Department of Defense Comments on
Benzoapyrene IASC draft Toxicological Review.pdf

Comments submitted by: Chemical Material Risk Management Directorate	Organization: Department of Defense	Date Submitted: 7/7/2011

*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.

Comment No.	Section	Pages	Comment	Suggested Action, Revision and References (if necessary)	*Category
1	2	3, line 27	References needed for these statements	Please provide references.	E
2	2	3, line 31	Same reference provided twice in one sentence.	Please eliminate one of the references.	E
3	2	4, lines 8 and 12	The text should mention that the percent absorbed depends on the concentration.	Please add that the amount absorbed is concentration dependant.	S
4	2	4, line 12	The antecedent of a pronoun should exist within the same paragraph.	Either reference “these studies” or connect with previous paragraph.	E
5	2	5	These inhalation exposures in Table 2-2 and associated pages do not indicate an exposure problem when compared with the RfC of 5 ng/m3. A reference to this fact would be appropriate to frame the exposure concentrations.	As per NAS (2009), EPA should start with a problem formulation statement: What is the problem with BaP, given these minimal exposures?	S
6	2	6	These oral exposures in Table 2-3 do not indicate an exposure problem when compared with the RfD of 500 ng/kg-day. A reference to this fact would be appropriate to frame the exposure concentrations.	EPA should start with a problem formulation statement here as well. What is the problem with BaP, given these minimal exposures?	S

7	Global		This document frequently assumes that results for any or every PAH are relevant to BaP. This document reviews the toxicity of BaP only; EPA has another document that demonstrates the differences among PAHs	All references to data that are not specifically about BaP should be deleted.	S/M
8	Global		DoD has documented in the comments below many instances where (1) the summary of a section is inconsistent with the statements in that section or (2) the conclusion in one section is inconsistent with that of another section. Given that the interagency review group was told to expect this draft in early January, it is unfortunate that a better quality review has not occurred prior to this release.	Improvements in internal consistency are necessary. It should not be left to readers to attempt to locate when conclusions either misstate or contradict the findings. The document should have undergone a quality review prior to release for interagency review. In particular, if people read only summaries of sections, they should have the same conclusion as those who read the whole section.	S/M
9	Global		Adjectives and adverbs are imprecisely used. Frequently, for example, a form of "rapid" is used when "increased amount" is meant. On page 38 of the text there are several more examples of sloppy presentation of information.	The document should be edited before it is released for interagency review to ensure the text is not misinterpreted.	S/M
10	Global	Global	EPA's 2005 cancer guidelines require quality human data to use the characterization "carcinogenic to humans." In this document, EPA has clearly stated that there are no quality studies of BaP alone that demonstrate human carcinogenicity. EPA has presented no data that the carcinogenicity of the PAH-containing mixtures is due to the BaP and not the combined effects of the other PAHs or other chemicals in those mixtures or both. EPA has a different	EPA should clearly present the data available on BaP alone with regard to the characterization of the weight of the evidence for carcinogenicity, as well as the requirements of its 2005 cancer guidelines. If EPA continues to include data on other PAHs and PAH-containing mixtures, it should clearly justify why this determination should be made in this document, rather than in its document on PAH mixtures.	S/M

			document that reviews PAHs. It also uses BaP as the index chemical. This means that if EPA determines that BaP is a human carcinogen, all PAHs will be determined to be human carcinogens as BaP-equivalents. Using data for PAH mixtures to determine information on BaP's weight of evidence for carcinogenicity and then using the BaP determination to assess PAH toxicity is circular reasoning that borders on a tautology.		
11	All	All	Please address the following editorial comments.	Page 246, line 24. "..divided by a total uncertainly factor of 1000 (factors of 3 for animal to human extrapolation, 10 for human interindividual variability, and 3 for database deficiencies)" Please correct the factors to make a multiple of 1000 i.e., 10 for animal to human extrapolation etc). Page 204, line 28 and following page. Please move the top half of the table onto the next page so that Table 5.1 is all on one page. Page 218, line 18. "s(1998) udies" should be changed to "(1998) studies" Page 235, line 5. "Average dose/day = (ug/application) x (number of exposures/week ÷ 7 days/week. Please add parenthesis to number of exposures /week) and make "7 days/week the	E

				sole denominator. Page 8, line 6. “samples. .” delete the second period Page 32, line 21. “benzo[a]pyrene should be capitalized at the beginning of the paragraph. This error occurs throughout the document Page 139, line 5. As above. Page 161, line4. As above. Page 166, line 36. As above. Page 38, line 3. There are three enzymes, "either" is incorrect. Page 32, line 15. The sentence is not written in proper English and needs editing. Page 170, line10. “caspace” should be spelled “caspase”. Page 176, line 7. “subchronicly” should be “subchronically” Page 178, line 5. Paragraph beginning with “In addition...” should be indented, for consistency. Page 181, line 32. “.Carfi e al.” please remove the leading period.	

				Page 181, line 21. “Spleen cell cultures…” should be indented. Page 186, line 20. “an alternant” should be “an alternate”. Page 244, line 6. Capitalized “b” in benzopyrene. Please search the document for this recurring error.	
12	Global	NA	1. Given that this is an updated profile of benzo[a]pyrene and that the EPA has promulgated the potential use of genomics in toxicology, it is surprising that there is no mention of microarrays or genomics in this review. There are 37 hits using the keywords benzopyrene and microarray in Pubmed many of which examine the carcinogenicity of BaP. See EPA Interim Genomics Policy: http://www.epa.gov/spc/genomics.htm “Genomics data may allow EPA to enhance its assessments and better inform the decision-making process”. Luo, W. et al. Phenotypic anchoring of global gene expression profiles induced by N-hydroxy-4-acetyaminobiphenyl and benzo[a]pyrene diol epoxide reveals correlations between expression profiles and mechanism of toxicity.	Review EPA Genomics Policy and studies for applicability.	S

			Chem Res Toxicol. 2005 18(4): 619-29. Bartosiewicz, M. et al. Applications of gene arrays in environmental toxicology: fingerprints of gene regulation associated with cadmium chloride, benzo[a]pyrene, and trichloroethylene. 2001. Environ Health Perspect. 109(1): 71-4.		
13	References	285	It would appear that the critical study for derivation of the RfD (Xu et al., 2010) is missing from the list of references	Please insert Xu et al., 2001 into references	S
14	3.1.1.1	9, line 27	If the concentration of BaP is low in the air, there is no particular reason to believe that either oral or dermal exposure will occur. Oral exposure would either be through consumption of food or water or via the mucociliary escalator, the latter of which is unexpected unless insoluble particles are involved. Dermal exposure, to the extent it occurs, would not only depend on the concentration but also the clothing of the person.	This statement should either have a reference or include data to support the assertion. Broad-based assertions with significant implications should be accompanied by a reference or primary data supporting them.	S
15	3.1.1.1	10, lines 1 through 28	The information about absorption of other PAHs is not relevant to the discussion of BaP unless the presence of other PAHs affects the absorption of BaP, in which case these data are confounded.	Please clarify the purpose of presenting information on other PAHs in a document that is only on BaP. IRIS has already released a document on PAH mixtures, and extra and redundant information unnecessarily lengthens an already long document. Please delete this and other references to PAHs unless their relevance is established.	S/M
16	3.1.1.1	11, lines 1 through 26	As the metabolism of BaP has not been discussed by this point in the document, please	Please add the requested information, and if this metabolite is related to other PAHs, please	S

			indicate the relevance of “trans-antibenzo[a]pyrene-tetrol in the urine” with regard to BaP absorption. Is this metabolite related to exposure to any other PAH?	explain how this confounding information was handled.	
17	3.1.1.1	11, line 28	Please explain the relevance of the comparison with other PAHs. Please also explain the conclusion that “benzo[a]pyrene is absorbed in an unpredictable fashion” given that, in all of the examples provided, there were significant confounders and the presence of materials that might affect the absorption of BaP, i.e., the presence of organic matter as discussed on page 9, line 8.	Please substantiate or remove the assertion that the absorption of BaP is “upredictable.” We believe that a more complete analysis of the data discussed in this section would demonstrate that the absorption of BaP can be reasonably and consistently predicted if the conditions of the absorption and the presence of other chemicals are included in the analysis.	S
18	3.1.1.1	11, line 33	We believe that instillation experiments are of limited value for assessing toxicokinetics and should not be used when inhalation data are available.	Please explain how the instillation data have been quantitatively adjusted or, preferably, merely reference them and indicate that they are of limited quantitative utility if inhalation data are available.	S
19	3.1.1.2	11, line 38 through 13, line 6	Please explain how a bolus administration of a very high concentration of BaP is relevant to absorption of ambient levels, as the previous discussions indicate that the extent of absorption is concentration dependant. Extensive discussions of data that are not relevant serve only to lengthen the document. Furthermore, the use of instillation limits the utility of these data.	Please explain or limit discussion of these experiments to one sentence.	S
20	3.1.1.2	12, line 6	The statement that “the clearance rate [from the lungs] was limited by diffusion through the	As recommended by several, recent external review panels, DoD recommends that EPA	S

			alveolar septa for highly lipophilic compounds such as benzo[a]pyrene" might also explain the asserted "unpredictability"of BaP that was discussed in the previous section. Since the majority of the experiments measured absorption as the appearance of urinary metabolites, the retention of BaP in the lungs (which is expected to be dose dependant as dose would affect the diffusion gradient) might cause lags and otherwise influence the appearance of urinary metabolites.	include more integration of the data it has been reviewing. As the toxicologists involved in these reviews have more time and knowledge about the chemical under review, DoD would expect that they would notice these correlations and present them in their analyses.	
21	3.1.1.2	14, line 11	It is not clear why dose administered as an aerosol concentration creates a limitation for the study.	As the cancer potency and RfD/Cs will be expressed as concentration in the air, please explain why "administered aerosol concentration" is a limitation of the study.	S
22	3.1.1.2	14, line 17	Use of triethylene glycol as the solvent will be a confounder, given all of the effects that have been discussed previously.	EPA should state that the differences between these results and those of saline – including the biphasic nature of the absorption – are likely due to the solvents, i.e., hydrophilic versus hydrophobic.	S
23	3.1.1.2	16, line 8	The word "particles" should be qualified, as EPA's definition of particles is quite broad.	Statement should read "some, insoluble particles."	S
24	3.1.1.2	17, line 21	Because most of the animal data presented are from instillation or other nonconventional methods, e.g., application to nasal tubinates, the conclusion that "absorbed rapidly (within minutes) and extensively" is insufficiently supported and should be qualified. Data from these non-conventional methods can not be	The text and associated conclusions should be appropriately qualified.	S

			used to quantitatively support absorption from inhalation. Similarly, the “more rapid absorption in the pulmonary regions” in line 23 can not be supported by instillation experiments, as the instillation procedure bypasses the nose.		
25	3.1.1.2	17, line 17	The statement that the variation in results is due to interindividual variability is neither referenced nor supported. As stated in previous comments, the perceived variability could be due to the lack of analysis on the part of EPA. None of the data presented in this document demonstrate that differences in subjects caused the variability. Furthermore, the statement that this is “most likely the result of different genetic makeups” has no support thusfar in the document. If they are discussed later, the section should be referenced here.	EPA should either provide data or a references of primary data to support these statements. If not, such speculation should be deleted.	S
26	3.1.2.2.	Global	As with inhalation, the experiments reported depend on non-conventional exposures, e.g., gavage (Ramesh et al., 2001b; O’Neill et al. 1990) or bolus (Foth et al., 1988)	The results from these experiments should be appropriately qualified and not used for quantitative analyses.	S
27	3.1.3.1.	21, lines 1 - 15	As reported, this study only reports results for “PAHs” not BaP, and therefore is not relevant to this document.	The study should be deleted from this section.	S
28	3.1.3.1	21, lines 33 and 37	The percentage absorbed is meaningless since the document does not report the dose applied and absorption of BaP is dose dependant.	The dose should be provided or the “results” should be deleted.	S
29	3.1.3.1	22, line 25	The adverb “rapidly” seems inappropriate, since	Perhaps the document means to say that not	S

			none of the studies examined uptake over time.	much was absorbed, at least as BaP.	
30	3.1.3.1	23, line 18, repeated on page 24m line 21	It is not clear whether this is the conclusion one of the authors" or that of EPA? Moreover, does the person who is speculating have data to differentiate the difference in viscosity versus a difference in hydrophobicity being the likely effecter? Hydrophobicity seems more likely, as the viscosity of soil should prevent any absorption.	The text should be clear as to which conclusions are those of the authors of the experiment and which are those made by EPA scientists. Moreover, speculations that are not supported by data should not be included.	S
31	3.1.3.1	23, line 30	It is highly unlikely that the soil, much less the BaP, was in a "monolayer".	The characterization should be corrected	S
32	3.1.3.1	23, line 38	Given that, in other radio-labeled experiments, not all of the radioactivity was recovered, if 34% was excreted, more than 34% was likely absorbed. The EPA authors should be careful about how they present the data and should not make their own, unsupportable conclusions.	The text should be corrected to reflect the reported data, not inferences.	S
33	3.1.3.1	24, line 7	Given that only metabolites were released and that this section is concerned with absorption, retention of the metabolites by the skin is not relevant for this section.	This document should avoid inference and speculation, especially that which is not relevant to the topic under discussion.	S
34	3.1.3.1	24, line 15	The "and" should be "or" or "and/or" as the experiment can not distinguish whether it is one or both that are saturated. Indeed, the next sentences suggest it is a saturation of metabolism, not absorption per se.	Change "and" to appropriately reflect the results of the experiment.	S
35	3.1.3.1	25, line 12	This sentence does not appear to be supported by the information provided in EPA's document.	The statement should be edited to accurately reflect the text it is summarizing.	S

			If “abundant” data are available, why is only one study presented. Furthermore, the data from that study are only given in terms of “PAHs” and as such provide no quantitative evaluation of human dermal absorption of BaP.		
36	3.1.3.1	24, line 18	The data do not indicate a change in rate, only amount.	The text should accurately reflect that more BaP was absorbed after induction; this document says nothing about a change in rate.	S
37	3.1.3.1	22, line 37	These tissues qualify as “dermal”, furthermore the text states that this data is not comparable to human skin data.	Discussion of these experiments should be moved to the appropriate section, or deleted.	S
38	3.1.3.1	25, line 15	As the dermal absorption is dose-dependant, the percent absorption should reflect this.	Add “dose-dependant” to this sentence.	S
39	3.1.3.1	25, line 17	Unless this sentence is specifically applicable to BaP, it should be deleted. The data demonstrate that absorption differs among PAHs; therefore, generalizations cannot be made.	The sentence should either replace “PAHs” with “benzo(a)pyrene”, if that is accurate, or it should be deleted.	S
40	3.1.3.1	26, line 26; 27, line 25; 31, line 38	The text should indicate if this is the authors’ or EPA’s conclusion. It appears that all three may be EPA’s speculations, as the data are open to other interpretations.	If the speculations about the mucocilary clearance are EPA’s speculations, they should either be deleted or clearly stated as such. If this is the case, the “is” in the last citation should be changed to “may” as it is speculative, not demonstrated.	S
41	3.2	31, line 8	This sentence is speculative and has no data to support it.	This sentence should be deleted.	S
42	3.2	31, line 8	Is EPA stating as a fact that particles (in this	As these documents all originate from NCEA,	S

			case with BaP adsorbed) do not circulate from the lungs to other parts of the body? If so, is this in agreement with EPA's conclusions regarding the distribution of particles in its documents on particulate matter? For example, section 4.3.3. "Particle Translocation" of EPA's "Integrated Science Assessment for Particulate Matter" (EPA/600/R-08/139F, December 2009) states, "There is evidence that particles may cross cell membranes and move from their site of deposition by other mechanisms [than mucociliary and macrophage mediated clearance]."	EPA should ensure agreement among their documents.	
43	3.2	31, line 34	It seems that "limited" be "absence of"; given (page 25, line 6) that there are "No adequate quantitative studies of benzo[a]pyrene tissue distribution in exposed humans."	Correct the the text to make it internally consistent.	S
44	3.2	31, line 36	Given that the previous section indicated that very little is absorbed, we are confused how "significant levels" can be in the tissues.	Recommend correction so the document is internally consistent.	S
45	3.2	32, lines 4 and 6	The dependent clause should be deleted, as EPA has already concluded that most of the BaP is metabolized as it is absorbed. Similarly, given the statement at line 4, shouldn't line 6 be about the metabolites?.	The independent clause makes the dependent clause unnecessary. Line 6 should say "metabolites".	S
46	3.3	32, line 10	Even if secondary sources are used, they should be referenced, as not all of the reviews will agree on all issues.	Please add the references.	S

47	3.3	32, line 34	Both steps in the metabolism should get equivalent treatment.	Phase II metabolism should also be displayed, perhaps in 3.3.2. Phase II Metabolism.	S
48	3.3.1.1	Global	Many statements in this section have specific references from the 1970s and 1980s, contradicting the earlier statement that “Key concepts have been adapted largely from these reviews and supplemented with recent findings.” Do these statements disagree with the reviews? Is this information based on what the reviews say about the articles (in which case the reference should so indicate) or based on a review of the primary article by EPA scientists?	The text should accurately reflect whether the EPA scientist read a review or the primary article. Disagreement between the introductory text and the main text should be resolved.	S
49	3.3.1.1	37, line 10	The singular in the parenthetical implies the lethality is only for one dose, but the sentence has 3 dose levels.	The sentence should be corrected to state clearly whether all or only the highest dose is lethal to the knock-out mice in 30 days.	S
50	3.3.1.1	37, line 27	This experiment is misplaced, as it addresses potential effects of the metabolites rather than metabolism.	The paragraph should be moved.	E
51	3.3.1.2	38, line 9	The term “bioactivation” either assumes metabolites are more active than the parent compound, which is demonstrably false for BaP, or that EPA is only concerned about metabolites that are more active, which would be unfortunate.	The text should be edited to reflect accurately the intention.	E
52	3.3.1.2	38, line 10	As the previous sentence stated that this paragraph would be about CYP-independent processes, the inclusion of “via CYPs” in the parenthetical is inconsistent.	The text should be carefully edited to avoid such apparent contradictions.	E

53	3.3.1.2	38, lines 11-18	This experiment is misplaced, as it addresses potential effects of the metabolites rather than metabolism.	The text should be moved,	E
54	3.3.1.2	38, line 24 through page 40, line 22	Without information about the concentration of BaP, it is not possible to judge whether these experiments were conducted at biologically relevant concentrations.	All in vitro data should be presented with a discussion of whether the doses are biologically relevant.	S/M
55	4.5.3	167-170	The discussion regarding AhR's role in BaP-mediated carcinogenesis is poorly written and inadequate. BaP-specific information needs to be clearly distinguished from general AhR biology and from evidence that was from PAH mixture studies. Figure 4-1 can be dramatically improved with more sophisticated and BaP-specific information; it is unnecessary to provide basic AhR biology. There are numerous sentences that are not clear within this section. AhR may be involved in regulating BaP metabolism AND/OR involved in the upregulation of genes involved in cell cycle and differentiation. These two distinct roles of AhR are not clearly described nor evaluated.	Recommend significant edits to improve the evaluation of BaP-mediated AhR activation, as it relates to both tumor initiation and promotion. A molecular biologist with expertise in nuclear receptor signaling should review this section and improve the accuracy and clarity of statements within.	S/M
56	4.5.3	169, line 21	It is unclear how the following statement is concluded: "The high levels of benzo[a]pyrene DNA adduct in organs other than the liver of AhR–/– mice may be the result of slow detoxification of benzo[a]pyrene in the liver, allowing high concentrations of the parent compound to reach distant tissues." It is also not	Recommend delete the sentence beginning on line 21.	S

			clear why this type of pontification is necessary or appropriate in the current section.		
57	4.6.2	179	According to EPA (1994) guidelines, the database shown in Table 4-28 is insufficient to develop an RfC. Specifically, lung effects were not sufficiently monitored, and the duration is too short. In addition to the studies shown in Table 4-28, Wolff et al., 1989 assessed lung injury after only a 4 week exposure, and Thyssen et al., 1981 did not report histological examination of the lung. A reproductive/developmental RfC could be developed, but it must be annotated as such and the lack of a general RfC must be clearly stated.	An RfC cannot be developed. Recommend removing the RfC development from the document, or deriving a reproductive/developmental RfC.	S/M
58	4.7.3.1	187	Figure 1 shows more than just the 4 key events described in the text, and yet is also missing other information discussed in the text. How does cytotoxicity play a role in tumor formation, for example? If it does not have a role, it is not clear why it was included it in the Figure. The arrows and boxes (key events) of the process should be specific to BaP, with supporting information to justify why the step is *necessary* in BaP-induced neoplasm formation. Figure 1 also does not differentiate between several potential MOAs (i.e. cytotoxicity versus mutation and promotion).	Figure 1 needs to be modified to represent the known key events specific to BaP-mediated carcinogenicity, as distinct from general steps in the carcinogenic process. Discuss cytotoxicity as a possible MOA for the cancer endpoint.	S/M
59	4.7.3.2	189	Section titled ***"Dose-response concordance and temporal relationship."*** Adduct formation	Please discuss specific data on mutations as a possible MOA for the cancer endpoint.	S/M

			does not equal mutation as EPA (2007) clearly states. EPA needs to provide information on how adduct formation's dose-response concordance and temporal relationship is relevant to the mutagenic MOA. There is no data presented for dose-response concordance and temporal relationship for mutations.		
60	4.7.3.2	190, line 21	It is agreed that there is temporal consistency between BPDE-DNA adducts and forestomach tumors, however a comparison of the **dose response** behavior of these two endpoints is inconsistent. If tumors were based on adduct formation, one questions why there is a sharp increase in tumor incidence between doses, and not a linear increase as for adducts. Moreover, EPA has not shown a temporal and dose response concordance between mutations and tumor formation.	The discussion amongst the various possible MOAs for the cancer endpoint needs to be balanced.	S/M
61	4.7.3.2	190, line 36	EPA has not demonstrated temporal and dose concordance between tumors and mutations. It is very unclear why these arguments are being made against alternative MOAs.	Please balance the discussion amongst the various possible MOAs for the cancer endpoint.	S
62	4.7.3.2	191, line 6	EPA needs to take all of the evidence on relevant MOAs, through the MOA/Human Relevance (MOA/HR) framework. Production of inflammatory cytokines and inhibition of GJIC should be analyzed for temporal and dose response concordance. Without doing this, the overall MOA conclusions made by EPA,	Balance the discussion amongst the various possible MOAs for the cancer endpoint. Apply the MOA/HR Framework to all potential MOA evidence.	S/M

			including the determination of inadequate information, cannot be evaluated.		
63	4.7.3.2	191, line 15	Not believed by whom? Please use a citation for this sentence. If it is speculation by the author, please state it as such.	Incorporate appropriate citation.	S
64	4.7.3.2	191, line 29	While support exists for gene mutations, the authors have not linked these mutations to tumor formation through the MOA/HR framework. For example, the section on "Dose-response concordance and temporal relationship" only gives evidence for DNA adducts.	Balance the discussion amongst the various possible MOAs for the cancer endpoint. Clearly differentiate between DNA adduct formation and mutagenicity.	S
65	4.8.1	192, line 11	This statement is not true. The expectation depends entirely on the full underlying MOA. EPA (2005, page 1-17) states that: "These empirical results are consistent with current understanding of the biological processes involved in carcinogenesis, which leads to a reasonable expectation that children can be more susceptible to <u>many</u> [*not all is implied in EPA's BaP text*] carcinogenic agents." Furthermore, EPA (2005, page 2-29) also states: "*Identifying and comparing metabolic process differences by age, sex, or other characteristic so that susceptible subpopulations can be*	Revise the ADAF discussion to be consistent with EPA cancer guidelines. Incorporate specific data that indicates that an ADAF for oral cavity tumors is not needed.	S/M

			recognized. For example, metabolic capacity with respect to P450 enzymes in newborn children is extremely limited compared to that in adults, so that a carcinogenic metabolite formed through P450 activity will have limited effect in the young, whereas a carcinogenic agent deactivated through P450 activity will result in increased susceptibility of this lifestage (Cresteil, 1998). A variety of changes in toxicokinetics and physiology occur from the fetal stage to post-weaning to young child. Any of these changes may make a difference for risk (Renwick, 1998)." BaP metabolites are formed in part by P450 enzymes, and thus, such metabolites are less likely to be formed in younger animals. So why is the ADAF for BaP being proposed?		
66	4.8.1	193, line 6	The authors lead this section off with the phrase "Increased childhood susceptibility..." presumably in comparison with adults, but then do not show any comparative data between adults and children. Without comparative information, any *difference (i.e. increase)* in susceptibility is impossible to evaluate. The data within section 4.8 merely demonstrates childhood susceptibility.	The comparative data between adults and children needs to be shown.	S
67	4.8.1	194, line 9	DoD does not agree with EPA's conclusions of the epidemiological studies assessing cigarette smoke. It is possible that exposure to other compounds within the cigarette smoke, such as	Evaluate the epidemiological studies of cigarette smoke to discuss the milieu of compounds within the smoke, not just PAHs. Clarify text.	S

			carbon monoxide, could cause the observed effect; the effects cannot be ascribed to PAHs alone without additional clarification or information. Also, the last sentence (line 14) does not make sense. Do the authors mean "missed period"?		
68	4.8.1	194, line 18	Clarify how overt toxicity or depression in fertility of F1 can be compared to parental toxicity when the exposure to the parental animals starts at GD 7.	Recommend correcting the summarization sentences to accurately reflect the available data; do not over interpret the data.	S
69	4.8.1	195, line 31	Rather than using default procedures, EPA needs to show these data (Vesselinovitch et al., 1975), since they appear to be a solid basis for the age dependent adjustment factor (ADAF). Note that these data may be useful to explore different ADAFs with different tumor types.	Please revise the ADAF discussion to be consistent with EPA cancer guidelines by incorporating specific data that indicates that an ADAF for oral cavity tumors is not needed. Such an ADAF may be needed for other tumors.	S/M
70	5.1	201	The document is lacking a candidate RfD array. These arrays are extremely useful in illustrating the variety of studies, the impact of POD and UF, and the range of potential values.	Please generate a candidate array for the potential RfDs to illustrate the PODs, UF and resulting potential RfDs for comparison.	S
71	5.1.2. Methods of Analysis	205, line 13	The reference cited is an External Review Draft that, in more than a decade, has not been finalized. Therefore, citing this guidance as a reason for any particular action is problematic.	Only final guidance or guidelines should be cited, or if EPA wishes to continue to cite this reference, the text should clearly state that this is not EPA guidance, i.e., "based on procedures suggested in EPA's draft technical guidance". Otherwise, some readers might assume that (1) this is formal, EPA guidance and (2) EPA must follow these procedures unless it can justify its deviance based on the weight of the evidence of	S/M

				scientific studies.	
72	5.1.2. Methods of Analysis	205, line 14	EPA's BMD software calculates a one-sided 95% lower limit that is equal to the more appropriate 90% two-sided confidence interval.	EPA should state the qualifications on this calculation clearly and transparently.	S
73	5.1.2	205, line 26	The rationale for the choice of principal study and critical effect(s) is clearly and logically presented. However, the decisions regarding principal study and the critical effect for developing the oral RfD for benzo[a]pyrene are not justified. The appropriate principal study and critical effect(s) were not selected. EPA's IRIS defines a "critical study" as "The study that contributes most significantly to the qualitative and quantitative assessment of risk. Also called Principal Study". The EPA also defines a "critical effect" as "The first adverse effect, or its known precursor, that occurs to the most sensitive species as the dose rate of an agent increases". Several studies [e.g., De Jong et al. (1999) 35-day rat gavage study, Kroese et al. (2001) 5-week, 90-day and 2-year rat gavage studies, Beland and Culp (1998) and Culp et al. (1998) mouse studies) have all reported dose-dependent and statistically significant increases in the incidence of forestomach hyperplasia in both rats and mice. Table 4-27 shows the NOAEL/LOAELs for this endpoint in both species. In several instances (e.g., pages 178, 201, etc), EPA has acknowledged that forestomach hyperplasia is a sensitive effect in	EPA does not provide sufficient justification for discounting forestomach hyperplasia as the critical effect, and needs to answer the question; how do the reproductive and fertility effects better characterize noncancer low dose effects? Please revise the critical effect choice and discussion to be consistent with EPA guidelines. Without appropriate justificatio to exclude forestomach hyperplasia, Beland and Culp (1998) would be the principal study and forestomach hyperplasia from this study would be the critical effect for developing the oral RfD for benzo[a]pyrene. EPA should appropriately qualify its discussion of this endpoint. If forestomach hyperplasia were chosen as the critical effect, then the toxicokinetic factor for experimental animal to human and within human variability might not be needed as per EPA's Acute Exposure Guideline Level methods. EPA authors need to explore this alternative or more clearly justify the selection of Xu et al. (2010) as the principal study and decreases in ovary weight, estrogen, and primordial follicles, and altered estrus cycling as the critical effects.	S/M

			the two chronic-duration studies in rats and mice. In addition, modeling results also identify increased forestomach hyperplasia in female B6C3F1 mice (Beland and Culp, 1998) (see Table 5-2) as the critical effect, i.e., observed at the lowest dose level. However, EPA did not select the forestomach effects as the most sensitive measure (p. 208, parag. 1). Instead, EPA selected the Xu et al. (2010) study as the principal study (see also page 245, line 7, and elsewhere). This latter study observed biologically and statistically significant decreases in ovary weight, estrogen, and primordial follicles, and altered estrus cycling in treated animals (see p. 208, parag. 1). The BMDL_{1SD} from the Xu et al. study is 1.5 mg/kg-day, a value that is 10x higher than the BMDL₁₀ of 0.115 mg/kg-day identified for forestomach effects in the Beland and Culp (1998) study. We acknowledge selection of rat forestomach hyperplasia as the critical endpoint for evaluating human non-cancer effects of BaP is problematic. DoD believes, based on EPA's definition of principal study and critical effects, the appropriate study and effect may not have been selected and therefore requires additional justification. It may be argued that rodent forestomach hyperplasia following gavage		

			administration of BaP is not relevant to humans; not only do humans not have a forestomach, but they also do not have an equivalent organ where ingested material is stored for long periods of time. Also, gavage may increase the concentration in the forestomach beyond that which would occur from an exposure through food or drinking water. The combination of the high concentration of BaP concurrent with the potential for gavage to cause physical irritation, may contribute to the hyperplasia. We understand that EPA has selected forestomach hyperplasia as a critical effect for other chemicals. If EPA feels these that the quantitative analysis of effects observed in the forestomach after exposure by gavage is not reasonable, this needs to be appropriately and transparently discussed. If EPA has used this endpoint for noncancer risk assessment for other chemicals, EPA should discuss why the conclusions differed for this chemical.		
74	5.1.2	209	EPA has recently recommended the use of animal to human body weight scaling as the default method in the development of the oral RfD for benzo[a]pyrene (see Risk Assessment Forum guidance, EPA/100/R11/0001). However, the Agency has not used this approach in calculating the human equivalent doses (HEDs) upon which the dose-response modeling can be based. In deriving an RfC (EPA, 1994), EPA routinely conducts dosimetric conversion of the	The corresponding HEDs for all noncancer effects data need to be calculated prior to the dose-response modeling as is done in deriving RfC. In this way, the principal study and the critical effect can be evaluated and compared and the appropriate HED-based POD selected for deriving the oral RfD.	S/M

			concentration to a human equivalent concentration (HEC) before the different adverse effects in the data array are evaluated and compared.		
75	5.1.2-5.1.3	209-210	Although EPA's rationale for the choice of UF values for derivation of the oral RfD is clearly and logically presented, it is based on the wrong critical effect. EPA has used a total UF of 3000: 10 each for UF_A, UF_H, and UF_S, 1 for UF_L, and 3 for UF_D. Furthermore, UF_A can be reduced to 3, if HEDs are calculated and used in the dose-response modeling to estimate the POD (e.g., $BMDL_{10}$). If the forestomach effects are used as the critical effect for benzo[a]pyrene (Beland and Culp, 1998), the total UF will likely be 30 to 100, instead of the 3000 used for the less sensitive endpoint of decreased ovarian weight (Xu et al., 2010).	Using the derived HED-based $BMDL_{10}$, for hyperplasia, the total UF will be 100, which is comprised of UF_A of 3 for the toxicodynamic component given that HED has been calculated, UF_H of 10 for human variability, UF_S of 1 because the principal study (if the Beland et al., 1998 turns out to produce the critical effect after HED calculation and BMD modeling) is a chronic study, UF_L of 1 for use of BMR for BMD modeling, and UF_D of 3 for lack of a standard multigeneration reproductive toxicity and a neurodevelopmental toxicity study. EPA scientists need to read the cancer bioassay reports, not just the published papers, for monitoring of other effects; this will aid in the judgment of UF_D. The overall factor should be either 30 or 300, depending on judgment of UF_D, and the toxicokinetic component of UF_D and UF_H. For example, if the hypeprplasia, the critical effect by EPA's own definition, is an irritant effect (EPA has not demonstrated this), then the toxicokinetic factor for experimental animal to human and within human variability might not be needed as per EPA's Acute Exposure Guideline Level methods.	S

76	5.1.3. RfD Derivation	Global	Selection of a subchronic study when EPA lists a number of high quality chronic studies in Table 5-2 that fit all of its criteria appears to be a method to increase the estimated toxicity of BaP for the following reasons: 1. Several of the chronic studies have BMDLs that are greater than the selected BMDL after the UF of 10 for subchronic to chronic is applied. 2. Reproductive studies, with the exception of mutigenerational studies, are of a duration appropriate to the chemical and the reproductive cycle of the animal. Thus, 60 days is sufficient for this type of study.	EPA must justify its deviation from standard, scientific practice, i.e., using a subchronic study to estimate a chronic RfD when high quality, chronic studies are available.	S/M
77	5.1.3	210, line 5	The database uncertainty factor may be 3-fold or it could be 10-fold, pending review of the chronic rat and mouse studies and determining the extent of noncancer effects monitoring. For example, if the chronic bioassays had little in the way of noncancer modeling, then a full factor of 10 might be needed. The key determinant here is to read the original research reports, and not just the published papers, since the authors of the published papers may have emphasized reporting on cancer endpoints, despite the fact that non cancer endpoints were monitored.	We recommend that the EPA authors evaluate the original research reports to make this determination.	S

78	5.2.3	214	The net difference in RfD and RfC is 380-fold. This difference is hugely problematic and needs to be addressed, especially since the critical effects, as determined by EPA, are both systemic and reproductive. Specifically: <u>RfC vs RfD</u> RfC= $4.6x10^{-6}$ mg/m^3 per day Assume inhalation rate of $20m^3/d$ = $92x10^{-6}$ mg/d = $0.92x10^{-4}$ mg/d RfD = $5x10^{-4}$ mg/kg-d = $350x10^{-4}$ mg/d RfD/RfC (assuming equivalent absorption between routes) = 380	Consider the huge difference in RfD/Cs, which is not anticipated based on toxicology of BaP. Our initial analysis supports that the RfC is incorrectly derived and, as suggested in other comments, should be dropped.	S/M
79	5.2.3	215, line 8	UF_D may need to be 10-fold if a review of the database suggests that the available bioassays in two species are insufficient for monitoring noncancer effects.	If the RfC is retained, recommend revising the UF_D to a 10 to reflect the lack of standard bioassays in two species.	S/M
80	5.4.1.3	221, line 8	One of the principal arguments EPA makes for mutagenic mode of action is early tumor occurrence in multiple tissues. In contrast, the forestomach tumors do not occur in the expected linear fashion (e.g., Neil and Rigdon; Culp et al., 1998, page 122). Moreover, Kroese et al (2001, page 32) state that adducts may not	Several MOAs may be operating to cause the tumor response. Authors need to follow EPA guidelines and explore joint action.	S/M

			be enough for tumor formation. EPA needs to discuss these possibilities, and perhaps judge that multiple modes of action may be occurring at different dose response areas. EPA's guidelines (page 3-22) allow for this: "Both linear and nonlinear approaches may be used when there are multiple modes of action. If there are multiple tumor sites, one with a linear and another with a nonlinear mode of action, then the corresponding approach is used at each site. If there are multiple modes of action at a single tumor site, one linear and another nonlinear, then both approaches are used to decouple and consider the respective contributions of each mode of action in different dose ranges. For example, an agent can act predominantly through cytotoxicity at high doses and through mutagenicity at lower doses where cytotoxicity does not occur. Modeling to a low response level can be useful for estimating the response at doses where the high-dose mode of action would be less important."		
81	5.4.2.4-5.4.2.1	223-225	There are no quantitative mathematical errors in concept or execution with the oral slope factor, and the approaches seem consistent with EPA 2005 Cancer Guidelines and the EPA report on the time-to-tumor model. The statistical method for combining tumor sites assuming independence and summing MLEs seems appropriate and correctly calculated, including	No action needed.	S

			the variance estimates, and in accord with the EPA report on use of the multistage-Weibull model for time to tumor data. In addition, the separate SFs (per species/sex/tumor type) are presented. This follows EPA cancer guidelines recommendations for displaying alternatives to express uncertainty ranges.		
82	5.4.2.4	227, lines 27-32	The inhalation slope factor matches very nicely with that derived from oral exposures, unlike for the oral RfD and inhalation RfC.	No action needed.	S
83	5.4.3.3	235, line 17	Disagree with the relaxation of the p≥0.1 rule for choosing models. The multistage model should not be preferred if it does not fit the data. Further justification is needed.	EPA needs to explain/further justify this exception to EPA methods, especially since other models, with p values greater than 0.1, are available as the basis of the slope factor. These models would result in lower risk, perhaps as much as 9-fold.	S/M
84	5.4.3.3	236, line 8	Agree with the point about incomplete mortality; however, the bolus dosing of the experimental protocols needs to be discussed, since such dosing may actually serve to decrease this same risk. Defense mechanisms might be more easily overwhelmed with bolus dosing when compared to dietary exposures, especially at higher doses.	EPA needs to balance conflicting science issues. Recommend adding a discussion regarding potential impact of bolus dosing protocols on cancer risk calculations.	S
85	5.4.3.3	236, line 11	The selected study is Sivak et al. (1997), C3H/HeJ male mice. There is no figure or text output in Appendix F for that sex and strain. Fig. F-12 on p 393 has the right data but wrong figure caption/title. The animal should be C3H/HeJ male mice. The BMD and BMDL	EPA needs to correct these errors.	S

			values in the text do not agree with those in Appendix F: Table 5-12: BMD = 0.12; BMDL = 0.066 Fig. F-12: BMD =0.109; BMDL = 0.058		
86	5.4.3.3	238, line 16	In the 2005 supplemental guidance (EPA/630/R-03/003F), Figure 3 shows that the assumption of early-life susceptibility is to be made only after five previous steps were completed that involve evaluations of MOA information. EPA needs to follow their guidanc and show these previous steps in order to properly evaluate early-life susceptibility.	Authors need to follow EPA guidelines; evaluate the carcinogenic MOA fully before determining early-life susceptibility.	S/M
87	5.4.3.3	238, line 17	The oral slope factor is reported as 1 per mg/kg-day elsewhere; the dermal slope factor is shown as 0.005. Be consistent regarding the number of significant figures shown.	Assign appropriate precision to all wrought values, and then be consistent with summary statements.	S
88	5.4.3.3	238, line 19	We disagree with the use of ADAF. The Vesselinovitch et al (1975) study---not 1984---clearly shows that the older experimental animals are similarly, or perhaps even more, sensitive to stomach tumors than younger animals (Vesselinovitch et al., Table 5, page 2951; this EPA text page 195). This argues contrary to the use of an ADAF here, since EPA's slope factor is based on tumors of the alimentary canal, including stomach tumors. An ADAF factor might be needed if liver tumors were the basis of the EPA slope factor.	The data clearly show that oral tumors are not more prevalent in the young. We recommend that the ADAF for this tumor endpoint be removed from the document.	S/M

89	5.4.5	240, line 8	The studies selected for use in the calculations do not have any data support provided; i.e., they are stand-alone data sets. For the oral slope factor, there is one rat study, one mouse study. For inhalation unit risk, there is only the study on Syrian golden hamsters. A much better sense of uncertainty would exist if there were calculations on studies by different researchers: a second rat and mouse study for oral, a different species for inhalation. Using only a single study falls within EPA guidelines, but it is still statistically weak.	The text needs to state the weaknesses of using limited data.	S

Department of Defense Comments on
Benzoapyrene IASC draft charge to peer reviewers.pdf

Comments submitted by: Chemical Material Risk Management Directorate	Organization: Department of Defense	Date Submitted: 7/7/2011

*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.

Comment No.	Section	Pages	Comment	Suggested Action, Revision and References (if necessary)	*Category
1	General Charge Question 1	1	The question asks about the clarity of EPA's synthesis, but not about accuracy nor relevance.	While clarity and transparency are important, neither is useful if the analyses are not accurate and relevant. Please add accuracy and relevance to the charge question.	S/M
2	A.1	1	This question does not specifically highlight the issue of using a subchronic study when high quality, chronic studies are available.	DoD believes that this issue should be specifically teed-up for the reviewers.	S/M
3	A. 2-4	1-2	These questions assume that the reviewers will agree that EPA chose the correct study. If the reviewers agree with DoD that a chronic study should have been selected, EPA will not have information necessary for completion of the document.	After the first question, EPA should have a branched series of questions. One branch would continue with the existing set of questions. The second branch would ask the reviewers to identify the study and endpoint that they recommend that EPA use and which UFs would be applied. Without this information, EPA would not be able to say that selection of another study had been peer reviewed	S/M
4	B	2	Both oral and inhalation noncancer toxicity values for BaP are based on systemic	The external reviewers should be asked to consider the difference between oral and	S/M

			reproductive critical effects, yet there is an approximate 380 fold difference between the two values (after unit conversion).	inhalation noncancer values and whether EPA has appropriately evaluated the BaP noncancer effects, the noncancer MOA, and the associated uncertainties within the RfD and RfC derivation.	
5	B.1	2	Given the database used for the RfC development we believe it should be teed up for the external reviewers.	Please add to the second sentence of this question: "Please comment on the sufficiency of the inhalation database used to derive the RfC, the selection of this study as the principal study...."	S/M
6	C.1	2	DoD understands this document to be evaluating BaP alone, as another IRIS document evaluates PAHs. Therefore, we believe that the reviewers should specifically be asked whether this WOE judgment is based on BaP alone or on all PAHs.	EPA should separate this question and ask if the reviewers would agree with this WOE based on only data from BaP and then if they would agree based on all data provided in the document.	S/M
7	C.2	2	DoD suggests that EPA specifically ask its external reviewers to consider whether the evaluation of the mutagenic MOA data followed the "Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens" and is sufficient to apply an ADAF to the derivation of cancer slope factors.	Please add a sub-question beneath question 2, asking external reviewers to comment on whether EPA appropriately followed the "Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens" in evaluating the MOA and applying an ADAF for oral, inhalation and dermal cancer risk.	S/M
8	C. 3-12	3	The wording of these questions assumes that the reviewers agree that a linear extrapolation should be used. If the reviewers were to disagree, the cancer potency would be determined by an RfD, and all of the questions	In this set of charge questions and in future sets, DoD strongly recommends that EPA ask about the “cancer potency” rather than the “slope factor” to allow the questions to be relevant even if the reviewers do not agree with a linear, no	S/M

			regarding “slope factor” could not be answered reasonably as written. DoD mentions this issue because it was the subject of much discussion during the external peer review of hexavalent chromium where most of the reviewers did not believe that a linear-to-zero extrapolation was appropriate, but felt compelled to answer the question as asked, i.e., that the procedure was appropriately done even if it should not have been done.	threshold extrapolation – or if they believe (as has occurred in several recent reviews) that both should be presented.	