

DATE

The Honorable Gina McCarthy
Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 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, cardiovascular toxicity, and adult nervous system toxicity are not potential human hazards.

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 made a sufficiently strong case 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 agency give more consideration to the available data on reproductive outcomes, including cervical hyperplasia and cervical inflammation, and provide a firmer justification for not selecting these as critical endpoints.

1 With respect to the application of uncertainty factors (UFs), the SAB recommends that the EPA consider
2 applying a body weight ^{3/4} (BW^{3/4}) adjustment factor for interspecies extrapolation from neonatal animal
3 to neonatal human. In addition, the agency should provide further justification for the application of a
4 database uncertainty factor of 3 that is based, in part, on the absence of a multi-generational study or
5 extended one-generation study, and the lack of a study examining functional neurological endpoints
6 following exposure from gestation through lactation.

7
8 For derivation of the inhalation reference concentration (RfC), the SAB found that the RfC value
9 provided in the assessment is not scientifically supported. While the endpoint (decreased fetal survival)
10 and key study selected are appropriate, the RfC is based only upon this one study that has some
11 technical deficiencies that decrease the confidence in the RfC derived using the data from this study.
12 Furthermore, the rationale for not employing a benchmark dose (BMD) approach to derive the point of
13 departure is unclear. Regarding UFs, the EPA application of an UF of 3 to address residual uncertainty
14 for interspecies extrapolation may be too low, since the regional deposited dose ratio (RDDR)
15 adjustment used with the key study may not completely account for systemic toxicokinetics following an
16 inhalation exposure. Additionally, because the effect was found at all exposure levels, the lowest-
17 observed-adverse-effect level (LOAEL) from this study provides a weaker basis than a no-observed-
18 adverse-effect level (NOAEL) for derivation of the RfC. The SAB recommends two studies that should
19 be considered by the EPA to develop a more comprehensive dose-response relationship for BaP.

20
21 For derivation of the oral slope factor for cancer, the SAB finds that appropriate studies and models
22 were selected for dose-response analysis. However, insufficient justification was provided for the
23 derivation of the final slope factor solely based on a single-sex mouse study that produced the largest
24 cancer slope factor. The SAB suggests that data from all studies be incorporated in the derivation of the
25 oral cancer slope factor. The SAB also questions the use of a default cross-species scaling factor applied
26 to all of the tumor sites identified in the two studies. The SAB recommends that the EPA provide a brief
27 explanation of the rationale for its use of the allometric scaling factor when deriving the BaP oral slope
28 factor, given what is known about the BaP mode of action for carcinogenicity, reaction rates,
29 toxicokinetics, and the portal of entry effect for alimentary tract tumors.

30
31 For the derivation of the inhalation unit risk (IUR) for cancer, the SAB finds that the EPA has selected
32 an appropriate study for dose-response analysis, and that appropriate models were used. The SAB
33 recommends additional discussion of key assumptions, conducting sensitivity analyses, and encourages
34 the agency to reconsider the decision not to use epidemiological data to support the derivation of the
35 IUR.

36
37 The SAB commends the agency's efforts in deriving the IRIS Program's first dermal slope factor (DSF).
38 However, the proposed DSF is not sufficiently supported scientifically. The SAB agrees that studies of
39 skin tumors in mice are relevant to humans based on evidence of a similar mode of action and can be
40 used to derive a DSF. However, the SAB recommends that the EPA include two additional studies for
41 review and consider combining results from the mouse skin tumor bioassays to strengthen the derived
42 DSF. The SAB also recommends that the EPA more thoroughly review the evidence of skin cancer in
43 studies of coke, steel and iron, coal gasification and aluminum workers given their relevance for
44 evaluating the appropriateness of using the mouse-based risk assessment model for predicting skin
45 cancer risk in humans.

46
47 The assessment used mass rather than mass/area as the dose metric for cancer risk at "low dose"
48 exposure to BaP. The SAB does not have a specific recommendation as to the dose metric, but strongly

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1 recommends that in the absence of empirical data, the decision be based upon a clearly articulated,
2 logical, scientific structure that includes what is known about the dermal absorption of BaP under both
3 conditions of the bioassays and anticipated human exposure, as well as the mechanism of skin
4 carcinogenesis of BaP. The SAB also recommends that cancer risk calculated from the derived DSF
5 should use the absorbed dose, and not the applied dose. Moreover, the SAB recommends that the EPA
6 describe what constitutes a “low dose” exposure when using the mass of BaP as the dose metric.
7

8 The SAB believes the cross-species scaling approach used in the assessment should be supported by a
9 coherent logical structure. In addition, differences between mouse and human skin should be considered,
10 such as thickness of and metabolic rates in the target tissue (i.e., the viable epidermis layer).
11

12 Finally, the SAB concludes that the available mechanistic studies in humans and animals support a
13 mutagenic mode of action for BaP-induced cancers, and the proposed use of age-dependent adjustment
14 factors is justified.
15

16 The SAB appreciates this opportunity to review EPA’s *Draft Toxicological Review of Benzo[a]pyrene*
17 and looks forward to the EPA’s response to these recommendations.
18

19 Sincerely,
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25 Enclosure
26

NOTICE

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Abbreviations and Acronyms

1		
2		
3	AhR	aryl hydrocarbon receptor
4	AIC	Akaike Information Criteria
5	ADAF	age-dependent adjustment factor
6	ADHD	attention deficit hyperactivity disorder
7	AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazole-propionate
8	ANOVA	analysis of variance
9	ATSDR	Agency for Toxic Substances and Disease Registry
10	BMC	benchmark concentration
11	BMCL	lower 95% confidence limit of the benchmark concentration
12	BMD	benchmark dose
13	BMDL	lower 95% confidence limit of the benchmark dose
14	BMR	benchmark response
15	BW	body weight
16	CAAC	Chemical Assessment Advisory Committee
17	CI	confidence interval
18	DSF	dermal slope factor
19	EPA	Environmental Protection Agency
20	ET	extrathoracic respiratory tract region
21	HED	human equivalent dose
22	HERO	Health and Environmental Research Online
23	HPBMC	human peripheral blood mononuclear cell
24	5-HT	5-hydroxytryptamine
25	IARC	International Agency for Research on Cancer
26	Ig	immunoglobulin
27	IRIS	Integrated Risk Information System
28	IUR	inhalation unit risk
29	LOAEL	Lowest-Observed-Adverse-Effect Level
30	MOA	mode of action
31	NAS	National Academy of Sciences
32	NCI	National Cancer Institute
33	NIOSH	National Institute for Occupational Safety and Health
34	NMDA	N-methyl-D-aspartate
35	NOAEL	No-Observed-Adverse-Effect Level
36	NRC	National Research Council
37	NTP	National Toxicology Program
38	OECD	Organization for Economic Co-operation and Development
39	OR	odds ratio
40	ORD	Office of Research and Development
41	PAH	polycyclic aromatic hydrocarbons
42	PFC	plaque forming cell
43	PHA	phytohemagglutinin
44	POD	point of departure
45	PU	pulmonary respiratory tract region
46	RfC	reference concentration
47	RfD	reference dose

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1	RDDR	regional deposited dose ratio
2	ROS	reactive oxygen species
3	RR	relative risk
4	TDAR	T-dependent antibody response
5	UCL	Upper Confidence Limit
6	UF	uncertainty factor
7	UF _D	Database uncertainty factor
8	UF _H	Human inter-individual variability uncertainty factor
9	UF _L	LOAEL-to-NOAEL uncertainty factor
10	UF _S	subchronic-to-chronic uncertainty factor
11	WHO	World Health Organization
12		
13		

1. EXECUTIVE SUMMARY

The Science Advisory Board (SAB) was asked by the EPA Integrated Risk Information System (IRIS) program to review the agency's *Draft IRIS Toxicological Review of Benzo[a]pyrene (September 2014)* (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.

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., cardio) and specific data gaps emerge.

The SAB appreciates that the agency is developing a handbook 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 are understood, but also that the strengths and weakness of the evaluated studies will be fully transparent. The SAB also requested 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 when evaluating epidemiology studies, as these studies would be relevant 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. Some of the studies of coal tar that were identified in the public comments were not included in the EPA review.

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

4 **Hazard Identification**

5 *Developmental Neurotoxicity and Developmental Toxicity*

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

16
17 The SAB concurs with the EPA that the available human studies support a contribution of BaP to human
18 developmental toxicity. Studies with PAH mixtures have shown a correlation between PAH exposure
19 and lower birth weights, increased risk of fetal death, and BaP DNA adducts. BaP exposure *in utero* has
20 been demonstrated to cause fetal death, lower fetal/offspring weights and affect fetal germ cells.
21 Additional studies that should be considered for inclusion include reported BaP-related effects on fetal
22 lung growth/function, and teratogenicity.

24 *Reproductive Toxicity*

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

32
33 BaP has a direct effect on adult rodent ovarian follicles. A recent study showed that *in vivo* exposure to
34 BaP induces significant DNA damage in mouse oocytes and cumulus cells. *In utero* exposure of
35 developing females to BaP provides compelling evidence that there is a sensitive window for exposure
36 to BaP for the developing ovary.

38 *Immunotoxicity*

39 The SAB finds that the available immunotoxicity data based on animal models of pure BaP and complex
40 PAH mixture exposures to humans (coke oven workers) support the claim that BaP is a human hazard
41 for the immune system. The evidence for immunotoxicity in humans is based upon complex PAH
42 mixture exposures. BaP as a pure chemical can cause suppression of human peripheral blood
43 mononuclear cell responses at low concentrations (10-100 nm) *in vitro*. Immunotoxicity is caused by a
44 combination of genotoxic (DNA adducts and p53-induced cell death) and non-genotoxic mechanisms
45 (signaling due to AhR activation and oxidative stress). Animal studies provide strong evidence that BaP
46 suppresses immune function leading to adverse consequences for host resistance to infections and
47 perhaps cancer. In addition to the evidence that BaP alters T cell development *in utero* and in adults,

1 there is evidence that BaP alters B cell development in the bone marrow of adults. It is likely that the
2 developing immune system is more sensitive to BaP exposures than adult exposures.

3 4 *Cancer*

5 The SAB finds that, in accordance with EPA's Cancer Guidelines (USEPA, 2005a), the EPA has
6 demonstrated that BaP is a human carcinogen. This conclusion was based primarily on: (1) extensive
7 evidence of carcinogenicity in animal studies, (2) the mode of carcinogenic action – mutagenic, and
8 associated key precursor events have been identified in animals, (3) strong evidence that the key
9 precursor events that precede the cancer response in animals are anticipated to occur in humans and
10 progress to tumors, and (4) strong support from an excess of lung cancer in humans who were exposed
11 to PAHs, although not to BaP alone. This conclusion is consistent with the evaluations by other
12 agencies, including the World Health Organization, International Agency for Research on Cancer (2010)
13 and Health Canada (2015).

14 15 *Other Toxicity*

16 Other potential hazards from BaP exposure are identified and discussed in Section 1.1.4; they include
17 forestomach toxicity, hematological toxicity, liver toxicity, kidney toxicity, cardiovascular toxicity, and
18 adult nervous system effects. Overall, the EPA concluded that the available evidence does not support
19 these non-cancer effects as potential human hazards. The SAB recommends that EPA's basis for
20 arriving at this conclusion be expanded for these health endpoints. In addition, the SAB finds that the
21 evidence presented in the assessment does not support EPA's conclusion that forestomach toxicity in
22 rodents, cardiovascular toxicity, and adult nervous system toxicity are not potential human hazards. The
23 SAB also notes that the literature search was not sufficiently comprehensive to identify studies relevant
24 to the characterization of cardiovascular system toxicity due to BaP exposure. Furthermore, the SAB
25 identifies adult and developmental pulmonary toxicity as non-cancer endpoints that can be credibly
26 associated with BaP exposure, but were not identified in the draft assessment.

27 28 **Dose-Response Analysis**

29 *Oral Reference Dose for Effects Other Than Cancer*

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

35
36 With respect to developmental toxicity as the most appropriate category of non-cancer effects, the SAB
37 suggests that the EPA give more consideration to the available reproductive outcomes including cervical
38 hyperplasia and cervical inflammation in Gao et al. (2011), and at least provide a firmer justification for
39 not selecting these as critical endpoints.

40
41 With respect to the choice of specific neurodevelopmental endpoints, the SAB recommends that the
42 EPA consider the overall picture of neurodevelopmental impact from all of the neurodevelopmental
43 endpoints in Chen et al. (2012)—including plus maze, reflex, locomotor activity and water maze—to
44 justify and support the choice of the critical endpoint. In particular, the SAB suggests that EPA
45 reconsider or provide stronger justification for not using escape latency from the Morris water maze.

1 With respect to the application of uncertainty factors, the SAB recommends that the EPA consider
2 application of a $BW^{3/4}$ adjustment as per the agency's 2011 allometric scaling guidance for extrapolation
3 from neonatal animal to neonatal human. In addition, the SAB recommends that the EPA further justify
4 the application of a database uncertainty factor of 3 that is based, in part, on the absence of a multi-
5 generational study, and the lack of a study examining functional neurological endpoints following
6 exposure from gestation through lactation.

7 8 *Inhalation Reference Concentration for Effects other than Cancer*

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

22 23 *Oral Slope Factor for Cancer*

24 The SAB finds that appropriate studies and models were selected for dose-response analysis. However,
25 an insufficient justification was provided for the selection of the final slope factor solely from the
26 Beland and Culp (1998) mouse study, instead of the slope factor from the Kroese et al. (2001) rat study,
27 or an average of the two, i.e., the EPA's choice of the single-sex mouse study that produces the largest
28 cancer slope factor instead of a slope factor that incorporates data from all studies. The SAB also has
29 questions regarding the choice of cross-species scaling factors. Using this approach, time-weighted daily
30 average doses are converted to human equivalent doses (HEDs) on the basis of $BW^{3/4}$ scaling. This
31 allometric scaling is based on current EPA guidelines and is surrounded by considerable uncertainty.
32 The SAB recommends that the EPA provide a brief explanation of the rationale for selecting an
33 allometric scaling factor for the BaP oral cancer slope factor given what is known about the BaP mode
34 of action for carcinogenicity, reaction rates, and toxicokinetics, and specifically, how the selection of the
35 allometric scaling factor applies when there is a portal of entry effect for alimentary tract tumors.

36 37 *Inhalation Unit Risk for Cancer*

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

1 *Dermal Slope Factor for Cancer*

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

5
6 • Choice of Studies:

7
8 The SAB agrees that studies of mouse skin tumors are relevant to humans based on evidence
9 for a similar mode of action. The draft BaP assessment reviewed 10 complete carcinogenicity
10 mouse skin tumor bioassays and Sivak et al. (1997) was chosen as the principal study. The
11 SAB recommends that the EPA consider adding Nesnow et al. (1983) and Levin et al. (1997)
12 for review and consider combining results from the different studies to strengthen the derived
13 DSF. The SAB also found the EPA's review of the epidemiological evidence of skin cancer
14 in humans was not adequate. The SAB recommends that the agency more thoroughly review
15 the evidence for skin cancer in studies of coke, steel and iron, coal gasification and aluminum
16 workers given their relevance for evaluating the appropriateness of using the mouse-based
17 risk assessment model for predicting skin cancer risk in humans. The SAB agrees with the
18 EPA that epidemiologic studies of therapeutic use of coal tar preparations do not provide an
19 adequate basis for either hazard identification or the derivation of a dermal slope factor.
20

21 • Dose-Response Analysis:

22
23 In evaluating the mouse (dermal) data, the EPA makes an adjustment if the dosing regimen is
24 less than the expected life span. Doses in studies known or assumed to be shorter than 104
25 weeks are adjusted by a factor of $(Le/104)^3$, where Le is exposure duration in weeks and 104
26 weeks is the life expectancy of a mouse. The EPA should explain how a coefficient of 3 was
27 chosen and whether nonlinear scaling by exposure duration has been used to derive other
28 cancer slope factors.
29

30 The draft BaP assessment used mass rather than mass/skin area as the dose metric for cancer
31 risk at "low doses" of BaP. Published dermal slope factors for BaP skin carcinogenesis have
32 used mass and mass/skin area as dose metrics and there do not appear to be any empirical
33 data available to inform a choice between these two dose metrics or another metric. The SAB
34 does not have a specific recommendation as to BaP dose metric, but strongly recommends
35 that in the absence of empirical data, the decision be based upon a clearly articulated, logical,
36 scientific structure that includes what is known about the dermal absorption of BaP under
37 both conditions of the bioassays and anticipated human exposures, as well as the mechanism
38 of skin carcinogenesis of BaP. The SAB recommends that cancer risk calculated from the
39 derived DSF should use absorbed dose, and not applied dose. The SAB also recommends that
40 the EPA describe what constitutes a "low dose" if the assumption that mass of BaP is the
41 appropriate dose metric for calculating the DSF from the skin cancer bioassay and for
42 estimating cancer risk in humans.
43

44 • Dermal Slope Factor Cross-Species Scaling:

45
46 Experimental cancer risk information for scaling from mouse to human skin cancer resulting
47 from dermal exposure is not available. The science for selecting the allometric scaling

1 approach employed by the EPA using body weight to the $\frac{3}{4}$ power is uncertain. However, the
2 chosen cross-species scaling approach should be supported by a coherent logical structure. In
3 addition, differences between mouse and human skin should be considered, such as thickness
4 of and metabolic rates in the target tissue (i.e., the viable epidermis layer).

5
6 The SAB has made other recommendations for describing the cancer risk calculated with the
7 DSF. The recommendations include the need to state clearly how the absorbed dose is
8 estimated from the exposed dose. In actual BaP exposures (from soil and other environmental
9 media), the absorbed dose should be estimated from the exposed dose and the exposure
10 scenario.

11 *Age-dependent Adjustment Factors for Cancer*

12 The SAB finds that the available mechanistic studies in humans and animals support a mutagenic mode
13 of action for BaP-induced cancers. Given that the EPA's *Supplemental Guidance for Assessing*
14 *Susceptibility from Early-Life Exposures to Carcinogens* establishes a rational approach for the
15 adjustment of tumor risk for exposures at different ages for carcinogens with a mutagenic mode of
16 action, the SAB concludes that the proposed use of age-dependent adjustment factors (ADAFs) is
17 justified.
18

19 **Executive Summary**

20 The SAB found that the major conclusions of the EPA draft assessment for BaP were clearly and
21 appropriately presented in the Executive Summary. Changes made to the body of the assessment in
22 response to the SAB recommendations regarding the derivation of the chronic RfD/RfC, or cancer slope
23 factors, should be incorporated into the Executive Summary. In addition, the SAB provides a number of
24 suggestions for improvement of the Executive Summary.
25

26 **Disposition of Public Comments**

27 The SAB found that most of the scientific issues raised by the public, as described in Appendix G, were
28 adequately addressed by the EPA. However, there were some issues on which the SAB differs from the
29 EPA responses or provides additional comments on the topic. These issues were identified and
30 referenced to relevant sections of the SAB report. The SAB encouraged EPA to provide additional
31 transparency and were supportive of a draft response summary table that was prepared in real time for
32 the SAB to review. The SAB thanks the public for these comments.
33
34
35
36

2. INTRODUCTION

The Science Advisory Board (SAB) was asked by the EPA Integrated Risk Information System (IRIS) program to review the agency'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 agency'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 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., cardio) 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 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 are 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 document or EPA references.

To increase transparency regarding excluded studies the SAB recommends that a table containing the list of excluded references, grouped by the applicable exclusion criteria, be included in the supplementary information. For the BaP 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 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

1 Tier 1 regarding exposure assessment, sample size, or follow-up period. The SAB finds requiring a
2 direct measure of BaP exposure unnecessarily restrictive, especially in regards to epidemiology studies,
3 as these studies would be relevant for hazard identification. Epidemiological studies of coke oven
4 workers and other occupational groups with known exposures to BaP are valuable sources of
5 information for determining causality even if they do not include quantification of BaP exposures. These
6 studies should at least be reviewed in the tables, if not the text. The assessment only considered that
7 three epidemiology studies met this criterion for Tier 1 for lung cancer (Armstrong and Gibbs 2009;
8 Spinelli et al. 2006; Xu et al. 1996) and four studies for bladder cancer (Gibbs and Sevigny 2007a,
9 2007b; Spinelli et al. 2006; Burstyn et al. 2007). The Tier 1 studies only included studies of the
10 aluminum and iron and steel manufacturing. It did not include any studies of workers from the coke
11 ovens, and roofing or asphalt industries which would have very high exposures to BaP and thus should
12 be relevant for determining causality even though they may not have had detailed exposure assessments
13 for BaP. Tier 2 studies are presented in a table in the assessment. However, there are many studies
14 missing from these tables (e.g., Romunstadt et al. 2000; Ronneberg 1999, that have been included in
15 prior assessments (i.e., see Table 1 in Bosetti et al. 2007 and Rota et al. 2014).

16
17 The review of epidemiology studies presented in the supplemental information section relied heavily on
18 a systematic review and meta-analysis reported by Bosetti et al. (2007) and by Armstrong et al. (2004).
19 It seems inappropriate for the EPA to rely solely on review articles rather than a review of the primary
20 literature. There is also a more recent meta-analysis that was not included in the assessment (Rota et al.
21 2014). Many of the epidemiologic studies cited in Bosetti and Rota are not discussed in the EPA
22 Supplemental Information document. For aluminum production workers the EPA only discusses the
23 studies by Spinelli et al. (1991, 2006), Romundstad et al. (2000a, 2000b) and Xu et al. (1996). There are
24 10 other studies of aluminum production workers cited in the Bosetti review (see Table 1 of Bosetti et
25 al. 2007), and five additional studies cited in the Rota review article (see Table 1 of Rota et al., 2014). It
26 is unclear why the EPA only included the few epidemiologic studies that they did review in their
27 assessment.

28
29 For asphalt workers and roofers, the Supplemental Information document refers the reader to the Bosetti
30 et al. (2007) review. Six papers were cited to provide evidence of an excess risk of lung cancer and weak
31 evidence for bladder cancer among asphalt workers and roofers (Burstyn 2007; Partanen and Boffetta
32 1994; Chiazzè et al. 1991; Hansen 1989, 1991; Hammond et al. 1976). Studies cited in Bosetti (see
33 Table 1) of roofers by Swaen et al. (1991) and of asphalt workers cited in Rota (see Table 1) by Behrens
34 et al. (2009) and Zanardi et al. (2013) seem to have been overlooked. For coke oven workers, coal
35 gasification, and iron and steel foundry workers the supplemental document relies entirely on the
36 reviews by Boffetta et al. (1997), Bosetti et al. (2007) and Armstrong et al. (2004). The more recent
37 review by Rota et al. (2014) identified two new studies of iron and steel workers (see Table 1) that were
38 not considered in the earlier reviews.

39
40 Finally, it is not clear why some of the studies of coal tar that were identified in the comments from the
41 American Coke and Coal Chemicals Institute were not included in the EPA assessment. In particular the
42 studies by Bhate et al. (1993), Hannuksela-Svahn et al. (2000), Jemec and Østerlind (1994), Jones et al.
43 (1985), Menter and Cram (1983), and Muller and Kierland (1964) seem to meet the criteria for review,
44 although the SAB noted that limitations in these studies make them of limited value for the assessment.

45
46 It also appears that *in vitro* studies (other than genotoxicity studies) and animal *in vivo* studies designed
47 to identify potential therapeutic agents that would prevent the carcinogenicity or genotoxicity of BaP

1 were not included. It would be expected that such studies might provide valuable additional information
2 on mode of action of BaP.

3
4 In Appendix B, the SAB recommends a number of additional peer-reviewed studies from the primary
5 literature, including some that are in HERO but were not used in the assessment, which the agency
6 should consider in the assessment of noncancer and cancer health effects of BaP.

7 **3.2. Hazard Identification**

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

14 **3.2.1. Developmental Toxicity**

15 *Charge Question 2a. The draft assessment concludes that developmental toxicity and developmental*
16 *neurotoxicity are human hazards of benzo[a]pyrene exposure. Do the available human and animal*
17 *studies support this conclusion?*

18
19 The SAB subdivided this Charge Question in two parts: developmental neurotoxicity; and
20 developmental toxicity other than neurodevelopment.

21 ***Developmental Neurotoxicity***

22 The SAB found the assessment to be thorough with regard to identifying studies pertaining to
23 developmental neurotoxicity and found no additional literature. The SAB concurs with the EPA that the
24 available human studies support the conclusion that BaP exposure contributes to human developmental
25 neurotoxicity. There are relevant human epidemiological studies on developmental effects on
26 neurodevelopment resulting from exposure to BaP-PAH mixtures (Perera et al. 2004, 2005, 2006, 2009,
27 2011, 2012a; 2012b; Tang et al. 2006, 2008). For example, in a prospective cohort study in New York
28 City, prenatal exposure to airborne PAH was found to affect children's IQ adversely (Perera et al. 2009).
29 When the cohort was followed to the age of 9 years, the investigators concluded that early life exposure
30 to environmental PAH may also contribute to attention deficit hyperactivity disorder (ADHD) behavior
31 problems in children (Perera et al. 2014). The EPA assessment appropriately notes that in human studies
32 the exposures are to PAH mixtures, and, therefore, the effects of BaP alone on child neurodevelopment
33 cannot be isolated and determined to be exclusively attributable to BaP rather than the sum, interaction,
34 or antagonist effect of multiple PAHs acting in concert. However, the human prospective cohort studies
35 have many strengths. These include the fact that (1) they are conducted in the target species (human), (2)
36 they are prospective, and (3) they are from two separate populations with one cohort followed from
37 before birth to the age of 9 years. An important aspect of the human studies that adds additional weight
38 to their validity is that they measured BaP-specific DNA adducts in maternal and umbilical cord blood
39 plasma and also used individually-worn air samplers on the mothers and found general agreement
40 between the air sampling and internal dose metrics (Perera et al., 2012b). Of importance is that the
41 method used for the BaP DNA adduct determinations in most of these studies was specific for BaP
42 adducts and not generic for other PAH DNA adducts. The fact that the New York City Children Study

1 (Perera et al., 2006; Perera et al., 2012b; Perera et al. 2014) used an assay for a specific BaP-DNA
2 adduct (Alexandrov et al. 1992) is a significant strength of these data.

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

15
16 The SAB has also identified weaknesses in Chen et al. (2012). The MWM was undersized for adult rats,
17 the reliance on latency as the sole index of performance on learning trials may be insufficient without
18 swim speed data; however, they report no swim speed differences on the probe trials. The use of the
19 Least Significant Difference (LSD) test is a concern as it over-emphasizes differences as significant that
20 may not be. The EPA assessment correctly notes the importance of the parallelism of the learning
21 curves. Learning rate was not shown to differ between groups. Rather the significant differences in
22 latency between treatment groups seen throughout testing was likely due to some other long-lasting
23 behavioral effect caused by developmental BaP exposure. The EPA also expressed concern about the
24 interpretative value of the probe trial data in light of the fact that the affected BaP groups never reached
25 the same level of proficiency on the learning trials as controls prior to being tested for memory and this
26 concern remains. The pup randomization and litter rotation among dams used in the study is an
27 unproven method of trying to prevent litter effects. It may work as intended or it may introduce
28 unknown effects. While effects, if any, would be expected to be randomly distributed across litters, there
29 exists the potential for interactions between groups created by this method of transferring pups between
30 dams. Concern was raised about having all dose groups within litters. This could cause cross
31 contamination of BaP from higher dose groups to lower dose or control groups. Further, it is unknown if
32 the dams could distinguish differences among the differently dosed pups and thereby differentially care
33 for her offspring.

34
35 Despite these concerns and despite issues concerning whether the data reflect a spatial learning deficit or
36 not, the Morris water maze (MWM) data show a BaP dose-dependent effect. Compared to the Elevated
37 Plus Maze (EPM) data, the increased escape latency in the MWM appears to be a more stable behavioral
38 change that was repeated over 4 days for two separate groups (cohorts) of animals. Rather than placing
39 reliance only on the EPM data and dismissing the MWM data, the SAB recommends taking into account
40 all the data in this study collectively and viewing them in their totality as evidence of a developmental
41 neurobehavioral effect of neonatal BaP exposure with long-term adverse central nervous system effects.

42
43 With regard to neurobehavioral assessment, it is important to focus on the mutually supportive effects
44 across behavioral domains in determining the reliability and pervasiveness of the low dose
45 neurodevelopmental BaP effects. With regard to the elevated plus maze specifically as a test of anxiety,
46 the significant effects of neurodevelopmental BaP exposure were found on all four measures used with
47 this test and showed increased movement of the BaP exposed groups into the open arms of the maze

1 relative to unexposed controls. This could be interpreted as decreased anxiety or increased risk taking of
2 the animals. However, with tests such as this, the anthropomorphic judgment of its meaning in human
3 terms is less important than the fact that it represents a persistent behavioral change caused by
4 developmental BaP exposure that is significantly different from control behavior and as such may be
5 regarded as an abnormal response. Given that BaP induced behavioral changes in other behavioral tests
6 ranging from reflex development to Morris water maze performance, the results of this study provide
7 converging evidence that shows a consistent pattern of alterations caused by developmental BaP
8 exposure that can be seen from early development to adulthood that may be irreversible.

9
10 The SAB understands the EPA's desire to use the Chen et al. (2012) data to generate an RfD. Given the
11 uncertainties identified, however, the assessment should consider if the resultant RfD emphasizing the
12 EPM effects is the most appropriate outcome, or using other end points, including the MWM results,
13 may be more stable and reliable.

14
15 The SAB further notes that the Chen et al. (2012) data are supported by other studies. Bouayed et al.
16 (2009) used mice treated with 0, 2 or 20 mg/kg BaP by gavage on postnatal day 0-14, that were assessed
17 at different ages, and appropriate statistical analyses were used. This is a low-quality study with
18 inadequate (small) sample size of five litters/dose, oversampling of four pups/litter without including
19 litter as a factor in the statistical analyses, and no mention of whether the observations were conducted
20 blind to treatment level and the order of testing counterbalanced across treatment level. Nevertheless,
21 many of the tests were affected and the data were generally in alignment with those of Chen et al.
22 (2012).

23
24 Tang et al. (2011) treated Wistar rats starting at weaning for 14 weeks with 1, 2.5, or 6.25 mg/kg BaP
25 i.p. from postnatal day 21 onward. Although the route of exposure is not directly relevant to humans,
26 they too found increases in MWM latency as their measure of learning and on the probe trial to test for
27 reference memory. They found effects at all doses of BaP. The study had reasonable group sizes
28 (9/group), reasonable learning curves, and the data were appropriately analyzed. These researchers also
29 relied on latency as their index of learning but their findings are in general agreement with those of Chen
30 et al. (2012).

31
32 Relevant to the derivation of the inhalation RfC, Wormley et al. (2004) is an inhalation developmental
33 neurotoxicity rat study in which exposure to BaP was on gestational days 11-21. The adult BaP-exposed
34 offspring showed reduced perforant pathway long-term potentiation and reduced hippocampal NMDA-
35 NR1 receptor expression. The exposure system used restraint and dams were also exposed to isoflurane
36 and minor surgery on gestational day 8 for which controls for these procedures were not included,
37 however, the sample size was adequate and the study supports the developmental neurotoxicity of BaP.

38
39 The SAB concurs with the EPA that there are plausible mechanistic studies identified for how BaP may
40 affect neurobehavioral development. Brown et al. (2007) and McCallister et al. (2008) treated rats with
41 BaP by gavage on gestational days 14-17 and found metabolites in higher concentrations in brain than
42 liver of the offspring. In addition, *in utero* BaP exposure reduced mRNA expression of glutamate
43 receptor subunits, NMDA-NR2A and NR2B, and AMPA receptor expression and protein concentrations
44 in hippocampus and inhibited NMDA-dependent cortical barrel field post-stimulation spikes by 50
45 percent. Bouayed et al. (2009) gave Swiss mice BaP on PND 0-14 and found effects on surface righting,
46 forelimb grip strength, and EPM similar to that found by Chen et al., along with reduced spontaneous
47 alternation and brain mRNA expression of 5-HT1A receptors. These findings implicate NMDA and

1 AMPA glutamate receptors, as well as 5-HT receptors as potentially mediating the neurobehavioral
2 effects seen by Chen et al. (2012) and others. They also support the view that developmental exposure to
3 BaP adversely affects brain development and behavior. There is also data that prenatal BaP treatment in
4 mice induces reactive oxygen species (ROS) (Winn and Wells 1997; Wells et al. 2010). The most salient
5 evidence for ROS-induced injury is BaP-induced increased generation of 8-oxoguanine that causes GC
6 to TA mutation in exposed embryos as another potential mechanism of BaP-induced developmental
7 neurotoxicity.

8
9 The SAB concluded that the EPA correctly identified BaP as a developmental neurotoxic agent in
10 animals with supporting evidence in humans. When reading across the human, animal, and mechanistic
11 data, there are sufficient studies that provide evidence of developmental neurotoxicity and the data are
12 convergent in showing BaP effects on brain development and behavior. While each study has
13 limitations, the weight of evidence supports BaP as developmentally neurotoxic.

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

30 ***Developmental Toxicity***

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

35
36 The SAB also concurs with the EPA that the animal studies presented support the conclusion that BaP is
37 a developmental toxicant in animals. BaP exposure *in utero* has been demonstrated to cause fetal death,
38 lower fetal/offspring weights and to affect fetal germ cells. The duration of oral BaP exposure included
39 the time of implantation through major organogenesis in the mouse (GD 7-16; Mackenzie and Angevine
40 1981). Duration of inhalation BaP exposure included the latter part of organogenesis and histogenesis
41 (GD 11- 20; Archibong et al. 2002). Additional studies that should be considered include reports on
42 BaP-related effects on fetal lung growth/function (Thakur et al. 2014) and teratogenicity (Shum et al.
43 1979; Rigdon and Rennels 1964; Nebert et al. 1977). The SAB further recommends that the EPA's
44 literature search include consideration of the relevant windows of prenatal development, recognizing
45 that appropriately powered, conducted, and reported teratology studies may have been conducted prior
46 to changes in testing guidelines that extended the dosing period to include the day prior to parturition.

1 Based on these literature searches, the EPA should include justification as to the
2 appropriateness/adequacy of the respective dosing paradigm, and the subsequent effects.

3
4 A brief survey of the literature indicates that there are additional reports that provide perspective on the
5 likely mode/mechanism of action leading to BaP-related developmental toxicity that are not mentioned
6 in the draft document. For example, there are studies on the formation of BaP adducts in rapidly
7 dividing cells, including fetal tissues (Lu et al. 1986), the severity of developmental toxicity associated
8 with Ah receptor status (Nebert et al. 1977), and the role of oxidative stress (Wells et al. 1997;
9 Nakamura et al. 2012; Thakur et al. 2014). Therefore, the SAB suggests that the EPA consider including
10 additional examples, as warranted, of mechanistic studies.

11
12 Toxicokinetic information regarding fetal exposures (Shendrikova and Aleksandrov 1974; Schlede and
13 Merker 1972) and lactational transfer should also be included as they inform the comparative doses to
14 developing organisms at different stages of development and exposed via different routes of
15 administration.

16
17 Regarding other windows of susceptibility and the potential for adverse developmental outcomes, the
18 SAB agrees that the postnatal development of other organ/systems may be impacted by BaP exposure;
19 specifically, the immune system (see Section 3.2.3, SAB Response for Charge Question 2c), lung
20 maturation/function, and cardiovascular changes (as identified in the EPA Toxicology Review). The
21 SAB encourages the EPA to further review the literature to identify potential additional studies that may
22 be useful in characterizing BaP-mediated developmental toxicity and dose-response relationships.

23 **3.2.2. Reproductive Toxicity**

24 *Charge Question 2b. The draft assessment concludes that male and female reproductive effects are a*
25 *human hazard of benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies*
26 *support this conclusion?*

27
28 The SAB agrees that the data support the conclusion that BaP is a male and female reproductive toxicant
29 through oral and inhalation routes of exposure. A sufficient number of appropriately conducted animal
30 studies are included that demonstrate a functional effect on reproductive endpoints indicative of BaP-
31 related reproductive toxicity and evidence for potential modes of action. The rodent data demonstrate
32 convincingly that BaP affects fertility and fecundity.

33 ***Male Reproductive Hazards***

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

43
44 The SAB recommends that the EPA consider the timing between the treatment with BaP and the
45 measurement of endpoints. Because it is a proliferative tissue, the testis has the potential to recover from

1 exposure to an insult after it is ended. Recovery can include but is not limited to restoration of normal
2 weight based on restoration of spermatogenesis and production of sperm with normal morphology with
3 subsequent waves of spermatogenesis. For sub-chronic studies, it could be informative to determine if
4 the testes had time to recover in the absence of continued exposure. There is the possibility of an
5 immediate effect from BaP or a PAH mixture that resolves with recovery time, could be dose dependent
6 and therefore could be missed depending on the timing of examination. The SAB requests that EPA
7 consider these factors as they assess the potential for male reproductive toxicity.

8
9 The SAB recommends that the EPA consider other hazard endpoints in addition to the classical
10 reproductive hazard endpoints included in the assessment. For example, BaP is mutagenic and
11 mutagenesis in the germline can be detrimental to reproductive health. Therefore, the SAB recommends
12 that the EPA give greater consideration to genotoxic effects on male germ cells as a possible mode of
13 action. The SAB recommends that the EPA consider inclusion of additional studies demonstrating that
14 exposure at different life stages (e.g., pre-adult vs. adult), can have differential effects on reproductive
15 health. References such as Liang et al. (2012) and Xu et al. (2014) could be used for this purpose.

16 ***Female Reproductive Hazards***

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

31
32 *In utero* exposure of developing females to BaP provides compelling evidence that there is a sensitive
33 window for exposure to BaP for the developing ovary (Mackenzie and Angevine 1981). Benzo[a]pyrene
34 $\geq 10\text{mg/kg}$ affects the developing fetal ovary, resulting in subsequent adult infertility (and in the absence
35 of additional BaP exposure). Because fetal oocyte numbers are fixed prior to birth, as compared with the
36 continual replenishment of sperm after puberty in males, BaP-related loss in oocytes indicates a
37 permanent adverse effect. In humans, tobacco smoke during *in utero* development produces similar
38 effects as BaP, including effects on subsequent adult fertility. Additional studies cited by the EPA
39 demonstrate that the human ovary is a target for BaP. The results reported from Wu et al. (2010) could
40 be considered relevant to developmental toxicity as well as reproductive toxicity due to early embryonic
41 death, an endpoint also observed in rodent experiments.

42 ***General Comments***

43 Germ cells are unique in that they will direct the development of the next generation. The success of the
44 developmental process in producing normal offspring is dependent on the quality of the germ cells and the
45 integrity of their DNA. The genotoxic effects of BaP have not been discussed in the assessment with regard

1 to reproductive toxicity. These genotoxic effects have the potential to result in miscarriages, birth defects and
2 genetic disease – all reproductive hazards. There are no direct studies of the effects of BaP on
3 spermatogonial stem cell mutagenesis, but there is a reference that implicates stem cell mutagenesis
4 (Olsen et al. 2010). Some papers discuss the mutagenic potential of BaP in somatic cells, but the
5 mechanism is likely the same in germ cells (Young et al. 2014). There are additional references on the
6 effects of BaP on adduct formation, mutagenesis, and gene expression in spermatogenic cells
7 (Verhofstad et al. 2010a; Verhofstad et al. 2010b; Verhofstad et al. 2011). Other papers discuss the
8 processing of BaP adducts during DNA replication and how different polymerases process the damage
9 differently (Starostenko et al. 2014); such differences could contribute to the genotoxic effects in
10 reproductive cells and during development. The Einaudi et al. (2014) study describes DNA damage in
11 oocytes emanating from BaP exposure. The implication of increased DNA damage and mutagenesis in
12 germ cells causes an increased risk of embryo-fetal death, birth defects and genetic disease among
13 offspring. The EPA should consider these points as they discuss the potential for female reproductive
14 impacts.

15 ***Recommendations:***

- 16 • The SAB recommends that genotoxic and mutagenic aspects of reproductive hazard be
17 addressed, especially as they provide perspective on likely mode of action, or a clear explanation
18 be provided as to why they are not addressed.
19
- 20 • The SAB recommends that the EPA consider additional endpoints (i.e., ovarian and testicular
21 effects) be considered for point of departure/BMD analyses and RfD derivation. The SAB
22 suggests that follicular counts be considered for females. For male studies, the SAB recommends
23 considering the recovery time after treatment prior to whatever endpoint is measured since the
24 testis is proliferative and new rounds of spermatogenesis could change the outcome. The SAB
25 also recommends that the EPA consider adding the biologically relevant endpoint of germline
26 mutagenesis, since BaP is a mutagen. The SAB recommends considering that the life stage at
27 which the animals are exposed to BaP and the life stage at which endpoints are measured be
28 added since the testis matures after birth. The abundance of BaP lesions incurred by germ cells is
29 another relevant measure for male and female studies that could be considered.
30
- 31 • The SAB recommends that the EPA provide additional clarity as to why certain studies, or parts
32 of studies, are brought forward while others are not; e.g., uterine hyperplasia/inflammation
33 observed in the Gao et al. (2011) study was not included. The EPA draft document does mention
34 effects on the ovary but little attention is paid to the actual mode of action (decreases in the
35 follicle pool) and there is a disconnect with linking this to the calculation of a point of departure.
36 The SAB recommends that the EPA either include these endpoints, or provide appropriate
37 justification as to why that they are not suitable for RfD determination (e.g., they support the
38 mode of action but—given limitations in experimental design -, such as appropriateness of the
39 route of administration and the short exposure duration— they are not suitable for generation of
40 an RfD).
41
- 42 • The EPA should provide context as to the likely applicability of the inflammatory cervical
43 response described in the Gao et al. (2011) study for BMD/RfD generation. The EPA may also
44 want to consider if this finding should be categorized under “reproductive effect” or “other
45 toxicity.”
46

- 1 • The following reference could be added to sperm effects: Jeng et al. 2015.
- 2
- 3 • The following references could be added to ovarian effects: Kummer et al. 2013; Mattison 1980;
- 4 Mattison et al. 1980; Sadeu and Foster 2011;
- 5
- 6 • The following reference could be added to mode of action-female reproductive effects: Sadeu
- 7 and Foster 2013; Young et al. 2014.

8 **3.2.3. Immunotoxicity**

9 *Charge Question 2c. The draft assessment concludes that immunotoxicity is a potential human hazard of*
10 *benzo[a]pyrene exposure. Do the available human, animal and mechanistic studies support this*
11 *conclusion?*

12
13 The SAB believes that the available immunotoxicity data based on animal models of pure BaP and
14 complex mixture exposures to humans (coke oven workers) support the claim that BaP is a human
15 hazard for the immune system.

16
17 The evidence for immunotoxicity in humans is based upon complex mixture exposures. There is no
18 doubt that BaP as a pure chemical can cause suppression of human peripheral blood mononuclear cell
19 (HPBMC) responses at low concentrations *in vitro* (10-100 nM, Davila et al. 1996). It is unclear whether
20 the levels of exposure demonstrated to have effects *in vitro* can be achieved from *in vivo* environmental
21 inhalation exposures or ingestion of cooked foods. Immunotoxicity can be caused by a combination of
22 genotoxic (DNA adducts and p53-induced cell death) and non-genotoxic mechanisms (signaling due to
23 AhR activation and oxidative stress, Burchiel and Luster 2001). Some of these mechanisms are similar
24 to cancer initiation and promotion, and there may, in fact, be a relationship between the carcinogenicity
25 of certain PAHs, such as BaP, and their immunotoxicity.

26
27 The effects of BaP can vary by dose and time and sometimes lead to complicated non-linear dose-
28 responses resulting in either increased or decreased immune parameters (Burchiel and Luster 2001). BaP
29 and other similar PAHs have specific structure-activity relationships that are associated with AhR
30 activation and increased P450 CYP1A1, CYP1A2, and CYP1B1 activities. BaP metabolites are likely
31 responsible for the immunotoxicity seen *in vivo*. Thus, complicated dose-response relationships can be
32 seen, which result from the actions of different metabolites of BaP (e.g., BP-diol-epoxides vs. BP-
33 quinones).

34 **Human Studies**

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

37
38 Szczeklik et al. (1994) reported decreased serum immunoglobulins (Igs) in coke workers with mg/m³
39 inhalation exposures. Zhang et al. (2012) studied 129 coke oven workers (compared to 37 warehouse
40 controls) for early and late apoptosis (Annexin V/PI) in HPBMC. The concentrations of BaP were 10-
41 1,600 ng/m³ in the working environment; 2.78-3.66 ng 1-hydroxypyrene (1-OHP) were measured in
42 urine. Karakaya et al. (1999) found an increase in serum Ig, which is not consistent with Szczeklik et al.
43 (1994), and may be associated with a difference in exposure dose and/or duration.

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

5
6 Because BaP is present in cigarette smoke, cigarette smoke studies are relevant for consideration.
7 Numerous cigarette smoking studies have demonstrated immune suppression, but the interpretation of
8 these effects is complicated by the strong action of nicotine, which in itself is immunosuppressive.
9 Therefore the inclusion of cigarette smoking studies is not recommended for this IRIS review. Cigarette
10 smoking can also be an important confounder for other environmental cohort studies, and must be
11 examined as an independent variable (Karakaya et al. 2004).

12 *Animal Studies*

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

17
18 Most immunotoxicity animal studies utilize mouse models (not rat) and they rely upon sensitive
19 functional assays, such as the T-dependent antibody response (TDAR). In the BaP assessment, the EPA
20 has acknowledged the mouse immune function studies (page 1-38, lines 20-28), but they have not been
21 included in the RfD calculation, presumably because these studies employed parenteral routes of
22 administration and did not utilize adequate numbers of animals per group and a sufficient number of
23 doses for evaluation. This is a common limitation of studies designed for assessing mechanism of action
24 rather than regulatory needs.

25
26 The dose required to produce thymic atrophy is known to be quite high in mice and rats compared to
27 that required to alter immune function (Luster et al. 1992). There is an overall consistency of findings
28 for BaP immunotoxicity in mice and some rat strains. Temple et al. (1993) showed decreased IgM
29 response and plaque forming cells (PFC) in mouse spleen at 5, 20, and 40 mg/kg and in F344 rats at 10
30 and 40 mg/kg 14 days subcutaneous injection, but the use of the rat model is limited by the lack of a
31 substantial immunotoxicity database.

32
33 Important structure-activity relationships established early on by Dean et al. (1983) showed suppression
34 of phytohemagglutinin (PHA)-induced T cell proliferation response of mouse spleen cells following
35 exposure of mice to 50 mg/kg BaP, but not by benzo(e)pyrene (BeP), a non-carcinogenic congener. In
36 mice, Ladics et al. (1992) have shown that BaP metabolites are responsible for suppression of the TDAR
37 in mouse spleen.

38
39 Immune function tests indicate that BaP is suppressive and might result in increased risk of infections
40 and perhaps cancer. This is evidenced by Munson et al. (1985) who showed a decreased resistance to
41 Strep, Herpes, and B16 melanoma by BaP but not by BeP. Influenza infectivity was not affected by BaP
42 and Listeria resistance was increased, thus demonstrating the complicated dose responses discussed
43 above. Kong et al. (1994) also demonstrated decreased lung resistance to tumor cell challenge in Fischer
44 344 (F-344) rats following intratracheal administration of BaP.

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

5 ***Developmental Immunotoxicity***

6 Developmental immunotoxicity is not well-addressed in the document. There is no recommendation for
7 calculation of an RfD based upon developmental immune exposures. Although BaP was found to
8 produce alterations in T cell development by several investigators (Urso and Gengozian 1982, 1984;
9 Urso and Johnson 1987; Rodriguez et al. 1999), these studies were limited by the use of a single high
10 dose (150 mg/kg) of BaP. Holliday and Smith (1994) found that 50 mg/kg total cumulative doses were
11 able to decrease thymus cellularity and inhibit T cell development in the thymus of mice exposed
12 gestationally. A decreased number of spleen cells was also seen by these investigators (Holladay and
13 Smith 1995).

14
15 In addition to the evidence that BaP alters T cell development *in utero* and in adults, there is also
16 evidence that BaP alters B cell development in the bone marrow of adults (Hardin et al. 1992). These
17 effects may be dependent on the expression and activity of the aryl hydrocarbon receptor (AhR).

18
19 It is likely that the developing immune system is more sensitive to BaP exposures than adult exposures
20 (Dietert et al. 2000, 2006; Luebke et al. 2006; WHO 2012). It is unclear whether the application of
21 uncertainty factors can address these concerns regarding the inadequacy of the database. It is generally
22 well known that developmental immunotoxicity is produced at much lower doses than those required to
23 produce immunotoxicity in adults. However, this may not be well documented for BaP in the present
24 literature used for the assessment.

25 ***Recommendations***

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

- 29
30 • There are concerns that sensitive immune function endpoints are not available to permit proper
31 evaluation of BaP immunotoxicity in animal models, including adult, developing and juvenile
32 animals, as well as assessing potential gender differences. These are data gaps that have been
33 identified.
- 34
35 • The EPA should discuss how the point of departure and uncertainty factors used in the oral RfD
36 derivation have addressed the potential for developmental immunotoxicity.
- 37
38 • EPA should consider developing Guidelines for immunotoxicity risk assessment, as has been
39 done by the WHO (2012).
- 40
41 • *In vitro* human PBMC studies should be included that support an understanding of mechanisms
42 of action that can guide the risk assessment.
- 43
44 • Humans are exposed to BaP in atmospheric mixtures associated with emissions from many
45 environmental sources. Associations between immunologically relevant endpoints and BaP

1 adducts have been found in some birth cohort studies (Jedrychowski et al. 2011; Tang et al.
2 2012; Jung et al. 2015). These studies are discussed elsewhere in this document in regard to
3 neurodevelopment in section 3.2.1 and should be linked with this discussion of developmental
4 immunotoxicity.

5 **3.2.4. Cancer**

6 *Charge Question 2d. The draft assessment concludes that benzo[a]pyrene is “carcinogenic to humans” by*
7 *all routes of exposure. Do the available human, animal, and mechanistic studies support this conclusion?*
8

9 The SAB finds that the EPA has demonstrated that BaP is a human carcinogen in accordance with the
10 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA 2005a). This conclusion was based primarily on
11 animal studies and mechanistic data, with strong support from an excess of lung cancer in humans who
12 are exposed to PAHs, but not to BaP alone. This conclusion is consistent with the evaluations by other
13 agencies, including the World Health Organization’s International Agency for Research on Cancer
14 (IARC 2010) and Health Canada (2015). Detailed consideration of the EPA criteria for whether or not a
15 compound is considered a human carcinogen, as applied to BaP, follows.

16
17 ***EPA Criterion 1 - The compound in question is “Carcinogenic to Humans” when there is convincing***
18 ***epidemiologic evidence of a causal association between human exposure and cancer.***
19

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

31
32 The BaP assessment focused on lung, bladder and skin cancers, but these are not the only organs for
33 which PAHs are carcinogenic. There is strong evidence for an association between PAH-exposure in
34 heavily char-broiled meat (Rothman et al. 1993) and colon adenoma risk (Sinha et al. 2005). In addition
35 there are strong associations between PAH-DNA adduct formation, cooked meat ingestion and colon
36 adenoma risk in the same population (Gunter et al. 2007).

37 The SAB suggests that the EPA reconsider the requirement for individual monitoring data (Tier 1
38 studies) in choosing to present epidemiological studies because some important papers have been
39 overlooked (see Appendix B). The Supplemental Information document summarizes six human studies
40 (Table D-33) which evaluated BaP-induced DNA adducts in humans. This is a small fraction of the
41 available studies that employ chemical class-specific methods to measure PAH-DNA or the major stable
42 DNA adduct of BaP, the r7,t8 ,t9-trihydroxy-c-10-(N²-deoxyguanosyl)-7,8,9,10-
43 tetrahydrobenzo[a]pyrene (BPdG), in human tissues. It is possible that some epidemiological studies
44 have been omitted by the EPA for lack of individual personal monitoring data. One could argue that for
45 biomarker association studies, and for establishing or supporting hazard identification in a workplace

1 known to be polluted, personal monitoring is not necessary. The presence of high ambient levels of BaP
2 and/or PAHs, high levels of urinary 8-hydroxy-pyrene, and/or high levels of BPdG are all strong
3 indicators of exposure. However, personal monitoring would be necessary for using epidemiological
4 data to support dose-response calculations.

5 There are a series of human epidemiological studies, involving cohorts of individuals, where subjects
6 have been stratified into quartiles or quintiles for their PAH-DNA adduct level (using chemical class-
7 specific methods). These studies have reported significant increases in cancer risk in individuals having
8 the highest PAH-DNA adduct levels, compared to those having the lowest levels. Compiling this data
9 into a table in the Supplemental Information would be very useful (see: Kyrtopoulos 2006; Poirier
10 2012).

11
12 The issue of the lack of an excess of skin tumors observed in most studies of therapeutic coal tar use
13 (Jones et al. 1985; Muller and Kierland 1964) was discussed by the SAB, and there appear to be two
14 major components to the overall consideration: (1) the hallmark characteristic of psoriatic skin is
15 hyperkeratosis caused by abnormally rapid proliferation; and (2) the clinical studies involving the use of
16 coal tar are incomplete. First, the skin of psoriasis patients who receive these treatments is not normal
17 skin, and therefore psoriasis patients are unlikely to experience the same risk from coal tar exposure as
18 the general population. In addition, psoriasis patients are known to shed skin cells at greatly increased
19 rates (Weinstein and McCullough 1973). Desquamation can reduce penetration of compounds past the
20 stratum corneum, so lipophilic materials, including the PAHs, may not reach the metabolically active
21 layers of the skin (Reddy et al. 2000). Both hyperkeratosis and desquamation could be protective with
22 respect to skin cancer risk by external PAH exposure. The finding by Roelofzen et al. (2012) of reduced
23 1-hydroxypyrene in urine and reduced PAH-DNA adducts in biopsied skin of psoriasis patients,
24 compared to healthy volunteers following dosing with coal tar ointments is consistent with this logic.
25 The second consideration is focused on the available clinical studies, and the SAB agrees with the EPA
26 that many of these studies suffer from small sample size, inadequate follow-up, undercounting of skin
27 cancers in particular, and a large potential for exposure misclassification. The limitations of these
28 studies, and the nature of psoriatic skin, make the available data largely uninformative with regard to the
29 question of whether BaP induces skin cancer in humans. The historic studies of an excess of scrotal
30 cancers in chimney sweeps, and more recent studies demonstrating an excess risk in asphalt workers, are
31 all consistent with BaP being a risk factor for skin cancer.

32
33 ***EPA Criterion 2 - The compound in question can be considered “Carcinogenic to Humans” when***
34 ***there is a lesser weight of epidemiological evidence but when all of the following conditions are met:***

- 35 ***a) strong evidence of an association between human exposure and either cancer or the key precursor***
36 ***events of the agent’s mode of action but not enough for a causal association***
37 ***b) extensive evidence of carcinogenicity in animals***
38 ***c) the mode(s) of carcinogenic action and associated key precursor events have been identified in***
39 ***animals***
40 ***d) there is strong evidence that the key precursor events that precede the cancer response in animals***
41 ***are anticipated to occur in humans and progress to tumors, based on available biological***
42 ***information***

43 The SAB agrees that the sum total of the mechanistic data show that all four of the required conditions
44 are met. Therefore, based on epidemiologic studies of cancer in humans and animal models, and on

1 mechanisms of action determined in both species, strong evidence of key precursor events related to BaP
2 exposure and found in humans indicates that BaP can be considered a human carcinogen.

3
4 The SAB agrees that BaP is metabolized/activated through three separate pathways: the diol-epoxide
5 pathway, the radical cation pathway and the *o*-quinone pathway. Furthermore, the SAB agrees that BaP-
6 induced tumors arise primarily through a mutagenic mode of action resulting from BaP-induced DNA
7 damage. Several studies over the last decade have shown that challenge of primary and transformed cells
8 with BaP increases retrotransposition of Long Interspersed Nuclear Element-1 (L1) (Stribinskis and
9 Ramos 2006). L1 retrotransposons are highly active mobile repetitive elements abundant in the human
10 genome (Ramos et al. 2013). Retrotransposition of L1 induces DNA strand breaks, increased frequency
11 of recombination and insertion mutations directly linked to various types of cancers (reviewed in Beck
12 et al. 2011), as well as disruption of local genome architecture and loss of transcriptional control of
13 neighboring genes (Raiz et al. 2012). As such, in addition to the mutational activity of reactive
14 electrophilic metabolites of BaP, the carcinogenic activity of BaP may involve genetic and epigenetic
15 events mediated by L1 reactivation (Teneng et al. 2011).

16
17 The most chemically stable DNA adducts of BaP are formed via the diol-epoxide pathway and persist in
18 human tissues for many years (VanGijssel et al. 2004). Much of the DNA damage generated by the
19 radical cation and *o*-quinone-ROS pathways is unstable, and some additional stable DNA damage (8-
20 OH-dG, ROS) is also caused by xenobiotics other than BaP. The steps connecting BaP exposure and
21 tumor formation by a mutagenic mechanism have been studied most completely in the diol-epoxide
22 pathway. However, because BaP is a complete carcinogen, the SAB emphasizes that the mechanism of
23 action must include both the initiating (mutagenic) effects and the promoting effects. The promoting
24 effects appear to occur largely through the radical cation and quinone metabolic pathways, which
25 increase cell proliferation, generate ROS and activate various growth factors and signaling pathways
26 (Burdick et al. 2003).

27
28 The SAB suggests that EPA could strengthen the statements in the assessment that describe the pathway
29 linking BaP exposure to tumor formation. The SAB recognizes that there is an overwhelming literature
30 available, and sorting out the critical original papers is daunting. The following is a series of findings
31 that highlight the critical steps in the diol-epoxide pathway connecting exposure to tumorigenesis via a
32 mutagenic mode of action. Statements are supported by original literature. This information might
33 clarify/enhance the statements in Table 1-17 on page 1-75, "Experimental support for the postulated key
34 events for mutagenic mode of action."

- 35
- 36 • Benzo[a]pyrene is metabolized/activated via the 7,8-diol to the diol-epoxide (r7,t8-dihydroxy-t-
37 9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene or BPDE) (Sims et al. 1974; King et al. 1976).
 - 38
 - 39 • BPDE interacts with the N2 position of guanine to form the stable r7,t8 ,t9-trihydroxy-c-10-(N²-
40 deoxyguanosyl)-7,8,9,10-tetrahydrobenzo[a]pyrene (BPdG) adduct (Daudel et al. 1975; Jeffrey
41 et al. 1976).
 - 42
 - 43 • BPdG forms in human cells and in mouse skin (Grover et al. 1976; Osborne et al. 1976).
 - 44
 - 45 • The BPdG adduct is mutagenic. Site-specific studies linked mutation hotspots with regions of
46 inefficient BPdG repair in modified DNA (Wei et al. 1995).
 - 47

- 1 • Formation of the BPdG adduct in an oncogene can mutate and activate that oncogene. Mutated
2 clones of the *c-Ha-ras* oncogene were formed as a result of *in vitro* reaction of the BPDE with
3 the *c-Ha-ras* proto-oncogene. The resulting activated *c-Ha-ras* oncogene caused malignant
4 transformation in NIH 3TC cells (Marshall et al. 1984).
5
- 6 • BaP caused dose-related increases in forestomach tumorigenesis and forestomach BPdG levels
7 during chronic lifetime (2 yr) feeding in mice (Culp and Beland 1994; Culp et al. 1998).
8
- 9 • Reduction in levels of the benzo[a]pyrene-7,8-diol metabolite, BPdG formation and tumor
10 formation was observed in mice treated with benzo[a]pyrene in the presence of the
11 chemopreventive agent benzyl-isothiocyanate (Sticha et al. 2000).
12
- 13 • First detection of a chemically-characterized BPdG adduct in human tissue DNA (Manchester et
14 al. 1988).
15
- 16 • In 39% of 705 human tissue DNA samples it was possible to detect the presence of BPdG
17 adducts, determined by chemical-specific methods (Boysen and Hecht 2003). In addition, PAH-
18 DNA adducts were localized in multiple human tissues by immunohistochemistry (Pratt et al.
19 2011).
20
- 21 • PAH exposures in humans are associated with a high frequency of GC→TA transversion
22 mutations, however this type of mutation can be caused by other xenobiotic agents and therefore
23 occurrence does not always provide a direct link to BaP exposure (Hussain et al. 2001).
24

25 BaP can either induce tumors after a single topical application to mouse skin followed by repeated tumor
26 promoter treatment or when given repeatedly in a complete carcinogenesis protocol (DiGiovanni 1992;
27 Abel et al. 2008). After topical application to mouse skin, BaP is metabolically activated to diol-
28 epoxides leading to formation of covalent DNA adducts, particularly the BPdG (described above and in
29 DiGiovanni 1992). The formation of BPdG leads to mutation in the *Ha-ras* gene of keratinocyte stem
30 cells, and constitutes an initiating event for tumor development in this tissue (DiGiovanni 1992; Abel et
31 al. 2008). Experimental evidence exists to show that BaP is metabolically activated to produce BPdG
32 and other similar types of minor DNA adducts in human skin (Rojas et al. 2001; Brinkman 2013), as
33 well as in skin, forestomach, lung, spleen, and esophagus of mice (Culp and Beland, 1994; John et al.
34 2012; Zuo et al. 2014). Additionally, BPdG was revealed in a variety of mouse and human tissues
35 exposed to PAH mixtures (Alexandrov et al. 1996; Rojas et al. 1998; Rojas et al. 2001). Lehman et al.
36 (1989) showed that human skin epithelial cells in culture treated with BaP produced the 7,8-diol
37 metabolite and BPdG. Watson et al. (1989) showed that epidermal DNA from human skin explants
38 treated with radiolabeled BaP had similar DNA adduct profiles to those seen in both mouse epidermis
39 and epidermal DNA samples from mouse skin explants. The major adduct was identified in all three
40 DNA samples as BPdG. Zhao et al. (1999) showed that treatment of a reconstituted human skin
41 equivalent model with BaP led to formation of BPdG and also led to the upregulation of *c-fos* and *p53*
42 proteins. The level of *p53* protein has also been shown to increase in mouse epidermis in association
43 with the formation of BPDE-DNA adducts (Serpi and Vahakangas 2003). Brinkman et al. (2013) also
44 recently demonstrated that BaP was metabolized to diol-epoxide metabolites in several different models
45 of human skin and showed that tetraols derived from BPDE could be readily detected in samples from
46 all of the model systems evaluated, including human skin explants. Brinkman et al. (2013) showed that
47 BaP was metabolized to genotoxic metabolites in both Normal Human Epidermal Keratinocytes and a

1 reconstituted skin equivalent system (EpiDermFT). Finally, in a study of atopic dermatitis patients
2 treated with coal tar, Rojas et al. (2001) demonstrated the presence of BPdG adducts in skin, that was
3 modulated by polymorphisms in the myelo-peroxidase gene. In conclusion, the available data suggest a
4 similar mutagenic mode of action for BaP in both mouse and human skin epidermis.

5
6 Whereas frequently we focus on a mutagenic mode of action (MOA) for BaP, as mentioned above, there
7 is additional evidence for the role of promotion/proliferation in BaP carcinogenesis. Furthermore, both
8 mutagenic and proliferative mechanisms occur simultaneously. A good example of this is the induction
9 of mouse forestomach tumors by oral exposure to BaP. The architecture of forestomach is similar to that
10 of skin, and the phenomenon of rodent forestomach tumors induced by oral BaP exposure is considered
11 to proceed via mechanisms similar to those in skin (see previous paragraph). In the forestomach, clearly
12 hyperplasia of the squamous epithelial cell layer plays a role (Culp et al. 2000), but one cannot discount
13 additional strong evidence of concomitant DNA damage leading to a mutagenic MOA. Culp and Beland
14 (1994) showed linearity for formation of BPdG, the major stable mutagenic DNA adduct induced by
15 BaP, in forestomachs of mice fed BaP for 21 days at 5 different dose levels. Furthermore, in a parallel
16 tumor study conducted under the same conditions, there was a dose-response relationship between BaP
17 concentration and forestomach tumors during 2 years of feeding mice three different levels of BaP in the
18 diet (Culp et al. 1998). Lastly, 78% of the forestomach tumors induced by lifetime feeding of BaP had
19 combined *H-ras* and *K-ras* mutations, further indicating that mutation-driven oncogene activation
20 played a role in the etiology of these tumors (Culp et al. 2000). Taken together these studies indicate that
21 both cell proliferation and DNA damage resulting in a mutagenic MOA contributed to the induction of
22 forestomach tumors in mice fed BaP in the diet for 21 days to 24 months. Therefore, the presence of
23 hyperplasia does not preclude a mutagenic MOA, particularly in the face of abundant evidence of DNA
24 damage, but may contribute to an enhancement of tumor incidence. Because there is clear evidence that
25 the ultimate active metabolite of BaP is a direct-acting genotoxin/mutagen, a linear extrapolation from
26 the point-of-departure is the appropriate approach for estimating the cancer potency of BaP, the
27 observation of hyperplasia notwithstanding.

28
29 Critical to our understanding of the published values for human BaP-induced DNA adducts and PAH-
30 DNA adducts is knowledge of what is being measured by a specific assay. The gold standard is the use
31 of structure-specific methods (Boysen and Hecht 2003.) Other assays have compound-class specificity.
32 For example, the various antibody-based methods (ELISA and immunohistochemistry) employ
33 monoclonal or polyclonal antibodies (termed BPDE-DNA antisera) raised against BaP-modified DNA.
34 These antisera cross-react with a family of carcinogenic PAHs bound to DNA. When evaluating human
35 tissue DNA, the data are expressed as “PAH-DNA adducts” because of the cross reactivity to DNA
36 samples modified with multiple carcinogenic hydrocarbons. Other assays are not BaP or PAH specific.
37 For example with ³²P-postlabelling, which detects adducts of many different chemical classes, it is not
38 possible to identify BPdG in human samples. Choice of an assay will impact the validity, reliability and
39 conclusions obtained from a particular study. In the original literature there is often confusion in the use
40 of nomenclature. The Toxicological Review (U.S. EPA 2014a) and Supplemental Information (U.S.
41 EPA 2014b) would be more user friendly with the addition of a table describing the characteristics and
42 nomenclature of the various methodologies used for BPdG and PAH-DNA adduct measurements.

43
44 The SAB found some of the text on page 1-72 of the assessment to be vague or inaccurate. For example,
45 line 25 “These results are consistent with evidence that BaP diol-epoxide is reactive with guanine bases
46 in DNA....” This statement is vague, despite the fact that there is actual experimental evidence in the
47 literature that would allow a more precise statement. In addition the sentence starting with

1 “Supporting. . .” on line 33 of that page, the statement that “. . .benzo[a]pyrene diol epoxide
2 (specifically[+]-anti-BPDE) is more potent than BaP itself. . .in producing lung tumors in newborn mice
3 following i.p. administration” is not correct (and is not supported by a reference). Despite the fact that it
4 is direct-acting, the diol-epoxide is too labile to be carcinogenic *in vivo*. The SAB asks the EPA to
5 clarify this text.

6 **3.2.5. Other Types of Toxicity**

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

10 The potential hazards identified and discussed in Section 1.1.4 are forestomach toxicity, hematological
11 toxicity, liver toxicity, kidney toxicity, cardiovascular toxicity, and (adult) nervous system effects.
12 Overall, the EPA concluded that the available evidence does not support these noncancer effects as
13 potential human hazards (Section 1.2.1). The SAB recommends that the basis for arriving at this
14 conclusion be expanded for each of these health endpoints. The current text does not provide an
15 adequate rationale for why the evidence does not support the listed effects as potential human hazards.
16 EPA needs to clarify whether this conclusion is due to insufficient data, inconsistent data, or sufficient
17 data to conclude that these health endpoints are not sensitive endpoints.

18
19 EPA has organized the summaries of human and animal studies in tables by target organ or system
20 effect (e.g., kidney toxicity, nervous system effects), and animal study tables include helpful
21 information on study design (species, strain, sex, number per group, dose levels, route of
22 administration and dosing regimen/duration) and study results. Additional context regarding the
23 overall study results is often needed to interpret the findings for a specific endpoint, including
24 available toxicokinetic information for the relevant dose range, if organ weight changes were or were
25 not accompanied by histopathological changes; and observations that inform the general health status
26 of animals under study.

27
28 With respect to the health endpoints discussed in section 1.1.4, the SAB concludes that the evidence
29 presented does not support liver, kidney, and hematological effects as human hazards; the EPA’s
30 rationale for those conclusions is incompletely described and the conclusions depend on the literature
31 search and study selection process, which was not considered to be sufficiently comprehensive to
32 identify all potential hazards credibly associated with BaP exposure (see response to Charge Question
33 1 – Literature Search/Study Selection and Evaluation). Notably, the list of search terms used indicates
34 that no queries were made that included the term “cardio” (i.e., cardiotoxicity; cardiovascular;
35 cardiopulmonary), “vascular,” “athero*,” etc. Similarly in the literature search secondary refinement,
36 it is noted that certain potential target organs (e.g., heart, liver, and kidney) were not included in the
37 search terms. Thus it is unclear that the assessment of all potential targets identified in the hazard
38 identification section (specifically section 1.1.4) was comprehensive. Moreover, it is unclear how the
39 information obtained from mechanistic studies was integrated into the assessment of hazards.

40
41 The SAB’s conclusion regarding target organ toxicities reviewed by the EPA is summarized below:

42
43 **Forestomach:** The available evidence presented does not support the EPA’s conclusion that
44 forestomach toxicity in rodents is not a potential human health hazard.
45

1 The document should be internally consistent regarding the human health hazard of forestomach
2 toxicity. The EPA did not consider human relevance to be an appropriate basis for excluding the
3 credible evidence of forestomach toxicity associated with BaP exposure, noting that humans do not
4 have a forestomach but do have similar squamous epithelial tissue in their oral cavity. This conclusion
5 is at odds with the overall conclusion for this section that the available evidence does not support
6 forestomach effects as representing a potential human hazard.

7
8 The decision not to consider forestomach toxicity further for dose-response analysis and the derivation
9 of reference values, as explained in section 1.2.1 (Weight of Evidence for Effects Other than Cancer)
10 should not be used as a justification for excluding forestomach toxicity as a hazard credibly associated
11 with BaP exposure. Forestomach toxicity may reflect a tumor-promoting key event in the tumorigenic
12 mode of action, and thus reflect part of a combination mode of action discussed by the EPA in the
13 section “other modes of action.”

14
15 For these reasons, forestomach toxicity is credibly associated with BaP exposure, so it is reasonable to
16 identify it as such in the hazard identification section of the document. The SAB recommends that the
17 EPA consider factors identified in IARC (2003) such as mode(s) of action and influencers of target
18 tissue residence time (viz., method and vehicle of BaP administration) in addressing the predictive
19 value for humans of forestomach effects in rodents.

20
21 ***Hematological toxicity:*** The available studies presented support the conclusion that hematological
22 toxicity is not a potential human hazard.

23
24 The summary of hematological toxicity is well done. The evidence provided for hematological
25 toxicity appears to be limited and suggests only a marginal effect on hematological parameters as the
26 magnitude of the alterations may not be biologically significant. The data presented suggest that dose
27 rate may influence blood cell parameters, but not in a reproducible fashion. Changes are minimal or
28 statistically insignificant at all but the highest dose levels (repeated oral dosing of 90 or 100 mg/kg-
29 day). Based on the evidence presented, the SAB agrees with the conclusion that the studies presented
30 do not provide convincing evidence that hematological effects are a human hazard of BaP exposure.

31
32 ***Liver toxicity:*** The available studies presented support the conclusion that liver toxicity is not a
33 potential human hazard.

34
35 The evidence provided for liver toxicity appears to be limited and suggests that while effects may be
36 observed at higher exposure levels it does not appear to be a sensitive health endpoint. The studies
37 described in this section reporting noncancer effects of BaP to the liver can be summarized as
38 identifying reproducible organ weight changes (all three studies) without associated histopathology in
39 two studies. In the third study, increased liver oval cell hyperplasia was reported only at the highest
40 dose level (90 mg/kg-day) following 35-day gavage dosing (DeJong et al. 1999). EPA should clarify
41 whether histopathology evaluations of the liver were performed by Knuckles et al. (2001). Based on
42 the evidence presented, the SAB agrees with the conclusion that these studies do not provide
43 convincing evidence that noncancer liver effects are a human hazard resulting from BaP exposure.
44 The results of Wester et al. (2012) (not cited in the assessment) should also be addressed which may
45 provide additional support for this conclusion.

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

4
5 In the three studies discussed by the EPA, there is no consistent finding indicative of kidney toxicity.
6 The evidence provided for kidney toxicity therefore appears to be limited and suggests that while
7 effects may be observed at higher exposure levels, it does not appear to be a sensitive health endpoint.
8 However, the SAB has identified relevant references regarding the effects of BaP on renal function in
9 rats (Alejandro et al. 2000; Parrish et al. 2002; Nanez et al. 2005; Valentovic et al. 2006), and the
10 intrauterine effects of BaP on kidney morphogenesis and late onset renal disease (Nanez et al. 2011).
11 The SAB recommends that these studies be reviewed to determine whether there is convincing
12 evidence that non-cancer kidney effects are a developmental and/or adult human hazard resulting from
13 BaP exposure.

14
15 ***Cardiovascular toxicity:*** The available studies do not support EPA's conclusion that cardiovascular
16 toxicity is not a potential human hazard and further explanation is needed as to the rationale for
17 reaching this conclusion.

18
19 The evidence provided for cardiovascular toxicity suggests potential toxicity at low dose levels,
20 recognizing that the data are too limited to be utilized quantitatively. It is not clear why evidence
21 pertaining to cardiovascular toxicity is not included in Table 1-9, and whether the designs of the
22 animal studies reviewed were suitable to identify adverse cardiovascular effects. There are multiple
23 modes of action by which chemicals may adversely impact the cardiovascular system, and it is unclear
24 if different lines of evidence (i.e., mechanistic, animal and human) were integrated for hazard
25 identification. Since cardiovascular effects were identified in rats and mice following gestational
26 exposures to BaP, the EPA should address whether such findings should be considered as part of the
27 weight of evidence for the cardiovascular system as a potential adult target of BaP exposure. Although
28 limited, the two epidemiology studies cited (Burstyn et al. 2005; Friesen et al. 2010) lend credence to
29 possible human relevance of this endpoint.

30
31 The SAB concludes that the literature search was not sufficiently comprehensive to identify studies
32 relevant to addressing the identification of cardiovascular system toxicity of BaP exposure (see
33 comments to charge question 1 – literature search/study selection and evaluation). Several studies
34 showing an influence of BaP on the severity and progression of atherosclerotic plaques in animal
35 models (as cited by Oesterling et al. 2008 – not included in this section) are not addressed. Other
36 studies to be considered as part of the weight of evidence evaluation, but not cited in this section, are
37 Knaapen et al. (2007) and Yang et al. (2009) which address the induction of atherosclerosis by BaP in
38 rodents; and Aboutabl et al. (2009, 2011), which examine cardiac hypertrophy and cardiac biomarkers
39 after BaP exposure. The induction of inflammatory cytokines by BaP (e.g., N'Diaye et al. 2009 – not
40 cited; and N'Diaye et al. 2006 – cited on p 1-77) should be included as part of the weight-of-evidence
41 discussion of cardiotoxicity. Other relevant recently published articles include Gan et al. (2012), Uno
42 et al. (2014) and Jayasundara et al. (2015).

43
44 The SAB recommends that EPA address the references that are missing. If they were excluded, the
45 basis for their exclusion should be provided. If not intentionally excluded, the missing references
46 should be included as part of the weight of evidence evaluation. The EPA should be explicit regarding

1 the rationale for concluding that the available evidence either does or does not support cardiovascular
2 system toxicity as a potential human hazard.

3
4 **Adult nervous system toxicity:** The available studies do not support EPA's conclusion that adult
5 nervous system toxicity is not a potential human hazard.

6
7 Further explanation is needed as to the rationale for concluding that the available evidence does not
8 support adult nervous system effects as a potential human hazard. The SAB notes that although EPA's
9 draft assessment concludes in Section 1.2.1 that adult nervous system is not a potential human target,
10 this conclusion was not explicitly stated in Section 1.1.4, where EPA indicates that the evidence for
11 "forestomach, liver, kidney, and cardiovascular system, as well as alter hematological parameters"
12 (page 1-44) does not support potential human hazards for these endpoints. "Nervous System Effects,"
13 however, are discussed in Section 1.1.4, which ends with the statement "These data suggest that
14 benzo[a]pyrene exposure could be neurotoxic in adults; however, only limited data are available to
15 inform the neurotoxic potential of repeated subchronic or chronic exposure to benzo[a]pyrene via the
16 oral route (Table 1-9)" (p.1-49). This section should be expanded to include a more rigorous
17 evaluation of the adult neurotoxicity evidence, especially since the EPA concludes that developmental
18 neurotoxicity is a potential human hazard. The EPA should clarify the conclusion with respect to adult
19 neurotoxicity and be consistent in Sections 1.1.4 and 1.2.1 of the assessment.

20
21 The evidence provided for adult neurotoxicity suggests potential toxicity at low dose levels,
22 recognizing that the data are too limited to utilize quantitatively for oral exposures. Decrements in
23 short term memory were reported in two studies of workers exposed occupationally to PAH mixtures
24 containing BaP (Niu et al. 2010; Qiu et al. 2013), lending possible credence to the human relevance of
25 this endpoint.

26
27 The SAB notes that Table 1-9 includes only two studies informing the neurotoxic potential of BaP
28 exposure in adult animals following subchronic or chronic oral exposures. If this is the case, the EPA
29 should indicate in the title of the table that only oral studies are included, because many more studies
30 are discussed in the text. Since hazard identification does not rely only on repeated subchronic or
31 chronic exposure scenarios alone, the EPA might consider developing a separate summary table just
32 for neurotoxicity studies that includes Saunders et al. (2001, 2002, 2006); Liu et al. (2002); Grova et
33 al. (2007, 2008); Maciel et al. (2014); Chen et al. (2011); Qiu et al. (2011); Xia et al. (2011); and
34 Bouayed et al. (2012). This summary table should include information on route, dose levels, and dose-
35 response relationship, including both positive and negative findings. Considering the relatively low
36 doses in laboratory animals at which behavioral alterations were reported, the rationale for not
37 considering the adult nervous system as a potential human target is unclear.

38
39 The section on adult neurotoxicity was not sufficiently rigorous in the analysis of oral neurotoxicity
40 studies in either the text or in the table. Bouayed et al. (2012), an oral study, was not included on
41 Table 1-9. The EPA may have mistaken this as an i.p. exposure study. The assessment should report
42 the negative finding on motor activity, and indicate that there were mixed results, rather than a
43 decreased depressive-like activity. The EPA should clarify that there was no dose-response
44 relationship (effects at 0.02 and 0.2, but not at 2 or 20 mg/kg/day), and that these effects could be
45 acute effects, because the behavioral tests were conducted 60 minutes after gavage dosing.

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

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

15
16 As reviewed in the EPA assessment, nervous system toxicity was assessed in animal studies where
17 BaP was administered starting at weaning, adolescence, or to adult rodents. The SAB concurs with the
18 EPA that these represent additional types of non-cancer BaP toxicity. However, the SAB suggests that
19 the EPA include these in its overall assessment of BaP as both a developmental and adult neurotoxic
20 agent. It was not clear in the assessment what the cutoff was for placing a study in the developmental
21 versus non-developmental category given that there are prenatal, neonatal, weaning, and adolescent
22 exposure studies, all of which are developmental in one sense or another even apart from the adult
23 neurotoxicity exposure studies. The EPA assessment clearly included the prenatal and early postnatal
24 studies in the developmental neurotoxicity section, but placed the weaning (starting exposure at P21)
25 and adolescent (starting exposure at P28) in the “other” non-cancer nervous system section. Further
26 justification of the boundaries would be useful.

27
28 The SAB recommends that the EPA be explicit as to the rationale for concluding that the available
29 evidence either does or does not support adult nervous system effects as a potential human hazard.

30 ***Other Toxicity:***

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

33
34 Adult and developmental pulmonary toxicity are not well addressed in the document. The SAB
35 identified references in regard to the effect of maternal exposure to BaP on fetal development, and
36 recent epidemiological studies that suggest an association between dietary BaP intake and lower birth
37 weight in children (Duarte-Salles et al. 2010, 2012, 2013). Also, there is little emphasis on the effects of
38 BaP on non-cancer pulmonary toxicity. Thakur et al. (2014) present evidence that maternal exposure of
39 mice to BaP leads to increased susceptibility of newborn mice to hyperoxic lung injury and chronic lung
40 disease (CLD). Supplemental oxygen therapy is frequently encountered in premature infants and very
41 low birth weight infants, and hyperoxia contributes to the development of bronchopulmonary dysplasia
42 (BPD), also known as CLD, in these infants. Maternal smoking is one of the risk factors for preterm
43 birth and for the development of BPD. This literature describing the effect of BaP on pulmonary toxicity
44 in infants as well as adults should be included.

1 **3.3. Dose-Response Analysis**

2 In section 2 of the draft assessment, the EPA uses the available human, animal, and mechanistic studies
3 to derive candidate toxicity values for each hazard that is credibly associated with benzo[a]pyrene
4 exposure in section 1, then proposes an overall toxicity value for each route of exposure. The SAB
5 comments on the EPA analyses in the sections that follow.

6 **3.3.1. Oral Reference Dose for Effects Other Than Cancer**

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

13
14 The SAB finds that developmental endpoints, and in particular neurodevelopmental endpoints, are in
15 principle an appropriate basis for deriving an RfD for BaP. However, the EPA has not made a
16 sufficiently strong case that the available developmental endpoints are the most appropriate non-cancer
17 endpoints for setting an RfD, or that among the available neurodevelopmental endpoints, the observed
18 results from the elevated plus maze test in Chen et al. (2012) are the most appropriate results.

19
20 With respect to developmental toxicity as the most appropriate category of non-cancer effects, the SAB
21 suggests that the EPA give more consideration to the available data on reproductive outcomes, including
22 cervical hyperplasia and cervical inflammation in Gao et al. (2011), or providing a firmer justification
23 for not selecting these critical endpoints. The Gao study is compelling in establishing a relationship
24 amongst BaP exposure, cervical hyperplasia and inflammation. Moreover, the apparent effect on ovary
25 weight reported by Xu et al. (2010) is inconsistent with the results reported by Knuckles et al. (2001)
26 and Kroese et al. (2001). Therefore, the EPA should clearly articulate the rationale for developing a
27 candidate RfD based on an apical, apparently inconsistent, ovarian response as compared to a single
28 study that characterizes multiple cervical responses resulting from BaP exposure.

29
30 Although cervical hyperplasia and its impact on fertility and fecundity are unclear (human literature
31 appears to focus on human papilloma virus, which causes proliferative lesions and decreased fecundity),
32 hyperplasia often precedes a tumor response. Nevertheless, disruption of cervical elasticity or a mass of
33 sufficient size would be expected to complicate parturition. As the EPA stated, cervical tumors were not
34 observed in animal studies, but this tissue was not examined for histopathological changes. Therefore,
35 microscopic changes may have gone unnoticed.

36
37 Dysregulation of anti-inflammatory cytokines has been suggested to be involved with cervical
38 ripening/preterm labor (MacIntyre et al. 2012) and sufficient perturbation would be expected to impact
39 birth outcome. Since BaP exposure was associated with alterations in inflammatory processes, this
40 suggests a potential link amongst BaP exposure, alterations in cytokine signaling and preterm labor.
41 Therefore this potential relationship, albeit speculative, is potentially relevant for risk assessment.

42
43 The SAB further recommends that (1) the EPA consider including their rationale for either exclusion or
44 inclusion to increase clarity and transparency, and (2) the EPA conduct the appropriate literature reviews

1 (as necessary) to support either inclusion or exclusion of endpoints for RfD determination. In addition
2 the EPA should better explain the reasons for not modeling immunotoxicity (IgM, IgA) endpoints.

3
4 With respect to the choice of specific neurodevelopmental endpoints, the SAB notes that there are
5 several important positive aspects to the Chen et al. (2012) study. These include: adequate numbers of
6 litters (40 litters, 10/dose group) were used; there was a well-defined dose-response for several
7 behavioral outcomes; the overall study presented multiple and well characterized tests; and the
8 subjective tests were conducted with observers blind to treatment level. However, the SAB also
9 identified several potentially significant negative aspects the study design and data analysis in Chen et
10 al. (2012) that were either not addressed or were not fully considered in the EPA assessment. These
11 include: potential dam and pup stress from repeated rotation of dams; potential nurturing bias against
12 high dose pups based on smell and/or behavioral differences especially following gavage doses; and the
13 total number of dams used and timing (e.g., litters redistributed to other dams who gave birth within 24
14 hrs of each other) to achieve 40 litters of 4 M and 4 F divided into 10 litters per track was not described.
15 Presumably, all 40 litters were not born in one day, so the details on how this was achieved (including
16 use of >40 litters initially, so that pups are exactly the same age in each litter) are a critical part of study
17 design that can impact study outcome and interpretation of data.

18
19 Given these concerns, the SAB recommends that the EPA should specifically consider the overall
20 picture of neurodevelopmental impact from all of the neurodevelopmental endpoints in Chen et al.
21 (2012), including plus maze, reflex, locomotor activity and water maze to justify and support the choice
22 of the critical endpoint. In particular, the SAB suggests that the EPA reconsider or provide stronger
23 justification for not using escape latency from the Morris water maze. This endpoint appears to be the
24 most stable behavioral difference that was repeated 4 days for 2 separate tracks (cohort) of animals. The
25 EPA is correct that this effect is not a learning or memory effect due to difference in baseline starting
26 from day 1, but it is some indication of an effect (even if that effect is a developmental effect on
27 locomotion). The EPA should explain how the BMD was calculated for escape latency since there are 4
28 different days for each track and each sex.

29
30 Although the BMD approach employed by the EPA for deriving the POD is not dependent on the
31 specific statistical tests used for group comparisons, the overall weight of evidence and evaluation of
32 Chen et al. (2012) is based on the original statistical analysis using the Least Significant Difference
33 (LSD) post hoc test. This test appears to be statistically inappropriate in this context.

34
35 The SAB agrees with the EPA's decision not to further consider the Xu et al. (2010) study, but given its
36 drawbacks, the SAB concludes that this study should not have been included in Table 2-2.

37
38 Regarding the discussion of uncertainty factors, the SAB suggests that the presentation of the UFs in the
39 assessment be reordered to start with LOAEL-NOAEL... and end with sensitive human, as this is the
40 logical flow when beginning with a POD from an animal study.

41
42 With respect to the application of uncertainty factors (UFs) in derivation of the RfD, the assessment
43 stated that the application of a full UF of 10 to the POD from the EPM for the animal to human
44 extrapolation in Chen et al. (2012) was needed. The EPA stated that this was because the allometric
45 $BW^{3/4}$ adjustment is not appropriate for extrapolating from neonate animal to adult humans. However,
46 given that this endpoint is a neurodevelopmental endpoint, it is unclear why the EPA considers the
47 extrapolation in question to be from neonatal animal to adult human, and not (as seems straightforward)

1 from neonatal animal to neonatal human. Therefore, the SAB recommends that the EPA consider
2 application of a BW^{3/4} adjustment as per EPA's 2011 allometric scaling guidance (U.S. EPA 2011).

3
4 The SAB also suggests that the EPA further justify the application of a UF of 3 for database deficiency
5 that is based, in part, on the absence of a multi-generational study or extended one-generation study
6 (OECD 443), and the lack of a study examining functional neurological endpoints following exposure
7 from gestation through lactation. The SAB suggests that the EPA more specifically address these issues
8 and provide a clearer rationale for its decision.

9
10 The SAB notes that BaP is also considered a hazard for several toxicological endpoints (e.g., immune,
11 cardiovascular) (see sections 3.2.3 and 3.2.5 above). The available information for these endpoints,
12 while sufficient for hazard identification, is insufficient for dose response assessment (e.g., insufficient
13 testing of effects on immune function, particularly in developing organisms). The SAB also
14 recommends that genotoxic aspects of reproductive hazard be addressed (see SAB response in 3.2.2.
15 Reproductive Toxicity). As part of the deliberation regarding application of a database uncertainty
16 factor, the EPA should also address whether the extent of residual uncertainty regarding these endpoints
17 is such that additional data for these endpoints are needed and if so, the EPA should consider whether
18 the existing database uncertainty factor of 3 is adequate.

19
20 The SAB identified two additional issues with the derivation of the RfD. Given the reproductive,
21 developmental and trans-placental effects of BaP, the SAB encourages the EPA to ensure that multi-
22 generational and one-generational effects are addressed to the extent that data are available. When
23 possible, the EPA should identify the sensitive sex in a given study and use the sensitive sex for dose-
24 response modeling.

25
26 The SAB found the last portion of charge question 3a (*Does the discussion of exposure scenarios*
27 *(section 2.1.5) reflect the scientific considerations that are inherent for exposures during a critical*
28 *window of development?*) somewhat vague. In section 2.1.5, the assessment notes that the most sensitive
29 endpoint for RfD development is based on "neurobehavioral changes in rats exposed to benzo[a]pyrene
30 during a susceptible lifestage," i.e., rats exposed during neurodevelopment. Thus, this endpoint is a
31 neurodevelopmental endpoint. The assessment notes in section 2.1.5 that " ..fluctuations in exposure
32 levels that result in elevated exposures during various lifestages could potentially lead to an appreciable
33 risk, even if average levels over the full exposure duration were less than or equal to the RfD." The
34 SAB agrees with this language as a statement of principle. However, as the RfD in this case is, in fact,
35 based on a susceptible lifestage that is shorter than a lifetime exposure, the statement in section 2.1.5 is
36 misleading as it seems to imply that this RfD does not specifically address this susceptible lifestage.

37 **3.3.2. Inhalation Reference Concentration for Effects Other Than Cancer**

38 *Charge Question 3b. The draft assessment proposes an overall reference concentration of 2×10^{-6}*
39 *mg/m³ based on decreased fetal survival during a critical window of development. Is this value*
40 *scientifically supported, giving due consideration to the intermediate steps of selecting studies*
41 *appropriate for dose-response analysis, calculating points of departure, and applying uncertainty*
42 *factors? Does the discussion of exposure scenarios (section 2.2.5) reflect the scientific considerations*
43 *that are inherent for exposures during a critical window of development?*

44
45 In the IRIS draft document, Archibong et al. (2002) is the critical study selected for the derivation of the
46 RfC. In this study, the BaP exposure occurred via particulate inhalation and the adverse effect identified

1 as the critical endpoint is decreased fetal survival (i.e., a non-respiratory endpoint). The SAB concludes
2 that the RfC value in the assessment is inadequately supported in light of concerns with the study design,
3 data analysis, and uncertainty factors, as discussed below.

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

23
24 The rationale for not employing a BMD approach is unclear. Unequal variances and lack of access to the
25 original datasets are not sufficient reason to avoid BMD modeling of the data in the key study. The EPA
26 has fit BMD models to epidemiological data summaries having these same attributes, and the agency
27 should consider those approaches in the current assessment.

28
29 Regarding use of UFs, the EPA applies a UF of 3 for interspecies extrapolation (rat-to-human) to the
30 LOAEL of $25 \mu\text{g}/\text{m}^3$ derived from the key study. This UF, rather than the full UF of 10, is intended to
31 address residual interspecies toxicodynamic uncertainty after interspecies toxicokinetic uncertainty has
32 been addressed. The EPA intended to address the toxicokinetic uncertainty by application of the regional
33 deposited dose ratio for extrarespiratory effects (RDDR_{er}) as set forth in its 1994 guidance on deriving
34 RfC (U.S. EPA 1994). "The RDDR_{er} is described in that document as follows:

35
36 4.3.5.2 Remote (Extrarespiratory) Effects. The respiratory tract might not be the target organ for an
37 inhaled compound. The dose actually delivered to other regions of the body will be affected by
38 metabolism, clearance, and distribution patterns. Particles depositing in the respiratory tract will
39 clear rapidly (ET can be within seconds of inhalation) or slowly (PU clearance may take weeks or
40 months) to the GI tract or be absorbed into the interstitium, lymphatics, or into the blood from the
41 respiratory tract. Once deposited, however, very few particles will clear by exhalation (sneezing or
42 coughing). Therefore, it is not unreasonable to estimate extrarespiratory deposition by total
43 deposition in the respiratory tract when information on dose delivered to nonrespiratory tract organs
44 is unavailable. The current default normalizing factor for extrarespiratory effects is body weight.

45
46 The SAB notes that while allometric scaling for the BaP RfC is based upon BW^1 (per above), for oral
47 and dermal BaP toxicity values the EPA selected an allometric scaling factor of $\text{BW}^{3/4}$. Although an

1 EPA guidance was cited as the basis for selection of the allometric scaling factor for each route of
2 exposure, the SAB is concerned that use of different EPA guidance documents spanning decades and
3 different exposure routes and endpoints (cancer and non-cancer) may have resulted in the application of
4 inconsistent scaling principles. Further, cross-species scaling depends upon the mode of action, the role
5 of metabolism and toxicokinetics, and the target organs and tissues; however, the draft assessment
6 provides no indication of the extent that these were considered in choosing the BaP scaling factor for
7 inhalation (and other routes). (See also the responses to Charge Questions 3c and 3e).

8
9 The SAB recommends that the EPA include a brief discussion of the rationale for selection of the
10 allometric scaling factor in the context of inhalation exposure to BaP leading to decreased fetal survival.
11 It would be helpful to clarify in this discussion the aspects of the BaP absorption, distribution,
12 metabolism, and elimination (ADME) that the scaling factor is intended to address. This is important not
13 only in justifying the allometric scaling of dose, but also the use of a UF of 3 instead of 10 as the use of
14 a UF of 3 for interspecies extrapolation is based on the assumption that issues related to interspecies
15 variability of toxicokinetics have been adequately addressed by the scaling factor and that the UF of 3 is
16 largely intended to solely address interspecies differences in toxicodynamics. The SAB notes that in its
17 1994 guidance (U.S. EPA 1994), the EPA recommends the application of an interspecies UF of 3 rather
18 than a full UF of 10 in the derivation of RfCs. The guidance states that this is "...due to the
19 incorporation of dosimetric adjustments." However, since the proposed RfC for BaP is derived from
20 particle deposition in the respiratory tract leading to extrarespiratory systemic effects, it is not entirely
21 clear that the dosimetric adjustment referred to in the 1994 document completely addresses the
22 variability in interspecies extrarespiratory systemic kinetics.

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

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

40
41 The Archibong et al. (2012) study explored the potential effects of BaP on the rat ovary, including
42 ovarian estrous cyclicity, hormone production, BaP metabolism, and subsequent effects on reproductive
43 outcomes. Female rats were exposed to 50, 75 or 100 $\mu\text{g}/\text{m}^3$ of BaP for 4h/d for 14 days and then mated
44 with unexposed males. During exposure, the 100 $\mu\text{g}/\text{m}^3$ exposure concentration group was associated
45 with an increase in cycle length, changes in hormone levels, and aryl hydrocarbon hydrolase activity.

1 When the exposure period was over and these animals were mated, this exposure group displayed a
2 lower ovulation rate, fewer pups born and decreased pup survival. Given that all the effects occurred in
3 the highest exposure group examined, and were consistent across endpoints, EPA may want to consider
4 the potential value of these endpoints for BMD analyses. These data suggest that although adult ovary is
5 a target, fetal development (as demonstrated in Archibong et al. 2002 and Wu et al. 2003) is more
6 sensitive to BaP-mediated toxicity under the exposure conditions employed.

7 **3.3.3. Oral Slope Factor for Cancer**

8 *Charge Question 3c. The draft assessment proposes an oral slope factor of 1 per mg/kg-d based on*
9 *alimentary tract tumors in mice. Is this value scientifically supported, giving due consideration to the*
10 *intermediate steps of selecting studies appropriate for dose-response analysis and calculating points of*
11 *departure?*
12

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

18 ***Analysis of Carcinogenicity Data (section 2.3.1)***

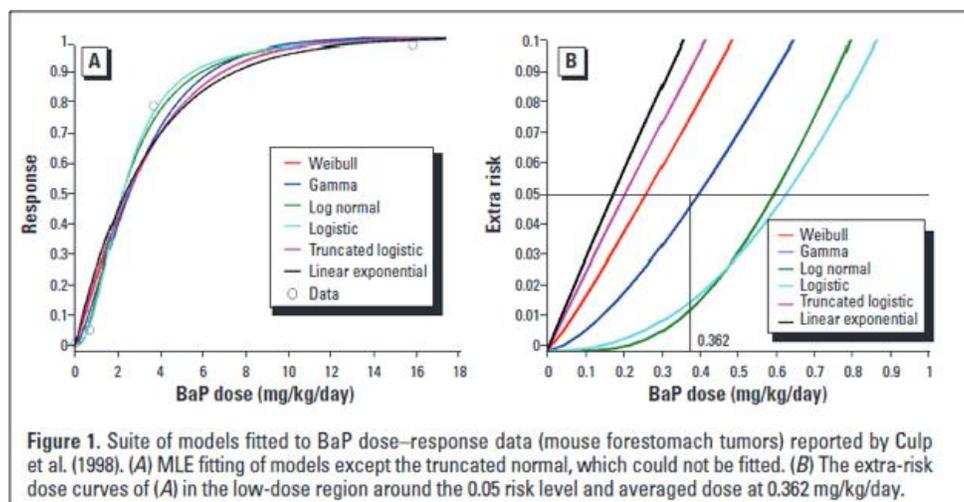
19 An oral slope factor for cancer was previously developed by EPA in 1992 and included on the IRIS
20 database. At that time, BaP was classified as a “probable human carcinogen.” The previous oral slope
21 factor (7.3 per mg/kg-day) was derived from the geometric mean of four slope factor estimates based on
22 studies of BaP oral carcinogenesis in Sprague-Dawley rats (2 years) and CFW Swiss mice (7 months)
23 from the combined incidence of forestomach, esophageal and laryngeal tumors. In the current
24 assessment, newer oral carcinogenesis studies were available for further refinement of the oral slope
25 factor (now proposed to be 1 per mg/kg-day), including two 2-year oral carcinogenesis bioassays that
26 associated lifetime BaP exposure with multiple tumor sites including: forestomach, liver, oral cavity,
27 jejunum, kidney, auditory canal, skin and mammary gland in male and female Wistar rats (Kroese et al.
28 2001) and forestomach, esophageal, tongue and larynx tumors in female B6C3F1 mice (Beland and
29 Culp 1998). The Kroese et al. (2001) and Beland and Culp (1998) studies were selected as the best
30 available for dose-response analysis and extrapolation to lifetime cancer risk following oral exposure to
31 BaP. These studies were conducted in accordance with Good Laboratory Practice (GLP) and showed
32 dose-related trends in most of the tumor sites. Neither of the studies used in the earlier oral slope factor
33 derivation were used for the current derivation.
34

35 The SAB finds that the two selected lifetime oral carcinogenesis studies were well done and appropriate
36 for the dose-response modeling used for cancer oral slope factor derivation. However, it is not clear why
37 only one of the studies, the study by Beland and Culp (1998), was ultimately used in the final derivation
38 of the oral slope factor and not both studies where a (weighted or unweighted geometric) mean or
39 median value might have been derived from the different oral slope factors calculated and presented in
40 the assessment. The SAB was concerned about the EPA's choice of the single-sex mouse study that
41 produces the largest cancer slope factor instead of some other slope factor that incorporates data from all
42 studies (rats and mice, males and females) previously judged to be of equal quality and relevance. This
43 decision was not clearly supported by the EPA Guidelines for Carcinogen Risk Assessment (USEPA,
44 2005a), which allows multiple studies to be combined and suggests "choosing a single dataset if it can
45 be justified as most representative of the overall response in humans."

1
2 The SAB acknowledges there are advantages and disadvantages to basing the oral slope factor for
3 cancer on a single mouse study that includes only one sex (female) versus basing it on a rat study that
4 includes both sexes; and, statistical bias that results from using extremity as a selection factor (i.e.,
5 always choosing the study that produces the largest slope factor). If no biological basis exists for
6 concluding that the mouse study is more representative of human response than the rat study, the EPA
7 should consider averaging over both studies (e.g., simple averaging as used in previous oral slope factor
8 derivation, or meta-analytic/Bayesian averaging as recommended in the 2014 NRC Review of IRIS
9 (NRC 2014). The oral slope factor for cancer presented in the 1992 BaP assessment was based on an
10 average of slope factors from two different studies, an estimation approach that could have been used in
11 this assessment. An approach similar to the one used in the 1992 BaP assessment should be considered

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

13 The oral slope factor for cancer is based on dose-response modeling that uses only the multistage-
14 Weibull model. This model incorporates both the time at which death occurs and the dose in estimating
15 the point of departure from which the cancer slope factor is calculated. This model is generally
16 considered appropriate for the available data, although confidence in the final estimates would be
17 increased if the reader were able to compare the multistage-Weibull model estimate to estimates
18 computed by fitting other dose-response models to the same data. These other estimates (and associated
19 deficiencies) could be summarized in an appendix along with the model that is finally chosen. For
20 example, Fitzgerald et al. (2004; their Figure 1 excerpted here) evaluated multiple models of tumor risk
21 and illustrated BMD estimates associated with a 5% extra risk ranged between roughly 0.15 and 0.6
22 BaP dose (mg/kg/day).
23



24
25
26 The adjustments for approximating human equivalent slope factors use the EPA cross-species scaling
27 methodology. Using this approach, time-weighted daily average doses are converted to HEDs on the
28 basis of $BW^{3/4}$ scaling, citing U.S. EPA (1992, 2005a). According to U.S. EPA (1992), $BW^{3/4}$ is used
29 as a default in the absence of chemical-specific information and is surrounded by considerable
30 uncertainty. It encourages the use of information on mode of action, reaction rates, pharmacokinetics,
31 and other factors as appropriate to derive a chemical-specific scaling factor, if sufficient data are
32 available. For example, it states, “Clearly, when data on metabolic conversion are available in a
33 particular case, they should be used in preference to the $BW^{3/4}$ default.” Consistent with the

1 recommendation given in response to Charge Question 3b, the SAB recommends that the EPA provide a
2 brief explanation of the rationale for its selection of an allometric scaling factor for the BaP oral cancer
3 slope factor given what is known about the BaP mode of action for carcinogenicity, reaction rates, and
4 toxicokinetics, and specifically, how the selection of the allometric scaling factor applies when there is a
5 portal of entry effect. Alimentary tract tumors (larynx, esophagus, forestomach) arguably meet the
6 definition of portal of entry effects, and the SAB suggests that the discussion include issues regarding
7 scaling of effects when many of the toxicokinetic processes that influence scaling of systemic effects do
8 not apply, or do not apply in the same way.

9
10 Also, for transparency, the impact of the change in allometric scaling from $BW^{2/3}$ used in the 1992 BaP
11 assessment to $BW^{3/4}$ in the present assessment should be discussed in the assessment. A comparison of
12 the results of using the two different scaling factors can be easily accomplished by demonstrating how
13 the scaling change impacts the estimate in the 1992 BaP assessment.

14
15 The assessment states that “the oral slope factor should only be used with lifetime human exposures of
16 <0.1 mg/kg-day, because above this level, the dose-response relationship is not expected to be
17 proportional to benzo[a]pyrene exposure” (p. 2-30, lines 23-25). How does the EPA expect this
18 limitation to be operationalized given that human BaP exposures typically occur within mixtures of
19 PAHs? How often, and in what situations might this condition be invalid?

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

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

24
25 First, the link between forestomach tumor incidence in mice and rats and cancer incidence in humans is
26 not clearly presented, and the assessment is incomplete without this discussion. The rodent forestomach
27 is highly sensitive to BaP carcinogenesis and represents a major organ for tumor development after oral
28 exposure to this PAH in both rats and mice. The mouse study of Beland and Culp (1998) is focused
29 almost exclusively on forestomach tumors. The rat study of Kroese et al. (2001) provided data on a
30 much broader range of tumor sites. Basing the oral slope factor for cancer on only the mouse study
31 increases the importance of describing the relevance of forestomach tumors in mice to human cancer.

32
33 Second, the SAB is concerned that the assessment does not discuss how the carcinogenicity of BaP and
34 use of the oral slope factor for cancer are impacted by the fact that humans are exposed to BaP as part of
35 PAH mixtures. Some discussion of this issue should be included in the “Uncertainties” section of the
36 assessment. The study by Culp et al. (1998) actually compares the oral carcinogenicity of BaP in a two-
37 year bioassay with two different coal tar mixtures of known content. The coal tar mixtures produce a
38 lower incidence of forestomach tumors compared to BaP, but higher incidence in lung tumors. These
39 data were further evaluated and modeled in the publication by Fitzgerald et al. (2004; their Figure 2
40 excerpted here). Some discussion and consideration of these data could be provided in more detail.

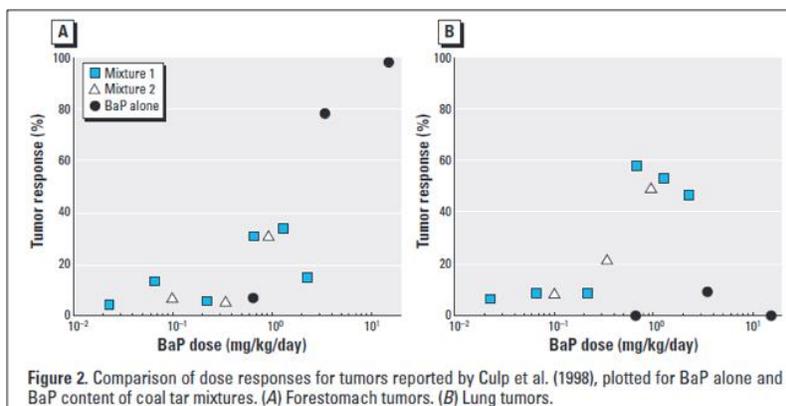


Figure 2. Comparison of dose responses for tumors reported by Culp et al. (1998), plotted for BaP alone and BaP content of coal tar mixtures. (A) Forestomach tumors. (B) Lung tumors.

1
2 **Previous IRIS Assessment Oral Slope Factor (section 2.3.5)**

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

8 **3.3.4. Inhalation Unit Risk for Cancer**

9 *Charge Question 3d. The draft assessment proposes an inhalation unit risk of 0.6 per mg/m³ based on a*
10 *combination of several types of benign and malignant tumors in hamsters. Is this value scientifically*
11 *supported, giving due consideration to the intermediate steps of selecting studies appropriate for dose-*
12 *response analysis and calculating points of departure?*

13
14 The SAB concluded that an appropriate study was selected for dose-response analysis and that
15 appropriate models were used to derive the inhalation unit risk (IUR). Although the IUR value is
16 scientifically supported, the SAB recommends additional discussion of key assumptions, several
17 sensitivity analyses, and reconsideration of the use of epidemiological data to derive inhalation unit risk
18 values. The SAB also suggests the need for an explicit conclusion statement regarding overall
19 uncertainty of the unit risk value, and a brief discussion of the applicability of this value to typical
20 environmental exposures (especially for sensitive subpopulations).

21
22 EPA identified Thyssen et al. (1981) as the only lifetime inhalation cancer bioassay available for
23 describing exposure-response relationships for cancer from inhaled BaP. The experimental design
24 utilized an adult, male hamster model and daily (3-4.5 hr/d) lifetime exposure to BaP via an inhalation
25 portal of entry (nose-only) for a submicronic sized BaP aerosol. Lifetime exposure had average survival
26 durations of 60 to 96 weeks and dose response outcomes included body weight, and incidence and
27 latency of tumors with segmental distributions, i.e., upper respiratory tract (URT), trachea, lung, oro-
28 pharynx, esophagus, and forestomach. The EPA relied on this study due to its merits as the “only study
29 of lifetime exposure to inhaled B(a)P.” Additional scientific support for Thyssen et al. (1981) arises
30 from a subsequent short communication by the same laboratory (Pauluhn et al. 1985). Although limited
31 in scope, the survival results and presence of neoplastic alterations demonstrate that the experimental
32 design using the hamster model can be replicated for low BaP aerosol concentrations employing an
33 inhalation portal of entry. Overall, the results of Thyssen et al. (1981) found tumors (benign and
34 malignant tumors of the pharynx, larynx, trachea, esophagus, nasal cavity, or forestomach) with
35 increasing BaP concentrations. The SAB identified strengths of the approach (durations of exposure to

1 natural death, histologic exam of tissues, monitoring of exposure concentrations) and limitations (lack of
2 distal lung tumors, variation in exposure concentrations, BaP exposure aerosol was developed using
3 sodium chloride condensation nuclei) and these issues were fully addressed in section 2.4.4 of the
4 assessment.

5
6 Due to the merits of a lifetime inhalation animal model study that demonstrated carcinogenicity results,
7 the EPA's selection of Thyssen et al. (1981) for dose-response assessment is appropriate. Dose-response
8 modeling and unit risk estimation for those data used appropriate methods, and the multistage Weibull
9 model fit was adequate. Although the SAB agrees with the EPA that the multistage Weibull model is
10 preferable due to incorporation of time-to-tumor data, the final unit risk value can be further supported
11 by: (1) supplemental sensitivity analyses using other dose-response models; (2) alternative assumptions
12 about latency and cross-species scaling of doses; and (3) not eliminating from the analysis all animals
13 without confirmed examination of one or more of the pharynx or respiratory tract tissues. The SAB also
14 recommends additional discussion of the assumptions used to derive the unit risk (that "any metabolism
15 of benzo(a)pyrene is directly proportional to breathing rate and that the deposition rate is equal between
16 species" on p. 2-35, lines 6-8, and selection of body weight scaling factors in relation to "portal of
17 entry," as discussed in the EPA Guidelines for Carcinogen Risk Assessment). EPA should also state a
18 conclusion regarding overall uncertainty or level of confidence for the IUR, as endorsed on p. 118 of the
19 NRC 2014 review of the IRIS program (NRC 2014).

20
21 Given the extensive human studies of lung cancer with airborne inhalation exposures to PAHs by coke
22 oven, and aluminum smelter workers (i.e., Table 1-11, summary of Tier 1 epidemiologic-based reports
23 of BaP in relation to lung cancer, pp. 1-55 to 1-56), and specifically, reports by Armstrong and Gibbs
24 (2009); Spinelli et al. (2006); Xu et al. (1996); and Gibbs and Labreche (2014), the SAB recommends
25 that the EPA give further consideration to selection of occupational studies (or meta-analysis of
26 occupational studies) to develop unit risk estimate(s) for inclusion in Table 2-9. Although interpretation
27 of the epidemiological evidence is challenging given that exposures were to mixtures of PAHs with
28 poorly understood interactions, a model using relative potency factors and an assumption of dose
29 additivity was reasonably accurate for some PAH mixtures and conservative for others in one
30 investigation (U.S. EPA 1990), and should be considered for adjustment of epidemiological results in
31 estimation of the unit risk attributable to BaP alone. Uncertainty and risk of bias due to exposure
32 measurement error, healthy worker effects, habituation, and/or co-exposure to cigarette smoke products
33 should also be considered and weighed against uncertainties regarding cross-species extrapolation of the
34 unit risk from hamsters to humans.

35
36 It may be helpful for the EPA to address how reasonable it is that lifetime exposures will be in the
37 approximately linear low dose region where the unit risk is applicable ($<0.3 \text{ mg/m}^3$, the human
38 equivalent POD). The SAB recognizes that a nationwide BaP exposure assessment is far beyond the
39 scope of the assessment, but reference to typical exposure ranges may be helpful to readers.

40 **3.3.5. Dermal Slope Factor for Cancer**

41 *Charge Question 3e. The draft assessment proposes a dermal slope factor of 0.006 per $\mu\text{g/day}$ based on*
42 *skin tumors in mice. Is this value scientifically supported, giving due consideration to the intermediate*
43 *steps of selecting studies appropriate for dose-response analysis, calculating points of departure, and*
44 *scaling from mice to humans? Does the method for cross-species scaling (section 2.5.4 and appendix E)*
45 *reflect the appropriate scientific considerations?*

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

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

5 Animal Studies:

6
7 The SAB agrees that studies of skin tumors in mice are relevant to humans based on evidence for a
8 similar mode of action as described in more detail in Section 3.2.4 (see discussion under *EPA Criterion*
9 2) of this report. In the choice of skin cancer bioassay studies for developing the dermal slope factor
10 (DSF), the BaP assessment reviewed 10 complete carcinogenicity mouse skin tumor bioassay studies
11 that repeated exposure over approximately 2 years from 1959 to 1997 (summarized in Tables 2-11 and
12 E-24) and the Sivak et al. (1997) study was chosen as the principal study. Other skin cancer bioassay
13 studies are mentioned and excluded for further analysis because, according to the Supplemental
14 Information document: (1) only one BaP dose level was considered; (2) all dose levels induced 90-100%
15 incidence of tumors; (3) dose applications were once/week or less; and (4) dose was delivered in a
16 vehicle that interacted with or enhanced BaP carcinogenicity. The Toxicological Review document
17 provided a different list of reasons for excluding studies from the dose-response analysis: (1) BaP dose
18 levels were insufficiently characterized; (2) only one BaP dose level was considered, (3) all dose levels
19 induced 90-100% incidence of tumors; and (4) studies were shorter (i.e., < 1 year). Nesnow et al. (1983)
20 and Levin et al. (1977) were not considered in the dose-response analysis because the study durations
21 were shorter (60 and 50-52 weeks, respectively) and dose applications were less than twice/week; i.e.,
22 once/week for the three lower dose levels in Nesnow et al. (1983) (the highest dose level was applied
23 twice/week) and once every two weeks in Levin et al. (1977). Based on the criteria listed in the
24 Toxicological Review document, Nesnow et al. (1983) and Levin et al. (1977) should have been
25 included in the dose-response analysis as the study durations were not less than 1 year. Related to the
26 criteria listed in the Supplemental Information document, the SAB questions excluding studies that
27 applied BaP less than once/week because it "is less useful for extrapolating to daily human exposure."
28 Dermal absorption measurements of BaP are consistent with nearly complete absorption of BaP into the
29 skin for all of the dosing regimens considered. Also, the daily human exposure doses used in risk
30 assessment studies are almost always daily averages of exposures that occur on a less than daily basis. If
31 the results of applying the same BaP dose by once/week or once every 2 weeks differ from applications
32 of more than once/week, then continuous daily exposure, which has been assumed in the analysis for the
33 dermal slope factor, is inappropriate because there would then be data indicating that dose-rate effects
34 cannot be ignored (page 2-41, lines 12-13).

35

36 The SAB notes the following errors in this section:

37

- 38 • The cited study for Grimmer et al. (1984) in the draft BaP assessment and the Supplemental
39 Information is a study on rat lung. The correct citation should be Grimmer, G; Brune, H;
40 Deutsch-Wenzel, R; Dettbarn, G; Misfeld, J; Abel, U; Timm, J. (1984). The contribution of
41 polycyclic aromatic hydrocarbons to the carcinogenic impact of emission condensate from coal-
42 fired residential furnaces evaluated by topical application to the skin of mice, *Cancer Lett*, 23:
43 167-176.
- 44 • The summary of the BMD model selection and BMDL₁₀ modeling results listed in Table E-24
45 are inconsistent with the selected model and POD values listed in Table 2-11 for Sivak et al.
46 1997 (Multistage 2^o and Multistage Weibull 2^o; BMDL₁₀ = 0.058 and POD = 0.060), Roe et al.

1 1970 (BMDL₁₀ = 0.48 and POD = 0.39) and Habs et al. 1980 (Multistage 3^o and Multistage 4^o;
2 BMDL₁₀ = 0.215 and POD = 0.24).
3

4 **Recommendation:**

- 5 • EPA should consider adding Nesnow et al. (1983) and Levin et al. (1977) to Table 2-11, with
6 comments regarding the lower dosing frequency and duration, and should consider combining
7 results from the different studies shown in Table 2-11. This would strengthen the derived DSF.
8 Skin cancer bioassay studies that examined only one BaP level or observed 90-100% incidence
9 of tumors are not suitable for estimating points of departure (POD). However, consistencies in
10 the observations of these studies with observations from the studies listed in Table 2-11 and
11 those used to develop the POD and DSF would strengthen the derived DSF. The criteria listed on
12 pages 2-39 and D-62 for excluding carcinogenicity mouse tumor bioassay studies from
13 consideration (and Table 2-11) should be revised for consistency. The selected model and
14 BMDL₁₀ and POD values listed in Tables 2-11 and E-24 should match.
15

16 Human Studies:
17

18 The EPA review of the epidemiologic evidence of skin cancer in humans is not sufficiently thorough.
19 The assessment cites evidence of an excess of skin cancer in studies of roofers (Hammond et al. 1976)
20 and workers exposed to creosote-treated wood (Karlehagen et al. 1992; Tornqvist 1986), but these
21 groups work outside and would thus have substantial exposure to UV. The assessment also notes that
22 recent studies of chimney sweeps do not demonstrate an increased skin cancer risk (Hogstedt et al.
23 2013). The assessment does not cite or discuss other studies that reported an excess of skin cancer in
24 destructive distillation of coal, shale oil extraction (Miller et al. 1986), tar refinery (Letzel and Drexler
25 1998), asphalt workers and roofers (Partanen and Boffetta 1994), workers exposed to creosote in brick
26 making and wood impregnation (Karlehagen et al. 1992) or studies of workers in other industries with
27 PAH exposure that were reviewed by Boffetta et al. (1997) and Gawkrödger (2004).

28 **Recommendation:**

- 29 • The EPA should more thoroughly review the evidence for skin cancer in studies of coke, steel
30 and iron, coal gasification and aluminum workers given their relevance for evaluating the
31 appropriateness of using the mouse-based risk assessment model for predicting skin cancer risk
32 in humans.
33

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

39 **Dose-response analysis (section 2.5.2)**

40 The draft assessment (p. 2-40, lines 18-20) states the following:
41

42 Although environmental dermal exposure may more likely occur intermittently than oral or
43 inhalation exposures, due to interruption of exposure through bathing or washing of affected areas,
44 the dermal slope factor was derived for use with estimates of constant daily lifetime exposure.
45 Therefore, all administered doses were converted to TWA daily doses using the equation:

1
2 Average daily dose/day = ($\mu\text{g}/\text{application}$) \times (number of applications/week \div 7 days/week)
3

4 This statement is misleading. In evaluating the mouse (dermal) data, the EPA makes an adjustment if the
5 dosing regimen is less than the expected life span. Doses in studies known or assumed to be shorter than
6 104 weeks are adjusted by a factor of $(L_e/104)^3$, where L_e is exposure duration in weeks and 104 weeks
7 is the life expectancy of a mouse (p. E-75). (This adjustment does not show up in the oral or inhalation
8 dose analyses as all studies were full lifetime.) The effect is transparent in the descriptions of the Roe et
9 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
10 of the Supplemental Information document, U.S. EPA 2014b). Per lines 31-33 on p. E-75, this
11 adjustment was also made for the grouped Sivak et al. (1997) data that are the basis for the selected
12 DSF. Presentation of the Sivak et al. data (Table E-24 on p. E-87) is dissimilar to that of the Roe et al.,
13 Habs et al., and Poe et al. datasets and the effect of the adjustment is obscured. Doll (1971) is cited as
14 the basis for the adjustment. Review of that document does provide some argument for non-linearly
15 increasing risk with increasing age and cumulative exposure. However multiple potential values of the
16 exponent describing dependence of risk on dose are discussed by Doll (1971) whereas a value of 3 is
17 selected in the BaP assessment without further explanation.

18 ***Recommendations:***

- 19
- EPA should make the Sivak et al. (1997) data adjustment transparent.
 - EPA should discuss why mouse dose is adjusted downward nonlinearly when ultimate human risk calculations assume a linear relationship between lifetime average daily dose and risk.
 - EPA should explain how a coefficient of 3 was chosen and whether nonlinear scaling by exposure duration has been used to derive other cancer slope factors.
- 23

24 ***Derivation of the dermal slope factor (section 2.5.3.)***

25 The BaP assessment states that mass rather than mass/area can be used as the appropriate dose metric for
26 cancer risk at “low doses” of BaP. The SAB notes that published dermal slope factors for BaP skin
27 carcinogenesis have used mass and mass/skin area as dose metrics and there do not appear to be any
28 empirical data available to inform a choice between these two dose metrics or to select another.
29

30 Experimental studies have demonstrated that equal masses of chemical absorb into the skin when the
31 area of direct chemical contact is less than the applied skin area (i.e., the mass of chemical applied is too
32 small to completely cover the application area). For example, Roy and Singh (2011) reported that the
33 percentage of BaP applied on contaminated soil that was absorbed was independent of the mass of soil
34 applied until the skin surface area was completely covered with soil; further increases in the mass of soil
35 applied caused the percent BaP absorption to decrease. The DSF derived from the skin cancer bioassay
36 in mice is based on the applied dose, which most probably closely approximates the absorbed dose. The
37 time between dose applications was long enough and the applied doses small enough in the mouse
38 studies for approximately 100% absorption. For example, Wester et al. (1990) observed 51% absorption
39 *in vivo* in monkey and 24% absorption *in vitro* in humans for $0.5 \mu\text{g}/\text{cm}^2$ in 24 h. The absorption rates
40 through mouse skin are faster than through humans and monkeys. The conclusion that absorbed dose
41 approximately equals the applied dose assumes that dose losses were minimal; therefore, study protocols
42 in the document should be evaluated for factors that may have affected losses of the applied dose (e.g.,
43 by grooming).

1 **Recommendations:**

- 2 • The SAB does not have a specific recommendation as to dose metric, but strongly recommends
3 that in the absence of empirical data, the decision be based upon a clearly articulated, logical,
4 scientific structure that includes what is known about the dermal absorption of BaP under both
5 conditions of the bioassay(s) and anticipated human exposures, as well as the mechanism of skin
6 carcinogenesis of BaP.
- 7 • The choice of dose metric needs to be better justified and the EPA should provide a convincing
8 argument for the use of mass as the dose metric.
- 9 • The SAB recommends that cancer risk calculated from the derived DSF should use **absorbed**
10 **dose** and not exposed applied dose.
- 11 • The EPA should describe what constitutes a “low dose” for the assumption that mass of BaP is
12 the appropriate dose metric for calculating the DSF from the skin cancer bioassay studies and for
13 estimating cancer risk in humans. This should be consistent with the proposed logical structure
14 for skin cancer from skin exposure to BaP, which is a solid at skin temperature. Issues to
15 consider include:
- 16 ○ For dermal absorption, the skin area with direct chemical contact must be less than the
17 total applied area; i.e., mass of BaP applied cannot completely cover the applied area. For
18 BaP deposited onto skin from a volatile solvent, the mass of BaP that would give a
19 theoretical uniformly thick film $<1 \mu\text{m}$ (i. e., $\sim 135 \mu\text{g}$ of BaP/cm²) would be too small to
20 completely cover the application area, where: Theoretical thickness of a uniform film on
21 the application area = [(BaP mass applied)/(application area)]/ ρ_{BaP} ; ρ_{BaP} = density of
22 BaP= 1.35 g/mL.
 - 23 ○ Metabolism in the target tissue (the viable epidermis) should not be saturated. The
24 document identifies the linear limit for using the slope factor to calculate cancer risk in
25 humans based on the human equivalent point-of-departure (PODHED = 17.9 $\mu\text{g}/\text{day}$)
26 estimated from the mouse PODM adjusted by the mouse-to-human scaling factor as the
27 $\text{BW}^{3/4}$. This is an appropriate limit that could be smaller than 17.9 $\mu\text{g}/\text{day}$ for different
28 scaling factor approaches.
- 29 • The EPA should consider adding diagrams illustrating the logical structure (physiological steps
30 to carcinogenesis) to facilitate choices of dose metric and cross-species scaling.
- 31 • The EPA should consider adding diagrams illustrating the steps involved in calculating human
32 cancer risk based on skin cancer bioassay studies in mice; for example
- 33 ○ Tumors observed in mouse studied as a function of time and exposed dose
 - 34 ○ Exposed dose \approx applied dose to estimate in mice: PODm and DSFm
 - 35 ○ DSFm scaled to the human DSFh
 - 36 ○ Estimate of absorbed dose from exposed dose and exposure scenario
 - 37 ○ Human cancer risk = DSFh x (Absorbed dose)

38 **Dermal slope factor cross-species scaling**

39 According to the assessment, the starting point is the dermal slope factor in the mouse (i.e., DSFm= 1.7
40 ($\mu\text{g}/\text{day}$)⁻¹), which is adjusted by the appropriate human to mouse ratio to obtain the dermal slope factor
41 in humans (DSFh). Experimental cancer risk information for scaling from mouse to human skin cancer
42 from dermal exposure is not available. It is unknown if the chosen approach for scaling of skin cancer
43 risk from BaP exposure to skin is similar to interspecies differences in whole body toxicokinetics, which
44 is the approach (i.e., allometric scaling using $\text{BW}^{3/4}$) adopted by the EPA. The assessment lists alternative

1 approaches for scaling however the SAB recognizes that the science for choosing the best approach is
2 uncertain. The EPA should clarify their choices in this section.

3 **Recommendations:**

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

12 **Uncertainties in the derivation of the dermal slope factor**

13 The cross-species mouse-to-human scaling of the DSF is a significant contributor to uncertainties.
14

15 **Other recommendations for describing cancer risk calculated with the DSF**

- 16 • The cancer risk calculation in mice (and therefore in humans) depends on absorbed dose; i.e.,
17 Cancer Risk = DSF x (Absorbed dose). The EPA should state clearly how the absorbed dose
18 estimates from exposed dose enters the calculation of cancer risk.
- 19 • In actual BaP exposures (from soil or other environmental media), the absorbed dose should be
20 estimated from the exposed dose and the exposure scenario.
- 21 • A soil-to-acetone absorption ratio as described in the response to public comments is
22 unnecessary.
- 23 • Cancer risk from BaP in soil should be calculated from the estimated absorbed dose from
24 exposure to BaP contaminated soil.
- 25 • Examples of cancer risk estimates from exposure to BaP contaminated soil will use an estimate
26 of the absorbed dose taken from the literature (or Risk Assessment Guidance for Superfund
27 (RAGS), Vol. 1, Part E). Because the assessment does not critically review this literature,
 - 28 ○ The literature of dermal absorption measurements from BaP contaminated soils should be
29 listed; and
 - 30 ○ The estimate of absorption used in the risk calculation should be identified as an example
31 (and not an endorsement of the value used).
- 32 • Each environmental media will have its own absorption characteristics that should be considered
33 in estimating an absorbed dose for estimating cancer risk.

34 **3.3.6. Age-Dependent Adjustment Factors for Cancer**

35 *Charge Question 3f. The draft assessment proposes the application of age-dependent adjustment factors*
36 *based on a determination that benzo(a)pyrene induces cancer through a mutagenic mode of action. Do*
37 *the available mechanistic studies in humans and animals support a mutagenic mode of action for cancer*
38 *induced by benzo(a)pyrene?*
39

40 The available mechanistic studies in humans and animals support a mutagenic mode of action for BaP-
41 induced cancers. Given that the EPA's *Supplemental Guidance for Assessing Susceptibility from Early-*
42 *Life Exposures to Carcinogens* (U.S. EPA 2005b) establishes a rational approach for the adjustment of

1 tumor risk for exposures at different ages to carcinogens with a mutagenic mode of action, the SAB
2 concludes that the proposed use of age-dependent adjustment factors (ADAFs) is justified.

3 **3.4. Executive Summary**

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

6
7 The SAB found that the major conclusions of the assessment were clearly and appropriately presented in
8 the Executive Summary. Changes made to the body of the assessment in response to the SAB
9 recommendations that impact the derivation of the chronic RfD/RfC or cancer slope factors should be
10 incorporated into the Executive Summary. In addition, the SAB had a number of suggestions for
11 improving the Executive Summary:

- 12
13 • The purpose of the gray box text at the beginning of the Executive Summary is not immediately
14 apparent. During the SAB panel meeting, the agency clarified that this box is intended to be a lay
15 language abstract for the report. That means that it has a different audience than the rest of the
16 document, and the SAB suggests that it stand alone from the Executive Summary and be clearly
17 identified as a lay language abstract or summary. The SAB further suggests that the gray box text
18 be examined to insure that the health literacy level is commensurate with the lay public as target
19 audience.
- 20 • For audiences that will focus on the Executive Summary, it is not clear in the narrative presented
21 why a toxicological review focusing on BaP is relevant. The SAB suggests adding introductory
22 text to the Executive Summary explaining the public health relevance of the assessment
23 especially related to the importance of evaluating hazard and risk from human exposures to BaP
24 present in PAH mixtures.
- 25 • Although the SAB has no specific advice regarding the appropriate length for the Executive
26 Summary, the agency should strive to capture the important conclusions in a summary that is of
27 readable length.
- 28 • The basis upon which levels of confidence in toxicity values (i.e., “low,” “medium,” or “high”)
29 are reached is not always apparent, and therefore the meaning of these descriptors as presented in
30 the Executive Summary will be unclear. The SAB suggests adding a few sentences in the
31 Executive Summary to explain how confidence levels are determined.

32 **3.5. Public Comments**

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

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

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

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

 - 18 • *Comment: Cross-species extrapolation of dermal slope factor (p. G-11).* Public commenters
19 stated that differences between mouse and human skin should be accounted for in cross-species
20 extrapolation. The EPA response notes that biological information is not currently sufficient to
21 develop robust models for cross-species extrapolation, and states that allometric scaling using
22 body weight to the $\frac{3}{4}$ power was selected based upon observed differences in the rates of dermal
23 absorption and metabolism of BaP. The SAB found that this cross-species scaling factor was not
24 sufficiently justified, as discussed in the response to Charge Question 3e.

 - 25 • *Comment: Uncertainties regarding implementation of the dermal slope factor (p. G-12).* Two
26 aspects of the public comments under this topic received significant discussion by the Panel. One
27 is a comment that a 13% dermal absorption factor for BaP may not be appropriate. The EPA
28 response explains the origin of the value, but acknowledges that it may be a high estimate. The
29 SAB also has concerns about the dermal absorption value, as discussed in the response to Charge
30 Question 3e. The SAB provides specific suggestions. The second comment is that the dose
31 metric of $\mu\text{g}/\text{d}$ is not appropriate for the slope factor in view of the mode of action. The EPA
32 response is that dermal bioassays report total dose applied to the skin but do not quantify the area
33 over which the dose is applied. The SAB concluded that the dose metric has not been sufficiently
34 justified by EPA, as explained in the response to Charge Question 3e.

 - 35 • *Comment: "Real world" validation of dermal slope factor (p. G12).* Public commenters
36 recommended that EPA perform calculations of risk from dermal exposure to PAHs using the
37 proposed dermal slope factor to determine whether the value is scientifically supportable.
38 Commenters discussed that this type of calculation shows skin cancer risks from common PAH

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.

1 exposures such as the use of pharmaceutical coal tar products that are unrealistically high. In
2 their response, the EPA indicated that sufficient details were not provided to allow the agency to
3 reproduce the calculations performed by the public commenters, and provided their own estimate
4 of risk from exposure to benzo(a)pyrene in soil showing a low excess cancer risk (6×10^{-6} for
5 average lifetime exposure that occurs during childhood and 1×10^{-6} for average lifetime
6 exposure that occurs during adulthood).

7 With respect to the dermal cancer slope factor, the SAB supports the application of a “fidelity exercise”
8 for proposed toxicity values to determine whether the toxicity values yield plausible upper bound risk
9 estimates. Generally, this exercise consists of using the proposed toxicity value to estimate risk from one
10 or more exposure scenarios and determine whether the results exceed lifetime risk estimates derived
11 from actual disease incidence (Howlader 2015) for the adverse effect(s) of interest. The SAB finds
12 limitations in the fidelity exercise approaches taken by both the public commenters and the EPA in its
13 response. For example, the EPA estimation of cancer risk from benzo(a)pyrene alone does not reflect
14 actual circumstances of exposure, which almost always occurs as a mixture of carcinogenic PAHs (BaP
15 plus others of varying potency). On the other hand, the limitations of coal tar therapeutics studies make
16 them largely uninformative with regard to the question of whether BaP induces skin cancer in humans.
17 The public commenter’s use of upper percentile exposure values to represent exposure of the overall
18 population tends to exaggerate risk, and the recognized under-reporting of skin cancer² was not taken
19 into account in comparisons. Further, the inherent conservative nature of toxicity values should be
20 recognized and taken into consideration in such analyses. The SAB suggests an improved fidelity
21 exercise to address concerns that the proposed dermal cancer slope factor may lead to unrealistic cancer
22 risk estimates.

23
24 As a general comment, the SAB supports the approach taken by the EPA in creating Appendix G in
25 which the most important scientific issues presented by public commenters are captured and arranged by
26 topic, with reference to the public commenters raising the issue. A more extensive approach, such as
27 providing comment-by-comment responses would be inefficient and cumbersome in a toxicological
28 review. The SAB is aware of contention by some public commenters that their comments were not
29 adequately captured and articulated in Appendix G. To minimize such concerns in future toxicological
30 reviews, the SAB urges the EPA to provide greater transparency in how public comments are distilled
31 into a list of scientific issues meriting an EPA response in the assessment. The EPA provided such a
32 draft table during the SAB deliberations and the SAB would encourage its addition to the document to
33 improve transparency about the review process. In particular, the SAB suggests that the EPA provide a
34 short description of the process used for deciding which comments to include in a public response
35 appendix and how comments are aggregated within the appendix. In particular, it would be helpful if the
36 EPA provided a table within the assessment showing the topics under which comments are aggregated,
37 which commenters provided comments within each topic, and the dates on which the comments were
38 made.

39

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,
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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 2015¹)

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

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

3
4 Charge questions on the draft Toxicological Review

5
6 **1. Literature search/study selection and Evaluation.**

7
8 The process for identifying and selecting pertinent studies for consideration in developing the assessment
9 is detailed in the *Literature Search Strategy/Study Selection and Evaluation* section. Please comment on
10 whether the literature search approach, screening, evaluation, and selection of studies for inclusion in the
11 assessment are clearly described and supported. Please comment on whether EPA has clearly identified
12 the criteria (e.g. study quality, risk of bias) used for selection of studies to review and for the selection of
13 key studies to include in the assessment. Please identify any additional peer-reviewed studies from the
14 primary literature that should be considered in the assessment of noncancer and cancer health effects of
15 benzo[a]pyrene

16
17 **2. Hazard identification.** In section 1, the draft assessment evaluates the available human, animal,
18 and mechanistic studies to identify the types of toxicity that can be credibly associated with
19 benzo[a]pyrene exposure. The draft assessment uses EPA's guidance documents (see
20 <http://www.epa.gov/iris/backgrd.html/>) to reach the following conclusions.

21
22 **2a. Developmental toxicity** (sections 1.1.1, 1.2.1). The draft assessment concludes that developmental
23 toxicity and developmental neurotoxicity are human hazards of benzo[a]pyrene exposure. Do the
24 available human, animal and **mechanistic** studies support this conclusion?

25
26 **2b. Reproductive toxicity** (sections 1.1.2, 1.2.1). The draft assessment concludes that male and female
27 reproductive effects are a human hazard of benzo[a]pyrene exposure. Do the available human, animal and
28 **mechanistic** studies support this conclusion?

29
30 **2c. Immunotoxicity** (sections 1.1.3, 1.2.1). The draft assessment concludes that immunotoxicity is a
31 potential human hazard of benzo[a]pyrene exposure. Do the available human, animal and **mechanistic**
32 studies support this conclusion?

33
34 **2d. Cancer** (sections 1.1.5, 1.2.2). The draft assessment concludes that benzo[a]pyrene is "carcinogenic
35 to humans" by all routes of exposure. Do the available human, animal, and mechanistic studies support
36 this conclusion?

37
38 **2e. Other types of toxicity** (section 1.1.4). The draft assessment concludes that the evidence does not
39 support other types of noncancer toxicity as a potential human hazard. Are there other types of noncancer
40 toxicity that can be credibly associated with benzo[a]pyrene exposure?

41
42 **3. Dose-response analysis.** In section 2, the draft assessment uses the available human, animal, and
43 mechanistic studies to derive candidate toxicity values for each hazard that is credibly associated
44 with benzo[a]pyrene exposure in section 1, then proposes an overall toxicity value for each route
45 of exposure. The draft assessment uses EPA's guidance documents (see
46 <http://www.epa.gov/iris/backgrd.html/>) in the following analyses.

1 3a. **Oral reference dose for effects other than cancer** (section 2.1). The draft assessment proposes an
2 overall reference dose of 3×10^{-4} mg/kg-d based on developmental toxicity during a critical window of
3 development. Is this value scientifically supported, giving due consideration to the intermediate steps of
4 selecting studies appropriate for dose-response analysis, calculating points of departure, and applying
5 uncertainty factors? Does the discussion of exposure scenarios (section 2.1.5) reflect the scientific
6 considerations that are **inherent** for exposures during a critical window of development?
7

8 3b. **Inhalation reference concentration for effects other than cancer** (section 2.2). The draft
9 assessment proposes an overall reference concentration of 2×10^{-6} mg/m³ based on decreased fetal survival
10 during a critical window of development. Is this value scientifically supported, giving due consideration to
11 the intermediate steps of selecting studies appropriate for dose-response analysis, calculating points of
12 departure, and applying uncertainty factors? Does the discussion of exposure scenarios (section 2.2.5)
13 reflect the scientific considerations that are **inherent** for exposures during a critical window of
14 development?
15

16 3c. **Oral slope factor for cancer** (section 2.3). The draft assessment proposes an oral slope factor of 1
17 per mg/kg-d based on alimentary tract tumors in mice. Is this value scientifically supported, giving due
18 consideration to the intermediate steps of selecting studies appropriate for dose-response analysis and
19 calculating points of departure?
20

21 3d. **Inhalation unit risk for cancer** (section 2.4). The draft assessment proposes an inhalation unit risk
22 of **0.6** per mg/m³ based on a combination of several types of benign and malignant tumors in hamsters. Is
23 this value scientifically supported, giving due consideration to the intermediate steps of selecting studies
24 appropriate for dose-response analysis and calculating points of departure?
25

26 3e. **Dermal slope factor for cancer** (section 2.5). The draft assessment proposes a dermal slope factor of
27 0.006 per ug/day based on skin tumors in mice. Is this value scientifically supported, giving due
28 consideration to the intermediate steps of selecting studies appropriate for dose-response analysis,
29 calculating points of departure, and scaling from mice to humans? Does the method for cross-species
30 scaling (section 2.5.4 and appendix E) reflect the appropriate scientific considerations?
31

32 3f. **Age-dependent adjustment factors for cancer** (section 2.6). The draft assessment proposes the
33 application of age-dependent adjustment factors based on a determination that benzo[a]pyrene induces
34 cancer through a mutagenic mode of action (see the mode-of-action analysis in section 1.1.5). Do the
35 available mechanistic studies in humans and animals support a mutagenic mode of action for cancer
36 induced by benzo[a]pyrene?
37

38 4. **Executive summary.** Does the executive summary clearly and appropriately present the major
39 conclusions of the assessment?
40

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

42
43 In August 2013, EPA asked for public comments on an earlier draft of this assessment. Appendix G
44 summarizes the public comments and this assessment's responses to them. Please comment on EPA's
45 responses to the scientific issues raised in the public comments. Please consider in your review whether

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- 1 there are scientific issues that were raised by the public as described in Appendix G that may not have
- 2 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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43 **Additional Peer-reviewed studies contained in HERO**

44 The SAB recommends that EPA consider the following peer-reviewed studies contained in HERO but
45 that are not cited within the BaP document:
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1 Gunter, MJ; Divi, RL; Kulldorff, M; Vermeulen, R; Haverkos, KJ; Kuo, MM; Strickland, P; Poirier,
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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.