materials, including the PAHs, may not reach the metabolically active layers of the skin (Reddy et al. 2000). Both hyperkeratosis and desquamation could be protective with respect to skin cancer risk by external PAH exposure. The finding by Roelofzen et al. (2012) of reduced 1-hydroxypyrene in urine and reduced PAH-DNA adducts in biopsied skin of psoriasis patients, compared to healthy volunteers, following dosing with coal tar ointments is consistent with this logic. The second consideration is focused on the available clinical studies, and the SAB agrees with the EPA that many of these studies suffer from small sample size, inadequate follow-up, undercounting of skin cancers in particular, and a large potential for exposure misclassification. The limitations of these studies, and the nature of psoriatic skin, make the available data largely uninformative with regard to the question of whether BaP induces skin cancer in humans. The historic studies of an excess of scrotal cancers in chimney sweeps, and more recent studies demonstrating an excess risk in asphalt workers, are all consistent with BaP being a risk factor for skin cancer.

EPA Criterion 2 - The compound in question can be considered "Carcinogenic to Humans" when there is a lesser weight of epidemiological evidence but when <u>all</u> 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
b) extensive evidence of carcinogenicity in animals
c) the mode(s) of carcinogenic action and associated key precursor events have been identified in animals
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 SAB agrees that the sum total of the mechanistic data show that all four of the required conditions are met. Therefore, based on epidemiologic studies of cancer in humans and animal models, and on mechanisms of action determined in both species, strong evidence of key precursor events related to BaP exposure and found in humans indicates that BaP can be considered a human carcinogen.

The SAB agrees that BaP is metabolized/activated through three separate pathways: the diol-epoxide pathway, the radical cation pathway and the *o*-quinone pathway. Furthermore, the SAB agrees that BaP-induced tumors arise primarily through a mutagenic mode of action resulting from BaP-induced DNA damage. Several studies over the last decade have shown that challenge of primary and transformed cells with BaP increases retrotransposition of Long Interspersed Nuclear Element-1 (L1) (Stribinskis and Ramos 2006). L1 retrotransposons are highly active mobile repetitive elements abundant in the human genome (Ramos et al. 2013). Retrotransposition of L1 induces DNA strand breaks, increased frequency of recombination and insertion mutations directly linked to various types of cancers (reviewed in Beck et al. 2011), as well as disruption of local genome architecture and loss of transcriptional control of neighboring genes (Raiz et al. 2012). As such, in addition to the mutational activity of reactive

electrophilic metabolites of BaP, the carcinogenic activity of BaP may involve genetic and epigenetic events mediated by L1 reactivation (Teneng et al. 2011).

The most chemically stable DNA adducts of BaP are formed via the diol-epoxide pathway and persist in human tissues for many years (VanGijssel et al. 2004). Much of the DNA damage generated by the radical cation and *o*-quinone-ROS pathways is unstable, and some additional stable DNA damage (8-OH-dG, ROS) is also caused by xenobiotics other than BaP. The steps connecting BaP exposure and tumor formation by a mutagenic mechanism have been studied most completely in the diol-epoxide pathway. However, because BaP is a complete carcinogen, the SAB emphasizes that the mechanism of action must include both the initiating (mutagenic) effects and the promoting effects. The promoting effects appear to occur largely through the radical cation and quinone metabolic pathways, which increase cell proliferation, generate ROS and activate various growth factors and signaling pathways (Burdick et al. 2003).

The SAB suggests that EPA could strengthen the statements in the draft assessment that describe the pathway linking BaP exposure to tumor formation. The SAB recognizes that there is an overwhelming literature available, and sorting out the critical original papers is daunting. The following is a series of findings that highlight the critical steps in the diol-epoxide pathway connecting exposure to tumorigenesis via a mutagenic mode of action. Statements are supported by original literature. This information might clarify/enhance the statements in Table 1-17 on page 1-75, "Experimental support for the postulated key events for mutagenic mode of action."

- Benzo[a]pyrene is metabolized/activated via the 7,8-diol to the diol-epoxide (r7,t8-dihydroxy-t-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene or BPDE) (Sims et al. 1974; King et al. 1976).

- BPDE interacts with the N2 position of guanine to form the stable r7,t8 ,t9-trihydroxy-c-10-(N^2-deoxyguanosyl)-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPdG) adduct (Daudel et al. 1975; Jeffrey et al. 1976).

- BPdG forms in human cells and in mouse skin (Grover et al. 1976; Osborne et al. 1976).

- The BPdG adduct is mutagenic. Site-specific studies linked mutation hotspots with regions of inefficient BPdG repair in modified DNA (Wei et al. 1995).

- Formation of the BPdG adduct in an oncogene can mutate and activate that oncogene. Mutated clones of the c-Ha-*ras* oncogene were formed as a result of *in vitro* reaction of the BPDE with the c-Ha-*ras* proto-oncogene. The resulting activated c-Ha-*ras* oncogene caused malignant transformation in NIH 3TC cells (Marshall et al. 1984).

- BaP caused dose-related increases in forestomach tumorigenesis and forestomach BPdG levels during chronic lifetime (2 yr) feeding in mice (Culp and Beland 1994; Culp et al. 1998).

- Reduction in levels of the benzo[a]pyrene-7,8-diol metabolite, BPdG formation and tumor formation was observed in mice treated with benzo[a]pyrene in the presence of the chemopreventive agent benzyl-isothiocyanate (Sticha et al. 2000).

- First detection of a chemically-characterized BPdG adduct in human tissue DNA (Manchester et al. 1988).

- In 39% of 705 human tissue DNA samples it was possible to detect the presence of BPdG adducts, determined by chemical-specific methods (Boysen and Hecht 2003). In addition, PAH-DNA adducts were localized in multiple human tissues by immunohistochemistry (Pratt et al. 2011).

- PAH exposures in humans are associated with a high frequency of GC→TA transversion mutations, however this type of mutation can be caused by other xenobiotic agents and therefore occurrence does not always provide a direct link to BaP exposure (Hussain et al. 2001).

BaP can either induce tumors after a single topical application to mouse skin followed by repeated tumor promoter treatment or when given repeatedly in a complete carcinogenesis protocol (DiGiovanni 1992; Abel et al. 2008). After topical application to mouse skin, BaP is metabolically activated to diol-epoxides leading to formation of covalent DNA adducts, particularly the BPdG (described above and in DiGiovanni 1992). The formation of BPdG leads to mutation in the Ha-*ras* gene of keratinocyte stem cells, and constitutes an initiating event for tumor development in this tissue (DiGiovanni 1992; Abel et al. 2008). Experimental evidence exists to show that BaP is metabolically activated to produce BPdG and other similar types of minor DNA adducts in human skin (Rojas et al. 2001; Brinkman 2013), as well as in skin, forestomach, lung, spleen, and esophagus of mice (Culp and Beland 1994; John et al. 2012; Zuo et al. 2014). Additionally, BPdG was revealed in a variety of mouse and human tissues exposed to PAH mixtures (Alexandrov et al. 1996; Rojas et al. 1998, 2001). Lehman et al. (1989) showed that human skin epithelial cells in culture treated with BaP produced the 7, 8-diol metabolite and BPdG. Watson et al. (1989) showed that epidermal DNA from human skin explants treated with radiolabeled BaP had similar DNA adduct profiles to those seen in both mouse epidermis and epidermal DNA samples from mouse skin explants. The major adduct was identified in all three DNA samples as BPdG. Zhao et al. (1999) showed that treatment of a reconstituted human skin equivalent model with BaP led to formation of BPdG and also led to the upregulation of c-fos and p53 proteins. The level of p53 protein has also been shown to increase in mouse epidermis in association with the formation of BPDE-DNA adducts (Serpi and Vahakangas 2003). Brinkman et al. (2013) also recently demonstrated that BaP was metabolized to diol-epoxide metabolites in several different models of human skin and showed that tetraols derived from BPDE could be readily detected in samples from all of the model systems evaluated, including human skin explants. Brinkman et al. (2013) showed that BaP was metabolized to genotoxic metabolites in both Normal Human Epidermal Keratinocytes and a reconstituted skin equivalent system (EpiDermFT). Finally, in a study of atopic dermatitis patients treated with coal tar, Rojas et al. (2001) demonstrated the presence of BPdG adducts in skin, that was modulated by polymorphisms in the myelo-peroxidase gene. In conclusion, the available data suggest a similar mutagenic mode of action for BaP in both mouse and human skin epidermis.

Whereas frequently we focus on a mutagenic mode of action (MOA) for BaP, as mentioned above, there is additional evidence for the role of promotion/proliferation in BaP carcinogenesis. Furthermore, both mutagenic and proliferative mechanisms occur simultaneously. A good example of this is the induction of mouse forestomach tumors by oral exposure to BaP. The architecture of forestomach is similar to that of skin, and the phenomenon of rodent forestomach tumors induced by oral BaP exposure is considered to proceed via mechanisms similar to those in skin (see previous paragraph). In the forestomach, clearly hyperplasia of the squamous epithelial cell layer plays a role (Culp et al. 2000), but one cannot discount

additional strong evidence of concomitant DNA damage leading to a mutagenic MOA. Culp and Beland (1994) showed linearity for formation of BPdG, the major stable mutagenic DNA adduct induced by BaP, in forestomachs of mice fed BaP for 21 days at 5 different dose levels. Furthermore, in a parallel tumor study conducted under the same conditions, there was a dose-response relationship between BaP concentration and forestomach tumors during 2 years of feeding mice three different levels of BaP in the diet (Culp et al. 1998). Taken together these studies indicate that both cell proliferation and DNA damage resulting in a mutagenic MOA contributed to the induction of forestomach tumors in mice fed BaP in the diet for 21 days to 24 months. Therefore, the presence of hyperplasia does not preclude a mutagenic MOA, particularly in the face of abundant evidence of DNA damage, but may contribute to an enhancement of tumor incidence. Because there is clear evidence that the ultimate active metabolite of BaP is a direct-acting genotoxin/mutagen, a linear extrapolation from the point-of-departure is the appropriate approach for estimating the cancer potency of BaP, the observation of hyperplasia notwithstanding.

Critical to our understanding of the published values for human BaP-induced DNA adducts and PAH-DNA adducts is knowledge of what is being measured by a specific assay. The gold standard is the use of structure-specific methods (Boysen and Hecht 2003.) Other assays have compound-class specificity. For example, the various antibody-based methods (ELISA and immunohistochemistry) employ monoclonal or polyclonal antibodies (termed BPDE-DNA antisera) raised against BaP-modified DNA. These antisera cross-react with a family of carcinogenic PAHs bound to DNA. When evaluating human tissue DNA, the data are expressed as "PAH-DNA adducts" because of the cross reactivity to DNA samples modified with multiple carcinogenic hydrocarbons. Other assays are not BaP or PAH specific. For example, with ^{32}P-postlabelling, which detects adducts of many different chemical classes, it is not possible to identify BPdG in human samples. Choice of an assay will impact the validity, reliability and conclusions obtained from a particular study. In the original literature there is often confusion in the use of nomenclature. The Toxicological Review (U.S. EPA 2014a) and Supplemental Information (U.S. EPA 2014b) would be more user friendly with the addition of a table describing the characteristics and nomenclature of the various methodologies used for BPdG and PAH-DNA adduct measurements.

The SAB found some of the text on page 1-72 of the draft assessment to be vague or inaccurate. For example, line 25 states that "These results are consistent with evidence that BaP diol-epoxide is reactive with guanine bases in DNA…" This statement is vague, despite the fact that there is actual experimental evidence in the literature that would allow a more precise statement. In addition, the sentence starting with "Supporting…." on line 33 of that page, the statement that "…benzo[a]pyrene diol epoxide (specifically[+]-anti-BPDE) is more potent than BaP itself…in producing lung tumors in newborn mice following i.p. administration" is not correct (and is not supported by a reference). Despite the fact that it is direct-acting, the diol-epoxide is too labile to be carcinogenic *in vivo*. The SAB asks the EPA to clarify this text.

Recommendations

- The Supplemental Material document contains only 6 papers in which DNA adduct formation has been measured in humans. There are many more such papers in the literature and this draft assessment would be more balanced if at least 20 of the most significant papers could be included.
- The current version of the draft assessment does not make a clear case for the pathway of BaP biotransformation that results in a mutagenic MOA. A series of the classical critical papers, and

their findings, have been listed as bullet points in our discussion of EPA Criterion 2, and this material should be included in the final BaP document.

- There is evidence of a strong association (Relative Risk or Odds Ratio) between increased human cancer risk in particular organs (lung [Tang et al., 1995], colon [Gunter et al., Carcinogenesis 2007]) and high levels of BPdG or PAH-DNA adduct formation in human nucleated blood cells. It would be useful to have these mentioned in a paragraph.
- A table describing the nomenclature, characteristics, specificity, sensitivity range, and detection limit for the various methodologies used for human BPdG and PAH-DNA adduct measurements could be easily assembled

3.2.5. Other Types of Toxicity

Charge Question 2e. 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 (BaP) exposure?

The potential hazards identified and discussed in Section 1.1.4 are 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 (Section 1.2.1). The SAB recommends that the basis for arriving at this conclusion be expanded for each of these health endpoints. The current text does not provide an adequate rationale for why the evidence does not support the listed effects as potential human hazards. The EPA needs to clarify whether this conclusion is due to insufficient data, inconsistent data, or sufficient data to conclude that these health endpoints are not sensitive endpoints.

The EPA has organized the summaries of human and animal studies in tables by target organ or system effect (e.g., kidney toxicity, nervous system effects), and animal study tables 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.

With respect to the health endpoints discussed in Section 1.1.4, the SAB concludes that the evidence presented does not support liver, kidney, and hematological effects as human hazards; the EPA's rationale for those conclusions is incompletely described and the conclusions depend on the literature search and study selection process, which was not considered to be sufficiently comprehensive to identify all potential hazards credibly associated with BaP exposure (see response to Charge Question 1 – Literature Search, Study Selection and Evaluation). Notably, the list of search terms used indicates that no queries were made that included the term "cardio" (i.e., cardiotoxicity; cardiovascular; cardiopulmonary), "vascular," "athero*," etc. Similarly in the literature search secondary refinement, it is noted that certain potential target organs (e.g., heart, liver, and kidney) were not included in the search terms. Thus it is unclear that the assessment of potential targets identified in the hazard identification section (specifically Section 1.1.4) was comprehensive. Moreover, it is unclear how the information obtained from mechanistic studies was integrated into the assessment of hazards.

The SAB's conclusion regarding target organ toxicities reviewed by the EPA is summarized below:

***Forestomach*:** The evidence presented does not support the EPA's conclusion that forestomach toxicity in rodents is not indicative of a potential human health hazard.

The draft assessment 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 implying a potential human hazard.

The decision not to 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 draft assessment. The SAB recommends that the EPA consider factors identified in IARC (2003) such as mode(s) of action and influencers of target tissue residence time (viz., method and vehicle of BaP administration) in addressing the predictive value for humans of forestomach effects in rodents.

***Hematological toxicity*:** The studies presented support the conclusion that hematological toxicity is not a potential human hazard.

The summary of hematological toxicity is well done. 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 data presented 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). Based on the evidence presented, the SAB agrees with the conclusion that the studies presented do not provide convincing evidence that hematological effects are a human hazard of BaP exposure.

***Liver toxicity*:** The studies presented support the conclusion that liver toxicity is not a potential human hazard.

The evidence provided for liver 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 studies described in this section reporting noncancer effects of BaP to the liver can be summarized as identifying reproducible organ weight changes (all three studies) without associated histopathology in two studies. In the third 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). EPA should clarify whether histopathology evaluations of the liver were performed by Knuckles et al. (2001). Based on the evidence presented, the SAB agrees with the conclusion that these studies do not provide

convincing evidence that noncancer liver effects are a human hazard resulting from BaP exposure. The results of Wester et al. (2012) (not cited in the draft assessment) should also be addressed and may provide additional support for this conclusion.

***Kidney toxicity*:** The studies presented support the conclusion that kidney toxicity is not a potential human hazard; however, adult and developmental renal toxicity are not fully addressed in the draft assessment.

In the three studies discussed in the draft assessment, there is no consistent finding indicative of kidney toxicity. The evidence provided for kidney toxicity therefore 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. However, the SAB has identified relevant references regarding the effects of BaP on renal function in rats (Alejandro et al. 2000; Parrish et al. 2002; Nanez et al. 2005; Valentovic et al. 2006), and the intrauterine effects of BaP on kidney morphogenesis and late onset renal disease (Nanez et al. 2011). The SAB recommends that these studies be reviewed to determine whether there is convincing evidence that non-cancer kidney effects are a developmental and/or adult human hazard resulting from BaP exposure.

Cardiovascular toxicity: The available studies do not support EPA's conclusion that cardiovascular toxicity is not a potential human hazard and further explanation is needed as to the rationale for reaching this conclusion.

The evidence provided for cardiovascular toxicity suggests potential toxicity at low dose levels, recognizing that the data are too limited to be utilized quantitatively. It is not clear why evidence pertaining to cardiovascular toxicity is not included in Table 1-9, and whether the designs of the animal studies reviewed were suitable to identify adverse cardiovascular effects. 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. Since cardiovascular effects were identified in rats and mice following gestational exposures to BaP, the EPA should address whether such findings should be considered as part of the weight of evidence for the cardiovascular system as a potential adult target of BaP exposure. Although limited, the two epidemiology studies cited (Burstyn et al. 2005; Friesen et al. 2010) lend credence to possible human relevance of this endpoint.

The SAB concludes that the literature search was not sufficiently comprehensive to identify studies relevant to addressing the identification of cardiovascular system toxicity of BaP exposure (see comments to Charge Question 1 – Literature Search, Study Selection and Evaluation). Several studies showing an influence of BaP 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 be considered as part of the weight of evidence evaluation, but not cited in this section, are Knaapen et al. (2007) and Yang et al. (2009) which address the induction of atherosclerosis by BaP in rodents; and Aboutabl et al. (2009, 2011), which examine cardiac hypertrophy and cardiac biomarkers after BaP exposure. The induction of inflammatory cytokines by BaP (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. Other relevant recently published articles include Gan et al. (2012), Uno et al. (2014) and Jayasundara et al. (2015).

The SAB recommends that EPA address the references that are missing. If they were excluded, the basis for their exclusion should be provided. If not intentionally excluded, the missing references should be included as part of the weight of evidence evaluation. The EPA should be explicit regarding the rationale for concluding that the available evidence either does or does not support cardiovascular system toxicity as a potential human hazard.

***Adult nervous system toxicity*:** The available studies do not support EPA's conclusion that adult nervous system toxicity is not a potential human hazard.

Further explanation is needed as to the rationale for concluding that the available evidence does not support adult nervous system effects as a potential human hazard. The SAB notes that although EPA's draft assessment concludes in Section 1.2.1 that adult nervous system is not a potential human target, this conclusion was not explicitly stated in Section 1.1.4, where EPA indicates that the evidence for "forestomach, liver, kidney, and cardiovascular system, as well as alter hematological parameters" (page 1-44) does not support potential human hazards for these endpoints. "Nervous System Effects," however, are discussed in Section 1.1.4, which ends with the statement "These data suggest that benzo[a]pyrene exposure could be neurotoxic in adults; however, only limited data are available to inform the neurotoxic potential of repeated subchronic or chronic exposure to BaP via the oral route (Table 1-9)" (p.1-49). This section should be expanded to include a more rigorous evaluation of the adult neurotoxicity evidence, especially since the EPA concludes that developmental neurotoxicity is a potential human hazard. The EPA should clarify the conclusion with respect to adult neurotoxicity and be consistent in Sections 1.1.4 and 1.2.1 of the draft assessment.

The evidence provided for adult neurotoxicity suggests potential toxicity at low dose levels, recognizing that the data are too limited to utilize quantitatively for oral exposures. Decrements in short term memory were reported in two studies of workers exposed occupationally to PAH mixtures containing BaP (Niu et al. 2010; Qiu et al. 2013), lending possible credence to the human relevance of this endpoint.

The SAB notes that Table 1-9 includes only two studies informing the neurotoxic potential of BaP exposure in adult animals following subchronic or chronic oral exposures. If this is the case, the EPA should indicate in the title of the table that only oral studies are included, because many more studies are discussed in the text. Since hazard identification does not rely only on repeated subchronic or chronic exposure scenarios alone, the EPA might consider developing a separate summary table just for neurotoxicity studies that includes Saunders et al. (2001, 2002, 2006); Liu et al. (2002); Grova et al. (2007, 2008); Maciel et al. (2014); Chen et al. (2011); Qiu et al. (2011); Xia et al. (2011); and Bouayed et al. (2012). This summary table should include information on route, dose levels, and dose-response relationship, including both positive and negative findings. Considering the relatively low doses in laboratory animals at which behavioral alterations were reported, the rationale for not considering the adult nervous system as a potential human target is unclear.

The section on adult neurotoxicity was not sufficiently rigorous in the analysis of oral neurotoxicity studies in either the text or in the table. Bouayed et al. (2012), an oral study, was not included on Table 1-9. The EPA may have mistaken this as an i.p. exposure study. The draft assessment should report the negative finding on motor activity, and indicate that there were mixed results, rather than a decreased depressive-like activity. The EPA should clarify that there was no dose-response

relationship (effects at 0.02 and 0.2, but not at 2 or 20 mg/kg/day), and that these effects could be acute effects, because the behavioral tests were conducted 60 minutes after gavage dosing.

The draft assessment indicates that Bouayed et al. (2009) reported an increase in aggressive behavior and consummatory sexual behavior in mice treated with 0.02 mg/kg-day, but should indicate in the text that there were no effects at 0.2 mg/kg-day (the highest dose tested). The EPA links this increase in aggressive behavior with decreased "anxiety" on the open-field test (pp. 2-3), yet the dose-response pattern is not consistent. The EPA should be more cautious about interpreting these findings because (1) the significance of four vs. two "attacks" is not clear, (2) Bouayed et al. (2009) provides no clear definition of how "attacks" were defined and distinguished from other social behaviors such as "play," and (3) the observers were not kept unaware of the treatment level.

The Grova et al. (2008) paper is an i.p. study that is not included in Table 1-9, presumably because Table 1-9 includes only oral studies. The EPA relates the increased time in the open arm of the plus maze in adult animals (Grova et al. 2008) to that observed in offspring (Chen et al. 2012) (p. 2-3). Yet the EPA does not indicate (pp. 1-49 and 2-3) that this was a high-dose effect that occurred at 200 mg/kg (i.p.) and not at the lower doses of 0.02–20 mg/kg.

As reviewed in the draft assessment, nervous system toxicity was assessed in animal studies where BaP was administered starting at weaning, adolescence, or to adult rodents. The SAB concurs with the EPA that these represent additional types of non-cancer BaP toxicity. However, the SAB suggests that the EPA include these in its overall assessment of BaP as both a developmental and adult neurotoxic agent. It was not clear in the draft assessment what the cutoff was for placing a study in the developmental versus non-developmental category given that there are prenatal, neonatal, weaning, and adolescent exposure studies, all of which are developmental in one sense or another even apart from the adult neurotoxicity exposure studies. The draft assessment clearly included the prenatal and early postnatal studies in the developmental neurotoxicity section, but placed the weaning (starting exposure at P21) and adolescent (starting exposure at P28) in the "other" non-cancer nervous system section. Further justification of the boundaries would be useful.

The SAB recommends that the EPA be explicit as to the rationale for concluding that the available evidence either does or does not support adult nervous system effects as a potential human hazard.

Other Toxicity: In addition, the SAB identified adult and developmental pulmonary toxicity as noncancer endpoints that can be credibly associated with BaP exposure, but were not identified in the draft assessment**.**

Adult and developmental pulmonary toxicity are not well addressed in the draft assessment. The SAB identified references in regard to the effect of maternal exposure to BaP on fetal development, and recent epidemiological studies that suggest an association between dietary BaP intake and lower birth weight in children (Duarte-Salles et al. 2010, 2012, 2013). Also, there is little emphasis on the effects of BaP on non-cancer pulmonary toxicity. Thakur et al. (2014) present evidence that maternal exposure of mice to BaP leads to increased susceptibility of newborn mice to hyperoxic lung injury and chronic lung disease (CLD). 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 BPD. This literature describing the effect of BaP on pulmonary toxicity in infants as well as adults should be included.

Recommendations

- The EPA should evaluate the missing references identified by the SAB on cardiovascular, kidney, and pulmonary toxicity of BaP.
- The EPA should be explicit as to the rationale for concluding that the available evidence either does or does not support adult nervous system effects as a potential human hazard.

3.3. Dose-Response Analysis

In Section 2 of the draft assessment, the EPA 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 SAB comments on the EPA analyses in the sections that follow.

3.3.1. Oral Reference Dose for Effects Other Than Cancer

Charge Question 3a. 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 inherent for exposures during a critical window of development?

The SAB finds that developmental endpoints, and in particular neurodevelopmental endpoints, are in principle an appropriate basis for deriving an RfD for BaP. However, the EPA has not made a sufficiently strong case that the available developmental endpoints are the most appropriate non-cancer endpoints for setting an RfD, or that among the available neurodevelopmental endpoints, the observed results from the elevated plus maze test in Chen et al. (2012) are the most appropriate results.

With respect to developmental toxicity as the most appropriate category of non-cancer effects, the SAB suggests that the EPA give more consideration to the available data on reproductive outcomes, including cervical hyperplasia and cervical inflammation in Gao et al. (2011), or providing a firmer justification for not selecting these critical endpoints. The Gao study is compelling in establishing a relationship amongst BaP exposure, cervical hyperplasia and inflammation. Moreover, the apparent effect on ovary weight reported by Xu et al. (2010) is inconsistent with the results reported by Knuckles et al. (2001) and Kroese et al. (2001). Therefore, the EPA should clearly articulate the rationale for developing a candidate RfD based on an apical, apparently inconsistent, ovarian response as compared to a single study that characterizes multiple cervical responses resulting from BaP exposure.

Although cervical hyperplasia and its impact on fertility and fecundity are unclear (human literature appears to focus on human papilloma virus, which causes proliferative lesions and decreased fecundity), hyperplasia often precedes a tumor response. Nevertheless, disruption of cervical elasticity or a mass of sufficient size would be expected to complicate parturition. As the EPA stated, cervical tumors were not observed in animal studies, but this tissue was not examined for histopathological changes. Therefore, microscopic changes may have gone unnoticed.

Dysregulation of anti-inflammatory cytokines has been suggested to be involved with cervical ripening/preterm labor (MacIntyre et al. 2012) and sufficient perturbation would be expected to impact birth outcome. Since BaP exposure was associated with alterations in inflammatory processes, this suggests a potential link amongst BaP exposure, alterations in cytokine signaling and preterm labor. Therefore, this potential relationship, albeit speculative, is potentially relevant for risk assessment. The EPA should consider including cervical hyperplasia and cervical inflammation from Gao et al. (2011) as a critical endpoint.

The SAB further recommends that the EPA (1)consider including their rationale for either exclusion or inclusion to increase clarity and transparency, and (2)conduct the appropriate study reviews (as necessary) to support either inclusion or exclusion of endpoints for RfD determination. In addition, the EPA should better explain the reasons for not modeling immunotoxicity (IgM, IgA) endpoints.

With respect to the choice of specific neurodevelopmental endpoints, the SAB notes that there are several important positive aspects to the Chen et al. (2012) study. These include: adequate numbers of litters (40 litters, 10/dose group) were used; there was a well-defined dose-response for several behavioral outcomes; the overall study presented multiple and well characterized tests; and the subjective tests were conducted with observers blind to treatment level. However, the SAB also identified several potentially significant negative aspects of the study design and data analysis in Chen et al. (2012) that were either not addressed or were not fully considered in the draft assessment. These include: potential dam and pup stress from repeated rotation of dams; potential nurturing bias against high-dose pups based on smell and/or behavioral differences especially following gavage doses; and the total number of dams used and timing (e.g., litters redistributed to other dams who gave birth within 24 hrs of each other) to achieve 40 litters of 4 M and 4 F divided into 10 litters per track was not described. Presumably, all 40 litters were not born in one day, so the details on how this was achieved (including use of >40 litters initially, so that pups are exactly the same age in each litter) are a critical part of study design that can impact study outcome and interpretation of data.

Given these concerns, the SAB recommends that the EPA should specifically consider the overall picture of neurodevelopmental impact from all of the neurodevelopmental endpoints in Chen et al. (2012), including plus maze, reflex, locomotor activity and water maze to justify and support the choice of the critical endpoint. In particular, the SAB suggests that the EPA reconsider or provide stronger justification for not using escape latency from the Morris water maze. This endpoint appears to be the most stable behavioral difference that was repeated 4 days for 2 separate tracks (cohort) of animals. The EPA is correct that this effect is not a learning or memory effect due to difference in baseline starting from day 1, but it is some indication of an effect (even if that effect is a developmental effect on locomotion). The EPA should explain how the BMD was calculated for escape latency since there are 4 different days for each track and each sex.

Although the BMD approach employed by the EPA for deriving the POD is not dependent on the specific statistical tests used for group comparisons, the overall weight of evidence and evaluation of Chen et al. (2012) is based on the original statistical analysis using the Least Significant Difference (LSD) post hoc test. This test appears to be statistically inappropriate in this context.

The SAB agrees with the EPA's decision not to further consider the Xu et al. (2010) study, but given its drawbacks, the SAB concludes that this study should not have been included in Table 2-2.

Regarding the discussion of uncertainty factors, the SAB suggests that the presentation of the UFs in the draft assessment be reordered to start with LOAEL-NOAEL… and end with sensitive human, as this is the logical flow when beginning with a POD from an animal study.

With respect to the application of uncertainty factors (UFs) in derivation of the RfD, the SAB supports the application of a UF of 10 for intrahuman variability. For interspecies extrapolation, the draft assessment stated that the application of a full UF of 10 to the POD from the EPM for the animal to human extrapolation in Chen et al. (2012) was needed. The EPA stated that this was because the allometric $BW^{3/4}$ adjustment is not appropriate for extrapolating from neonate animal to adult humans. However, given that this endpoint is a neurodevelopmental endpoint, it is unclear why the EPA considers the extrapolation in question to be from neonatal animal to adult human, and not (as seems straightforward) from neonatal animal to neonatal human. Therefore, the SAB recommends that the EPA consider application of a $BW^{3/4}$ adjustment as per EPA's 2011 allometric scaling guidance (U.S. EPA 2011).

The SAB also suggests that the EPA further justify the application of a 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), and the lack of a study examining functional neurological endpoints following exposure from gestation through lactation. The SAB suggests that the EPA more specifically address these issues and provide a clearer rationale for its decision.

The SAB notes that BaP is also considered a hazard for several toxicological endpoints (e.g., immune, cardiovascular) (see Sections 3.2.3 and 3.2.5 above). The available information for these endpoints, while sufficient for hazard identification, is insufficient for dose response assessment (e.g., insufficient testing of effects on immune function, particularly in developing organisms). In addition, the genotoxic effects of BaP have the potential to result in miscarriage, birth defects, and genetic disease (see SAB response in 3.2.2. Reproductive Toxicity). The SAB recommends that genotoxic aspects of reproductive hazard be addressed. As part of the deliberation regarding application of a database uncertainty factor, the EPA should also address whether the extent of residual uncertainty regarding these endpoints is such that additional data for these endpoints are needed and if so, the EPA should consider whether the existing database uncertainty factor of 3 is adequate.

The SAB identified two additional issues with the derivation of the RfD. Given the reproductive, developmental and trans-placental effects of BaP, the SAB encourages the EPA to ensure that multi-generational and one-generational effects are addressed to the extent that data are available. When possible, the EPA should identify the sensitive sex in a given study and use the sensitive sex for dose-response modeling.

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 window of development?*) somewhat vague. In Section 2.1.5, the draft 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 during neurodevelopment. Thus, this endpoint is a neurodevelopmental endpoint. The draft assessment notes in Section 2.1.5 that "*…fluctuations in exposure levels that result in elevated exposures during various lifestages could potentially lead to an appreciable risk, even if average levels over the full exposure duration were less than or equal to the RfD.*" The SAB agrees with this language as a statement of principle. However, as

the RfD in this case is, in fact, based on a susceptible lifestage that is shorter than a lifetime exposure, the statement in Section 2.1.5 is misleading as it seems to imply that this RfD does not specifically address this susceptible lifestage.

Recommendations

- The EPA should specifically consider the overall picture of neurodevelopmental impact from all of the neurodevelopmental endpoints in Chen et al. (2012), including plus maze, reflex, locomotor activity and water maze to justify and support the choice of the critical endpoint. In particular, the SAB suggests that the EPA reconsider or provide stronger justification for not using escape latency from the Morris water maze.
- The EPA should explain how the BMD was calculated for escape latency.
- The EPA should consider application of a $BW^{3/4}$ adjustment for extrapolation from neonatal animal to neonatal human.
- The EPA should further justify the application of a UF of 3 for database deficiency.

3.3.2. Inhalation Reference Concentration for Effects Other Than Cancer

Charge Question 3b. The draft assessment proposes an overall reference concentration of 2 x 10^{-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 inherent for exposures during a critical window of development?

In the draft assessment, Archibong et al. (2002) is the critical study selected for the derivation of the RfC. In this study, the BaP exposure occurred via particulate inhalation and the adverse effect identified as the critical endpoint is decreased fetal survival (i.e., a non-respiratory endpoint). The SAB concludes that the RfC value in the draft assessment is inadequately supported in light of concerns with the study design, data analysis, and uncertainty factors, as discussed below.

The key study selected (Archibong et al. 2002) has technical limitations and specific deficiencies which decreases the confidence in an RfC based upon this one study. These include: uncertainty in the dosing schedule (gestation day 8-17 vs. 11-20), laparotomy on gestation day 8, confinement to nose-only exposure chambers for 4 hrs/day, potential impact of anesthesia on hormone secretion and stress from collection of blood samples from the orbital plexus, ambiguity on the rationale for comparator control selection for hormone measurements, and the apparent effect of carbon black on fetal weight. Stress resulting from these procedures would be expected to affect hormone levels and may have contributed to other responses attributed to BaP. Although the carbon black control exposure does not appear to affect fetal survival, it does appear to have an effect on progesterone levels. The gestation day 17 plasma progesterone levels are unexpectedly different in the unexposed and carbon black control groups, suggesting that the carrier (carbon black) used in the BaP dose groups may have impacted the purported effect on progesterone levels. The authors' selection of the unexposed air control as the comparator for BaP-attributed effects on prolactin levels is also unclear. A decrease in fetal weight of ~17% was observed between the unexposed air and carbon black groups suggesting that carbon black exposure affects fetal weight (10.6 ± 0.1 vs. 8.8 ± 0.1, respectively). Fetal weight is considered to be one of the most sensitive and relevant indicators of developmental toxicity (correlate to small for gestational age in humans). The SAB suggests that the EPA consider these factors in assessing the utility of this study for determination of an RfC.

The rationale for not employing a BMD approach is unclear. Unequal variances and lack of access to the original datasets are not sufficient reason to avoid BMD modeling of the data in the key study. The EPA has fit BMD models to epidemiological data summaries having these same attributes, and the agency should consider those approaches in the current draft assessment.

Regarding use of UFs, the EPA applies a UF of 3 for interspecies extrapolation (rat-to-human) to the LOAEL of 25 $\mu g/m^3$ derived from the key study. This UF, rather than the full UF of 10, is intended to address residual interspecies toxicodynamic uncertainty after interspecies toxicokinetic uncertainty has been addressed. The EPA intended to address the toxicokinetic uncertainty by application of the regional deposited dose ratio for extrarespiratory effects ($RDDR_{er}$) as set forth in its 1994 guidance on deriving RfC (U.S. EPA 1994). The $RDDR_{er}$ is described in that document as follows:

> 4.3.5.2 Remote (Extrarespiratory) Effects. The respiratory tract might not be the target organ for an inhaled compound. The dose actually delivered to other regions of the body will be affected by metabolism, clearance, and distribution patterns. Particles depositing in the respiratory tract will clear rapidly (ET can be within seconds of inhalation) or slowly (PU clearance may take weeks or months) to the GI tract or be absorbed into the interstitium, lymphatics, or into the blood from the respiratory tract. Once deposited, however, very few particles will clear by exhalation (sneezing or coughing). Therefore, it is not unreasonable to estimate extrarespiratory deposition by total deposition in the respiratory tract when information on dose delivered to nonrespiratory tract organs is unavailable. The current default normalizing factor for extrarespiratory effects is body weight.

The SAB notes that while allometric scaling for the BaP RfC is based upon BW^1 (per above), for oral and dermal BaP toxicity values the EPA selected an allometric scaling factor of $BW^{3/4}$. Although an EPA guidance was cited as the basis for selection of the allometric scaling factor for each route of exposure, the SAB is concerned that use of different EPA guidance documents spanning decades and different exposure routes and endpoints (cancer and non-cancer) may have resulted in the application of inconsistent scaling principles. Further, cross-species scaling depends upon the mode of action, the role of metabolism and toxicokinetics, and the target organs and tissues; however, the draft assessment provides no indication of the extent that these were considered in choosing the BaP scaling factor for inhalation (and other routes). (See also the responses to Charge Questions 3c and 3e).

The SAB recommends that the EPA include a brief discussion of the rationale for selection of the allometric scaling factor in the context of inhalation exposure to BaP leading to decreased fetal survival. It would be helpful to clarify in this discussion the aspects of the BaP absorption, distribution, metabolism, and elimination (ADME) that the scaling factor is intended to address. This is important not only in justifying the allometric scaling of dose, but also the use of a UF of 3 instead of 10 as the use of a UF of 3 for interspecies extrapolation is based on the assumption that issues related to interspecies variability of toxicokinetics have been adequately addressed by the scaling factor and that the UF of 3 is largely intended to solely address interspecies differences in toxicodynamics. The SAB notes that in its 1994 guidance (U.S. EPA 1994), the EPA recommends the application of an interspecies UF of 3 rather than a full UF of 10 in the derivation of RfCs. The guidance states that this is "…due to the incorporation of dosimetric adjustments." However, the SAB also notes that 55% of the particulate aerosol used in Archibong et al. (2002) had a cumulative mass less than 2.5 µm, which would deposit in the upper respiratory tract of rats but would deposit in the deeper lungs in humans. Since the proposed

RfC for BaP is derived from particle deposition in the respiratory tract leading to extrarespiratory systemic effects, it is not entirely clear that the dosimetric adjustment referred to in the 1994 document completely addresses the variability in interspecies extrarespiratory systemic kinetics.

The Archibong et al. (2002) study found effects at all levels of exposure; thus the use of the LOAEL from this study provides a weaker basis than a NOAEL for derivation of the RfC. The EPA should consider the studies of Wu et al. (2003) and Archibong et al. (2012). Although these two studies are not replicates of the key study, they may be useful in developing a more comprehensive dose-response relationship for BaP and, thus, perhaps increased confidence in the proposed RfC.

In the Wu et al. (2003) study, female rats were exposed for 4h/d to 25, 75, and 100 $\mu g/m^3$ of BaP for 10 days from gestation days 11-20. Dams were allowed to litter, birth index calculated, and pups were subsequently euthanized at various time points. Additional endpoints included collection of brains and livers of F1 pups for measurement of BaP metabolites and mRNA expression profiles for AhR and CYP1A1. The most likely apical endpoint appropriate for determining a POD/BMD is birth index. The authors report that the birth index in the low exposure group (25 $\mu g/m^3$) was not statistically different from the concurrent control (although it appears lower), whereas the 75 and 100 $\mu g/m^3$ exposure groups were statistically lower than the concurrent controls. This suggests that 25 $\mu g/m^3$ may be the NOAEL for this endpoint, under the conditions of this study. However, BMD approaches should also be considered (and contrasted to BMD results of the study by Archibong et al. 2002). Nevertheless, this effect on birth-index is consistent with the effects on pup survival and litter size reported by Archibong et al. (2002).

The Archibong et al. (2012) study explored the potential effects of BaP on the rat ovary, including ovarian estrous cyclicity, hormone production, BaP metabolism, and subsequent effects on reproductive outcomes. Female rats were exposed to 50, 75 or 100 $\mu g/m^3$ of BaP for 4h/d for 14 days and then mated with unexposed males. During exposure, the 100 $\mu g/m^3$ exposure concentration group was associated with an increase in cycle length, changes in hormone levels, and aryl hydrocarbon hydrolase activity. When the exposure period was over and these animals were mated, this exposure group displayed a lower ovulation rate, fewer pups born and decreased pup survival. Given that all the effects occurred in the highest exposure group examined, and were consistent across endpoints, EPA may want to consider the potential value of these endpoints for BMD analyses. These data suggest that although adult ovary is a target, fetal development (as demonstrated in Archibong et al. 2002 and Wu et al. 2003) is more sensitive to BaP-mediated toxicity under the exposure conditions employed.

Recommendations

- In addition to Archibong et al. (2002), the EPA should also consider Wu et al. (2003) and Archibong et al. (2012) for RfC derivation. Collectively, these three studies may be useful in developing a more comprehensive dose-response relationship for BaP and, thus, perhaps increased confidence in the proposed RfC.
- EPA should explore if these three studies are amenable to BMD approaches.
- The application of respective UFs needs further justification.
- SAB recommends that the EPA include a brief discussion of the rationale for selection of the allometric scaling factor in the context of inhalation exposure to BaP leading to decreased fetal survival.

3.3.3. Oral Slope Factor for Cancer

Charge Question 3c. 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?

The SAB concludes that appropriate studies and models were selected for dose-response analysis. However, insufficient justification was provided for selection of the final slope factor solely from the Beland and Culp (1998) mouse study, instead of the slope factor from the Kroese et al. (2001) rat study or an average of the two. The SAB also raised questions regarding the choice of cross-species scaling factors, and secondary analyses and other additions to the report to improve transparency.

Analysis of Carcinogenicity Data (section 2.3.1)

An oral slope factor for cancer was previously developed by EPA in 1992 and included on the IRIS database. At that time, BaP was classified as a "probable human carcinogen." The previous oral slope factor (7.3 per mg/kg-day) was derived from the geometric mean of four slope factor estimates based on studies of BaP oral carcinogenesis in Sprague-Dawley rats (2 years) and CFW Swiss mice (7 months) from the combined incidence of forestomach, esophageal and laryngeal tumors. In the current draft assessment, newer oral carcinogenesis studies were available for further refinement of the oral slope factor (now proposed to be 1 per mg/kg-day), including two 2-year oral carcinogenesis bioassays that associated lifetime BaP exposure with multiple tumor sites including: forestomach, liver, oral cavity, jejunum, kidney, auditory canal, skin and mammary gland in male and female Wistar rats (Kroese et al. 2001) and forestomach, esophageal, tongue and larynx tumors in female B6C3F1 mice (Beland and Culp 1998). The Kroese et al. (2001) and Beland and Culp (1998) studies were selected as the best available for dose-response analysis and extrapolation to lifetime cancer risk following oral exposure to BaP. These studies were conducted in accordance with Good Laboratory Practice (GLP) and showed dose-related trends in most of the tumor sites. Neither of the studies used in the earlier oral slope factor derivation were used for the current derivation.

The SAB finds that the two selected lifetime oral carcinogenesis studies were well done and appropriate for the dose-response modeling used for cancer oral slope factor derivation. However, it is not clear why only one of the studies, the study by Beland and Culp (1998), was ultimately used in the final derivation of the oral slope factor and not both studies where a (weighted or unweighted geometric) mean or median value might have been derived from the different oral slope factors calculated and presented in the draft assessment. The SAB was concerned about the EPA's choice of the single-sex mouse study that produces the largest cancer slope factor instead of some other slope factor that incorporates data from all studies (rats and mice, males and females) previously judged to be of equal quality and relevance. This decision was not clearly supported by the EPA *Guidelines for Carcinogen Risk Assessment* (USEPA, 2005a), which allows multiple studies to be combined and suggests "choosing a single dataset if it can be justified as most representative of the overall response in humans."

The SAB acknowledges there are advantages and disadvantages to basing the oral slope factor for cancer on a single mouse study that includes only one sex (female) versus basing it on a rat study that includes both sexes; and, statistical bias that results from using extremity as a selection factor (i.e., always choosing the study that produces the largest slope factor). If no biological basis exists for concluding that the mouse study is more representative of human response than the rat study, the EPA should consider averaging over both studies (e.g., simple averaging as used in previous oral slope factor

derivation, or meta-analytic/Bayesian averaging as recommended in the 2014 NRC review of IRIS). The oral slope factor for cancer presented in the 1992 BaP assessment was based on an average of slope factors from two different studies, an estimation approach that could have been used in this draft assessment. An approach similar to the one used in the 1992 BaP assessment should be considered

Dose-Response Analysis (section 2.3.2) and Derivation of the Oral Slope Factor (section 2.3.3)

The oral slope factor for cancer is based on dose-response modeling that uses only the multistage-Weibull model. This model incorporates both the time at which death occurs and the dose in estimating the point of departure from which the cancer slope factor is calculated. This model is generally considered appropriate for the available data, although confidence in the final estimates would be increased if the reader were able to compare the multistage-Weibull model estimate to estimates computed by fitting other dose-response models to the same data. These other estimates (and associated deficiencies) could be summarized in an appendix along with the model that is finally chosen. For example, Fitzgerald et al. (2004; their Figure 1 excerpted here) evaluated multiple models of tumor risk and illustrated BMD estimates associated with a 5% extra risk ranged between roughly 0.15 and 0.6 BaP dose (mg/kg/day).

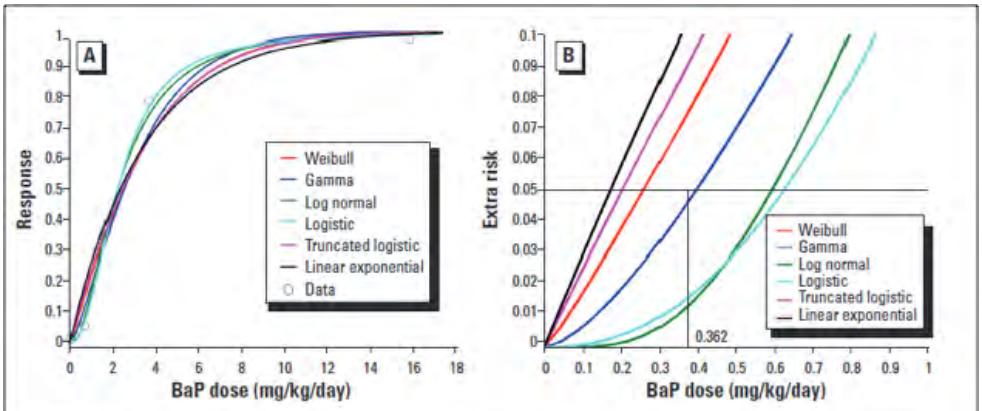

Figure 1. Suite of models fitted to BaP dose–response data (mouse forestomach tumors) reported by Culp et al. (1998). (*A*) MLE fitting of models except the truncated normal, which could not be fitted. (*B*) The extra-risk dose curves of (*A*) in the low-dose region around the 0.05 risk level and averaged dose at 0.362 mg/kg/day.

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, citing U.S. EPA (1992, 2005a). According to U.S. EPA (1992), $BW^{3/4}$ is used as a default in the absence of chemical-specific information and is surrounded by considerable uncertainty. It encourages the use of information on mode of action, reaction rates, pharmacokinetics, and other factors as appropriate to derive a chemical-specific scaling factor, if sufficient data are available. For example, it states, "Clearly, when data on metabolic conversion are available in a particular case, they should be used in preference to the $BW^{3/4}$ default." Consistent with the recommendation given in response to Charge Question 3b, the SAB recommends that the EPA provide a brief explanation of the rationale for its selection of an allometric scaling factor for the BaP oral cancer slope factor given what is known about the BaP mode of action for carcinogenicity, reaction rates, and toxicokinetics, and specifically, how the selection of the allometric scaling factor applies when there is a portal of entry effect. Alimentary tract tumors (larynx, esophagus, forestomach) arguably meet the definition of portal of entry effects, and the SAB suggests that the discussion include issues regarding

scaling of effects when many of the toxicokinetic processes that influence scaling of systemic effects do not apply, or do not apply in the same way.

Also, for transparency, the impact of the change in allometric scaling from $BW^{2/3}$ used in the 1992 BaP assessment to $BW^{3/4}$ in the present draft assessment should be discussed in the assessment. A comparison of the results of using the two different scaling factors can be easily accomplished by demonstrating how the scaling change impacts the estimate in the 1992 BaP assessment.

The draft assessment states that "the oral slope factor should only be used with lifetime human exposures of <0.1 mg/kg-day, because above this level, the dose-response relationship is not expected to be proportional to benzo[a]pyrene exposure" (p. 2-30, lines 23-25). How does the EPA expect this limitation to be operationalized given that human BaP exposures typically occur within mixtures of PAHs? How often, and in what situations might this condition be invalid?

Uncertainties in the Derivation of the Oral Slope Factor (section 2.3.4)

A number of uncertainties were discussed in the draft assessment related to derivation of the oral slope factor for cancer and provided in Table 2-8. Overall, this section was well written. However, the SAB suggests additional discussion in the draft assessment on two important points.

First, the link between forestomach tumor incidence in mice and rats and cancer incidence in humans is not clearly presented, and the assessment is incomplete without this discussion. The rodent forestomach is highly sensitive to BaP carcinogenesis and represents a major organ for tumor development after oral exposure to this PAH in both rats and mice. The mouse study of Beland and Culp (1998) is focused almost exclusively on forestomach tumors. The rat study of Kroese et al. (2001) provided data on a much broader range of tumor sites. Basing the oral slope factor for cancer on only the mouse study increases the importance of describing the relevance of forestomach tumors in mice to human cancer.

Second, the SAB is concerned that the draft assessment does not discuss how the carcinogenicity of BaP and use of the oral slope factor for cancer are impacted by the fact that humans are exposed to BaP as part of PAH mixtures**.** Some discussion of this issue should be included in the "Uncertainties" section of the draft assessment. The study by Culp et al. (1998) actually compares the oral carcinogenicity of BaP in a two-year bioassay with two different coal tar mixtures of known content. The coal tar mixtures produce a lower incidence of forestomach tumors compared to BaP, but higher incidence in lung tumors. These data were further evaluated and modeled in the publication by Fitzgerald et al. (2004; their Figure 2 excerpted here). Some discussion and consideration of these data could be provided in more detail.

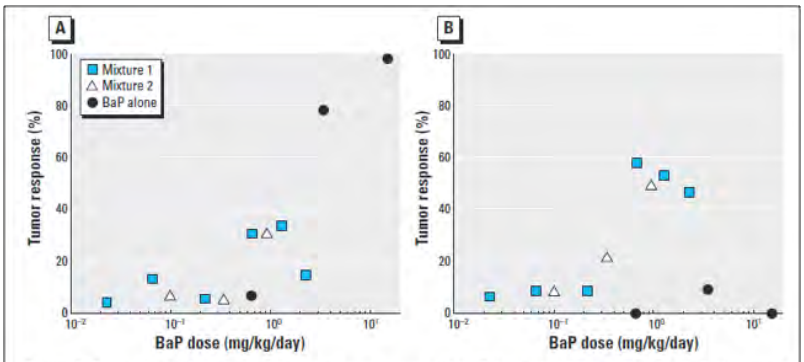

Figure 2. Comparison of dose responses for tumors reported by Culp et al. (1998), plotted for BaP alone and BaP content of coal tar mixtures. (*A*) Forestomach tumors. (*B*) Lung tumors.

Previous IRIS Assessment Oral Slope Factor (section 2.3.5)

A brief description of the derivation of the previous oral slope factor for cancer is given on page 2-32 of the draft assessment. The SAB suggests that additional discussion comparing the previous analysis with the current analysis might be useful, especially in light of the comments above regarding the use of a single carcinogenicity study for the current slope factor calculation and the differences in scaling between the current and previous slope factor derivation.

Recommendations

- If no biological basis exists for concluding that the mouse study is more representative of human response than the rat study, the EPA should consider averaging over both studies to derive the oral slope factor for BaP.
- The SAB recommends that the EPA provide an explanation of the rationale for its selection of an allometric scaling factor for the BaP oral cancer slope factor given what is known about the BaP mode of action for carcinogenicity, reaction rates, and toxicokinetics, and specifically, how the selection of the allometric scaling factor applies when there is a portal of entry effect.

3.3.4. Inhalation Unit Risk for Cancer

Charge Question 3d. The draft assessment proposes an inhalation unit risk of 0.6 per mg/m3 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?

The SAB concluded that an appropriate study was selected for dose-response analysis and that appropriate models were used to derive the inhalation unit risk (IUR). Although the IUR value is scientifically supported, the SAB recommends additional discussion of key assumptions, several sensitivity analyses, and reconsideration of the use of epidemiological data to derive inhalation unit risk values. The SAB also suggests the need for an explicit conclusion statement regarding overall uncertainty of the unit risk value, and a brief discussion of the applicability of this value to typical environmental exposures (especially for sensitive subpopulations).

EPA identified Thyssen et al. (1981) as the only lifetime inhalation cancer bioassay available for describing exposure-response relationships for cancer from inhaled BaP. The experimental design utilized an adult, male hamster model and daily (3-4.5 hr/d) lifetime exposure to BaP via an inhalation portal of entry (nose-only) for a submicronic sized BaP aerosol. Lifetime exposure had average survival durations of 60 to 96 weeks and dose response outcomes included body weight, and incidence and

latency of tumors with segmental distributions, i.e., upper respiratory tract (URT), trachea, lung, oro-pharynx, esophagus, and forestomach. The EPA relied on this study due to its merits as the "only study of lifetime exposure to inhaled B(a)P." Additional scientific support for Thyssen et al. (1981) arises from a subsequent short communication by the same laboratory (Pauluhn et al. 1985). Although limited in scope, the survival results and presence of neoplastic alterations demonstrate that the experimental design using the hamster model can be replicated for low BaP aerosol concentrations employing an inhalation portal of entry. Overall, the results of Thyssen et al. (1981) found tumors (benign and malignant tumors of the pharynx, larynx, trachea, esophagus, nasal cavity, or forestomach) with increasing BaP concentrations. The SAB identified strengths of the approach (durations of exposure to natural death, histologic exam of tissues, monitoring of exposure concentrations) and limitations (lack of distal lung tumors, variation in exposure concentrations, BaP exposure aerosol was developed using sodium chloride condensation nuclei) and these issues were fully addressed in section 2.4.4 of the draft assessment.

Due to the merits of a lifetime inhalation animal model study that demonstrated carcinogenicity results, the EPA's selection of Thyssen et al. (1981) for dose-response assessment is appropriate. Dose-response modeling and unit risk estimation for those data used appropriate methods, and the multistage Weibull model fit was adequate. Although the SAB agrees with the EPA that the multistage Weibull model is preferable due to incorporation of time-to-tumor data, the final unit risk value can be further supported by: (1) supplemental sensitivity analyses using other dose-response models; (2) alternative assumptions about latency and cross-species scaling of doses; and (3) not eliminating from the analysis all animals without confirmed examination of one or more of the pharynx or respiratory tract tissues. The SAB also recommends additional discussion of the assumptions used to derive the unit risk (that "any metabolism of BaP is directly proportional to breathing rate and that the deposition rate is equal between species" on p. 2-35, lines 6-8, and selection of body weight scaling factors in relation to "portal of entry," as discussed in the EPA *Guidelines for Carcinogen Risk Assessment*). EPA should also state a conclusion regarding overall uncertainty or level of confidence for the IUR, as endorsed on p. 118 of the NRC 2014 review of the IRIS program (NRC 2014).

Given the extensive human studies of lung cancer with airborne inhalation exposures to PAHs by coke oven, and aluminum smelter workers (i.e., Table 1-11, summary of Tier 1 epidemiologic-based reports of BaP in relation to lung cancer, pp. 1-55 to 1-56), and specifically, reports by Xu et al. (1996); Spinelli et al. (2006); Armstrong and Gibbs (2009); and Gibbs and Labreche (2014), the SAB recommends that the EPA give further consideration to selection of occupational studies (or meta-analysis of occupational studies) to develop unit risk estimate(s) for inclusion in Table 2-9. Although interpretation of the epidemiological evidence is challenging given that exposures were to mixtures of PAHs with poorly understood interactions, a model using relative potency factors and an assumption of dose additivity was reasonably accurate for some PAH mixtures and conservative for others in one investigation (U.S. EPA 1990), and should be considered for adjustment of epidemiological results in estimation of the unit risk attributable to BaP alone. Uncertainty and risk of bias due to exposure measurement error, healthy worker effects, habituation, and/or co-exposure to cigarette smoke products should also be considered and weighed against uncertainties regarding cross-species extrapolation of the unit risk from hamsters to humans.

It may be helpful for the EPA to address how reasonable it is that lifetime exposures will be in the approximately linear low-dose region where the unit risk is applicable (<0.3 mg/m^3, the human

equivalent POD). The SAB recognizes that a nationwide BaP exposure assessment is far beyond the scope of the draft assessment, but reference to typical exposure ranges may be helpful to readers.

Recommendations

- The EPA should conduct supplemental sensitivity analyses using other dose-response models, alternative assumptions, and not eliminating from the analysis all animals without confirmed examination of one or more of the pharynx or respiratory tract tissues.
- The EPA should give further consideration to selection of occupational studies (or meta-analysis of occupational studies) to develop unit risk estimate(s) for inclusion in Table 2-9
- The SAB also suggests the inclusion of an explicit conclusion statement regarding overall uncertainty of the unit risk value, and a brief discussion of the applicability of this value.

3.3.5. Dermal Slope Factor for Cancer

Charge Question 3e. The draft assessment proposes a dermal slope factor of 0.006 per µg/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?*

Neither the proposed dermal slope factor nor the proposed method for cross-species scaling is sufficiently scientifically supported. Discussion is provided below that explains the SAB's concerns with the justifications of these two analyses in the draft assessment.

Analysis of carcinogenicity data (choice of Studies) (section 2.5.1)

Animal Studies:

The SAB agrees that studies of skin tumors in mice are relevant to humans based on evidence for a similar mode of action as described in more detail in Section 3.2.4 (see discussion under *EPA Criterion 2*) of this report. In the choice of skin cancer bioassay studies for developing the dermal slope factor (DSF), the draft assessment reviewed 10 complete carcinogenicity mouse skin tumor bioassay studies that repeated exposure over approximately 2 years from 1959 to 1997 (summarized in Tables 2-11 and E-24) and the Sivak et al. (1997) study was chosen as the principal study. Other skin cancer bioassay studies are mentioned and excluded from further analysis because, according to the Supplemental Information document: (1) only one BaP dose level was considered; (2) all dose levels induced 90-100% incidence of tumors; (3) dose applications were once/week or less; and (4) dose was delivered in a vehicle that interacted with or enhanced BaP carcinogenicity. The draft assessment provided a different list of reasons for excluding studies from the dose-response analysis: (1) BaP dose levels were insufficiently characterized; (2) only one BaP dose level was considered, (3) all dose levels induced 90-100% incidence of tumors; and (4) studies were shorter (i.e., < 1 year). Nesnow et al. (1983) and Levin et al. (1977) were not considered in the dose-response analysis because the study durations were shorter (60 and 50-52 weeks, respectively) and dose applications were less than twice/week; i.e., once/week for the three lower dose levels in Nesnow et al. (1983) (the highest dose level was applied twice/week) and once every two weeks in Levin et al. (1977). Based on the criteria listed in the draft assessment, Nesnow et al. (1983) and Levin et al. (1977) should have been included in the dose-response analysis as the study durations were not less than 1 year. Related to the criteria listed in the Supplemental Information document, the SAB questions excluding studies that applied BaP less than once/week because they are "less useful for extrapolating to daily human exposure." Nearly complete absorption of BaP into the skin

can be reasonably assumed for all of the mouse dosing regimens considered. Also, the daily human exposure doses considered in risk assessments are daily averages of exposures that are not uniformly distributed over lifetimes. If the results of applying the same BaP dose by once/week or once every 2 weeks differ from applications of more than once/week, then continuous daily exposure, which has been assumed in the analysis for the dermal slope factor, is inappropriate because there would then be data indicating that dose-rate effects cannot be ignored (page 2-41, lines 12-13).

The SAB notes the following errors in this section:

- The cited study for Grimmer et al. (1984) in the draft assessment and the Supplemental Information is a study on rat lung. The correct citation should be Grimmer, G; Brune, H; Deutsch-Wenzel, R; Dettbarn, G; Misfeld, J; Abel , U; Timm, J. (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.
- The summary of the BMD model selection and/or BMD_{10} and $BMDL_{10}$ modeling results listed in Tables E-23 [for Sivak et al. (1997)] and E-24 are inconsistent with the selected model and POD values listed in Table 2-11. The comparative modeling results are as follows: Sivak et al. (1997) (BMD_{10} = 0.0985 vs. 0.11), Roe et al. (1970) (BMD_{10} = 0.748 vs. 0.69; $BMDL_{10}$ = 0.48 vs. POD = 0.39) and Habs et al. (1980) (Multistage 3^o vs. Multistage 4^o; BMD_{10} = 0.294 vs. 0.36; $BMDL_{10}$ = 0.215 vs. POD = 0.24).

Recommendation

- EPA should consider adding Nesnow et al. (1983) and Levin et al. (1977) to Table 2-11, with comments regarding the lower dosing frequency and duration, and should consider combining results from the different studies shown in Table 2-11. This would strengthen the derived DSF. Skin cancer bioassay studies that examined only one BaP level or observed 90-100% incidence of tumors are not suitable for estimating points of departure (POD). However, consistencies in the observations of these studies with observations from the studies listed in Table 2-11 and those used to develop the POD and DSF would strengthen the derived DSF. The criteria listed on pages 2-39 and D-62 for excluding carcinogenicity mouse tumor bioassay studies from consideration (and Table 2-11) should be revised for consistency. The selected model and $BMDL_{10}$ and POD values listed in Tables 2-11, E-23 and E-24 should match.

<u>Human Studies</u>:

The EPA review of the epidemiologic evidence of skin cancer in humans is not sufficiently thorough. The draft assessment cites evidence of an excess of skin cancer in studies of roofers (Hammond et al. 1976) and workers exposed to creosote-treated wood (Karlehagen et al. 1992; Tornqvist 1986), but these groups work outside and would thus have substantial exposure to UV. The draft assessment also notes that recent studies of chimney sweeps do not demonstrate an increased skin cancer risk (Hogstedt et al. 2013). The draft assessment does not cite or discuss other studies that reported an excess of skin cancer in destructive distillation of coal, shale oil extraction (Miller et al. 1986), tar refinery (Letzel and Drexler 1998), asphalt workers and roofers (Partanen and Boffetta 1994), workers exposed to creosote in brick making and wood impregnation (Karlehagen et al. 1992) or studies of workers in other industries with PAH exposure that were reviewed by Boffetta et al. (1997) and Gawkrodger (2004).

Recommendation

- The EPA should more thoroughly review the evidence for skin cancer in studies of coke, steel and iron, coal gasification and aluminum workers given their relevance for evaluating the appropriateness of using the mouse-based risk assessment model for predicting skin cancer risk in humans.

The SAB notes that epidemiologic studies of therapeutic use of coal tar preparations do not provide an adequate basis for either hazard identification or the derivation of a dermal slope factor due to uncertainties regarding the PAH dose, deficiencies in the study data, and the relevance of psoriatic skin, which is characterized by abnormally rapid proliferation. (See discussion in Section 3.2.4, Cancer, under EPA Criterion 1.)

Dose-response analysis (section 2.5.2)

The draft assessment (p. 2-40, lines 18-20) states the following:

> Although environmental dermal exposure may more likely occur intermittently than oral or inhalation exposures, due to interruption of exposure through bathing or washing of affected areas, the dermal slope factor was derived for use with estimates of constant daily lifetime exposure. Therefore, all administered doses were converted to time-weighted average (TWA) daily doses using the equation:
>
> Average daily dose/day = (μg/application) × (number of applications/week ÷ 7 days/week)

This statement is applicable to the Multistage-Weibull analysis (pp. E-82 to E-83 of the Supplemental Information document) of the Sivak et al. (1997) data from which the selected DSF was ultimately derived. Evaluation of dermal dose response for the Sivak et al. dataset (and all of the other datasets considered, pp. E-86 to E-111) by the Multistage Cancer Model also includes a dose adjustment if the duration of the dosing regimen was less than the expected remaining life span. Doses in studies known or assumed to be shorter than 104 weeks were adjusted (p. E-75) by a factor of $(Le/104)^3$, where Le is exposure duration in weeks and 104 weeks is the default life expectancy of a mouse post study initiation. (This adjustment does not appear in the oral or inhalation dose analyses, which were conducted using the Multistage-Weibull approach.) The effect is transparent in the descriptions of the Roe et al. (1970), Habs et al. (1980) and Poel et al. (1959) studies in Tables E-20 and E-21 (pp. E-79 and E-80 of the Supplemental Information document, U.S. EPA 2014b). Presentation of the Sivak et al. (1997) data (Table E-24 on p. E-87) is dissimilar to that of the Roe et al. (1970), Habs et al. (1980), and Poel et al. datasets and the adjustment is obscured (i.e., the assumed length of exposure is not reported). While the result of the Multistage Cancer Model analysis of the Sivak et al. (1997) data was ultimately not used to derive the recommended DSF, the numerical value generated by that method is very similar to the selected result, is potentially supportive, and should be clearly explained.

The dose adjustment described above is attributed to Doll (1971). That document does support non-linearly increasing risk with increasing age and cumulative exposure. However multiple potential values of the exponent describing dependence of risk on age are discussed by Doll (1971) whereas the selected value of 3 is apparently a default value specified in EPA's 1980 water quality criteria documents guidelines (U.S.EPA, 1980). Given that the default value is quite old and that Sivak et al. (1997) provided time-to-tumor data, reevaluation of the numerical value of the exponent appears both feasible and warranted.

Recommendations

- The EPA should make the Sivak et al. (1997) dose adjustment transparent.
- The EPA should explain why a default coefficient of 3 was chosen and how well it describes temporal dependence of the time-to-tumor data from the Sivak et al. study.
- The EPA should cite prior examples in which dose adjustment for truncated study duration has been incorporated in derivation of cancer slope factors.

Derivation of the dermal slope factor (section 2.5.3.)

The draft assessment states that mass rather than mass/area can be used as the appropriate dose metric for cancer risk at "low doses" of BaP. The SAB notes that published dermal slope factors for BaP skin carcinogenesis have used mass and mass/skin area as dose metrics and there do not appear to be any empirical data available to inform a choice between these two dose metrics or to select another.

Experimental studies have demonstrated that equal masses of chemical absorb into the skin when the area of direct chemical contact is less than the applied skin area (i.e., the mass of chemical applied is too small to completely cover the application area). For example, Roy and Singh (2011) reported that the percentage of BaP applied on contaminated soil that was absorbed was independent of the mass of soil applied until the skin surface area was completely covered with soil; further increases in the mass of soil applied caused the percent BaP absorption to decrease. The DSF derived from the skin cancer bioassay in mice is based on the applied dose, which most probably closely approximates the absorbed dose in the case at hand. The time between dose applications was long enough (> 3 days for a 2-times/week exposure protocol or >2 days for a 3-times/week exposure protocol) and the applied doses small enough in the mouse studies for approximately 100% absorption. Wester et al. (1990) observed 51% absorption *in vivo* in monkeys and 24% absorption *in vitro* for human skin at 0.5 $\mu g/cm^2$ in 24 h and absorption rates through mouse skin are generally faster than through human and monkey skin (Bronaugh et al. 1982; Kao et al. 1985; Vecchia and Bunge 2006). Also, the application site was not cleaned before new applications, so the dose remained on the skin to be completely absorbed. The conclusion that absorbed dose approximately equals the applied dose does assume that dose losses were minimal; therefore, study protocols in the draft assessment should be evaluated for factors that may have affected losses of the applied dose (e.g., by grooming).

For many human BaP exposure scenarios, it is likely that a significant fraction of the gross BaP dose will never be absorbed into the skin. For humans, the timing of exposure may be more frequent than the 2-3 days interval between dose applications in the mouse studies and humans wash periodically, potentially removing surface doses. In human exposure, BaP is nearly always in a matrix such as soil, sediment, soot or tar, which can reduce dermal absorption by one or more mechanisms. For example, Wester et al. (1990) observed that dermal absorption in monkey from soil was 25% of the amount absorbed when BaP was applied directly to the skin. This observation is the basis of the 0.25 value for the soil to skin transfer coefficient (Ksoil) used to adjust exposed soil doses in the example shown in Appendix G (p. G-12 and p. G-14). Given the likelihood that the DSF derived from the skin cancer bioassay in mice is effectively based on the absorbed dose, then skin cancer risk in humans should be calculated using an estimate of the absorbed dose for the given exposure scenario.

Recommendations

- The SAB does not have a specific recommendation as to dose metric, but strongly recommends that in the absence of empirical data, the decision be based upon a clearly articulated, logical, scientific structure that includes what is known about the dermal absorption of BaP under both

conditions of the bioassay(s) and anticipated human exposures, as well as the mechanism of skin carcinogenesis of BaP.

- The choice of dose metric needs to be better justified and the EPA should provide a convincing argument for the use of mass as the dose metric.
- The derived DSF is based on applied doses that likely closely approximate absorbed doses. Therefore, the SAB recommends that cancer risk calculated from the derived DSF should use **absorbed dose** and not applied dose.
- The EPA should describe what constitutes a "low dose" for the assumption that mass of BaP is the appropriate dose metric for calculating the DSF from the skin cancer bioassay studies and for estimating cancer risk in humans. This should be consistent with the proposed logical structure for skin cancer from skin exposure to BaP, which, in pure form, is a solid at skin temperature. Issues to consider include:
    - For dermal absorption, the skin area with direct chemical contact must be less than the total applied area; i.e., mass of BaP applied cannot completely cover the applied area. For BaP deposited onto skin from a volatile solvent, the mass of BaP that would give a theoretical uniformly thick film <1 μm (i. e., ~135 μg of BaP/cm^2) would be too small to completely cover the application area, where: Theoretical thickness of a uniform film on the application area = [(BaP mass applied)/(application area)]/$\boldsymbol{\rho}$BaP; $\boldsymbol{\rho}$BaP= density of BaP= 1.35 g/mL.
    - Metabolism in the target tissue (the viable epidermis) should not be saturated. The draft assessment identifies the linear limit for using the slope factor to calculate cancer risk in humans based on the human equivalent point-of-departure (POD_{HED} = 17.9 μg/day) estimated from the mouse POD_M adjusted by the mouse-to-human scaling factor as the $BW^{¾}$. This is an appropriate limit that could be smaller than 17.9 μg/day for different scaling factor approaches.
- The EPA should consider adding diagrams illustrating the logical structure (physiological steps to carcinogenesis) to facilitate choices of dose metric and cross-species scaling.
- The EPA should consider adding diagrams illustrating the steps involved in calculating human cancer risk based on skin cancer bioassay studies in mice; for example
    - Tumors observed in mouse studied as a function of time and exposed dose
    - Exposed dose ≈ applied dose to estimate point of departure in mice (POD_M) and dermal slope factor in mice (DSF_M)
    - DSF_M scaled to the dermal slope factor in humans (DSF_H)
    - Estimate of absorbed dose from exposed dose and exposure scenario
    - Human cancer risk = DSF_H x (Absorbed dose)

Dermal slope factor cross-species scaling

According to the draft assessment, the starting point is the dermal slope factor in the mouse (i.e., DSF_M= 1.7 $(\mu g/day)^{-1}$), which is adjusted by the appropriate human to mouse ratio to obtain the DSF_H. Experimental cancer risk information for scaling from mouse to human skin cancer from dermal exposure is not available. It is unknown if the chosen approach for scaling of skin cancer risk from BaP exposure to skin is similar to interspecies differences in whole body toxicokinetics, which is the approach (i.e., allometric scaling using $BW^{¾}$) adopted by the EPA. The draft assessment lists alternative approaches for scaling, however the SAB recognizes that the science for choosing the best approach is uncertain. The EPA should clarify their choices in this section.

Recommendations

- The chosen scaling approach should be supported by a coherent logical structure. Consistent with recommendations on cross-species scaling in response to Charge Questions 3b and 3c, this should be clearly articulated in the document. Differences between mouse and human skin should be considered in light of the proposed logical structure for skin cancer risk; for example:
    - Thickness of and metabolic rates in the target tissue (i.e., the viable epidermis layer).
    - Differences in stratum corneum thickness will affect the absorbed dose from a given exposed dose applied to humans compared with mice. However, it may not affect the cross-species scaling of the DSF, which is based on absorbed dose.

Uncertainties in the derivation of the dermal slope factor

The cross-species mouse-to-human scaling of the DSF is a significant contributor to uncertainties.

Other recommendations for describing cancer risk calculated with the DSF

- The cancer risk calculation in mice (and therefore in humans) depends on absorbed dose; i.e., Cancer Risk = DSF x (Absorbed dose). The EPA should state clearly how the absorbed dose estimates from exposed dose enters the calculation of cancer risk.
- In actual BaP exposures (from soil or other environmental media), the absorbed dose should be estimated from the exposed dose and the exposure scenario.
- A soil-to-acetone absorption ratio as described in the response to public comments is unnecessary.
- Cancer risk from BaP in soil should be calculated from the estimated absorbed dose from exposure to BaP contaminated soil.
- Examples of cancer risk estimates from exposure to BaP contaminated soil will use an estimate of the absorbed dose taken from the literature (or Risk Assessment Guidance for Superfund (RAGS), Vol. 1, Part E). Because the draft assessment does not critically review this literature,
    - The literature of dermal absorption measurements from BaP contaminated soils should be listed; and
    - The estimate of absorption used in the risk calculation should be identified as an example (and not an endorsement of the value used).
- A "fidelity exercise" for the proposed DSF to determine whether the toxicity value yields a plausible upper bound risk estimate should be helpful.
- Each environmental media will have its own absorption characteristics that should be considered in estimating an absorbed dose for estimating cancer risk.

3.3.6. Age-Dependent Adjustment Factors for Cancer

Charge Question 3f. 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. Do the available mechanistic studies in humans and animals support a mutagenic mode of action for cancer induced by benzo(a)pyrene?

The available mechanistic studies in humans and animals support a mutagenic mode of action for BaP-induced cancers. Given that the EPA's *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposures to Carcinogens* (U.S. EPA 2005b) establishes a rational approach for the adjustment of tumor risk for exposures at different ages to carcinogens with a mutagenic mode of action, the SAB concludes that the proposed use of age-dependent adjustment factors (ADAFs) is justified.

3.4. Executive Summary

Charge Question 4. Does the executive summary clearly and appropriately present the major conclusions of the assessment?

The SAB found that the major conclusions of the draft assessment were clearly and appropriately presented in the Executive Summary. Changes made to the body of the draft assessment in response to the SAB recommendations that impact the derivation of the chronic RfD/RfC or cancer slope factors should be incorporated into the Executive Summary. In addition, the SAB had a number of suggestions for improving the Executive Summary:

- The purpose of the gray box text at the beginning of the Executive Summary is not immediately apparent. During the SAB panel meeting, the EPA clarified that this box is intended to be a lay language abstract for the report. That means that it has a different audience than the rest of the draft assessment, and the SAB suggests that it stand alone from the Executive Summary and be clearly identified as a lay language abstract or summary. The SAB further suggests that the gray box text be examined to insure that the health literacy level is commensurate with the lay public as target audience.

- For audiences that will focus on the Executive Summary, it is not clear in the narrative presented why a toxicological review focusing on BaP is relevant. The SAB suggests adding introductory text to the Executive Summary explaining the public health relevance of the draft assessment, especially related to the importance of evaluating hazard and risk from human exposures to BaP present in PAH mixtures.

- Although the SAB has no specific advice regarding the appropriate length for the Executive Summary, the EPA should strive to capture the important conclusions in a summary that is of readable length.

- The basis upon which levels of confidence in toxicity values (i.e., “low,” “medium,” or “high”) are reached is not always apparent, and therefore the meaning of these descriptors as presented in the Executive Summary will be unclear. The SAB suggests adding a few sentences in the Executive Summary to explain how confidence levels are determined.

3.5. EPA's Response to Public Comments

Charge Question 5. 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.

The SAB found that most of the scientific issues raised by the public, as described in Appendix G of the Supplemental Information document, were adequately addressed by EPA.[1] However, there were some issues for which the SAB requested additional clarification from EPA. These issues are identified below with reference to relevant sections of the SAB report.

- *Comment: Metric used to characterize results in the elevated plus maze* (p. G-5). Public commenters noted that the way the maze response was quantified is not the preferred way. The EPA response agrees with the point raised, but explains that data necessary to quantify response in the preferred way were not available, but there was enough information available to conclude that the results presented are valid (i.e., were not unduly influenced by changes in general locomotor or exploratory behaviors). The SAB's discussion regarding these results is summarized in the response to Charge Question 2a.

- *Comment: Use of decreased anxiety-like effects as a critical effect (p. G-6).* Public commenters questioned whether decreased anxiety-like effects are adverse effects. The EPA response explains that decreased anxiety represents a clear change in nervous system function and can impair an organism's ability to react to a potentially harmful situation. The SAB's discussion on this endpoint is provided in the response to Charge Question 2a.

- *Comment: Cross-species extrapolation of dermal slope factor (p. G-11).* Public commenters stated that differences between mouse and human skin should be accounted for in cross-species extrapolation. The EPA response notes that biological information is not currently sufficient to develop robust models for cross-species extrapolation, and states that allometric scaling using body weight to the ¾ power was selected based upon observed differences in the rates of dermal absorption and metabolism of BaP. The SAB found that this cross-species scaling factor was not sufficiently justified, as discussed in the response to Charge Question 3e.

- *Comment: Uncertainties regarding implementation of the dermal slope factor* (p. G-12). Two aspects of the public comments under this topic received significant discussion by the Panel. One is a comment that a 13% dermal absorption factor for BaP may not be appropriate. The EPA response explains the origin of the value, but acknowledges that it may be a high estimate. The SAB also has concerns about the dermal absorption value, as discussed in the response to Charge Question 3e. The SAB provides specific suggestions. The second comment is that the dose metric of μg/d is not appropriate for the slope factor in view of the mode of action. The EPA

[1] The Draft Toxicological Review for Benzo[a]pyrene that the SAB was asked to review contained only those public comments received by EPA prior to the completion of the document (i.e., responses EPA received on the 2013, draft). Thus, the SAB's comments in response to this charge question relate to the EPA's responses to those earlier public comments.

response is that dermal bioassays report total dose applied to the skin but do not quantify the area over which the dose is applied. The SAB concluded that the dose metric has not been sufficiently justified by EPA, as explained in the response to Charge Question 3e.

- *Comment: "Real world" validation of dermal slope factor* (p. G12). Public commenters recommended that EPA perform calculations of risk from dermal exposure to PAHs using the proposed dermal slope factor to determine whether the value is scientifically supportable. Commenters discussed that this type of calculation shows skin cancer risks from common PAH exposures such as the use of pharmaceutical coal tar products that are unrealistically high. In their response, the EPA indicated that sufficient details were not provided to allow the EPA to reproduce the calculations performed by the public commenters, and provided their own estimate of risk from exposure to BaP in soil showing a low excess cancer risk (6×10^{-6} for average lifetime exposure that occurs during childhood and 1×10^{-6} for average lifetime exposure that occurs during adulthood).

With respect to the dermal cancer slope factor, the SAB supports the application of a "fidelity exercise" for proposed toxicity values to determine whether the toxicity values yield plausible upper bound risk estimates. Generally, this exercise consists of using the proposed toxicity value to estimate risk from one or more exposure scenarios and determine whether the results exceed lifetime risk estimates derived from actual disease incidence (Howlader 2015) for the adverse effect(s) of interest. The SAB finds limitations in the fidelity exercise approaches taken by both the public commenters and the EPA in its response. For example, the EPA estimation of cancer risk from BaP alone does not reflect actual circumstances of exposure, which almost always occurs as a mixture of carcinogenic PAHs (BaP plus others of varying potency). On the other hand, the limitations of coal tar therapeutics studies make them largely uninformative with regard to the question of whether BaP induces skin cancer in humans. The public commenter's use of upper percentile exposure values to represent exposure of the overall population tends to exaggerate risk, and the recognized under-reporting of skin cancer[2] was not taken into account in comparisons. Further, the inherent conservative nature of toxicity values should be recognized and taken into consideration in such analyses. The SAB suggests an improved fidelity exercise to address concerns that the proposed dermal cancer slope factor may lead to unrealistic cancer risk estimates.

As a general comment, the SAB supports the approach taken by the EPA in creating Appendix G in which the most important scientific issues presented by public commenters are captured and arranged by topic, with reference to the public commenters raising the issue. A more extensive approach, such as providing comment-by-comment responses would be inefficient and cumbersome in a toxicological review. The SAB is aware of contention by some public commenters that their comments were not adequately captured and articulated in Appendix G. To minimize such concerns in future toxicological reviews, the SAB urges the EPA to provide greater transparency in how public comments are distilled into a list of scientific issues meriting an EPA response in the draft assessment. The EPA provided such

[2] ACS, 2015, American Cancer Society, Cancer Facts & figures 2015. Atlanta: American Cancer Society; 2015. p 21. "Skin cancer is the most commonly diagnosed cancer in the United States. However, the actual number of the most common types – basal cell and squamous cell skin cancer (i.e., keratinocyte carcinoma), more commonly referred to as nonmelanoma skin cancer (NMSC) – is very difficult to estimate because these cases are not required to be reported to cancer registries. The most recent study of NMSC occurrence estimated that in 2006, 3.5 million cases were diagnosed among 2.2 million people. NMSC is usually highly curable."

a draft table during the SAB deliberations and the SAB would encourage its addition to the document to improve transparency about the review process. In particular, the SAB suggests that the EPA provide a short description of the process used for deciding which comments to include in a public response appendix and how comments are aggregated within the appendix. In particular, it would be helpful if the EPA provided a table within the draft assessment showing the topics under which comments are aggregated, which commenters provided comments within each topic, and the dates on which the comments were made.

Recommendations

- As suggested in Appendix C, major science issues pointed out by public commenters should be included in the relevant charge questions. The SAB can then comment on whether EPA's approach is scientifically supported. The SAB should not be asked if EPA has adequately addressed all public comments.

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APPENDIX A: EPA'S CHARGE QUESTIONS

Charge to the Science Advisory Board for the IRIS Toxicological Review of Benzo[a]pyrene

September 2014 (Updated March 2015[1])

Introduction

The U.S. Environmental Protection Agency (EPA) is seeking a scientific peer review of a draft Toxicological Review of Benzo[a]pyrene developed in support of the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD).

IRIS is a human health assessment program that evaluates scientific information on effects that may result from exposure to specific chemical substances in the environment. Through IRIS, EPA provides high quality science-based human health assessments to support the Agency's regulatory activities and decisions to protect public health. IRIS assessments contain information for chemical substances that can be used to support hazard identification and dose- response assessment, two of the four steps in the human health risk assessment process. When supported by available data, IRIS provides health effects information and toxicity values for health effects (including cancer and effects other than cancer) resulting from chronic exposure. IRIS toxicity values may be combined with exposure information to characterize public health risks of chemical substances; this risk characterization information can then be used to support risk management decisions.

An existing assessment for benzo[a]pyrene, which includes an oral slope factor (OSF) and a cancer weight of evidence descriptor, was posted on IRIS in 1987. The IRIS Program is conducting a reassessment of benzo[a]pyrene. The draft Toxicological Review of Benzo[a]pyrene is based on a comprehensive review of the available scientific literature on the noncancer and cancer health effects in humans and experimental animals exposed to benzo[a]pyrene. Additionally, appendices for chemical and physical properties, toxicokinetic information, summaries of toxicity studies, and other supporting materials are provided as *Supplemental Information* (see Appendices A to E) to the draft Toxicological Review.

The draft assessment was developed according to guidelines and technical reports published by EPA (see *Preamble*), and contains both qualitative and quantitative characterizations of the human health hazards for benzo[a]pyrene, including a cancer descriptor of the chemical's human carcinogenic

[1] The charge questions were modified (as shown in bold font) as a result of panel discussions during the March 4, 2015 preliminary teleconference

potential, noncancer toxicity values for chronic oral (reference dose, RfD) and inhalation (reference concentration, RfC) exposure, and cancer risk estimates for oral, inhalation, and dermal exposure.

Charge questions on the draft Toxicological Review

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

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?

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?

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 **inherent** 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 **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^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?

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?

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 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.

APPENDIX B: ADDITIONAL PEER-REVIEWED STUDIES ON HEALTH EFFECTS OF BaP

The SAB recommends the following 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:

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Additional Peer-reviewed studies contained in HERO

The SAB recommends that EPA consider the following peer-reviewed studies contained in HERO but that are not cited within the BaP document:

Gunter, MJ; Divi, RL; Kulldorff, M; Vermeulen, R; Haverkos, KJ; Kuo, MM; Strickland, P; Poirier, MC; Rothman, N; Sinha, R. (2007). Leukocyte polycyclic aromatic hydrocarbon-DNA adduct formation and colorectal adenoma. *Carcinogenesis* 28(7):1426-1429.

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APPENDIX C: SUGGESTIONS ON THE FORMAT FOR EPA's CHARGE QUESTIONS

The format for EPA's charge questions for the SAB review of the IRIS Toxicological Review of Benzo[a]pyrene is different than that for previous IRIS assessments. The CAAC-BaP panel would like to offer the following suggestions based on the experience during panel review of this assessment:

1) Charge questions on hazard identifications should not consist of a separate charge question for all critical endpoints. This is because the first step in the development of toxicity values involves the selection of critical studies and endpoints. Thus, the discussion on critical effects became redundant during the review meeting.

2) Charge questions on the development of RfD, RfC, oral slope factor, IUR, and dermal slope factor actually involve many subparts that should be reviewed by panel members with very different expertise. Separate charge questions should be provided for each subpart (e.g., selection of critical studies and effect, determination of the point of departure, derivation of the toxicity value, uncertainty analysis) arranged in a logical sequence. This will make the assignment of lead discussants for each subpart of the charge question clearer.

3) For the charge question on EPA's response to public comments, the major science issues pointed out by public commenters should be included in the relevant charge questions (or subparts of the charge question). The SAB can then comment on whether EPA's approach is scientifically supported. The SAB should not be asked if EPA has adequately addressed all public comments.