EPA's Response to Selected Major Interagency Comments on the Interagency Science Consultation Draft IRIS Toxicological Review of n-Butanol

September 8, 2011

Purpose:

The Integrated Risk Information System (IRIS) assessment development process of May 2009, includes two steps (Step 3 and 6) where White House offices and other federal agencies can comment on draft assessments. The following are EPA's responses to selected major interagency review comments received during the Interagency Science Consultation step (Step 3) for the draft IRIS Toxicological Review of n-Butanol (dated June 2011). All interagency comments provided were taken into consideration in revising the draft assessment prior to posting for public comment and external peer review. The complete set of interagency comments is attached as an appendix to this document.

For a complete description of the IRIS process, including Interagency Science Consultation, visit the IRIS website at www.epa.gov/iris.

June 2011 Interagency Science Consultation Draft IRIS Assessment—Selected Major Comments and Responses:

Topic #1: Critical Effect Description for the Reference Dose (RfD)

OMB Comment: In describing effects of Sitarek et al, EPA refers to visceral malformations. It would be helpful throughout the document to be clear that EPA means dilation of the lateral ventricle and of the third ventricle in the brain and of the subarachnoid space. Does Sitarek refer to this dilation as a 'visceral malformation' or is this EPA terminology? We suggest simply describing the effect whenever it is mentioned rather than grouping it into a class of malformations. It would also be helpful to discuss what the implications of this dilation are and why this endpoint is of concern. Page 63 states that this was the most sensitive toxicological effect, but there is no discussion of what it means or even if it is adverse. Discussion of what constitutes abnormal dilation could not be found in the document. We also note that Table 4-6, shows a NOAEL at 300 mg/kg-day for dilation of the unilateral and bilateral renal pelvis. Page 62 states that renal pelvis effects were seen at 300 mg/kg-day, but this is not consistent with findings presented in table 4-6.

> **EPA Response:** Sitarek et al. (1994) refer to the dilations as "visceral observations" to encompass dilation of the lateral ventricle and/or the third ventricle in the brain, the

subarachnoid space and dilation of renal pelvis. EPA used the term "visceral malformations" to encompass these and other developmental effects as described in Ema et al. (2005). The text has been checked to ensure that consistent terminology is used throughout the document, being explicit about the reported total versus individual organ dilation data where available.

EPA has augmented the text in the discussion of the critical effect to include more specific information regarding the adversity of the effect, including clarification of the discussion of the biological significance and human relevance of these brain dilation effects (Section 4.6.1 of the Toxicological Review).

Table 4-6 (now Table 4-4 in the external review draft) accurately presents the data reported in the Sitarek et al. (1994) study. The study authors reported a statistically significant increase in the percentage of litters with dilation at doses ≥ 300 mg/kg-day which incorporates both the brain and kidney dilations and was noted by EPA in Sections 4.3.1, 4.6.1, and 5.1.1 of the Toxicological Review. The study authors also reported, as shown in Table 4-4 of the Toxicological Review, a statistically significant increase in dilation of the subarachnoid space and lateral ventricle and/or third ventricle of the brain at ≥ 300 mg/kg-day and dilation of the unilateral and bilateral renal pelvis at 1000 mg/kg-day. EPA identified a LOAEL of 300 mg/kg-day based on the increased incidence of visceral malformations in the brain (dilation of the subarachnoid space and dilation of the lateral ventricle and/or third ventricle) from the Sitarek study (noted in the study summary in Section 4.3.1 and Table 4-13 of the Toxicological Review). EPA has checked the text throughout the document to ensure accuracy whenever the endpoints and doses are discussed.

Topic#2: BMD Modeling

OMB Comment: In appendix B it is not clear how EPA has done the BMD modeling. EPA appears to be grouping all the dilation changes together rather than modeling each endpoint separately when they clearly have different NOAEL/LOAEL values. We suggest that EPA calculate BMD/BMDL values for each endpoint separately rather than grouping them. It is not clear that providing "a better dose response estimate" should be the driver for combining unequal endpoints if such an approach is not supported by the data. Once data for each endpoint are presented, it would then be helpful to have a science based discussion about the appropriate endpoint to choose. It is likely that the overall values are being driven by the dilation in the brain

(since effects occurred here at lower doses compared to the kidney) however since the modeling is not presented for each endpoint, it is difficult to confirm this.

> **EPA Response:** EPA concluded that the developmental neurotoxicity endpoints (dilation of the subarachnoid space and dilation of the lateral ventricle and/or third ventricle of the brain) were the most sensitive and that the combined brain malformation data would provide a better estimate of the overall neurodevelopmental toxicity. However, the developmental neurotoxicity endpoints could not be modeled together because the data in the principal study (Sitarek et al., 1994) were presented as either total dilations (i.e., any fetus or litter identified as having dilation in the brain and/or renal pelvis) or presented as separate areas of dilation in the brain and kidney (i.e., subarachnoid space [brain], lateral ventricle and/or third ventricle [brain], unilateral renal pelvis [kidney], bilateral renal pelvis [kidney]). Because it is not possible to model the developmental neurotoxicity endpoints in combination, EPA has included the benchmark dose modeling of the individual brain dilation effects. This analysis indicated that the dilation of the lateral and/or third ventricle of the brain is the most sensitive effect, and was therefore selected as the critical effect for the derivation of the RfD. The selection of this endpoint is based on the EPA *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991) and is further discussed in Section 5.1. of the Toxicological Review. Additionally, Section 5.1.2 in the Toxicological Review has been revised to describe the modeling for the individual brain dilation effects and to further characterize the rationale for considering the individual brain dilation effects versus the total dilations.

Topic #3: Critical Effect Description for the Reference Concentration (RfC)

OMB Comment: Page B-1 (which should probably be correctly labeled page 68), states on line 32 that there were changes in neurological function. It would be helpful for EPA to be more explicit regarding what the actual effect was. Page 32 states: “There were dose- and duration-related increases in the percentage of rotarod test failures, indicating impaired neuromuscular function and learned avoidance behavior, in the rats.” Based on this, should the effect be changes in neuromuscular function and learned avoidance behavior? As EPA relies on this endpoint, more clarity is needed throughout the tox review and when describing the RfC. Similarly, it would be helpful to provide a discussion about what effect is expected to be seen in humans. Has rotarod testing in rodents been shown to be a predictor of learned avoidance behavior in humans or would something else be expected?

EPA Response: The changes in neurological function referred to on page B-1 (p68) are impairment of rotorod performance as conducted and reported by Korsak et al. (1994). Rotorod performance tests, in general, are designed to assess neuromuscular function; therefore, the changes in rotorod performance observed by Korsak et al. (1994) are considered to be indicative of impaired neuromuscular function. In the Korsak et al. (1994) study, prior to n-butanol exposure, all rats were trained on the rotorod task for 10 days which included learned avoidance behavior to discourage voluntary jumps from the rod. Neuromuscular function was then evaluated in the rotorod task following exposure to n-butanol. A decrease in neuromuscular function is considered adverse as per EPA's *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998). Additionally, this change in neurological function is considered to be relevant to humans. The selection of this critical effect is supported by other neurotoxic effects (e.g., decreased immobility in the swim despair test and decreased response) observed in acute animal studies at concentrations $\geq$1,420 mg/m^3 (Frantik et al., 1994; DeCeaurriz et al., 1983). This critical effect is further supported by other neurotoxic effects observed in occupationally-exposed humans (e.g., ataxia, hearing impairment and vertigo; Velazquez et al., 1969; Seitz, 1972). EPA has revised the text to be explicit about the critical effect throughout the document.

Appendix
Comments on the Interagency Science Consultation Draft
IRIS Toxicological Review of n-Butanol

Office of Management and Budget (OMB) Comments on the Interagency Science Consultation Draft IRIS Toxicological Review of n-Butanol (dated June 2011)

OMB Staff Working Comments on EPA's draft n-Butanol Toxicological Review (page numbers refer to the draft dated June 2011) **and Draft Charge to External Reviewers**
Aug 4, 2011

General Science Comments:

- Page 19, section 4.1.2.1 mentions that all the occupational studies had limitations. Consistent with NAS recommendations, it would be useful, throughout Section 4, if EPA provided clear criteria and protocols for the review of the data. Clear guidelines for study selection and exclusion would also be helpful. It seems that sometimes co-exposures are treated as confounders and an analysis of impacts is done; however, in other cases, co-exposures are used as a rationale to discount a study completely. Understanding EPAs criteria and approach to evaluating the studies would be useful as it is not clear exactly what design limitations caused EPA to eliminate studies.

- In describing effects of Sitarek et al, EPA refers to visceral malformations. It would be helpful throughout the document to be clear that EPA means dilation of the lateral ventricle and of the third ventricle in the brain and of the subarachnoid space. Does Sitarek refer to this dilation as a 'visceral malformation' or is this EPA terminology? We suggest simply describing the effect whenever it is mentioned rather than grouping it into a class of malformations. It would also be helpful to discuss what the implications of this dilation are and why this endpoint is of concern. Page 63 states that this was the most sensitive toxicological effect, but there is no discussion of what it means or even if it is adverse. Discussion of what constitutes abnormal dilation could not be found in the document. We also note that Table 4-6, shows a NOAEL at 300 mg/kg-day for dilation of the unilateral and bilateral renal pelvis. Page 62 states that renal pelvis effects were seen at 300 mg/kg-day, but this is not consistent with findings presented in table 4-6.

- Page 63, line 18, please use more detail to describe the neurotoxicological effects. As this is a critical endpoint, more discussion of the implications and whether or not they are adverse or precursors for some other effect is needed. It would also be helpful to have a table presenting the findings. Page 62 states that it is described in table 4-11, but the RTI study is not in that table. We don't see any substantive discussion of this study in Section 4. We suggest EPA correct this oversight and also include discussion of how they have ensured quality of this unpublished study.

- Page B-1 (which should probably be correctly labeled page 68), states on line 32 that there were changes in neurological function. It would be helpful for EPA to be more explicit regarding what the actual effect was. Page 32 states: "There were dose- and duration-related increases in the percentage of rotarod test failures, indicating impaired neuromuscular function and learned avoidance behavior, in the rats." Based on this, should the effect be changes in neuromuscular function and learned avoidance behavior? As EPA relies on this endpoint, more clarity is needed throughout the tox review and when describing the RfC. Similarly, it would be helpful to provide a discussion about what effect is expected to be seen in humans. Has rotarod testing in rodents been shown to be a predictor of learned avoidance behavior in humans or would something else be expected?

- Section 4.6.3, the mode of action discussion does not seem to be tied to the critical endpoints of concern (impaired neuromuscular function, learned avoidance behavior and dilation of lateral ventricle and subarachnoid space) for non cancer effects. We suggest adding a clear discussion regarding what is known, predicted, or expected linking mechanistic information to the chosen critical endpoints.

- Section 5.1.1, RfD derivation:
    - In determining the critical effect for the RfD, this section repeatedly states that renal pelvis effects were seen at 300 mg/kd-day. This is not consistent with the data presented in Table 4-6. Looking at this table, it does not support a similar BMDL value for dilation in brain and kidney, thus we have concerns about the characterization of the chosen critical effect.
    - In appendix B it is not clear how EPA has done the BMD modeling. EPA appears to be grouping all the dilation changes together rather than modeling each endpoint separately when they clearly have different NOAEL/LOAEL values. We suggest that EPA calculate BMD/BMDL values for each endpoint separately rather than grouping them. It is not clear that providing "a better dose response estimate" should be the driver for combining unequal endpoints if such an approach is not supported by the data. Once data for each endpoint are presented, it would then be helpful to have a science based discussion about the appropriate endpoint to choose. It is likely that the overall values are being driven by the dilation in the brain (since effects occurred here at lower doses compared to the kidney) however since the modeling is not presented for each endpoint, it is difficult to confirm this.
    - Page 73 mentions that this dilation "may be irreversible". Are there any data to support this? As this is the critical endpoint, further discussion of available data would be helpful. Additionally, EPA states "The functional significance of dilation of the lateral ventricles and subarachnoid space appears to be related to the rate and severity of the dilation, as well as the developmental stage at which it occurs (Weichert et al., 2010; Del Bigio,

2001).” EPA also notes that no information on severity was provided, however at higher doses authors referred to “frank hydrocephalus”. It would be helpful for EPA to add some discussion of exactly what the functional significance might be regarding the dilation in the brain seen at 300 mg/kg-day and why EPA considers this to be biologically significant as stated on page 73. A rational for this statement is necessary; without this discussion it is unclear as to why EPA is choosing this as a critical effect.

- When choosing a critical endpoint based on fetal effects, it is important to have a clear discussion regarding whether or not there was maternal toxicity. It would be helpful for EPA to have a discussion regarding what maternal endpoints were examined and what was seen. Table 4-13 states that there were no maternal effects, but it is not clear what was examined.
- Page 75, in discussing modeling, states “the combined data for the dilation incidences in the brain would provide a better estimate of the overall developmental toxicity associated with n-butanol since dilation of subarachnoid space and the lateral ventricle are reported precursors of hydrocephalus (Raimondi, 1994).” Does this mean that the critical effect is actually a precursor effect rather than an adverse effect? It would be helpful to clarify this.
- Page 76, in discussing the BMD modeling, instead of mentioning only how EPA chose the best fit based on AIC and inspection of plots, it would be helpful to have some discussion of the biological plausibility of each of the models considering the endpoint being examined.
- Page B-2, footnote e of table B-2, states that the log logistic model was the best fitting model and the only model with adequate fit. However EPA chooses to use the log-probit model. The rational for this is not clear.
- Page 77, it would be helpful to provide more discussion regarding EPA’s choice of the database uncertainty factor. Based on the available information and the developmental studies that are available, would EPA expect to see effects at lower doses in a multigenerational study or is it simply that EPA always applies a 3x factor for a missing multigenerational study? More clarity on the EPA criteria for applying this factor would be helpful.

- Section 5.2.3 RfC derivation:
  - Page 84 refers to the endpoint as “impaired motor coordination”. Is this the same as impaired neuromuscular function and learned avoidance behavior? We suggest that EPA use consistent terminology to describe the critical effect, being as specific as possible. EPA may want to consider asking the peer reviewers to comment on appropriate terminology to describe the rodent effects.
  - EPA applies an UF of 10 for subchronic to chronic. Looking at Table 4-5, it is not clear that there were statistically significant increases in rotarod

failures between 2 and 3 months. This suggests that perhaps there could be a plateau or learning occurring. Therefore, are there grounds to suggest that with longer exposure the failure count would be significantly worse? Further discussion of why 10x is justified, based on the available data would be helpful.

- It would be helpful to provide more discussion regarding EPA's choice of the database uncertainty factor. Based on the available information and the developmental and neurodevelopmental studies that are available, would EPA expect to see effects at lower doses in a multigenerational study or is it simply that EPA always applies a 3x factor for a missing multigenerational study? More clarity on the EPA criteria for applying this factor would be helpful.
- Considering that there are 3 orders of magnitude of uncertainty, how can the overall confidence in the RfC be anything but low? Page 93 states that it is low-medium.

- Section 5.3, appears to be more of a discussion of data limitations, rather than uncertainties. We suggest EPA add the uncertainty tables that have been provided in previous assessments and provide information and discussion regarding critical decision points EPA has made regarding species, sex, model choice, exposure assumptions, etc and how changes to these choices could impact the final derivations. It would also be helpful to provide readers any information that may exist regarding other existing cancer
  - On page 88, lines 26-37, it is not clear how this discussion of data gaps in the database tracks with the database uncertainty values that were chosen and noncancer values as determined by other government agencies or international bodies.

- The foreword of the tox review states that Section 6 is intended to convey the overall limitations of the assessment. We could not find any discussion of limitations in this section.

Editorial Comments (with Scientific Impacts):

- Page 5, line 3, suggest using plain language to describe the "vinous odor"

- Page 6, line 3, suggest using primary reference in place of HSBD. HSBD is simply a compendium of information.

- Chapter 2, it would be helpful to add information discussing what current exposure sources are and what current typical exposures are. This is important information which we have seen in other recent tox profiles. It would also be useful to have this information in Section 6 as well.

- Page 7, lines 6-14, it is not clear in these sentences which results are rodent and which are human. Suggest clarifying throughout so that there is no confusion for readers. Please also clarify that the Boman study was *in vitro*.

- Page 13, lines 6-14, from the description it is unclear how Deters measured liver toxicity and how it was proven that hepatotoxicity was not related to ADH metabolism.

- Page 15, line 24, is scaling to body weight to the ¼ standard or was this simply done for model fitting purposes? Please clarify.

- Page 30, lines 19-20, please use primary citations in place of EPA 1988 and HSBD.

- Page 41, line 16, please clarify that this is an EPA determination.

- Page 46, since neurotoxicity is a key endpoint, it would be helpful to have a table arraying the different studies, dose levels, endpoints and effects seen.

- Table 4-12, states that is provides study details in support of the MOA for neurological effects. However, it is not clear where we can find a discussion of these studies on the MOA for neurological effects. Some discussion of the doses in the mechanistic studies and how this relates to dose levels where neurological effects are seen would be helpful. Since EPA also considers studies looking at dilation in the brain, it would also be helpful to have some discussion of how mechanistic data provide information to support a MOA leading to these effects.

- Figures 5-1 and 5-2, while these visual plots are helpful, it would also be very helpful to see visual plots of the candidate studies after the UF's are applied. The application of UFs can certainly change how the candidate studies relate to each other and thus carrying through each of these candidates to derive a possible RfC/RfD (including UFs) would be useful for reviewers. Perhaps a second set of tables showing this would be most helpful.

Comments on the Draft Charge:

[Note: some suggestions for charge questions are provided in comments in the above sections. Many of those comments have not been reiterated here, but should be considered as equally important.]

- General Questions 2: It is unclear how reviewers will be able to determine if additional studies "would have a significant impact on the conclusions." Suggest reframing this to simply ask about relevant studies and then EPA can conduct further evaluation to determine if the studies will have a significant impact.

- A2, please clarify if the critical affect is adverse or a precursor and ask reviewers to comment on this. Please also clarify that the endpoint is dilation of the lateral ventricle and subarachnoid space rather than using the broad, more poorly defined term ‘visceral malformations’. This change should be made throughout the charge.

- A3, please add a specific question regarding the appropriateness of EPA grouping all dilation effects together for the purposes of BMD modeling. To inform reviewers’ decision, EPA should present BMD results for each organ separately.

- B2, please clarify that the endpoint is impaired neuromuscular function and learned avoidance behavior rather than using the broader term of ‘neurobehavioral function’. Please also ask the reviewers to comment on whether this effect is a precursor or is adverse. This change should be made throughout the charge.

- Section B, please also add a question asking for comments on using the AUC for dosimetry.

Agency for Toxic Substances and Disease Registry (ATSDR) Comments on the Interagency Science Consultation Draft IRIS Toxicological Review of n-Butanol (dated June 2011)

Date: July 14, 2011

From: Agency for Toxic Substances and Disease Registry

Subject: Comments on EPA's Toxicological Review of n-Butanol

To: Environmental Protection Agency

We appreciate the opportunity to review EPA's Toxicological Review of n-Butanol. Overall, this document is well-written, concise, and makes reasonable assertions based on the literature. The document reads well and the flow of information is logical, with the pertinent studies laid out in a fluid manner.

The use of Benchmark Dose (BMD) modeling methods for the RfD was explicitly described for understanding and appropriately used to derive the point of departure (POD) for the present RfD.

The use of a NOAEL for RfC derivation is appropriate. The UF of 1000 seems high, but is explained very well in the document.

There is obviously a "data need" for carcinogenicity data. A quantitative estimate of the cancer potential of this substance could not be derived based on the unavailability of data.

Editorial Comments:

- Page 19, Line 30: The "e" has been left off of the word **exposure.**

- Page 39, Line 3: There should be double-spacing after the period, before the sentence "According to McLain (2008)…"

- Page 41, Line 1: Should there be a hyphen between "mid concentration"; Should there be a space between 1 st indicating 1st?