UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C. 20460

OFFICE OF THE ADMINISTRATOR
SCIENCE ADVISORY BOARD

August 26, 2011

EPA-SAB-011-014

The Honorable Lisa P. Jackson
Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, D.C. 20460

Subject: SAB Review of EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments (May 2010)

Dear Administrator Jackson:

EPA's Office of Research and Development (ORD) requested that the Science Advisory Board (SAB) review the Agency's draft report entitled *EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments (May 2010)* ("Report"). The Report contains EPA's technical response to key comments in the 2006 National Academy of Sciences (NAS) report, *Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment.* The NAS reviewed EPA's 2003 exposure and human health reassessment of dioxin and recommended that the Agency should: more thoroughly justify and communicate its approaches to dose-response modeling for the health effects of dioxin, taking into consideration both nonlinear and linear methods for characterizing cancer risk; improve the transparency and clarity of the selection of key data sets for the dioxin dose-response analysis; reevaluate its cancer weight-of-evidence determination for dioxin based on the Agency's *2005 Guidelines for Carcinogen Risk Assessment*; consider using physiologically-based pharmacokinetic (PBPK) modeling in the dioxin risk assessment; and improve transparency, thoroughness and clarity in quantitative uncertainty analysis. The NAS also encouraged EPA to calculate a reference dose (RfD), which had not been derived in the 2003 reassessment.

In response to EPA's request, the SAB convened an expert panel to review the Agency's Report. The SAB Panel was asked to comment on the scientific soundness of EPA's responses to the NAS recommendations. The enclosed SAB report provides the consensus advice and recommendations of the Panel, with the exception of one member who offered a dissenting opinion mainly on 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) carcinogenicity.

The SAB finds that EPA's Report is generally clear, logical, and responsive to many but not all of the recommendations of the NAS. We have provided recommendations to further enhance the transparency, clarity, and scientific integrity of the Report. The SAB has identified deficiencies in EPA's Report with respect to the completeness of its consideration of two critical elements of the TCDD assessment: 1)

SDMS DocID 492980

nonlinear dose-response for TCDD carcinogenicity, and 2) uncertainty analysis of TCDD toxicity. Our major comments and recommendations are provided below:

- The SAB commends EPA for the comprehensive and rigorous process that was used to identify, review, and evaluate the TCDD literature. The criteria for study selection have been clearly articulated, well justified, and applied in a scientifically sound manner. To further improve clarity and transparency of the Report, we recommend that EPA include a better means of tracking and describing which studies did not satisfy inclusion criteria. Similarly, we recommend that EPA strengthen its justification for excluding studies of dioxin-like compounds. The Report can be enhanced by incorporating information from studies with dioxin-like compounds into a qualitative discussion of the weight-of-evidence for cancer and noncancer endpoints.

- EPA used the Emond physiologically-based pharmacokinetic model to evaluate the internal dose of TCDD in human and rodent tissue, and to estimate the continuous daily TCDD intake over the relevant period of exposure. The SAB agrees with EPA that this model provides the best available basis for the dose metric calculations. We also support EPA's use of blood TCDD concentrations as the relevant dose metric. However, we recommend that EPA expand the discussion of other published models, evaluate the impact of model selection on dose metric prediction, provide a more quantitative uncertainty analysis, and conduct an external peer review of the mouse model because it has not been published in the peer-reviewed literature.

- The SAB agrees with EPA's characterization of TCDD as carcinogenic to humans in accordance with EPA's *2005 Guidelines for Carcinogen Risk Assessment*. The SAB recommends that in the weight-of-evidence characterization EPA build upon all available data to support its decision and clearly indicate how different types of data support each other. One dissenting Panel member, however, expressed the view that at best, there is equivocal evidence for TCDD classification as a human carcinogen.

- The SAB agrees with EPA's selection of the Cheng et al. (2006) study, which analyzed the National Institute for Occupational Safety and Health (NIOSH) occupational cohort, as the critical study for the quantitative cancer assessment. The SAB also agrees that it is appropriate to use all-cancer mortality as the basis of the oral slope factor because of the extensive dose-response information and because in the case of TCDD there appear to be multiple targets for carcinogenic action.

- The SAB finds that the Report did not respond adequately to the NAS recommendation to adopt both linear and nonlinear methods of extrapolation in order to account for the uncertainty of the dose-response curve for TCDD. The Report states that only a linear approach could be justified. We recommend that EPA revise the Report to provide a discussion of evidence of possible modes of action that include both linear and nonlinear alternatives for the cancer endpoint. In the absence of a definitive nonlinear mode of action, estimates based on the linear option can serve as the baseline for comparison with other estimates.

- The SAB supports EPA's selection and use of two co-critical epidemiologic studies for the derivation of the RfD for TCDD. These studies evaluated the effects of human exposure to TCDD following accidental release at a chemical plant near Seveso, Italy. The SAB finds that the study endpoints used by EPA to determine the RfD (decrease in sperm count and motility and increased thyroid stimulating hormone in blood) are relevant to public health. The selection of

these endpoints also resolves the critical issue of differing windows of susceptibility to environmental toxic agents over the course of the life cycle, with pre- and periconceptional exposures comprising the window of greatest susceptibility. We recommend, however, that EPA provide a discussion of the strengths and weaknesses of the studies and an indication of whether these weaknesses affect the RfD determination. The SAB agrees with the benchmark dose modeling approaches used by EPA in the Report and the decision to use human data as preferred to animal data for the RfD determination.

- EPA's Report discusses a broad range of philosophical and methodological issues to be considered in conducting an uncertainty analysis for TCDD toxicity. The SAB acknowledges the challenges of a unified quantitative uncertainty analysis. However, we do not agree with the position taken in the Report that such an analysis is unfeasible and we have suggested a number of methods that could be used for this purpose.

- Finally, EPA's Report could be improved by editing and restructuring to better integrate the material presented in various sections, eliminate redundancies, and move some material into appendices to provide more succinct responses to NAS concerns. In addition, we recommend including a glossary in the Report to help minimize confusion and misinterpretation among diverse users.

The SAB appreciates the opportunity to provide EPA with advice on this important subject. EPA should move in a proficient and expeditious manner to finalize the IRIS document for dioxin. We look forward to receiving the Agency's response.

Sincerely,

/Signed/

Dr. Deborah L. Swackhamer, Chair
EPA Science Advisory Board

/Signed/

Dr. Timothy J. Buckley, Chair
SAB Dioxin Review Panel

Enclosure

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[*] Did not participate in the discussion of the charge questions specifically pertaining to use of the Mocarelli et al. (2008) study in deriving the reference dose.

[**] Dissenting opinion in Appendix B.

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ABBREVIATIONS AND ACRONYMS

AhR	aryl hydrocarbon receptor
BMD	benchmark dose
BMDL	benchmark dose lower bound
BMR	benchmark response level
CYP	cytochrome P450
DLC	dioxin-like compound
ED	effective dose
EPA	U.S. Environmental Protection Agency
HED	human equivalent dose
IRIS	integrated risk information system
LASC	lipid-adjusted serum concentrations
LOAEL	lowest-observed-adverse-effect level
MOA	mode of action
NAS	National Academy of Sciences
NHEERL	National Health and Environmental Effects Research Laboratory
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
OSF	oral slope factor
PBPK	physiologically-based pharmacokinetic
PCDDs	polychlorinated dibenzo-*p*-dioxin
PCDFs	polychlorinated dibenzofuran
POD	point of departure
RfD	reference dose
RR	relative risk
SAB	Science Advisory Board
T3	triiodothyronine
T4	thyroxine
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TEF	toxicity equivalence factor
TEQ	toxicity equivalence
TSH	thyroid stimulating hormone
UF	uncertainty factor
WHO	World Health Organization

1. EXECUTIVE SUMMARY

In 2003, EPA, along with other federal agencies, asked the National Academy of Sciences (NAS) to review aspects of the science in EPA's draft dioxin reassessment entitled, *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds* and, in 2004, EPA sent the 2003 draft dioxin reassessment to the NAS for review. In 2006, the NAS released the report of its review entitled, *Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment*. The NAS recommended that EPA should: more thoroughly justify and communicate its approaches to dose-response modeling for the health effects of dioxin, taking into consideration both nonlinear and linear methods for characterizing cancer risk; improve the transparency and clarity of the selection of key data sets for the dioxin dose-response analysis; reevaluate its cancer weight-of-evidence determination for dioxin based on the Agency's *2005 Guidelines for Carcinogen Risk Assessment*; consider using physiologically-based pharmacokinetic (PBPK) modeling in the dioxin risk assessment; and improve transparency, thoroughness and clarity in quantitative uncertainty analysis. The NAS also encouraged EPA to calculate a reference dose (RfD), which had not been derived in the 2003 reassessment.

EPA's Office of Research and Development (ORD) prepared the draft report, entitled *EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments (May 2010)* (EPA, 2010) (hereafter referred to as the *Report*), and requested that the EPA Science Advisory Board (SAB) conduct an independent external peer review of the *Report*. This Executive Summary highlights the findings and recommendations of the SAB Dioxin Review Panel (the "Panel") in response to charge questions concerning each of the six sections of the *Report*. EPA's charge questions are provided in Appendix A.

General Charge *(Charge Questions 1.1 - 1.2)*

EPA asked the SAB to comment on whether the *Report* is clear and logical and whether there are other critical studies that would make a significant impact on the conclusions of the hazard characterization or dose-response assessment of the chronic noncancer and cancer health effects of TCDD.

As further discussed in the responses to the charge questions in Section 3.1 of this report, the SAB finds that the *Report* is generally clear, logical, and responsive to many but not all of the recommendations of the NAS. The SAB has, however, provided many recommendations to further improve the clarity, organization, and responsiveness of various parts of the *Report*. The SAB was impressed with the process that EPA used to identify, review, and evaluate the relevant literature. The SAB finds that EPA's process was comprehensive and rigorous and included public participation. However, the SAB recommends that the *Report* be improved by: incorporating text to better integrate the material presented in the individual chapters, providing greater clarity and transparency in indicating which studies do not satisfy criteria for inclusion in EPA's assessment of TCDD, and editing the *Report* to provide greater clarity in writing and make it more concise by moving some material into appendices.

During its deliberation, the SAB did not identify any additional studies that would make a significant impact on the conclusions of the hazard characterization and dose-response assessment. The SAB recommends that EPA provide more discussion and clarity concerning the exclusion of null epidemiologic studies. In addition, as further discussed in the responses to the relevant charge questions, the SAB has identified deficiencies in the *Report* with respect to the completeness of its consideration of

two critical elements: 1) nonlinear dose-response for TCDD carcinogenicity, and 2) uncertainty analysis. As discussed below, the SAB has provided recommendations to improve the *Report* in these areas.

Transparency and Clarity in the Selection of Key Data Sets for Dose-Response Analyses *(Charge Questions 2.1 – 2.3)*

The NAS recommended that EPA develop a clear and readily understandable methodology for evaluating and including epidemiologic and animal bioassay data sets in dose-response evaluations. The SAB Panel was asked to comment on whether EPA had been responsive to NAS concerns and had applied epidemiology and animal bioassay study criteria considerations in a scientifically sound manner.

Section 2 of the *Report* contains a clear presentation of the process EPA used to select key data sets for dose-response analysis and is thus responsive to NAS recommendations in this area. The *Report* also clearly identifies the studies that were used for dose-response analysis. However, the SAB has provided recommendations to further enhance the overall clarity and transparency of Section 2 of the *Report*. The SAB recommends careful and extensive editing to revise and consolidate Section 2.

The SAB also finds that EPA's epidemiology and animal bioassay study criteria and considerations are scientifically justified, clearly described, and applied in a scientifically sound manner. However, the SAB has provided recommendations to improve and strengthen the scientific justification and clarity of description of EPA's study criteria and considerations. EPA should provide better justification of the rationale for using only studies where the exposure was primarily to TCDD for derivation of the reference dose. This justification should include both scientific and practical reasons. EPA should incorporate information from studies with dioxin-like compounds (DLCs) into a qualitative discussion of the weight-of-evidence for cancer and noncancer endpoints. There is a wealth of literature on these compounds that can be used to support mode of action determination as well as the plausibility of individual biological effects that are used as the basis for risk assessment. In addition, the SAB has provided a number of specific recommendations to further clarify the justifications for some of the study inclusion and exclusion criteria.

Use of Toxicokinetics in Dose-Response Modeling for Cancer and Noncancer Endpoints *(Charge Questions 3.1 – 3.5)*

Section 3 of EPA's *Report* discusses the use of a physiologically-based pharmacokinetic (PBPK) model (Emond et al., 2004, 2005, 2006) with blood concentration as the dose metric rather than first-order body burden. EPA asked the SAB to comment on the scientific justification for application of this model. EPA also developed a PBPK model to estimate TCDD concentration in mouse tissues. The SAB was asked to comment on the scientific rationale for development of the mouse model and the performance of the model.

The SAB agrees with EPA's use of blood TCDD concentration as a surrogate for tissue exposure to TCDD. Blood TCDD concentration is a better choice than using body burden (as in the 2003 Reassessment) because it is more closely related to the biologically relevant dose metric: the free concentration of dioxin in the target tissues. It is important to recognize, however, that TCDD distribution within tissues such as the liver can be non-uniform. The SAB further agrees that the PBPK model developed by Emond et al. (2004, 2005, 2006) provides the best available basis for the dose metric calculations in the assessment. However, EPA should clarify how the model was used in studies

that reported the concentrations of dioxin in plasma, serum, blood, or blood fat:blood measurements. The SAB also recommends additional discussion of: other published models, the intended use of the Emond model in the assessment, and the basis for selection of the Emond model. The EPA modifications to the published Emond model are minor and appropriate. However, the use of 0.6 as the Hill coefficient in the Emond model for CYP1a2 induction is well outside the confidence interval of 0.78 and 1.14 reported by Walker et al. (1999). The use of a Hill coefficient value well below unity would lead to a nonlinear model behavior that is biologically implausible. As a result, when the human model was used for extrapolation to lower doses (in the calculation of risk-specific doses), the model would tend to estimate a lower exposure level for a given blood concentration. The SAB recommends repeating the human Emond model calculations with multiple values for the Hill coefficient to characterize the resulting uncertainty in the exposure estimates.

A more quantitative uncertainty analysis needs to be conducted for the PBPK model. Methods that could be useful and informative for such analysis are suggested in the response to Charge Question 6.2. The sensitivity analysis in the *Report* left out the Hill coefficient, which is one of the most important parameters in the model for low-dose extrapolation. Model sensitivities are species, dose, and dose-scenario dependent, so they need to be determined under the same exposure conditions that dose metrics are calculated.

The mouse model developed by EPA based on the published rat model (Emond et al., 2004, 2005, 2006) is appropriate. However, EPA should conduct an external peer review of the mouse model. The SAB agrees with the average daily dose calculation approaches described in the *Report*. However, the EPA should carefully explain how the early life stage internal doses were calculated because serum thyroid stimulating hormone (TSH) levels in newborns are used as a critical effect.

Reference Dose *(Charge Questions 4.1 – 4.8)*

In Section 4 of the *Report* EPA discusses the use of two co-critical studies (Mocarelli et al., 2008 and Baccarelli et al., 2008) for development of the reference dose for TCDD. EPA asked the SAB to comment on the scientific justification for selection and use of these studies to develop the reference dose.

Selection of Critical Studies and Effects

The SAB supports EPA's selection of the Mocarelli et al. (2008) and Baccarelli et al. (2008) studies for identifying "co-critical" effects for the derivation of the reference dose (RfD). These two human epidemiological studies are well designed and provide sufficient exposure information, including biological concentrations that could be used to establish acceptable lifetime daily exposure levels. The rationale for selecting these two studies over numerous other available studies for determining the RfD is clearly described but study weaknesses are not clearly delineated. The SAB recommends that EPA provide a discussion of the strengths and weaknesses of these studies with an indication of whether the weaknesses affect determination of the RfD. In addition, EPA should make use of the comprehensive data base of both animal and human epidemiological studies to demonstrate a consistent and integrative signal of toxicity across species and endpoints for TCDD. The coherence of evidence provided by the studies should be made stronger in the *Report* by including discussion of both human and experimental animal studies that have examined the effects of dioxin and DLCs on other reproductive and endocrine endpoints. In this regard, dose-response relationships as well as comparisons of no-observed-adverse-

effect levels (NOAELs) and lowest-observed-adverse-effect levels (LOAELs) should be discussed. With respect to TCDD, the SAB agrees with EPA's assertion that traditional (e.g., immune, endocrine, reproductive) endpoints are more appropriate than biochemical endpoints for establishing points of departure (PODs). The associations of traditional endpoints with health outcomes have been well studied and they are more tightly associated with adverse outcomes than biochemical endpoints. However, because of the wealth of data on P450s and their importance in chemical response to exogenous agents, EPA should discuss biochemical endpoints relevant to establishing and strengthening the proposed reference dose.

Estimation of Continuous Exposure for Mocarelli et al. (2008)

Mocarelli et al. (2008) reported male reproductive effects (decrease in sperm count and motility) observed later in life for boys with high acute exposure to TCDD between the ages of 1 and 9 (average age 5 years), followed by low level background dietary exposure. EPA identified a 10 year critical exposure window and estimated the continuous TCDD intake as the average of the high acute exposure and the 5 year average exposure during the critical exposure window. The SAB finds that the pattern of exposure in the study at the city of Seveso (high acute exposure) posed some extrapolation issues for the EPA, particularly whether the same endpoints and or dose-response from high acute exposures would be expected when extrapolating to low-dose chronic exposures. It would be useful for EPA to provide a discussion of published examples in which dioxin studies were conducted using both high-dose acute and low-dose chronic exposures in animals for the same endpoint, and how the outcomes compare both qualitatively and quantitatively. The life stage-specific approach to hazard and dose-response characterization for children's health risk assessment in EPA's *Framework for Assessing Health Risks of Environmental Exposures to Children* (EPA, 2006) is also relevant to addressing this issue and should be discussed.

Designation of a 20% Decrease in Sperm Count as a LOAEL for Mocarelli et al. (2008)

The SAB supports the use of the designated change from normal sperm counts and sperm motility for determining an RfD. While the shifts observed in sperm counts may or may not pose a significant health effect in a single individual, such shifts on a population basis could presumably lead to an increased incidence of adverse health outcomes. The SAB recommends that World Health Organization (WHO) reference values for male reproductive parameters and life stage differences in sperm counts in humans be discussed in the *Report*.

Determination of Effective Exposure Estimate for the Baccarelli et al.(2008) Study

EPA determined the maternal intake at the LOAEL from the maternal serum-TCDD vs. neonatal TSH regression model by finding the maternal TCDD lipid-adjusted serum concentrations (LASC) at which neonatal TSH exceeded 5 µ-units/ml (µU/ml). EPA then used the Emond PBPK model under the human gestational scenario to estimate the continuous daily oral TCDD intake that would result in a TCDD LASC corresponding to a neonatal TSH of 5 µU/ml at the end of gestation. EPA estimated the effective maternal intake as 0.024 ng/kg-day. The SAB supports EPA's decision to use the Baccarelli et al. (2008) estimates of the relevant effective doses. However, EPA should clarify how these measurements relate to ranges and variations in exposure *in utero*.

Designation of 5µ-U TSH per ml blood as a LOAEL for Baccarelli et al. (2008)

EPA selected a LOAEL of 5µU TSH per ml blood in neonates. The SAB supports EPA's designation of the TSH endpoint within the context of the broader dioxin literature. While the shift observed in TSH levels may or may not pose a significant health effect in a single individual, such a shift on a population basis could presumably lead to an increased incidence of adverse health outcomes. There is a need to better describe the potential adverse health outcomes related to altered neonatal TSH levels. For example, in addition to effects on growth, both cognitive and motor deficits have been found in young adults with congenital hypothyroidism (Oerbeck et al., 2003; Oerbeck et al., 2007). The *Report* could better describe the consequences of transient hypothyroidism on reproductive outcomes (Anbalagan et al., 2010).

Selection of Uncertainty Factors

A composite uncertainty factor of 30 (an uncertainty factor of 10 for the lack of a NOAEL, and an uncertainty factor of 3 for human interindividual variability) was applied to the LOAEL of 0.020 ng/kg-day from Mocarelli et al. (2008) to obtain the RfD. The SAB agrees that EPA has used the appropriate uncertainty factors for the derivation of the RfD. However, a discussion of the decision not to include an uncertainty factor for data quality is needed.

Benchmark Dose (BMD) Modeling of animal bioassay data and EPA's Choice of POD from These Studies

The SAB supports the use of the BMD modeling approaches and agrees that the animal data have sufficient limitations that preclude their use to establish a RfD.

Cancer Assessment *(Charge Questions 5.1 – 5.8)*

In Section 5 of the *Report*, EPA has provided: a weight-of-evidence characterization of TCDD as a known human carcinogen, conclusions regarding the mode of carcinogenic action for TCDD, EPA's selection of data sets for cancer dose-response modeling, and consideration of approaches for assessment of TCDD carcinogenicity. The SAB was asked to comment on the scientific soundness of these aspects of EPA's cancer assessment.

Weight-of-Evidence Cancer Descriptor

The SAB agrees with EPA's conclusion that TCDD is "Carcinogenic to Humans." However, one Panel member expressed a dissenting view that at best, there is equivocal evidence for the carcinogenicity of TCDD in the occupational setting where body burdens were much higher than current or previous background levels (see dissenting opinion in Appendix B). The SAB recommends that the Agency provide more discussion of the power of the studies used and the difficulties involved when assessing rare tumors. EPA should consider including studies with substantial DLC exposure where toxicity equivalence factors (TEFs) can be calculated in the weight-of-evidence discussion. EPA should also attempt to characterize the uncertainty regarding the carcinogenicity of TCDD at low human exposures, since the minimum dose at which carcinogenic effects would be expected to occur cannot be clearly

delineated from the current epidemiological human data. EPA has concluded that AhR activation is a necessary but not sufficient precursor event in the carcinogenic activity of TCDD. Therefore, it would be beneficial if the Agency could evaluate available data on AhR activation and related effects in human cells and animal models to help inform the doses at which these precursor events are observed for comparison to the epidemiological data.

Mode of Action

The SAB asserts that the mode of action for TCDD toxicity should be "reasonably well known" rather than "largely unknown," although the exact mechanism of action has not been fully delineated for any distinct TCDD toxicity endpoint. EPA should provide a discussion of the evidence for possible modes of action that include both linear and nonlinear alternatives, and the description of the nature of a receptor mediated dose-response should be expanded by including more evidence regarding the nonlinearity of the receptor mediated dose-response for dioxin.

Selection of Critical Study for Cancer Endpoint

The SAB supports the use of the Cheng et al. (2006) study for quantitative cancer assessment. This study incorporated information on gradation of exposure. However, expanded discussion of several other studies would support the weight-of-evidence for carcinogenicity in less common cancers such as lymphomas and soft tissue sarcoma. It is appropriate to use all-cancer mortality for the assessment because TCDD appears to have multiple targets for carcinogenic action and because of the extensive dose-response information. The SAB agrees that the use of the Emond model to estimate risk-specific doses from Cheng et al. (2006) dose-response modeling results is scientifically justified and clearly described. However the value of the Hill coefficient used in the model is problematic. The Cheng et al. (2006) study did not provide completely clear information regarding risks below current background exposure levels. The SAB therefore suggests that EPA expand the discussion to consider the possibility that mode of action considerations could help indicate whether linear extrapolation of the Cheng et al. (2006) data is appropriate to obtain risk estimates in this range of exposures.

Nonlinear Approach for Assessment of TCDD Carcinogenicity

The SAB finds that the *Report* did not respond adequately to the NAS recommendation to adopt "both linear and nonlinear methods of risk characterization to account for the uncertainty of dose-response relationship shape below the ED01." EPA should present both linear and nonlinear risk assessment approaches. In the absence of a definitive nonlinear mode of action, the linear option results can serve as the baseline for comparison with other estimates. The examples in the current document should be formalized and extended to allow for such a comparison.

Quantitative Uncertainty Analysis *(Charge Questions 6.1 – 6.3)*

Section 6 of the *Report* discusses a broad range of philosophical and methodological issues to be considered in conducting an uncertainty analysis for TCDD toxicity. The SAB was asked to comment on the scientific soundness of this section of the *Report.*

Section 6 of EPA's *Report* provides many useful insights for EPA's dioxin reassessment, but it is not scientifically justified. As further discussed in the responses to the charge questions in Section 3.6 of this

report, the SAB does not agree with EPA's argument that conducting a unified quantitative uncertainty analysis for TCDD toxicity is unfeasible. EPA's decision to not conduct an integrated quantitative uncertainty analysis may be based primarily on grounds of practicality or timeliness. In particular, EPA argues that a complete quantitative uncertainty analysis would require data and resources not available. The SAB disagrees with this logic. While EPA may lack an adequate empirical basis for full Monte-Carlo propagation of input distributions, there are other options available. More limited evaluations can, and should, be implemented to inform critical issues in the dioxin reassessment. In the response to Charge Question 6.2 the SAB offers a number of methods that could be used. The SAB recommends that EPA revise its argument that quantitative uncertainty analysis for dioxin toxicity is unfeasible.

EPA's document contrasted volitional uncertainty with cognitive uncertainty. The SAB recommends that the term "volitional uncertainty," which might also have been called "decisional uncertainty," be dropped from the Agency's document. EPA should display different modeling choices and the consequences of making them. It is recommended that EPA apply standard tools and techniques for analysis of model uncertainty.

In addition, the SAB finds that the sensitivity studies EPA has already completed (e.g., sensitivity analysis and uncertainty analysis of dose metrics derived for the risk assessment) are useful. While there is a need to minimize further delay of the finalization of EPA's dioxin assessment, the SAB recommends that these completed sensitivity studies be integrated into whatever overall uncertainty analysis the Agency elects to undertake.

2. INTRODUCTION

EPA has been preparing an assessment of the potential health impacts of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) for many years. In 2003, EPA released an external review draft report entitled, *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds* (U.S. EPA, 2003) (hereafter referred to as the *2003 Reassessment*). The EPA Science Advisory Board (SAB) had previously reviewed the Agency's draft *Reassessment* in 1995 and 2000 (EPA SAB, 1995, 2001), and the 2003 *Reassessment* was reviewed by the National Academy of Sciences (NAS). In 2006, the National Research Council (NRC) of the National Academies published their evaluation of EPA's reassessment, *Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment* (NRC, 2006).

The NAS identified key recommendations that they believed would result in substantial improvement to the *2003 Reassessment* and thus support a scientifically robust characterization of human responses to exposures to TCDD. The NAS recommended that EPA should: more thoroughly justify and communicate its approaches to dose-response modeling for the health effects of dioxin, taking into consideration both nonlinear and linear methods for characterizing cancer risk; improve the transparency and clarity of the selection of key data sets for the dioxin dose-response analysis; reevaluate its cancer weight-of-evidence determination for dioxin based on the Agency's *2005 Guidelines for Carcinogen Risk Assessment*; consider using physiologically-based pharmacokinetic (PBPK) modeling in the dioxin risk assessment; and improve transparency, thoroughness and clarity in quantitative uncertainty analysis. The NAS also encouraged EPA to calculate a reference dose (RfD), which had not been derived in the *2003 Reassessment*.

In 2010, EPA's Office of Research and Development (ORD) prepared the draft report, *EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Responses to NAS Comments* (EPA, 2010) (hereafter referred to as the *Report*) and requested that the EPA Science Advisory Board (SAB) conduct an independent external peer review of the draft document. In its review, the SAB was asked to consider the accuracy, objectivity, and transparency of EPA's reanalysis and responses to the NAS.

In response to ORD's request, the SAB convened an expert panel to conduct the review. The Panel held an initial public teleconference on June 24, 2010 to receive an orientation to EPA's *Report*. The Panel then held two public face-to-face meetings (July 13 – 15, 2010 and October 27 – 29, 2010) to deliberate on the charge questions (see Appendix A) and two public teleconferences (March 1 and 2, 2011) to discuss its draft report. The SAB Panel's draft report was considered and approved by the chartered SAB on a public teleconference call on June 6, 2011. Oral and written public comments were considered throughout the advisory process.

EPA asked the SAB to specifically focus its review on six sections of the dioxin assessment. The charge questions (provided in Appendix A) focused on: transparency and clarity in the selection of key data sets for dose-response analysis, the use of physiologically-based pharmacokinetic (PBPK) modeling in dose-response modeling for cancer and noncancer endpoints, derivation of a proposed oral reference dose (RfD) for noncancer endpoints, cancer weight-of-evidence classification, mode of action of dioxin carcinogenicity, derivation of oral slope factor (OSF) for dioxin, and quantitative uncertainty analysis. This SAB report provides the consensus advice and recommendations of the Panel, with the exception of one member who offered a dissenting opinion mainly on the TCDD carcinogenicity (see Appendix B).

3. RESPONSES TO EPA'S CHARGE QUESTIONS

3.1. General Charge Questions

The Panel was asked to comment on: whether the EPA's *Report* was clear and logical, whether the Agency had objectively and clearly presented the key National Academy of Sciences (NAS) recommendations, and whether there were other critical studies that would make a significant impact on the conclusions of the hazard characterization or dose-response assessment of the chronic noncancer and cancer health effects of TCDD.

> *Charge Question 1.1. Is the draft Response to Comments clear and logical? Has EPA objectively and clearly presented the three key NRC recommendations?*

Response:

Although the Panel has provided some editorial suggestions to improve the clarity of EPA's *Report*, in general the Panel finds that the Agency has developed a report that is clear, logical and responsive to many but not all of the recommendations of the NAS. While we provide a general assessment of opportunities for improvement in the context of this overview charge question, most of the issues related to clarity, organization, and responsiveness are addressed more completely and specifically in the context of the subsequent, more specific charge questions.

With respect to the first question, the Panel finds that EPA was effective in developing a clear, transparent, and logical response to the NAS. The Panel is particularly impressed with the process that EPA used for identifying, reviewing, and evaluating the relevant literature. EPA's process was comprehensive, rigorous, and included public participation. The Agency's report, *EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments (May 2010)*, consists of two volumes. The first volume contains the main text of the *Report* and is 690 pages long (including 37 pages of references). The second volume contains appendices and is 1,159 pages long. Because of the size and complexity of EPA's *Report*, the Panel finds the Executive Summary to be particularly important and valuable in providing a concise and accurate summary. As described in detail in the responses to the charge questions in Section 3.2 of this report, the Panel has identified the need for better integration across chapters and greater clarity and transparency in indicating which studies did not satisfy inclusion criteria, and therefore were not carried forward for further consideration in subsequent chapters of the *Report* to meet particular needs. In contrast, the Panel is satisfied that the inclusion criteria and the associated retained studies are well described and transparent. Given the enormity of the dioxin published literature, the Panel recognizes that it is not a trivial matter to characterize the studies that were not considered. Therefore the Panel suggests that the *Report* be revised to generally indicate how this issue was considered. Information about studies that were excluded could be provided in an appendix.

The *Report* is long and dense with a considerable amount of jargon and in some places it is quite repetitive. These features, while a necessity of this type of document, at times detract from clarity or make the EPA's logic difficult to discern. The Panel finds some instances where the *Report* would benefit from greater clarity in writing. For example, topic sentences are sometimes not easily connected to paragraph content. A specific example of this is in the second paragraph on page xxvii of Volume 1

where the text does not clearly identify separate EPA activities to address NAS comments. Another example of a *Report* section that should be edited to improve clarity is the qualitative discussion of the uncertainty in the RfD (Section 4.4 of Volume 1). The clarity of this section could be improved by including bullet points to highlight and separate key points and/or provide links to information in other sections of the document (e.g., Section 6 – Feasibility of Quantitative Uncertainty Analysis). A careful review by a qualified technical editor is needed. Similarly, the clarity and accessibility of the *Report* should be enhanced by the inclusion of a glossary to help minimize confusion and misinterpretation among the diverse users of the document. At 690 pages, Volume 1 is a formidable report. The Panel appreciates the dilemma of preparing a report that is both complete and rigorous and at the same time succinct and efficient. EPA should make greater use of appendices and eliminate redundancies in order to provide a more approachable document.

With respect to the second part of Charge Question 1.1 (i.e., objectivity and clarity of presentation of the three key NAS recommendations), the Panel finds that EPA has been successful. EPA's *Report* clearly presents the key NAS recommendations. However, as described more fully in responses to the relevant specific charge questions below, the Panel has identified deficiencies in the *Report* with respect to the completeness of its consideration of two critical elements: 1) nonlinear dose-response for TCDD carcinogenicity and 2) uncertainty analysis.

Recommendations

- As further discussed in the responses to the charge questions in Section 3.2 of this report, the Panel recommends that the *Report* be revised to provide greater clarity and transparency in the discussion of studies that did not satisfy inclusion criteria for use in the dioxin assessment. The Panel recommends that the *Report* be revised to provide an overview of reasons why studies were excluded.

- The *Report* would benefit from greater clarity in writing. The Panel therefore recommends that the *Report* be carefully reviewed by a qualified technical editor and revised to incorporate such improvements as better integration across chapters, better connection between topic sentences and paragraph content, and elimination of repetition.

- The Panel recommends that the clarity and accessibility of EPA's *Report* be enhanced by the inclusion of a glossary to help minimize confusion and misinterpretation among the diverse users of the document.

- The Panel recommends that EPA find additional efficiencies (e.g., greater use of appendices and elimination of redundancies) to yield more succinct and approachable document. In this regard, we recommend that EPA develop a summary document, longer than the current executive summary but shorter than the full Report, that captures the highlights of the response to the NAS.

- As discussed in the responses to other charge questions in this report, the Panel has identified deficiencies in EPA's *Report* with respect to the completeness of its consideration of two critical elements: 1) nonlinear dose-response for TCDD carcinogenicity, and 2) uncertainty analysis. In the relevant charge question responses below, the Panel provides recommendations to improve the *Report* in these areas.

Charge Question 1.2. Are there other critical studies that would make a significant impact on the conclusions of the hazard characterization and the dose-response assessment of the chronic noncancer and cancer health effects of TCDD?

Response:

During its deliberation, the Panel did not identify any additional studies that would impact the hazard characterization or the dose-response assessment. However, EPA's *Report* should provide more discussion of null epidemiologic studies and a clearer indication of the reasons for excluding these studies.

Recommendations

- The Panel recommends that EPA's *Report* provide more discussion of null epidemiologic studies and a clearer indication of the reasons for excluding these studies.

3.2. Transparency and Clarity in the Selection of Key Data Sets for Dose-Response Analysis

The NAS committee recommended that EPA develop a clear and readily understandable methodology for evaluating and including epidemiologic and animal bioassay data sets in dose-response evaluations. Section 2 of EPA's *Report* describes the Agency's approach to ensuring transparency and clarity in the selection of the studies for dose-response analyses. The Panel was asked to comment on: whether EPA had been responsive to NAS concerns about transparency and clarity in data set selection, whether the epidemiology and animal bioassay study criteria and considerations had been scientifically justified and clearly described, and whether EPA had applied the epidemiology and animal bioassay study criteria considerations in a scientifically sound manner.

EPA developed and applied two sets of criteria for the animal bioassays and epidemiologic data. The Agency collected and evaluated these studies, including studies from the *2003 Reassessment* and newer studies found through literature searches and through public submissions. The Panel views with favor all of the efforts made by EPA to develop this section of the document. The Panel compliments the Agency for its efforts to present the nuanced differences and complicating issues surrounding this subject in a comprehensive and logical manner. The intention of the comments and recommendations provided below is to assist the EPA in further improvement of Section 2.

Charge Question 2.1. Is this section responsive to the NAS concerns about transparency and clarity in data set selection for dose-response analysis?

Response:

Members of the Panel find that Section 2 of the *Report* is responsive to NAS concerns about transparency and clarity. Moreover, it is perceived and appreciated that, in addressing these concerns, EPA has improved the approach in the original *2003 Reassessment*. The EPA's collaboration with Argonne National Laboratory, and invitation to the public to engage in updating the literature search to identify all appropriate studies for evaluation, as well as the conduct of the Dioxin Workshop in February of 2009, were instrumental in enhancing the transparency and clarity regarding the process of

selection of studies for the dose-response analysis. The development of clear criteria for study evaluation and inclusion was crucial in addressing the concerns raised by the NAS.

EPA's *Report* presents a clear identification of the study selection process and the studies that were used for dose-response analysis. For example, the process and criteria used to select key data sets for dose-response analyses is described in Section 2.3 of the *Report* and in the Executive Summary. Flow diagrams (e.g., ES-1 and ES-2) clearly demonstrate how studies were chosen for inclusion. Likewise, Appendix B, which includes a point-by-point evaluation of which epidemiological studies were included and excluded, is useful and provides a detailed rationale explaining why the EPA used the particular studies selected in the *Report*. However, more discussion is needed to clearly explain exclusion of null epidemiologic studies. In addition, the results of the literature search performed by EPA are available online. Clarity could be improved by providing search words used for the MedLine searches. A clear case for including high-quality human studies over animal studies is also made.

While Section 2 of the *Report* is deemed responsive to NAS concerns, the Panel finds that overall clarity and transparency regarding dataset selection would be further and markedly enhanced if EPA were to make Section 2 (and the document as a whole) more concise. In its present form, Section 2 is viewed by the Panel as overly verbose, to the detriment of overall clarity and we provide the following recommendations to improve the *Report*.

Recommendations

- The Panel strongly recommends careful and extensive editing to revise and consolidate Section 2 and the *Report* as a whole. Specifically, editing should include aspects of English grammar and syntax, minimizing redundancies, and efforts to provide more succinct responses to NAS concerns.

- The Panel recommends restructuring Section 2 to make it easier to follow a study used by EPA from one section of the *Report* to another. In other words, EPA should improve overall document integration using Section 2 as the foundation for this integration.

Charge Questions 2.2 and 2.3

> *<u>Charge Question 2.1.</u> Are the epidemiology and animal bioassay study criteria/considerations scientifically justified and clearly described?*
>
> *<u>Charge Question 2.3.</u> Has EPA applied the epidemiology and animal bioassay study criteria/considerations in a scientifically sound manner? If not, please identify and provide a rational for alternative approaches.*

Response:

The Panel's discussion of Charge Questions 2.2 and 2.3 was highly integrated. Therefore, comments and specific recommendations that stem from these two questions are presented together.

The Panel finds that EPA's study criteria and considerations are scientifically justified and clearly described, and that these were generally applied in a scientifically sound manner. Thus, Section 2 is deemed responsive to NAS concerns regarding the scientific justification and clarity of description for

epidemiology and animal bioassay study criteria/considerations. However, several concerns were discussed by the Panel, and are summarized here.

The Panel's major concern pertains to improving clarity with regard to the decision to include or exclude particular studies and groups of studies from the data sets to be used. The rationale for distinct criteria for epidemiological and animal studies should be made stronger, and data set selection for noncancer and cancer endpoints has room for further clarification and justification. There was discussion, with differences of opinion among members of the Panel, regarding EPA's scientific justification and clarity of description concerning the Agency's decision to exclude dioxin-like compounds. There is consensus among Panel members that the following recommended improvements would strengthen this section, and thus the document as a whole.

Recommendations

Rationale for excluding dioxin-like compounds

- EPA should better justify the rationale for using studies where the exposure is primarily to TCDD (or for animal studies only to TCDD) to calculate the reference dose. This justification should include scientific and practical reasons.
- There is a wealth of literature on dioxin-like compounds that can be used to support mode of action determination as well as the plausibility of individual biological effects that are used for risk assessment. EPA should incorporate information from studies with dioxin-like chemicals into a qualitative discussion of the weight-of-evidence for cancer and noncancer endpoints.

Study inclusion and exclusion criteria and considerations

- While the Panel generally finds that EPA's study criteria and considerations are scientifically justified and clearly described, the Agency should further clarify the justifications for some specific study inclusion and exclusion criteria/considerations. To be clear, this recommendation does not indicate that the Panel suggests that a different approach to data set selection is needed. However, the approach used should be explained more effectively and clearly. In this regard, the following specific recommendations are provided to address points of concern raised by Panel members about the study inclusion and exclusion criteria:
    - EPA should remove the criterion that studies must contain an explicit statement of TCDD purity. For research purposes, TCDD is available from a limited set of vendors, and all sell it as a highly purified compound. Thus, for the animal studies, it is highly unlikely that any study would be conducted using impure TCDD. Therefore, excluding a study simply due to absence of statements regarding TCDD purity runs the risk of excluding high quality studies because the author or journal editorial staff did not elect to include this piece of information.
    - EPA should revise the explanation of the in vivo mammalian bioassay evaluation indicating that the "study design is consistent with standard toxicological practices." This is too vague as it likely has different meaning to readers from different backgrounds. In addition to defining this more clearly, it is recommended that, if possible, a reference should be provided to an EPA document in which these practices are described in detail.

- EPA should define the phrase "common practices," and if possible cite appropriate Agency documents to which the reader can refer for further detail. To provide further context, this recommendation refers specifically to statements such as the following one on page 2-5: "The study criteria shown below and in Figure 2-3 for animal bioassay data reflect EPA's preferences for TCDD-specific study inclusion, some of which are based on common practices and guidance for POD selection and RfD and OSF derivation."

- EPA should provide a more thorough (albeit concise) discussion of data set limitations to educate the reader regarding Agency decisions about study inclusion/exclusion criteria. For instance, consider adding an expanded discussion on suitability of studies of immunological effects and/or thyroid and diabetes (e.g., Calvert,1999; Steenland, 2001; Baccarelli et al., 2002, 2004).

Considerations concerning selection of epidemiology studies

- The Panel recommends that EPA better justify and explain considerations relating to the selection of epidemiology studies. The following specific recommendations are provided. Many of these specifically address the use of more standard epidemiology vocabulary and descriptors.

  - EPA evaluated the available epidemiologic cohorts and studies based on five considerations presented on pages 2-6 and 2-7 of the *Report*. The Panel finds that Consideration #2 (page 2-6) is worded awkwardly and that epidemiologic terms are misspecified. The Panel therefore recommends that EPA revise Consideration #2 as follows:

    - Define "susceptible to important biases." This is a non-specific term and the biases should be explained.

    - Clarify what is meant by "control for potential confounding exposures." Does this refer to only exposure to dioxin-like compound exposures or was it meant to more broadly refer to other exposures as well (NIOSH cohort studies)? Does the text "bias arising from study design" refer to selection bias or is this phrase used more broadly to describe how exposure and outcome are measured and covariate data collected?

    - Define what is meant by the phrase "bias arising from statistical analyses." It is unclear if bias is the correct term, rather this may refer to model misspecification.

  - With regard to scientific justification and application of Consideration #3 (listed on page 2-7), the Panel recommends that EPA provide more discussion and clarity on the exclusion of null epidemiologic studies.

  - In Exclusion Criterion #3 (listed on page 2-7) EPA should define "reported dose."

  - The Panel recommends that the discussion in Section 2 of the consideration of "confounding and other potential sources of bias" be clarified. The differences between males and females with regard to TCDD half-life are discussed, but the description of the number of males and females in each study population were often missing or very difficult to determine. Also, in the occupational cohort studies, the possibility of men and women performing different job tasks also increased the possibility that the men and women were exposed at different levels. However, when the job categories with assigned TCDD exposure levels were presented, there was often no discussion of the numbers by gender in the categories. For example, the

Manz et al. study (1991) of the Hamburg cohort (1,583 men and 399 women) does not describe the TCDD categories by gender. In addition, the validity of the TCDD exposure levels assigned to the categories was examined "in a group of 48 workers who provided adipose tissue samples" (Page 2-41, lines 18-19). How were these workers selected? How many were approached but refused to provide a sample? Assessment of selection bias in this and other similar circumstances was lacking in some of the studies. This is particularly notable in the lack of overall response rates reported for several of these studies. Inclusion of these factors in the study review would be very helpful.

- The Panel recommends that discussion of the consideration that "statistical precision, power, and study follow-up are sufficient" be clarified. These metrics can be difficult to determine with the smaller sample size populations, but there are studies that can be very useful even given the small samples. For example, the relative risks calculated for increasing TCDD exposure and risk of breast cancer in the Seveso study were greatly increased in the 3rd and 4th highest exposure categories, but the relative risks were not statistically significant (page 2-56, lines 1-8 of the *Report*).

3.3. The Use of Toxicokinetics in the Dose-Response Modeling for Cancer and Noncancer Endpoints

EPA used a physiologically-based pharmacokinetic (PBPK) model (Emond et al., 2004, 2005, 2006) with blood concentration as the dose metric rather than first-order body burden. The Panel was asked to comment on the scientific justification for EPA's application of this model, the model modifications that EPA implemented, and EPA's characterization of uncertainty in the model. EPA also developed a PBPK model to estimate TCDD concentration in mouse tissues. The Panel was asked to comment on the scientific rationale for development of the mouse model, the performance of the mouse model, and whether model uncertainty had been adequately characterized. In addition, the Panel was asked to comment on the use of the Emond PBPK model to estimate human intake based on internal exposure measures, EPA's sensitivity analysis of the kinetic modeling, and EPA's estimate of lifetime average daily dose

<u>Charge Question 3.1.</u> The 2003 Reassessment utilized first-order body burden as the dose metric. In the draft Response to Comments document, EPA used a physiologically-based pharmacokinetic (PBPK) model (Emond et al., 2004, 2005, 2006) with whole blood concentration as the dose metric rather than first-order body burden. This PBPK model was chosen, in part, because it includes a biological description of the dose-dependent elimination rate of TCDD. EPA made specific modifications to the published model based on more recent data. Although lipid-adjusted serum concentrations (LASC) for TCDD are commonly used as a dose metric in the literature, EPA chose whole blood TCDD concentrations as the relevant dose metric because serum and serum lipid are not true compartments in the Emond PBPK models (LASC is a side calculation proportional to blood concentration).

Please comment on the following:

<u>Charge Question 3.1.a.</u> Please comment on the justification of applying a PBPK model with whole blood TCDD concentration as a surrogate for tissue TCDD exposure in lieu of using first-order body burden for the dose-response assessment of TCDD.

Response:

The use of body burden in the *2003 Reassessment* represents an improvement over the usual default metric of administered dose (mg/kg/d) because the default metric would not properly reflect the accumulation of dioxin in the tissues over time. However, because the accumulation of dioxin in liver is dose-dependent, body burden would not serve as a direct surrogate for tissue exposure. The use of blood concentration is a better choice than body burden because it is more closely related to the biologically relevant dose metric: the free concentration of dioxin in the target tissues (liver, fetus, etc.). It is important to recognize, however, that TCDD distribution within tissues such as the liver can be non-uniform. Blood concentrations are routinely used to estimate biologically effective exposures for pharmaceuticals.

The rationale for the use of blood concentration rather than lipid adjusted serum concentration (LASC) should not be based on the Emond model structure. It would be trivial to change the model so that LASC could be predicted. Indeed, the model is apparently used to estimate LASCs in the RfD calculations (e.g., page xli, line 21 in the Executive Summary of the *Report*). The question that should be addressed is only whether blood concentrations or LASCs provide better surrogates for cross-species and cross-study comparisons of free dioxin concentration in the target tissues. LASC is the preferred measure for reporting dioxin biomonitoring data, and is the measurement reported in most of the human epidemiological studies. A metric that considers blood lipid content is also more likely to reflect free dioxin concentration in the plasma, and hence free concentration in the target tissue. The EPA pointed out (page xxxiv in the Executive Summary of the *Report*) that the LASC was related to the blood concentration by a scalar; however, EPA incorrectly concluded that the metrics are equivalent and later (page 3-511, line 6 of the *Report*) discussed the fact that the relationship between them was subject to inter-individual and inter-species variation. If the LASC were used to drive the distribution of TCDD to tissues, the pharmacokinetic outcome would be different from using blood as the driver because the tissue:blood ratio would differ. If the blood fat:blood and tissue:blood values were accounted for in the model, the use of blood and LASC would be similar. It is not clear at this point how this issue was addressed in the dose metric calculations. Consideration of this issue is unlikely to drastically affect the outcome of the risk calculations, but it would be important for a quantitative uncertainty analysis.

***Recommendation*s**

- The use of the blood metric is acceptable for the PBPK model. EPA should clarify how the model deals with studies that report the concentration of dioxin in plasma, serum, blood or blood fat:blood measurements.

  Charge Question 3.1.b. Please comment on the scientific justification for using the Emond et al. model as opposed to other available TCDD kinetic models.

Response:

The Emond model provides the best available basis for the dose metric calculations in the assessment. It is the product of a high-caliber, multi-year research effort at EPA's National Health and Environmental Effects Research Laboratory, and represents a significant effort in terms of data collection. This model builds on prior PBPK modeling efforts conducted by Andersen et al. (1997). However, additional

discussion of other published models and quantitative evaluation of the impact of model selection on dose metric predictions should also be provided.

Recommendations

- The *Report* should discuss how the model was intended to be used in the assessment, which would then dictate why a particular model was selected. That is, for the intended purposes, was the Emond model more robust and/or simpler than other models (such as Andersen et al., 1993, Andersen et al., 1991, and Simon et al. 2009), and did it contain sufficient details for biological determinants deemed important by the Agency?

Charge Question 3.1.c. Please comment on the modifications implemented by EPA to the published Emond et al. model.

Response:

The EPA modifications to the published Emond model (modifications described on page 3-44 of the *Report* account for volume of plasma and describe urinary clearance using blood concentration and not a lumped compartment) are minor and appropriate. The model changes are scientifically appropriate and well supported.

Charge Question 3.1.d. Please comment on whether EPA adequately characterized the uncertainty in the kinetic models.

Response:

The *Report* presents a reasonably thorough qualitative characterization of the uncertainty in the kinetic models that is sufficient to support their use in the assessment. A more quantitative uncertainty analysis is needed. Methods that could be useful and informative for such an analysis are suggested in the response to Charge Question 6.2. It is critical to demonstrate the dependence of human equivalent dose (HED) and risk predictions on uncertainty and variability in the model parameters, particularly those with high sensitivity (Evans and Andersen, 2000). Moreover, dose metric uncertainty needs to be determined under the same exposure conditions that dose metrics are calculated: both for the various studies that serve as the basis for the dose-response assessments and for human exposures at the corresponding HEDs and risk specific doses.

The Hill coefficients for CYP1a1 and CYP1a2 induction used in the Emond model were 1.0 and 0.6, respectively, based on fitting of kinetic data from single doses of dioxin (Wang et al., 1997; Santostefano et al., 1998). However, Walker et al. (1999) subsequently estimated a Hill coefficient of 0.94 for both CYP1a1 and CYP1a2 induction using chronic exposures which were more relevant to the use of the Emond model in the dioxin risk assessment. The value of 0.6 used in the Emond model is well outside the confidence interval of 0.78 to 1.14 reported by Walker et al. (1999). The use of a Hill coefficient value well below unity would lead to a nonlinear model behavior that is biologically implausible (hypersensitivity to induction at doses near zero). As a result, when the human model was used for extrapolation to lower doses (as in the calculation of risk-specific doses) the model would tend to estimate a lower exposure level for a given blood concentration. This effect can be seen in Table ES-1 of the *Report*, where a 5 order-of-magnitude change in risk is associated with a 6 order-of-magnitude

change in risk specific dose. That is, the model-estimated risk specific doses in the vicinity of 10^{-6} risk are about a factor of 10 lower (more conservative) than linear extrapolation. The evidence for this parameter needs to be carefully reviewed and the reasonable range of values determined. At the least, the human Emond model calculations will need to be repeated with multiple values of the Hill coefficient to characterize the resulting uncertainty in the estimates. When this is done, the Agency should also consider increasing the fat:blood partition in the human model from 100 to 200 to be more consistent with the human data (Patterson and Mackay, 1987; Schecter and Ryan, 1989; Schecter et al., 1989; Iida et al., 1999; Maruyama et al., 2002). The Hill coefficient is not likely to have as significant an effect on calculations with the animal models, since low-dose extrapolation was not performed in the animals, but this should also be verified by sensitivity/uncertainty analysis of the animal models. Public comments were submitted to the Panel recommending consideration of a Hill coefficient value of 1.0 and pointing out why lower values are inappropriate.

Recommendations

- The Panel recommends additional efforts to fully characterize the uncertainty in the models with special consideration of the Hill coefficient value.

  Charge Question 3.2. Several of the critical studies for both noncancer and cancer dose-response assessment were conducted in mice. A mouse PBPK model was developed from an existing rat model in order to estimate TCDD concentrations in mouse tissues, including whole blood.

  Please comment on the following:

  Charge Question 3.2.a. Please comment on the scientific rationale for the development of EPA's mouse model based on the published rat model (Emond et al., 2004, 2005, 2006).

Response:

The Panel agrees that an appropriate approach was used to develop the mouse model on the basis of the published rat model and the available mouse kinetic data. It should be noted that the NAS recommendation to use human data for dose metric could be accomplished because dose-dependent elimination of TCDD has been described in humans, albeit in just a few cases. Dose-dependent elimination has been reported repeatedly in animals and the PBPK model reflected this dose-dependence. Using CYP1A2 data from humans (caffeine metabolism) and mice would offer an opportunity to validate and/or adjust the mouse model.

Recommendations

- An external peer review of the mouse model should be conducted because this model has not been published in the peer-reviewed literature. This is typically a requirement for models to be used by the Agency.

  Charge Question 3.2.b. Please comment on the performance of the mouse model in reference to the available data.

Response:

The Panel finds that the mouse model performed reasonably well, apart from under-prediction of urinary excretion data. The urinary excretion data can be improved by taking into account the fact that urine contains metabolites only, which partition differently from the parent compound. The model appears to be adequate for use in estimating dose metrics for the assessment, but with greater uncertainty than the rat and human models. This is considered a reasonable approach to solve a deficiency in published PPBK models to meet the needs of this assessment.

The EPA's suggestion in the RfD chapter that the clustering of mouse points of departure (PODs) at the lowest doses was due to mouse model failure is inappropriate and should be rewritten.

Recommendations

- EPA should use the mouse model. The scientific credibility of the model will be enhanced by its publication in an appropriate peer reviewed journal.

  Charge Question 3.2.c. Please comment on whether EPA adequately characterized the uncertainty in the mouse and rat kinetic models. Please comment specifically on the scientific justification of the kinetic extrapolation factor from rodents to humans.

Response:

EPA provides an adequate characterization of the qualitative uncertainty in the mouse and rat kinetic models sufficient to justify their use, together with the human model, to estimate rodent-to-human extrapolation factors. On the other hand, formal recalibration of the PBPK model parameters using a Hierarchical Bayesian approach such as Markov chain Monte Carlo analysis is not considered necessary or particularly useful. However, a more quantitative uncertainty analysis is needed.

Recommendations

- A more quantitative uncertainty analysis is recommended. Methods that could be useful and informative for such an analysis are suggested in the response to Charge Question 6.2 in this report.

  Charge Question 3.3. Please comment on the use of Emond et al. PBPK model to estimate human intakes based on internal exposure measures.

Response:

The modified Emond model is the best available approach for estimating exposures on the basis of internal exposure measurements. Nevertheless, there is considerable uncertainty associated with attempting to reconstruct prior exposures in a human population (e.g., Seveso).

Recommendations

- Model simulations of the Cheng et al. (2006), Moccarelli et al. (2008), and Bacarelli et al. (2008) studies need to be described in more detail. The impact of model parameter and exposure uncertainties on model predicted dose metrics should be evaluated quantitatively and reported.

  Charge Question 3.4. Please comment on the sensitivity analysis of the kinetic modeling (see Section 3.3.5).

Response:

The *Report* only presents the sensitivity analysis published by Emond et al. (2006), which is not entirely adequate for the purposes of this assessment. The analysis left out the Hill coefficient, which is one of the most important parameters in the model for low dose extrapolation (Evans and Andersen, 2000). Moreover, model sensitivities are species, dose, and dose-scenario dependent, so they need to be determined under the same exposure conditions as those for which dose metrics are calculated: both for the various studies that serve as the basis for the dose-response assessments and for human exposures at the corresponding HEDs and risk specific doses. This represents the most pragmatic path forward for an evaluation of model sensitivity as it relates to potential environmental regulation.

Recommendations

- In order to authenticate the model for its intended purpose, EPA should provide a sensitivity analysis of the model under the same exposure conditions for which dose metrics were calculated.

  Charge Question 3.5. Both EPA's noncancer and cancer dose-response assessments are based on a lifetime average daily dose. Did EPA appropriately estimate lifetime average daily dose? If not, please suggest alternative approaches that could be readily developed based on existing data.

Response:

The Panel agrees with the average daily dose calculation approaches described in the *Report*. It was not clear to some Panel members how the computational estimates of internal dose for newborns were carried out since a lactation model was not used. This is important because of the use of TSH in newborns as a critical effect. EPA, and Baccarelli et al. (2008), developed an empirical description of the relationship between maternal TCDD levels (lipid adjusted) in serum at birth of neonate and the measured serum TSH in the newborns up to 3 days of age. The Emond et al. model was run in an iterative fashion by adjusting chronic daily intake (ng/kg/day) in the human gestation model to predict maternal serum level of TCDD at term that was associated with infant serum thyroid stimulating hormone (TSH) concentration of 5 µ-units/ml (µU/ml) (by using the regression equation). The result was 0.024 ng/kg bw/day.

Recommendations

- EPA should carefully explain how the early life stage internal doses are calculated.

3.4. Reference Dose

EPA selected two co-critical studies (Mocarelli et al., 2008 and Baccarelli et al., 2008) for development of the reference dose for TCDD. The Panel was asked to comment on the scientific justification for selection and use of these studies to develop the reference dose.

> *Charge Question 4.1. The Mocarelli et al. (2008) and Baccarelli et al. (2008) studies were selected as co-critical studies for the derivation of the RfD. Is the rationale for the choice of Mocarelli and Baccarelli scientifically justified and clearly described? Please identify and provide the rationale for any other studies that should be selected, including the rationale for why the study would be considered a superior candidate for the derivation of the RfD. In addition, male reproductive effects and changes in neonatal thyroid hormone levels, respectively, were selected as the co-critical effects for the RfD. Please comment on whether the selection of these critical effects is scientifically justified and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.*

Response:

The Panel finds that use of the Mocarelli et al. (2008) and Baccarelli et al. (2008) studies is appropriate for identifying "co-critical" effects for the RfD calculation. These are human epidemiological studies that were well designed and executed. The studies provide sufficient exposure information, including biological concentrations that could be used to help establish acceptable life-time daily exposure levels. Some of the strengths of the human studies include the use of a well-characterized human cohort, conducted by dioxin epidemiology experts, and the fact that similar PODs were found across a broad spectrum of other reported dioxin toxicities in multiple species. The rationale for selecting these two studies over numerous other available studies is clearly described and, overall, EPA has provided a well-considered and rational discussion of why these two human studies were selected for determining the RfD. However, one issue discussed by the Panel is that, while the strengths of the two human studies are well-described, the study weaknesses are not always clearly delineated. For example, in the Baccarelli (2008) study there is limited discussion of how the presence of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and coplanar polychlorinated biphenyls (PCBs) that were also found in the blood might confound the interpretation of TCDD association with elevated TSH levels. In addition there is no discussion of the potential impact of residential histories (e.g., individuals who may have moved in and out of Zone A after the accident). The Panel believes that it is important to discuss both the strengths and weaknesses of these two studies. More discussion of the weaknesses of the studies is needed.

As indicated above, the Panel agrees that the major strengths of the human studies are the use of a well-characterized dioxin-exposed human cohort, conducted by dioxin epidemiology experts, and the fact that similar PODs were found across a broad spectrum of other reported dioxin toxicities in multiple species. However, in isolation from each other, and lacking a description of supportive animal and epidemiological studies, the studies are less useful for setting the RfD. The Panel emphasizes the need to consider these other supportive studies within the context of the weight of the dioxin and dioxin-like compound (DLC) database. The strength of the RfD should not be based solely on these two human epidemiology studies, but rather should be supported by integration with other similar supporting dioxin and DLC studies. The Panel strongly supports looking at the comprehensive data base of both animal

and human epidemiological studies together to demonstrate a consistent and integrative signal of toxicity across species and endpoints for TCDD. It is suggested that similar studies with DLCs should also be included as these would be supportive, at least for a semi-quantitative comparative analysis. This "collective" impact of the studies is stated in the *Report* but the coherence of evidence needs to be made stronger as it represents the contextual framing for understanding dioxin health impacts. This response would include discussions of both human and experimental animal studies that have examined the effects of dioxin or DLCs on other reproductive and endocrine endpoints and should, for example, include discussion of dose-response relationships as well as comparisons of no-observed-adverse effect levels (NOAELs) and lowest-observed-adverse effect levels (LOAELs).

The Panel notes that Figures 4.3 and 4.4 in the *Report* show quantitative comparisons across the RfDs and benchmark dose lower bounds (BMDLs) calculated from the animal and epidemiological studies. These figures are useful in understanding the quantitative similarities (to the PODs in the chosen studies) in these calculations. The Panel also notes that since the figures do not have an indication of endpoints being measured, just the reference to the publications, the consistency in signal (i.e., the similarities in PODs determined) is not as readily apparent as it could be.

Although it has been addressed in the *Report*, the Panel recommends expanding the discussion of the known human age-specific variability in endpoints such as sperm counts, though the data from Moccarelli et al. (2008) do show ranges and variance (in Figure 3/Table 2), and neonatal TSH levels.

Recommendations

- EPA should provide a discussion of the strengths and weaknesses of the Mocarelli et al. (2008) and Baccarelli et al. (2008) studies with an indication of whether the weaknesses affect determination of the RfD.

- EPA should label the endpoints for studies included in Figures 4.3 and 4.4.

- The comprehensive data base of both animal and human epidemiological studies, including studies with DLCs (e.g., studies cited in Goodman et al., 2010), should be discussed together to demonstrate a consistent and integrative signal and coherence of evidence of toxicity across species and endpoints for TCDD.

> *<u>Charge Question 4.2.</u> In the Seveso cohort, the pattern of exposure to TCDD is different from the average daily exposure experienced by the general population. The explosion in Seveso created a high dose pulse of TCDD followed by low level background dietary exposure in the exposed population. In the population, this high dose pulse of TCDD was slowly eliminated from body tissues over time. There is uncertainty regarding the influence of the high-dose pulse exposure on the effects observed later in life.*
>
> *<u>Charge Question 4.2.a.</u> Mocarelli et al. (2008) reported male reproductive effects observed later in life for boys exposed to the high dose pulse of TCDD between the ages of 1 and 10. EPA identified a 10 year critical exposure window. In the development of the candidate RfD, EPA used an exposure averaging approach that differs from the typical approach utilized for animal bioassays. EPA determined that the relevant exposure should be calculated as the mean of the*

pulse exposure and the 10-year critical exposure window average. Please comment on the following:

Charge Question 4.2.a.i. Please comment on EPA's approach for identifying the exposure window and calculating average exposure for this study.

Response:

The Panel discussed extensively, both as part of the deliberations on Section 4 of the *Report* and also as part of the discussion on Section 3, extrapolation issues posed by the pattern of exposure found in the study at the city of Seveso. Issues raised included the question of whether the same endpoints and or dose-response would be expected from such exposure scenarios with high acute exposures when extrapolating to low-dose chronic exposures**.** It would be useful for EPA to provide a discussion of published examples in which dioxin studies were conducted using both high-dose acute and low-dose chronic exposures in animals for the same endpoint and how the outcomes compare both qualitatively and quantitatively (see further discussion and recommendations in the response to Charge Question 4.8). It would be important to determine whether similar results were observed for similar endpoints. Several Panel members indicated that there were sufficient data in the immunological or reproductive areas that may allow such a comparison. Several chronic dioxin animal studies may be useful in this regard (Yoshizawa et al., 2009; Sand et al., 2010; Yoshizawa et al., 2010). The life stage-specific approach to hazard and dose-response characterization for children's health risk assessment found in EPA's *Framework for Assessing Health Risks of Environmental Exposures to Children* (EPA, 2006), is also relevant to addressing this issue and should be discussed. The Panel also recommends that the publication of Bell et al., (2010), which summarized and presented data on some differences about chronic vs. acute exposure in maternal transfer, be considered in this discussion.

Charge Question 4.2.a.ii. Please comment on EPA's designation of a 20% decrease in sperm count (and an 11% decrease in sperm motility) as a LOAEL for Mocarelli et al. (2008).

Response:

The Panel finds that the designated changes from normal sperm counts and sperm motility are of public health relevance and therefore of interest for determining an RfD. Collectively, there is support for these endpoints within the context of the broader dioxin literature. The Panel discussed whether the magnitude of these changes would represent an adverse health effect. While the shifts observed in sperm counts may or may not pose a significant health effect in a single individual, such shifts on a population basis could presumably lead to an increased incidence of adverse health outcomes. Designation of the decreases in sperm count and motility as a LOAEL for Mocarelli et al. (2008) is appropriate. Although there was concern expressed about the sample size used for sperm number and known variability in the biological endpoint, the Panel finds that sample collection was conducted consistently across subjects and the differences in groups were apparent.

The Panel recommends that further discussion of World Health Organization (WHO) reference values for male reproductive parameters be included in the *Report*, as was done for the relevant TSH levels. Several references are available which provide background information and current values recommended by WHO regarding sperm counts (e.g., Skakkebaek, 2010). The Panel recommends that

the standard deviations or range of changes from Mocarelli et al. (2008) be discussed in the *Report* because this provides a better understanding of the potential magnitude of effect.

Life stage differences in sperm counts were discussed by the Panel. Members of the public also provided comments on this issue. It would be appropriate to indicate in the *Report* that life stage differences clearly exist in sperm counts in humans and to cite and discuss the EPA life stage document (EPA, 2006).

Recommendations

- Discussion on WHO reference values for male reproductive parameters should be included in the *Report* (e.g., Skakkebaek, 2010).

- EPA should indicate and discuss within the *Report* that life stage differences clearly exist in sperm counts in humans as documented (for example) in EPA, 2006.

- The standard deviations or range of changes from the Mocarelli (2008) study should be discussed in the *Report* to provide a better understanding of the potential magnitude of effect.

> *Charge Question 4.2.b. For Baccarelli et al. (2008), the critical exposure window occurs long after the high-dose pulse exposure. Therefore, the variability in the exposure over the critical exposure window is likely to be less than the variability in the Mocarelli et al. subjects. EPA concluded that the reported maternal exposures from the regression model developed by Baccarelli et al. provide an appropriate estimate of the relevant effective dose as opposed to extrapolating from the measured infant TCDD concentrations to maternal exposure. Additionally, EPA selected a LOAEL of 5 µU TSH per ml blood in neonates; as this was established by World Health Organization (WHO) as a level above which there was concern about abnormal thyroid development later in life. Please comment on the following:*
>
> *Charge Question 4.2.b.i. Please comment on EPA's decision to use the reported maternal levels and the appropriateness of this exposure estimate for the Baccarelli et al. study.*

Response:

The Panel supports EPA's decision to use the Baccarelli et al. (2008) estimates of the relevant effective doses. However, it should be made clearer how these measurements relate to ranges and variations in exposure *in utero*.

Recommendations

- EPA should clarify how the Baccarelli et al. (2008) exposure measurements relate to ranges and variations in exposure *in utero.*

> *Charge Question 4.2.b.ii. Please comment on EPA's designation of 5 µU TSH per ml blood as a LOAEL for Baccarelli et al., (2008).*

Response:

The change in TSH levels reported by Baccarelli et al. (2008) is of public health relevance and therefore of interest for determining an RfD. EPA's designation of 5µU TSH/ml blood as a LOAEL for Baccarelli et al. is appropriate. As discussed above, collectively, there is support for this endpoint within the context of the broader dioxin literature. There was discussion on whether the magnitude of these changes would represent an adverse health effect. The Panel notes that the shift observed in TSH levels may or may not pose a significant health effect in a single individual, but such a shift on a population basis could presumably lead to an increased incidence of adverse health outcomes. The Panel also discussed the variability in neonatal TSH levels but concerns about this issue were minimized by the fact that samples were all collected on the same postnatal day. The Panel suggests that if any follow-up data on thyroid hormone levels, such as T3, T4 or TSH levels, are available from the population studied, then these results should be discussed in the *Report*. The Panel discussed several studies describing health effects associated with elevated neonatal TSH levels not always recognized as associated with congenital hyperthyroidism (CH). There is a need to better describe the potential adverse health outcomes related to altered neonatal TSH levels. For example, in addition to effects on growth, both cognitive and motor deficits have been found in young adults with congenital hypothyroidism (Oerbeck et al., 2003; Oerbeck et al., 2007). The *Report* could better describe the consequences of transient hypothyroidism on reproductive outcomes e.g., see Anbalagan et al. (2010). Other references that relate to this question include: Chevrier et al. (2007), Dimitropoulos et al. (2009), and Yr (2008).

Recommendations

- EPA should better describe the potential adverse health outcomes related to altered neonatal TSH levels (e.g., effects on both cognitive and motor deficits).

> *Charge Question 4.3. Please comment on the rationale for the selection of the uncertainty factors (UFs) for the RfD. If changes to the selected UFs are proposed, please identify and provide a rationale.*

Response:

A composite uncertainty factor of 30 (an uncertainty factor of 10 for the lack of a NOAEL, and an uncertainty factor of 3 for human interindividual variability) was applied to the LOAEL of 0.020 ng/kg-day from Mocarelli et al. (2008) to obtain the RfD. The Panel agrees that the appropriate uncertainty factors (UFs) were included. The exclusion or inclusion of the UFs in the *Report* is obvious, clearly discussed, and adequately rationalized. The *Report* would be more transparent if EPA included a short discussion of the basis for the decision not to include a UF for data quality.

Recommendations

- EPA should include in the Report a short discussion of the basis for the decision not to include a UF for data quality.

> *Charge Question 4.4. EPA did not consider biochemical endpoints (such as CYP induction, oxidative stress, etc.) as potential critical effects for derivation of the RfD for TCDD due to the uncertainties in the qualitative determination of adversity associated with such endpoints and*

quantitative determination of appropriate response levels for these types of endpoints in relation to TCDD exposure. Please comment on whether the decision not to consider biochemical endpoints is scientifically justified and clearly described.

Response:

Biochemical endpoints such as P450 activation, increased oxidative stress, etc. may be acceptable endpoints to establish PODs, particularly when the quantitative relationship between the biochemical endpoint and an adverse health outcome is clearly evident. However, with respect to TCDD, the Panel agrees that more traditional endpoints (e.g., immune, endocrine, reproductive) are more appropriate because associations of these endpoints with health outcomes are well studied and provide a stronger association to an adverse outcome than biochemical endpoints. However, because of the wealth of data on P450s and their importance in disease development, normal development, and chemical response to exogenous agents, EPA should discuss biochemical endpoints, particularly P450s, relevant to strengthening the proposed reference dose.

Recommendations

- Because of the wealth of data on P450s and their importance in chemical response to exogenous agents, EPA should discuss biochemical endpoints (particularly P450s) relevant to strengthening the proposed reference dose.

Charge Question 4.5. In using the animal bioassays, EPA averaged internal blood TCDD concentrations over the entire dosing period, including 24 hours following the last exposure. Please comment on EPA's approach for averaging exposures including intermittent and one-day gestation exposure protocols.

Response:

For animal studies it has been shown that for some effects acute exposure could give different results than chronic exposure. For TCDD, however, its persistence might suggest that such differences may be less pronounced. In Baccarelli et al. (2008) there is extensive discussion regarding the use of the exposure average time for the TCDD concentrations. This is of biological significance as several papers have indicated the unique aspects of high peak exposure of TCDD as occurred in Seveso and in several of the animal studies. The endpoints affected as a result of these peaks do not always translate to impacts from lower chronic exposures. As previously stated, it would be helpful to discuss any available animal studies comparing high-dose acute vs. low-dose chronic effects on similar endpoints for dioxin or DLCs. By returning to the broader animal literature and using time and dose-response studies from the dioxin and DLC studies, biological support for the two critical endpoints might be found.

Charge Question 4.6. Please comment on the benchmark dose (BMD) modeling conducted by EPA to analyze the animal bioassay data and EPA's choice of points of departure (PODs) from these studies.

Response:

The Panel agrees with the BMD modeling approaches used in Section 4 of the *Report*. EPA conclusions that the animal data had sufficient limitations that precluded their use to establish a RfD are adequately justified. The reasons provided, however, are quite diverse, (e.g., no NOAEL, not considered an adverse effect, the effect at the LOAEL is too divergent from the control group, insufficient dose groups at the low-end of the dose-response curve, monotonic responses) and there is no way for the reader to determine which study has particular deficiencies without going back to the original paper. To help address this gap, the Panel suggests that several of the best animal studies be discussed in some detail so these limitations are more apparent to the reader. As indicated previously, the EPA authors need to better cite the endpoint guidance that is present within EPA documents for defending these approaches and application of BMD models for the critical effects. This is especially necessary given public comments that EPA was not following its own guidelines.

Recommendations

- EPA conclusions that animal data had limitations that precluded their use in establishing the RfD are adequately justified, but there is no way for the reader to determine which study has particular deficiencies without going back to the original paper. To help address this gap, several of the best animal studies should be discussed in some detail so the limitations are more apparent.

> *Charge Question 4.7. For the animal bioassay modeling, EPA applied the kinetic extrapolation at the level of the POD prior to applying the uncertainty factors because EPA has less confidence in the kinetic model output at lower doses reflective of the RfD. Please comment on whether this approach was scientifically justified and clearly described.*

Response:

The EPA approach of applying the kinetics on the actual data present at the POD is preferred in this assessment (see additional discussion in the responses to the charge questions in Section 3.3 of this report - The use of toxicokinetics in the dose-response modeling for cancer and noncancer endpoints).

> *Charge Question 4.8. Please comment as to whether EPA's qualitative discussion of uncertainty in the RfD is justified and clearly described.*

Response:

The Panel agrees that EPA's discussion of the uncertainties in deriving the RfD using the Seveso cohort is justified and clearly described. Section 4 of the *Report* discusses study limitations regarding the need to adjust from acute exposure to average daily dose, the issue of critical windows, co-exposure to DLCs, and the strength/weaknesses of the animal data. The Panel agrees with EPA that the major limitation of the Seveso cohort is the uncertainty arising from how well the effects resulting from high-dose acute exposure translate to low-dose daily exposures. Again, it would be useful to re-review the animal studies to identify whether there are any studies where dioxin or DLCs were administered by acute as well as chronic (or even subchronic) exposure and comparable endpoints were examined. If so, the information can be used to help confirm or refute the accuracy of the "average daily dose" adjustment. This is of particular concern in the Mocarelli study as "time periods of susceptibility" appear in male reproductive

development and these periods (windows) may be very short. Again, animal studies, particularly those involving male reproduction, may be helpful.

It would also be useful to include a discussion of potential uncertainty in the exposure estimates from the Baccarelli study. Serum dioxin levels were only established in a subset of the cohort (approximately 51) at the time of the study while dioxin levels from the main cohort were estimated from data collected from zone of residence (A or B) at a much earlier time.

The discussion in the *Report* of whether the background DLC exposure may have a significant impact, particularly at the lower TCDD exposure levels, is important. While the Panel agrees that the true DLC impact cannot be determined, it would be helpful to provide some general estimates of the variability that may occur at the proposed RfD.

Recommendations

- EPA should re-review the animal studies to identify whether there are any studies where dioxin or DLCs were administered by acute as well as chronic (or even subchronic) exposure and comparable endpoints were examined. If so, the information can be used to help confirm or refute the accuracy of the "average daily dose" adjustment.

- EPA should include in the *Report* a discussion of potential uncertainty in the exposure estimates from the Baccarelli study. Serum dioxin levels were only established in a subset of the cohort (approximately 51) at the time of the study while dioxin levels from the main cohort were estimated from data collected from zone of residence (A or B) at a much earlier time.

- Background DLC exposure may have a significant impact, particularly at the lower TCDD exposure levels. While the Panel agrees that the true DLC impact cannot be determined, we recommend that EPA provide some general estimates of the variability that may occur at the proposed RfD.

3.5. Cancer Assessment

In the *Report* EPA has provided: a weight-of-evidence characterization of TCDD as a known human carcinogen, conclusions regarding the mode of carcinogenic action for TCDD, EPA's selection of data sets for cancer dose-response modeling, and consideration of approaches for assessment of TCDD carcinogenicity. The Panel was asked to comment on the scientific soundness of these aspects of EPA's cancer assessment.

> *<u>Charge Question 5.1.</u> Weight-of-Evidence Cancer Descriptor: The 2003 Reassessment concluded that TCDD is a "known human carcinogen." In the current draft Response to Comments document, EPA concluded that under the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005) TCDD is "carcinogenic to humans." Is the weight-of-evidence characterization scientifically justified and clearly described?*

Response:

The Panel agrees with the characterization that "TCDD is carcinogenic to humans" under EPA's 2005 *Guidelines for Carcinogen Risk Assessment.* Available occupational epidemiologic studies (e.g., studies

of chemical manufacturing workers in the National Institute for Occupational Safety and Health, NIOSH, cohort) provide convincing evidence of an association between TCDD and human cancer that cannot be reasonably attributed to chance or confounding and other types of bias, and with a demonstration of temporality, strength of association, consistency, biological plausibility, and a biological gradient. The findings of these studies have been previously described in EPA's 2003 Reassessment and in the *Report.* Additional evidence from animal studies and from mechanistic studies provides support for the characterization of TCDD as carcinogenic to humans. One Panel member expressed a dissenting opinion (see Appendix B of this report) that at best, there is equivocal evidence for the carcinogenicity of TCDD in the occupational setting where body burdens were much higher than current or previous background levels. The Panel provides the following recommendations to strengthen the *Report*.

Recommendations

- EPA should provide more discussion of the power of studies used and the difficulties involved when assessing rare tumors. Thoroughly addressing these aspects will make the weight-of-evidence characterization in Section 5 of the *Report* more clear and transparent.

- In the weight-of-evidence characterization, EPA should build on all the available data to support the decision. It needs to be made clear how different types of data (in vitro, in vivo, human) support each other; or not.

- EPA should consider including studies with substantial DLC exposure where TEFs can be calculated. Specific experimental studies include Li and Rozman (1995), Rozman et al. (1993, 2005), and Viluksela et al. (1994, 1997a,b, 1998a,b).

- EPA should attempt to characterize the uncertainty regarding the carcinogenicity of TCDD at low human exposures, since the minimum dose at which carcinogenic effects would be expected to occur cannot be clearly delineated from the current epidemiological human data. The Agency has concluded that AhR activation is a necessary but not sufficient precursor event in the carcinogenic activity of TCDD. Therefore, it would be beneficial if the Agency could evaluate available data on AhR activation and related effects in human cells and animal models to help inform the doses at which these precursor events are observed for comparison with the epidemiological data.

  *<u>Charge Question 5.2.</u> Mode of Action**:** The mode of action of a carcinogen can inform identification of hazards and approaches used for a dose-response assessment. The mode of carcinogenic action for TCDD has not been elucidated for any tumor type. EPA concluded that, while interaction with the Ah receptor is likely to be a necessary early event in TCDD carcinogenicity in experimental animals, the downstream events involved are unknown.*

  <u>Charge Question 5.2.a.</u> Are the available data related to mode(s) of action for the carcinogenicity of TCDD appropriately characterized and clearly presented?

Response:

The Panel appreciates the attempts by the Agency to further develop cancer mode of action concepts based on available dioxin liver, lung, and thyroid toxicity data. Such innovative and explorative work is clearly fundamental to the continued need to further develop risk assessment sciences and to make more detailed and integrated use of already existing and published data.

The Panel compliments the Agency for providing an up-to-date dioxin cancer mode of action section in its response to NAS comments. It could, however, be improved by incorporating additional data on linear and nonlinear modes of action in different target tissues and life stages. A large amount of data related to the mode of action for the carcinogenicity of TCDD is described in the *Report*, but the focus appears to be on presenting evidence that supports the use of a default linear approach rather than providing a balanced evaluation of alternative mode of action hypotheses.

The discussion of the likely dose-response for receptor mediated processes focuses only on the first step, binding of the agonist to the receptor, which is ultimately linear at low concentrations. However, no discussion is given to the nature of the dose-response for the down-stream sequelae of receptor activation, for which there is evidence of nonlinearity. It is, in fact, the fundamentally nonlinear nature of the dose-response for receptor mediated processes that underlies the conviction of a large segment of the scientific community that a nonlinear approach should be preferred for the risk assessment for dioxin.

Recommendations

- EPA should further expand the discussion of mode of action data available to delineate linear versus nonlinear modes of action and effects in different target tissues at different life stages.

  Charge Question 5.2.b. Do the available data support EPA's conclusion that the overall mode(s) of action for TCDD-induced carcinogenesis is largely unknown? Please comment on whether this evaluation is clearly described.

Response:

The Panel notes that much is known about TCDD toxicity and mode of action. Some Panel members indicated that the characterization of the mode of action should be "reasonably well known" rather than "largely unknown." Nevertheless, the Panel agrees that the exact mechanism-of-action has not been fully delineated for any distinct TCDD-toxicity end-point. For example, it was pointed out that most TCDD toxicities are mediated by activation of the AhR. Many studies have demonstrated that TCDD can activate or interfere with the activity of estrogen receptors, as well as other steroid receptors. Such interference can disrupt the regulation of cell proliferation, cell death and tissue differentiation. By disrupting these cell functions, TCDD can have profound and lasting effects as demonstrated by studies showing that TCDD exposure during development produces adult neural dysfunctions.

Recommendations

- EPA should provide a discussion of the evidence for possible modes of action that include both linear and nonlinear alternatives.

- EPA should describe the receptor mediated nonlinear mode of action for dioxin (e.g., Van den Heuvel et al., 1994; Li and Rozman 1995; Andersen et al., 1997; Bhattacharya et al., 2010; Gim et al., 2010) and DLCs (e.g., Rozman et al., 1993, 2005; Stahl et al., 1994; Viluksela et al., 1994, 1997a,b, 1998a,b), as well as evidence regarding the fundamentally nonlinear nature of receptor mediated cellular responses (e.g., Andersen et al., 1999; Louis and Becskei, 2002; Zhang et al., 2010).

  Charge Question 5.3. Is EPA's approach for selecting data sets from the key epidemiologic studies and animal bioassays identified for cancer dose response modeling scientifically justified and clearly described?

Response:

EPA derived candidate oral slope factors (OSFs) for all cancer mortality from human epidemiological studies as well as for individual and combined tumor incidence from rodent cancer bioassays. The study inclusion criteria outlined in the Report were used to select the studies for TCDD dose-response modeling. EPA chose to give higher consideration to the human epidemiological data in developing the OSF for TCDD and decided to use the results of the Cheng et al. (2006) study based on total cancer mortality. The Panel agrees with this approach because the Cheng et al. (2006) study incorporated information on gradation of exposure and because in the case of TCDD there appear to be multiple targets for carcinogenic action. Expanded discussion of several other studies would support the weight-of-evidence for carcinogenicity in less common cancers such as lymphomas and soft tissue sarcoma. The Panel discussed the possible value of including studies with DLCs in the evaluation of the weight-of-evidence, in light of the small number of studies involving primarily exposure to TCDD. There are a numerous studies in the literature involving the health effects of DLCs. These include rice oil poisoning incidents in Japan and Taiwan. These incidents have been described, and additional references have been provided, in Schecter and Gasiewicz (2003).

Recommendations

- EPA should present in a clear and visible format, for example in a table, an indication of which studies were carried forward or not, and the reasons for the decisions made. The weight-of-evidence discussion should be expanded to include evidence from studies of individual cancers for which precise gradation of exposure data is lacking.

  Charge Question 5.4. For the animal bioassay data, potential cancer oral slope factors (OSFs) were calculated by linear extrapolation (using a linear, non threshold cancer approach) from the point of departure (POD). EPA also estimated the composite risk of the occurrence of several tumor types from the animal cancer bioassay data.

  Please comment on the following:

  Charge Question 5.4.a. Please comment on whether the approach for estimating cancer risk, including the use of tumor modeling of the TCDD animal cancer bioassay data, is scientifically justified and clearly described.

Response:

The Panel agrees that the approach for estimating cancer risk from animal studies was scientifically justified and clearly described.

> *Charge Question 5.4.b. Please comment on the choice of using a $BMDL_{01}$ as the POD for the development of candidate oral slope factors derived from the TCDD animal cancer bioassays.*

Response:

The Panel notes the consistency of the selection of the $BMDL_{01}$ as the POD with Agency guidelines and has no further comments.

> *Charge Question 5.5. EPA selected Cheng et al. (2006) – an analysis of the NIOSH occupational cohort – as the critical study for oral slope factor (OSF) development. This study was chosen because it considers dose-dependent elimination of TCDD rather than first-order kinetics.*
>
> *Charge Question 5.5.a. Please comment on whether the rationale for this selection is scientifically justified and clearly described. Please identify and provide the rationale for any other studies that should be considered and provide a critical evaluation of the study and of its suitability for meeting the goals of a quantitative cancer assessment.*

Response:

The Panel agrees that Cheng et al (2006) is the appropriate study for OSF development. The selection of this study is well described.

> *Charge Question 5.5.b. Cheng et al. (2006) analyzed all-cancer mortality. Please comment on the use of all-cancer mortality as the basis of the OSF.*

Response:

The Panel agrees that in this case, it is appropriate to use the Cheng et al. (2006) study, which analyzed all-cancer mortality, to derive the OSF because of the extensive dose-response information and because in the case of TCDD there appear to be multiple targets for carcinogenic action.

> *Charge Question 5.5.c. Please comment on whether the use of the Emond PBPK model in the estimation of risk-specific doses from the Cheng et al. dose-response modeling results is scientifically justified and clearly described.*

Response:

The Panel agrees that the use of the Emond model to estimate risk-specific doses from the Cheng et al. (2006) dose-response modeling results is scientifically justified and clearly described. This is because the "concentration-and-age-dependent elimination model" (CADM) used in Cheng et al. (2006) was considered to be less useful for predicting reliable metrics for sensitive human subpopulations. Also, the dose conversions were consistent with those used in the derivation of the RfD. However, as discussed in

the response to Charge Question 3.1.d, the Panel is concerned about the value of the Hill coefficient used.

> *Charge Question 5.5.d. EPA elected to use the log linear relationship of fat concentration and rate ratio to estimate risk-specific doses at all risk levels. EPA could have estimated a POD for cancer risk itself at a single risk level (BMR) for extrapolation to the origin. Please comment on EPA's choice of extrapolation approach.*

Response:

Since the fat concentrations generated by CADM were not linear with the oral exposure at higher doses, a single oral slope factor to be used for all risk levels could not be obtained. EPA used the upper 95% bound on the slope (from Cheng et al., 2006) of the linear relationship between the natural logarithm of the rate ratio and the cumulative fat TCDD concentration (fat-AUC) to estimate risk-specific doses for TCDD at all risk levels. The Panel agrees that the Agency has chosen the appropriate extrapolation approach.

> *Charge Question 5.5.e. The slope factor derived from Cheng et al. (2006) was extrapolated below the background TCDD exposure levels experienced by the NIOSH cohort. Please comment on this extrapolation.*

Response:

The ability of the Cheng study to be informative regarding risks below current background exposure levels is not completely clear. Consideration of both linear and nonlinear mode of action may clarify the extrapolation of risks below background exposure levels.

Recommendations

- EPA should expand the discussion in the *Report* to consider the possibility that mode of action considerations could help to inform whether linear extrapolation of the Cheng data to obtain risk estimates in this range of exposures is appropriate.

> *Charge Question 5.6. Please comment on whether EPA has clearly described the major qualitative uncertainties in the derivation of the OSF.*

Response:

The Panel finds the description of qualitative uncertainties in the derivation of the OSF to be clear and adequate.

> *Charge Question 5.7. EPA did not consider dioxin-like compounds (DLCs) in the cancer dose-response modeling because the occupational exposures in the available cohorts were primarily to TCDD. Background DLC exposures were not incorporated in the dose-response modeling because EPA judged that it was not possible to disaggregate the responses from background exposure to DLCs and occupational exposure to TCDD. Please comment on whether this approach is scientifically justified and clearly described.*

Response:

While the Panel finds that it is important to include DLC studies in the weight-of-evidence analysis, we are conflicted on their use as a source of dose-response estimates for TCDD. The Panel notes the scientific importance and regulatory relevance of including a coordinated TEQ/DLC discussion in the *Report*. Including TEQ/DLC aspects in the evaluation would allow for the use of additional studies with dose-response information that more closely mirror environmental exposures. On the other hand, the Panel recognizes the complications associated with developing a TCDD risk estimate that is dependent on current TEF values.

Recommendations

- DLC studies should be considered in the weight-of-evidence discussion. There is a wealth of literature on these compounds that can be used to support mode of action determination as well as the plausibility of individual biological effects that are used as the basis for risk assessment.

  Charge Question 5.8. The NRC suggested that EPA consider nonlinear approaches for the assessment of TCDD carcinogenicity. In the Response to Comments, EPA presents two illustrative nonlinear approaches for cancer, but considers both inappropriate to use because lack of MOA information.

  Charge Question 5.8.a. Please comment on these two illustrative nonlinear approaches including EPA's conclusions regarding the limitations of these approaches.

Response:

EPA's *Report* does not respond adequately to the NAS recommendation to adopt "both linear and nonlinear methods of risk characterization to account for the uncertainty of dose-response relationship shape below the ED_{01}." Instead of adopting both linear and nonlinear methods, the EPA argues that only a linear approach can be justified, and derives two examples of RfD development using a nonlinear approach that is characterized as an illustrative exercise only. The choice not to include both linear and nonlinear risk assessment approaches for TCDD is inconsistent with the Agency's *2005 Guidelines for Carcinogen Risk Assessment* (EPA, 2005) (page 3-23/24):

> "Nonlinear extrapolation having a significant biological support may be presented in addition to a linear approach when the available data and a weight-of-evidence evaluation support a nonlinear approach, but the data are not strong enough to ascertain the mode of action applying the Agency's mode of action framework."
>
> "In the absence of data supporting a biologically based model for extrapolation outside of the observed range, the choice of approach is based on the view of mode of action of the agent arrived at in the hazard assessment. If more than one approach (e.g., both a nonlinear and linear approach) are supported by the data, they should be used and presented to the decision maker."

Recommendations

- EPA should present both linear and nonlinear risk assessment approaches. In the absence of a definitive nonlinear mode of action, the linear option results can serve as the baseline for comparison with other estimates. The examples in the current document should be formalized and extended to allow for such a comparison.

  Charge Question 5.8.b. Are there other nonlinear approaches that could be readily developed based on existing data for the assessment of TCDD carcinogenicity? If so, please suggest alternative approaches and describe their utility and suitability for meeting the goals of a quantitative cancer assessment.

Recommendations

- Since the EPA nonlinear analysis only used studies in S-D rats that were identified in Section 2 of the *Report* for potential noncancer dose-response modeling, additional alternative PODs should be added. For example, Simon et al. (2009), which was cited in EPA's *Report*, provided a number of alternative PODs for a nonlinear approach that should be included in the EPA risk assessment.

3.6. Feasibility of Quantitative Uncertainty Analysis

In its evaluation of EPA's *2003 Reassessment*, the NAS committee recommended that EPA improve the transparency, thoroughness, and clarity in quantitative uncertainty analysis (QUA). Section 6 of EPA's Response to NAS Comments document addresses NAS comments regarding QUA. The Panel was asked to comment on: whether Section 6 of EPA's *Report* was clearly presented and scientifically justified; EPA's conclusion that a QUA is not feasible; the discussion of volitional uncertainty, and the utility of the limited sensitivity studies presented by EPA.

Charge Question 6.1. Please comment on the discussion in this Section. Is the response clearly presented and scientifically justified?

Response:

As discussed below, the Panel finds that Section 6 of EPA's *Report* is clearly presented, but it is not scientifically justified. In particular, the Panel disagrees with EPA's argument that, since the most detailed and complete available methods for conducting a QUA are unfeasible, no analysis can be conducted at all. There are a number of approaches for conducting a QUA, some of which are feasible given current knowledge and data. Specific methods that can be implemented in a timely manner using available data and knowledge are suggested.

Clarity of the EPA response to the NAS presented in Section 6 of the Report

The EPA response is clearly presented and provides many useful insights for the Agency's dioxin reassessment. The *Report* addresses a broad range of philosophical and methodological issues in conducting an uncertainty analysis for TCDD toxicity, specifically for estimates of cancer oral slope factors and noncancer reference doses. Section 6 is successful in identifying the challenges involved in

assessing uncertainty in toxicity estimates based on a small set of available models for toxicokinetics, dose-response relationships, and low dose extrapolation, with limited application, testing, and verification; and a small set of animal bioassay, epidemiological or clinical/case studies, many with differing endpoints, dose metrics, and (in the case of the human studies) uncertain exposure and subject data. In its discussion of available methods, the *Report* is somewhat biased in its treatment of certain statistical methods (discussed below) which could address some of these issues (though the *Report* does note the potential contribution of the methods at the end of Section 6 as part of ongoing or future studies) and overly pessimistic regarding our ability to provide improved quantitative estimates for certain portions of the toxicity assessment.

Some Panel members note that the whole section should be rewritten to make it more understandable to non-statisticians. As further discussed in the editorial comments on Section 6 in Appendix D of this report, some phrasing and word choices in the text should be reconsidered, in particular "exotic methods," "volitional uncertainty," and "epistemic uncertainty." The Panel finds that the definition of "quantitative uncertainty analysis" is overly narrow and should be expanded to embrace other common and useful methods discussed below. In a few other places, the *Report's* wording in Section 6 is strongly at variance with the literature on uncertainty analysis (see editorial comments in Appendix D of this report).

Scientific justification of the arguments presented in Section 6

The Panel finds that the arguments in Section 6 are not scientifically justified. In Section 6, EPA's decision to not do an integrated quantitative uncertainty analysis is presented and a variety of theoretical issues are discussed, but EPA's decision may be based primarily on grounds of practicality or timeliness. EPA indicates that a complete quantitative uncertainty analysis would require data and resources not available. We disagree with this logic. More limited evaluations can, and should, still be implemented to inform critical issues in the dioxin reassessment. EPA should be methodical in considering what variables and components of the assessment would be included in the analysis. The Panel finds that the uncertainty narratives and sensitivity analyses already in the document are an excellent beginning and may constitute the lion's share of the work necessary to implement quantitative uncertainty analysis based on simple bounding.

The Panel does not concur with the specific argument EPA used to justify not doing a unified QUA. If the answer to the question of why EPA did not undertake one is that it was not possible to specify precise marginal distributions and dependence functions from existing data, then the conclusion would be that EPA has not been responsive to the NAS criticism, because there are many possible approaches that could be used that do not depend on such specifications. If the argument is that EPA guidance does not require a QUA, then one might agree that the NAS criticism is perhaps itself unreasonable. If EPA had asserted that it actually had done an uncertainty analysis in the form of uncertainty factors (UFs) and the limited sensitivity studies that were performed, then that might be understandable, though not consistent with the current state-of-the-art in risk and uncertainty analysis. Even if the argument had been that mounting a QUA is a significant and controversial undertaking itself and that doing one should not delay the finalization of the *Report*, then such a practicality argument would be understandable given the protracted delay in completing the dioxin reassessment.

Instead, EPA asserts that "Data are the ultimate arbiter of whether quantitative uncertainty analysis … has sufficient evidentiary support." This flies in the face of how uncertainty analyses are normally

conceived. Of course, the absence of data is never a substantive reason *not* to conduct an uncertainty analysis; it is the reason *to* do one.

In its *Report*, EPA indicates that it needs an "underlying distribution from which to sample" in order to conduct a quantitative uncertainty analysis. The Panel notes that this is not necessarily true, and it is facile to shrug off a call to characterize and account for important uncertainties in the assessment process on these grounds alone. If one can *estimate* the value of a quantity, then one should be able to express the uncertainty about the value, otherwise one does not really have a scientific measurement in the first place. One is not forced to identify precise probability distributions and dependence functions for everything that is to be characterized as uncertain. Even when the uncertainty is volitional (or decisional or just model uncertainty), there can be relevant ranges that are interesting to decision makers and stakeholders. In some cases, the analysis may be formally closer to a sensitivity analysis, but some appropriate response is usually possible, if not always practicable. To its credit, EPA has acknowledged the legitimacy of the call for QUA by NAS and undertaken some efforts in this direction.

In the *Report*, EPA calls uncertainty analysis an "emerging area in science" and this is inarguably true, but it does not seem reasonable to hold that methodological research is necessary for EPA to do anything more comprehensive to respond to NAS's criticism, even if we disallow the use of expert elicitation. Under a commitment to the idea that analyses be *data-driven*, it is possible to do something that is useful, even if it is not predicated on precise distributions. There are a variety of ways to conduct a quantitative uncertainty analysis, even an entirely probabilistic one that obeys the Kolmogorov axioms (Gillies, 2000) that require neither extensive data nor expert elicitation. The response to Charge Question 6.2 below provides a list of various ways (with references) to accomplish this. The list includes probability trees or model choice trees that articulate the structure of the model and dependencies, sensitivity analyses, simple interval analysis that just propagates the plausible ranges, and the supervaluation approach that uses nested inner and outer intervals (with the inner range representing the values that most everyone considers to be plausible values and the outer range representing conservatively broad ranges). There is also a continuous and unbounded version of nesting intervals in an approach known as info-gap analysis that would be useful if one cannot develop finite bounds on some of the inputs. One can also propagate *bounds* on distribution functions, so whatever imperfect information about each input variable's distribution is available, one can fashion bounds on distribution functions and propagate them through the calculations, with or without assumptions or information about the dependencies among variables.

The Panel notes that the approaches mentioned above require EPA to make certain modeling judgments, in the same way that developing any analysis requires judgments. However, this does not mean that analysts would be required to make up numbers or elicit any expert opinion. Such an analysis does not necessarily require a lot of extra work by EPA. These methods can be simple to develop, and they are mostly computationally trivial. Of course, the more comprehensive the analysis is, the harder it is to complete. But the analysis does not have to be fully comprehensive to provide useful insights.

We note that there was not perfect consensus among Panel members about the value of a quantitative uncertainty analysis. Some on the Panel agree that an uncertainty analysis is not an absolute good. For instance, if the final answer is already clear, an uncertainty analysis can be a waste of time and resources. It would not be reasonable to insist on another analysis which would merely waste time and resources. Likewise, if the analysis is done poorly, or without appeal to available evidence from the real world, it can be misleading. For instance, the idea, mentioned in footnote 66 on page 6-20 of EPA's

Report, of arbitrarily converting uncertainty factors to independent lognormal random variables in a scattered attempt to mount a QUA would entail a suite of unjustified and probably untenable assumptions rendering the exercise nearly pointless. Finally, if the analysis is used *strategically* to avoid rendering or finalizing a decision that is proper, it can be counterproductive. However, most members of the Panel indicate that quantitative uncertainty analysis is an integral part of any good assessment, and that one is essential to address the many empirically unresolved questions and issues that have arisen in this assessment which beg for explicit consideration in the context of an uncertainty analysis. In its discussion of the other charge questions, the Panel has identified a number of important issues that should be addressed in an eventual uncertainty analysis.

Other methods to be considered

The Panel finds that relevant Bayesian methods have been inadequately addressed and improperly dismissed in Section 6. In particular, methods that should be given a more extensive and balanced discussion with more citations to the literature include: 1) Bayesian hierarchical modeling (Coull et al., 2003; Axelrad et al., 2007; Ryan, 2008; Choi et al., 2010;) which is used for combining information from multiple studies, and 2) Bayesian model averaging (Morales et al., 2006; Viallefont et al., 200l; Wheeler and Bailer 2007, 2009) which would be useful for considering more than one dose-response equation, while allowing the data to weight their *relative* likelihood and contribution to the estimate. These Bayesian methods should not be referred to as "exotic." For example, in agreeing with the Section 6 authors that these methods should be pursued in ongoing and future case studies, White et al. (2009) refer to them as "advanced," rather than exotic. Specifically, they recommend that health scientists should explore statistical approaches to model selection and suggest that "improvements to statistical approaches for model selection, such as model averaging, should be pursued. Case study applications of these advanced statistical approaches will identify potential strengths and weaknesses of the approaches and their significance for risk characterization" (White et al., 2009).

Recommendations

- The Panel recommends that EPA revise Section 6 of the *Report* because, as discussed above, the arguments in this section are not scientifically justified. In particular, EPA should consider revising its argument that quantitative uncertainty analysis is unfeasible for the dioxin assessment. Specific suggestions regarding feasible methods for quantitative uncertainty analysis are provided herein.

  Charge Question 6.2. Please comment on EPA's overall conclusion that a comprehensive quantitative uncertainty analysis is not feasible.

Response:

As discussed above, the Panel rejects EPA's argument that a quantitative uncertainty analysis is unfeasible. Although a quantitative uncertainty analysis is challenging, the Panel does not agree that it is impossible or even impractical to undertake one. While it may well be true that we lack an adequate empirical basis for full Monte-Carlo propagation of input distributions, there are many other options available. Many on the Panel indicated that the present circumstances warrant a compromise approach that would be simple and achievable with modest effort by the Agency. Various bounding approaches, sensitivity studies, uncertainty set analyses, and event trees (probability trees without the probabilities)

are suggested as possible approaches that could be used. With such methods, legitimate and comprehensive uncertainty analyses (including even fully probabilistic analyses) are possible. They would be useful and sufficient to respond to NAS' criticism.

The Panel agrees with EPA's assertion that expert elicitation would be problematic and should be "off the table." However, many on the Panel further suggest that value-of-information methods would also be very useful, although feedback from EPA included reservations about this idea. A discussion of value of information methods is provided in Appendix C of this report.

The Panel considered the use of bounding approaches for quantitative uncertainty analysis and asked EPA to provide information about the limitations of bounding approaches. In response, EPA asked Dr. Roger Cooke (an author of EPA's *Report*) to send the Panel a document on bounding analysis. The short bounding analysis document provided to the Panel by Dr. Cooke focused on the features of interval analysis, although this is not by any means the only approach that might be useful in the context of the dioxin assessment. The bounding analysis document mentions one issue that could be construed as a disadvantage of this simplest bounding approach. It is the idea that the ranges are supposed to be absolute bounds on the possible values of each input variable. So, for instance, the only thing one can say about a percentage is that it is between zero and 100%, or the only thing one can say about a dispersal distance is that it is between zero and the circumference of the Earth (these are Dr. Cooke's examples). But the Panel finds that this criticism seems to represent a misunderstanding of the word "absolute." Vacuous (e.g., physically limiting) bounds are not the only bounds that can be used in interval analysis. In fact, they are meant to be informed by observed study results. Furthermore, one is not necessarily limited to interval ranges and interval analysis.

The Panel suggests that there are in fact a variety of methods that, with proper application, could be useful and informative, including:

- **Sensitivity analysis studies** (even if not completely comprehensive) (Saltelli et al., 2000a,b; Frey and Patil, 2002),
- **Interval analysis** (Moore 1966; Neumaier, 1990) which has been widely used for decades and can be applied to complex models and even blackbox models (Trejo and Kreinovich, 2001),
- **Nesting of intervals**, e.g., two levels, wide and narrow can give conservative and optimistic characterizations of overall uncertainty (van Fraassen, 1966, 1980),
- **Probability bounds analysis** (Ferson and Long, 1995; Ferson et al., 2003) including Bayesian p-boxes (Montgomery, 2009), which has been used in a variety of applications (Regan et al., 2002a,b; Aughenbaugh and Paredis, 2007; Dixon, 2007; Karanki et al., 2009; Minnery et al., 2009), including assessments at two Superfund sites (EPA, 2002-2005, 2007),
- **Info-gap decision theory** (Ben-Haim, 2006) which has been used in several applications, (Regan et al., 2005; Davidovitch et al., 2009; Hall and Harvey, 2009; Rout et al., 2009; Yokomizo, 2009),
- **Robust optimization** (Bertsimas and Brown, 2009; Bertsimas et al., 2009; Bertsimas et al., 2010; Ben-Tal et al., 2010), and
- **Probability trees**, which are distributional methods for considering alternative assumptions and models at various stages of the toxicity assessment. Small (2008) explains that the distributional approach for characterizing uncertainty in cancer risk assessment was developed by Evans, Sielken, and co-workers beginning in the 1990s (Sielken, 1990; Holland and Sielken, 1993; Evans et al., 1994a,b, 1995; Sielken et al., 1995; Sielken and Valdez-Flores, 1996, 1999) and has

also been referred to as information analysis, weight-of-evidence analysis, the comprehensive methodology, and comprehensive realism (Sielken, 1990; Sielken et al.,1995). The method has since been acknowledged in a number of reviews of cancer risk assessment practice and research needs (Boyce, 1998; Moschandreas and Karuchit, 2002; Zeise et al., 2002), and applied in various forms for risk assessment of different chemical compounds (Crump, 1994; Humphreys et al., 2001; Rai et al., 2002; Kirman et al., 2004; Starr et al., 2006; David et al., 2006). The distributional approach enables consideration of a "portfolio-of-mechanisms" that may contribute to carcinogenesis (Cox, 2006).

These methods are nontrivial and potentially valuable alternatives to traditional probabilistic uncertainty analysis, and they are able to provide insights on critical uncertainties in the assessment endpoints and the ongoing and future research needed to achieve their resolution. The motivation for all of these approaches is the recognition that the use of a single set of assumptions for the components of a cancer risk assessment, whether default, conservative, or otherwise, fails to capture the full range of plausible or likely relationships, how these relationships depend upon our current state of knowledge, the implications for computed values of potency or unit risk, and the opportunities for improved estimates. The methods require modeling judgment as any analysis does, but they can provide a basis for ongoing integration and value of information assessment as new studies and knowledge accumulate over time (Brusick et al., 2008). These methods can at least provide useful bounds on the plausible risks and on the value of information (VOI) of reducing uncertainties further (especially, perhaps, on whether the dose-response relation has a threshold).

There are, of course, many significant benefits to undertaking a quantitative uncertainty analysis. Although a completely comprehensive analysis might indeed be too much to expect, it is possible and practical to provide readers with much more useful information about uncertainty. A policy maker might reasonably expect the *Report* to provide insight into major uncertainties and questions such as the following:

- How likely is it that TCDD is not a human carcinogen at current exposure levels? Full discussion of this uncertainty may help to overcome probability neglect and action bias (Patt and Zeckhauser, 2000).
- How likely is it that TCDD at current exposure levels has health effects that have not yet been identified in the toxicological or epidemiological literature (Diamanti-Kandarakis et al., 2009; Soto and Sonnenschein, 2010)?
- What is the probability that reducing TCDD exposures would not reduce cancer risk at all, or only by amounts that would not be measurable, based on recent epidemiological studies and updates such as Pesatori et al. (2009)?
- What is the probability that reducing TCDD exposures would reduce cancer risk in the whole U.S. population, or targeted subpopulations, by amounts significantly greater than a prediction derived from the cancer slope factor estimated by EPA?
- What is the probability that reducing TCDD exposures would increase cancer risk (e.g., if the dose-response relation is J-shaped or U-shaped)?
- What is the decision-analytic value of information (VoI) from collecting more information on AhR kinetics and dose-response before making risk management decisions?
- What is the probability that TCDD interacts with other compounds to which U.S. or targeted subpopulations are exposed, increasing cumulative risk for cancer or other health effects (Carpenter et al., 2002)?

Although many members of the public believe that it is imprudent or even morally wrong to delay tighter regulation of TCDD exposures (perhaps reflecting beliefs that TCDD is a potent carcinogen, developmental toxin, etc.) many on the Panel expressed the view that EPA should provide a thorough quantitative decision analysis that makes explicit the current uncertainties and trade-offs and that shows the conditions under which acting now or postponing action are the optimal actions. Without such quantitative analysis, risk management decisions for TCDD will not be adequately informed, and principles other than those of rational decision making (e.g., the biases discussed in Sunstein and Zeckhauser, 2010) may dominate risk management decisions for TCDD. EPA's uncertainty analysis should provide the scientific basis for improved decision making. The current decision, in effect, to "punt" on quantitative uncertainty analysis is not adequate for informing responsible risk management decision and policy-making, and is not justified.

Recommendations

- The Panel recommends that EPA reconsider the argument for not doing a quantitative uncertainty analysis, or undertake one. EPA could follow the recommendation of the NAS on this point by using one or more of the techniques suggested above.

  Charge Question 6.2.a. Please comment on the discussion in Section 6 regarding volitional uncertainty and how this type of uncertainty limits the ability to conduct a quantitative uncertainty analysis.

Response:

In the *Report*, EPA contrasts volitional uncertainty with cognitive uncertainty. The Panel recommends that the term "volitional uncertainty," which might also have been called "decisional uncertainty," should be dropped from the *Report*. EPA should instead display the different modeling choices and the consequences of making them. The decisions mentioned in the discussion in Section 6 of volitional uncertainty are modeling choices, and they should be dealt with using techniques for model uncertainty. Standard tools and techniques for analysis of model uncertainty can be applied.

Recommendations

- The Panel recommends that EPA delete from the *Report* the notion of "volitional uncertainty." EPA should display the different modeling choices and the consequences of making them.

  Charge Question 6.3. Throughout the document (including the Appendices), EPA presents a number of limited sensitivity analyses (e.g., toxicokinetic modeling, RfD ranges, cancer OSF ranges, cancer RfD development). Please comment on the approaches used, and the utility of these sensitivity analyses in clarifying potential significant uncertainties.

Response:

The Panel congratulates EPA on the sensitivity studies that it has already done and considers them to be very useful. These studies should be integrated and unified in an overall uncertainty analysis. The Panel emphasizes that EPA has already done the lion's share of the effort needed in their considerations

described in the uncertainty narratives. EPA should take credit for this hard work and extend the sensitivity studies to respond fully to the NAS criticism.

Recommendations

- The Panel recommends that sensitivity studies that have already been completed be integrated into whatever additional uncertainty analysis EPA elects to undertake.

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APPENDIX A: EPA'S CHARGE QUESTIONS

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR ENVIRONMENTAL ASSESSMENT
WASHINGTON, DC 20460

May 27, 2010

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

SUBJECT: Request for Science Advisory Board Review of the Draft Report, "EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments"

FROM: Becki Clark, Deputy Director
National Center for Environmental Assessment (8601P)
Office of Research and Development

TO: Vanessa T. Vu, Ph.D., Director
EPA Science Advisory Board (1400F)

This is to request a review by the Science Advisory Board of the draft report entitled "EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments" (EPA/600/R-10/038A). This draft report details the Environmental Protection Agency's (EPA) response to key comments and recommendations included in the 2006 NAS report ("Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment") on their review of the (EPA) 2003 draft dioxin reassessment. This draft report also includes significant new analyses on both the potential cancer and noncancer human health effects that may result from chronic exposures to dioxins.

Attached is the Charge that provides background information as well as questions that are to be the focus of the Science Advisory Board review of this draft report.

Please let me know if you have any questions. Thank you.

Attachment: Charge for EPA's Science Advisory Board – Review of the Draft Report, "EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments"

cc: Peter W. Preuss
Annette Gatchett
Glenn Rice
Cheryl Itkin

Charge to the Science Advisory Board for Peer Review of Draft Report
"EPA's Reanalysis of Key Issues Related to Dioxin Toxicity
and Response to NAS Comments"

May, 2010

EPA has been preparing an assessment of the potential health impacts of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) for many years. In 2003, EPA released an external review draft report entitled, *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds* (U.S. EPA, 2003) (herein referred to as *"2003 Reassessment"*) that was reviewed by the EPA Science Advisory Board (SAB), and then by the National Academy of Sciences (NAS). In 2006, the National Research Council (NRC) of the National Academies published their report of EPA's reassessment, *Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment* (NRC, 2006).

The current Report *EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments* (*"Response to Comments"*) before the SAB is a response to the review by the NRC, and includes new analyses completed in response to the NRC recommendations and recently published literature, as well as a discussion of topics where our views differed. The draft *Response to Comments* document is not an assessment per se; it is designed to supplement the information provided in *the 2003 Reassessment*. However, the draft *Response to Comments* provides a noncancer reference dose and updated cancer values. Detailed discussions of many of the issues addressed in the draft *Response to Comments* are available in the *2003 Reassessment* and have not been reproduced in the current Report – whenever appropriate; the reader is directed to the pertinent chapters of the *2003 Reassessment*.

The NRC identified three key recommendations that they believed would result in substantial improvement to the EPA *2003 Reassessment* and thus support a scientifically robust characterization of human responses to exposures to TCDD. These three key areas are (1) improved transparency and clarity in the selection of key data sets for dose-response analysis, (2) further justification of approaches to dose-response modeling for cancer and noncancer endpoints, and (3) improved transparency, thoroughness, and clarity in quantitative uncertainty analysis. The NRC Report also encouraged EPA to calculate a reference dose (RfD), which had not been derived in the *2003 Reassessment*. The draft *Response to Comments* document addresses each of these issues. Please consider the accuracy, objectivity, and transparency of EPA's reanalysis and responses in your review.

General Charge Questions

1.1 Is the draft *Response to Comments* clear and logical? Has EPA objectively and clearly presented the three key NRC recommendations?

1.2 Are there other critical studies that would make a significant impact on the conclusions of the hazard characterization or dose-response assessment of the chronic noncancer and cancer health effects of TCDD?

Specific Charge Questions

Section 2. Transparency and Clarity in the Selection of Key Data Sets for Dose-Response Analysis

2.1. Is this Section responsive to the NAS concern about transparency and clarity in data-set selection for dose-response analysis?

2.2. Are the epidemiology and animal bioassay study criteria/considerations scientifically justified and clearly described?

2.3. Has EPA applied the epidemiology and animal bioassay study criteria/considerations in a scientifically sound manner? If not, please identify and provide a rationale for alternative approaches.

Section 3. The Use of Toxicokinetics in Dose-Response Modeling for Cancer and Noncancer Endpoints

3.1 The *2003 Reassessment* utilized first-order body burden as the dose metric. In the draft *Response to Comments* document, EPA used a physiologically-based pharmacokinetic (PBPK) model (Emond et al., 2004, 2005, 2006) with whole blood concentration as the dose metric rather than first-order body burden. This PBPK model was chosen, in part, because it includes a biological description of the dose-dependent elimination rate of TCDD. EPA made specific modifications to the published model based on more recent data. Although lipid-adjusted serum concentrations (LASC) for TCDD are commonly used as a dose metric in the literature, EPA chose whole blood TCDD concentrations as the relevant dose metric because serum and serum lipid are not true compartments in the Emond PBPK models (LASC is a side calculation proportional to blood concentration).

Please comment on:

3.1.a. The justification of applying a PBPK model with whole blood TCDD concentration as a surrogate for tissue TCDD exposure in lieu of using first-order body burden for the dose-response assessment of TCDD.

3.1.b. The scientific justification for using the Emond et al. model as opposed to other available TCDD kinetic models.

3.1.c. The modifications implemented by EPA to the published Emond et al. model.

3.1.d. Whether EPA adequately characterized the uncertainty in the kinetic models.

3.2. Several of the critical studies for both noncancer and cancer dose-response assessment were conducted in mice. A mouse PBPK model was developed from an existing rat model in order to estimate TCDD concentrations in mouse tissues, including whole blood.

Please comment on:

3.2.a. The scientific rationale for the development of EPA's mouse model based on the published rat model (Emond et al., 2004, 2005, 2006).

3.2.b. The performance of the mouse model in reference to the available data.

3.2.c. Whether EPA adequately characterized the uncertainty in the mouse and rat kinetic models. Please comment specifically on the scientific justification of the kinetic extrapolation factor from rodents to humans.

3.3 Please comment on the use of the Emond et al. PBPK model to estimate human intakes based on internal exposure measures.

3.4. Please comment on the sensitivity analysis of the kinetic modeling (see Section 3.3.5).

3.5. Both EPA's noncancer and cancer dose-response assessments are based on a lifetime average daily dose. Did EPA appropriately estimate lifetime average daily dose? If not, please suggest alternative approaches that could be readily developed based on existing data.

Section 4. Reference Dose

4.1. The Mocarelli et al. (2008) and Baccarelli et al. (2008) studies were selected as co-critical studies for the derivation of the RfD. Is the rationale for this selection scientifically justified and clearly described? Please identify and provide the rationale for any other studies that should be selected, including the rationale for why the study would be considered a superior candidate for the derivation of the RfD. In addition, male reproductive effects and changes in neonatal thyroid hormone levels, respectively, were selected as the co-critical effects for the RfD. Please comment on whether the selection of these critical effects is scientifically justified and clearly described. Please identify and provide the rationale for any other endpoints that should be selected as the critical effect.

4.2. In the Seveso cohort, the pattern of exposure to TCDD is different from the average daily exposure experienced by the general population. The explosion in Seveso created a high dose pulse of TCDD followed by low level background dietary exposure in the exposed population. In the population, this high dose pulse of TCDD was slowly eliminated from body tissues over time. There is uncertainty regarding the influence of the high-dose pulse exposure on the effects observed later in life.

4.2.a. Mocarelli et al. (2008), reported male reproductive effects observed later in life for boys exposed to the high dose pulse of TCDD between the ages of 1 and 10. EPA identified a 10 year critical exposure window. In the development of the candidate RfD, EPA used an exposure averaging approach that differs from the typical approach utilized for animal bioassays. EPA determined that the relevant exposure should be calculated as the mean of the pulse exposure and the 10-year critical exposure window average. Please comment on the following:

4.2.a.i. EPA's approach for identifying the exposure window and calculating average exposure for this study.

4.2.a.ii. EPA's designation of a 20% decrease in sperm count (and an 11% decrease in sperm motility) as a LOAEL for Mocarelli et al. (2008).

4.2.b. For Baccarelli et al. (2008), the critical exposure window occurs long after the high-dose

pulse exposure. Therefore, the variability in the exposure over the critical exposure window is likely to be less than the variability in the Mocarelli et al. subjects. EPA concluded that the reported maternal exposures from the regression model developed by Baccarelli et al. provide an appropriate estimate of the relevant effective dose as opposed to extrapolating from the measured infant TCDD concentrations to maternal exposure. Additionally, EPA selected a LOAEL of 5 µ-units TSH per ml blood in neonates; as this was established by World Health Organization (WHO) as a level above which there was concern about abnormal thyroid development later in life. Please comment on the following:

4.2.b.i. EPA's decision to use the reported maternal levels and the appropriateness of this exposure estimate for the Baccarelli et al. study.

4.2.b.ii. EPA's designation of 5 µ-units TSH per ml blood as a LOAEL for Baccarelli et al. (2008).

4.3. Please comment on the rationale for the selection of the uncertainty factors (UFs) for the RfD. If changes to the selected UFs are proposed, please identify and provide a rationale.

4.4. EPA did not consider biochemical endpoints (such as CYP induction, oxidative stress, etc.) as potential critical effects for derivation of the RfD for TCDD due to the uncertainties in the qualitative determination of adversity associated with such endpoints and quantitative determination of appropriate response levels for these types of endpoints in relation to TCDD exposure. Please comment on whether this decision is scientifically justified and clearly described.

4.5. In using the animal bioassays, EPA averaged internal blood TCDD concentrations over the entire dosing period, including 24 hours following the last exposure. Please comment on EPA's approach for averaging exposures including intermittent and one day gestation exposure protocols.

4.6. Please comment on the benchmark dose (BMD) modeling conducted by EPA to analyze the animal bioassay data and EPA's choice of points of departure (PODs) from these studies.

4.7. For the animal bioassay modeling, EPA applied the kinetic extrapolation at the level of the POD prior to applying the uncertainty factors because EPA has less confidence in the kinetic model output at lower doses reflective of the RfD. Please comment on whether this approach was scientifically justified and clearly described.

4.8. Please comment as to whether EPA's qualitative discussion of uncertainty in the RfD is justified and clearly described.

Section 5. Cancer Assessment

5.1. Weight of Evidence Cancer Descriptor: The *2003 Reassessment* concluded that TCDD is a "known human carcinogen." In the current draft *Response to Comments* document, EPA concluded that under the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005) TCDD is "carcinogenic to humans." Is the weight-of-evidence characterization scientifically justified and clearly described?

5.2. Mode of Action: The mode of action of a carcinogen can inform identification of hazards and approaches used for a dose-response assessment. The mode of carcinogenic action for TCDD has not been elucidated for any tumor type. EPA concluded that, while interaction with the Ah receptor is likely to be a necessary early event in TCDD carcinogenicity in experimental animals, the downstream events involved are unknown.

5.2.a. Are the available data related to mode(s) of action for the carcinogenicity of TCDD appropriately characterized and clearly presented?

5.2.b. Do the available data support EPA's conclusion that the overall mode(s) of action for TCDD-induced carcinogenesis is largely unknown? Please comment on whether this evaluation is clearly described.

5.3. Is EPA's approach for selecting data sets from the key epidemiologic studies and animal bioassays identified for cancer dose response modeling scientifically justified and clearly described?

5.4. For the animal bioassay data, potential cancer oral slope factors (OSFs) were calculated by linear extrapolation (using a linear, nonthreshold cancer approach) from the point of departure (POD). EPA also estimated the composite risk of the occurrence of several tumor types from the animal cancer bioassay data.

5.4.a. Please comment on whether the approach for estimating cancer risk, including the use of tumor modeling of the TCDD animal cancer bioassay data, is scientifically justified and clearly described.

5.4.b. Please comment on the choice of using a $BMDL_{01}$ as the POD for the development of candidate oral slope factors derived from the TCDD animal cancer bioassays.

5.5. EPA selected Cheng et al. (2006) – an analysis of the NIOSH occupational cohort – as the critical study for oral slope factor (OSF) development. This study was chosen because it considers dose-dependent elimination of TCDD rather than first-order kinetics.

5.5.a. Please comment on whether the rationale for this selection is scientifically justified and clearly described. Please identify and provide the rationale for any other studies that should be considered and provide a critical evaluation of the study and of its suitability for meeting the goals of a quantitatively cancer assessment.

5.5.b. Cheng et al. (2006) analyzed all-cancer mortality. Please comment on the use of all-cancer mortality as the basis of the OSF.

5.5.c. Please comment on whether the use of the Emond PBPK model in the estimation of risk-specific doses from the Cheng et al. dose-response modeling results is scientifically justified and clearly described.

5.5.d. EPA elected to use the log linear relationship of fat concentration and rate ratio to estimate risk-specific doses at all risk levels. EPA could have estimated a POD for cancer risk itself at a single risk level (BMR) for extrapolation to the origin. Please

comment on EPA's choice of extrapolation approach.

5.5.e. The slope factor derived from Cheng et al. (2006) was extrapolated below the background TCDD exposure levels experienced by the NIOSH cohort. Please comment on this extrapolation.

5.6. Please comment on whether EPA has clearly described the major qualitative uncertainties in the derivation of the OSF.

5.7. EPA did not consider dioxin-like compounds (DLCs) in the cancer dose-response modeling because the occupational exposures in the available cohorts were primarily to TCDD. Background DLC exposures were not incorporated in the dose-response modeling because EPA judged that it was not possible to disaggregate the responses from background exposure to DLCs and occupational exposure to TCDD. Please comment on whether this approach is scientifically justified and clearly described.

5.8. The NRC suggested that EPA consider nonlinear approaches for the assessment of TCDD carcinogenicity. In the *Response to Comments*, EPA presents two illustrative nonlinear approaches for cancer, but considers both inappropriate to use because of the lack of MOA information.

5.8.a. Please comment on these two illustrative nonlinear approaches including EPA's conclusions regarding the limitations of these approaches.

5.8.b. Are there other nonlinear approaches that could be readily developed based on existing data for the assessment of TCDD carcinogenicity? If so, please suggest alternative approaches and describe their utility and suitability for meeting the goals of a quantitative cancer assessment.

Section 6. Feasibility of Quantitative Uncertainty Analysis from NAS Evaluation of the 2003 Reassessment

6.1. Please comment on the discussion in this Section. Is the response clearly presented and scientifically justified?

6.2. Please comment on EPA's overall conclusion that a comprehensive quantitative uncertainty analysis is not feasible.

6.2.a. Please comment on the discussion in Section 6 regarding volitional uncertainty and how this type of uncertainty limits the ability to conduct a quantitative uncertainty analysis.

6.3. Throughout the document (including the Appendices), EPA presents a number of limited sensitivity analyses (e.g., toxicokinetic modeling, RfD ranges, cancer OSF ranges, cancer RfD development). Please comment on the approaches used, and the utility of these sensitivity analyses in clarifying potential significant uncertainties.

APPENDIX B: DISSENTING OPINION FROM KARL ROZMAN, PH.D.

The University of Kansas Medical Center

Karl K. Rozman, Ph.D.
Professor
School of Medicine
Department of Pharmacology,
Toxicology and Therapeutics

December 9, 2010

Thomas Armitage, Ph.D.
Designated Federal Officer
USEPA Science Advisory Board (1400R)
1200 Pennsylvania Ave., N.W.
Washington, D.C. 20460

RE: A Dissenting Opinion

Dear Tom,

As I have indicated in my previous written and oral opinions to this panel, I disagree with the panel conclusions regarding the carcinogenicity of TCDD and the adequacy of the EPA response to the criticisms of the NAS report.

There is at best equivocal evidence (statistically not significant) for the carcinogenicity of TCDD (or DLCs) in the occupational setting where the body burdens were at least 100 or 1000 times higher than the current or previous background levels. Therefore, the consideration of a practical threshold for any defined population requires acceptance of the compelling scientific conclusion that there is negligible (essentially zero) carcinogenic risk at current background levels which are much lower than past levels. Any other conclusion is incompatible with sound science and no amount of modeling or data manipulation will transform a non-existing effect at occupational exposure levels into a risk at current background levels other than the non-scientific, policy-driven, non-threshold extrapolation by EPA.

Further, it is my opinion that the EPA document (2010 Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments, 600/-10/038A) is deliberately non-responsive to the recommendations of the NAS report.

Respectfully,

Karl K.Rozman, Ph.D., D.A.B.T.
Professor

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Additional Information Provided by Dr. Karl Rozman to Support Dissenting Opinion

The gist of my conclusion based on the evidence presented is that TCDD and its congeners is/are **not known human carcinogens** at occupational and high environmental exposure levels; indeed not even at levels that induce clinical chloracne (ATSDR, 1998; ATSDR, 2011). There is no question that chronic TCDD exposures are carcinogenic for rodents at levels that are comparable to or somewhat higher than those that cause chloracne in humans. Therefore, I have never taken issue with TCDD as a rodent carcinogen or even as a possible human carcinogen. It is my conclusion (after more than 100 peer-reviewed publications of mine dealing with this class of chemicals) that given sufficiently high (greater than the chloracnegenic dose) exposures, dioxins may very well cause cancer in humans just as they do in rodents. Rather, my dissent with the Panel is based on the unassailable logic that if even very high exposures (reviewed in ATSDR, 2011) do not cause cancer in human beings, then past exposures (about 7ppt) and far lower current exposure levels (2 ppt or less) do not produce carcinogenic risk to humans. As you can see from the ATSDR (1998, 2011) summaries, the un-manipulated Fingerhut Study failed to detect an increase in total cancer, although there were many cases of chloracne. Analyses of several hundred, perhaps more than a thousand blood samples revealed the presence of some very high levels of TCDD. Manipulation of those data by U.S. EPA following the lead of IARC (1997) and NTP (2001) resulted in U.S. EPA declaring TCDD as a **known human carcinogen**. From that point on, there was no limit to extrapolation between species and from high-to-low doses. But modeling of an effect non-existent in humans is nothing more than fiction.

There are numerous major scientific objections to the application of U.S. EPA´s policy forcing default linear treatment of all carcinogenicity dose-responses. In the case of TCDD and congeners, linear treatment of the dose-response cannot be justified given the abject failure of these materials to elicit genotoxicity in a wide variety of test systems (e.g., ATSDR, 1998). As such, the U.S. EPA default assumption of linearity in the case of TCDD and congeners is policy masquerading as science.

I for one cannot accept a “policy” pretending to be rigorous science when the intent is to hide non-scientific assumptions for the purpose of short-term political, perceived gain.

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APPENDIX C: VALUE OF INFORMATION

When human health risk assessments include an explicit representation of uncertainty, the potential value of new information (VOI) can be estimated by its ability to reduce uncertainties that matter most to the assessment target. While methods for determining VOI are most commonly associated with the decision analysis literature in the context of informing management or regulatory decisions (Raiffa, 1968; Keeney, 1982; Winkler and Murphy, 1985; Finkel and Evans, 1987; Taylor et al., 1993; Clemen, 1996; Chao and Hobbs, 1997), there are many steps in a scientific assessment well before (or even without subsequent) decision support and decision making where VOI evaluations can be of benefit in characterizing current scientific knowledge and the potential for its improvement. EPA should integrate these methods into their current and ongoing assessments of dioxin toxicity.

When uncertainty in a scientific assessment is measured by the variance of model predictions, a first measure of VOI is the extent to which this variance might be reduced by new or additional data (e.g., Patwardhan and Small, 1992; Brand and Small, 1995; Abbaspour et al., 1996; Chao and Hobbs, 1997; Sohn et al., 2000; Bosgra et al., 2005; Cooke, 2009). The relative contribution of different model assumptions and parameter uncertainties to the variance of the estimated effect (e.g., the BMD), or the cancer slope factor) provides an indication of which of these uncertainties would be most beneficial to address. In addition, a VOI assessment considers the potential for the component uncertainties to be reduced, based on the feasibility, resource requirements (time and funding), and likelihood of success of the studies that would be needed to achieve the necessary improvement in scientific knowledge.

A scientific VOI study may also target a key classification inference that results from a risk assessment, for example, whether a compound is genotoxic. Assuming the current assessment leads one to assign an inconclusive probability to this outcome (e.g., between 10% and 90%, so that neither inference can be rejected with a high degree of confidence), then potentially valuable studies are those able to shift subsequent probabilities to high values (e.g., above 90, 95, or 99%) with a positive result (e.g., providing support for genotoxicity) and/or to low values (below 10, 5, or 1%) with a negative result.

To illustrate, Small (2008) presents a simple probability tree model (a “distributional approach”) for assessing genotoxicity based on studies of DNA damage response caused by naphthalene and its metabolites. In the proposed studies a series of isogenic cell lines deficient in various DNA metabolism pathways are used to characterize the DNA damage responses caused by the targeted compounds. Following results from the cultured cells, mice deficient in the specific DNA damage responses would be exposed to naphthalene. Possible inferences are identified based on the assessed sensitivity and selectivity of study results to the genotoxicity of naphthalene. Study outcomes considered include: i) DNA damage responses in the isogenic cells; ii) increased numbers of stable DNA adducts in the DNA repair deficient mouse lung; and iii) heightened Clara cell toxicity in the DNA repair deficient mouse lung. Illustrative results using Netica are presented as follows:

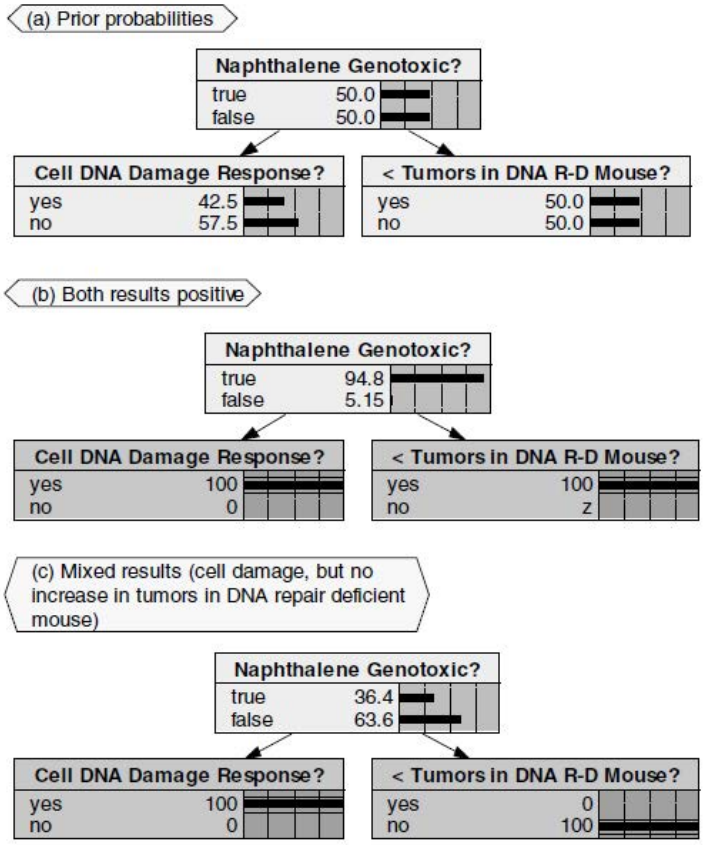

Fig. 5. Illustrative consideration of selected experimental results for naphthalene Study IV (outcomes assumed independent, prior probability of genotoxicity set to 0.5, and sensitivities and selectivities chosen by author solely for illustration of methodology): (A) Prior probability before study; (B) Positive outcomes for both study results; and (C) Positive results for cell DNA damage, but negative results for increased tumors in DNA repair-deficient mouse.

As noted, the results shown above are intended solely to demonstrate the way in which study results can be combined to support or refute targeted inferences.

Even when the uncertainty tree method is only used to delineate the set of possible outcomes and relationships among steps and assumptions in the risk assessment (i.e., mode of action; dosimetry measures for exposure; the mathematical form of the dose-response relationship; the experimental data set(s) used to fit the relationship; and the procedure used for interspecies extrapolation) *without the assignment of probabilities to the tree branches*, key assumptions and the experiments needed to support or refute them can still be identified. These will typically involve elements of the assessment that, depending on their resolution, effectively restrict the set of possible outcomes to either a positive or a negative inference regarding the endpoint of the risk assessment. Establishing a procedure of this type will allow the Agency to put in place a more formal mechanism for identifying, conducting, and integrating the results of key studies for future assessments.

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APPENDIX D: EDITORIAL COMMENTS AND CORRECTIONS

The Panel's advice and responses to the charge questions are contained in the body of this report. However, in the course of the review, the following editorial comments and corrections have been provided. This is not intended to be an exhaustive list.

a. Section 2

Minor suggestions to further improve clarity regarding data set inclusion/exclusion criteria.

Page xxxvii, Lines 16-19. The sentence needs clarification. It currently gives the impression that studies that were eliminated for further analysis would have NOAELs available.

Pages 2-234 – 2-247. EPA should consider adding information to the appendices and/or tables to provide readers with clarification regarding the exclusion of particular studies. For example, an extra column in Table 2-7 listing, by numbered reference, the criteria that were or were not met for each study would be helpful.

b. Section 6

Page 6-2. Add NRC (1996).

Page 6-3, bottom: The word "margins" should be "marginals."

Page 6-3, line 26: If EPA wants to use the adverb "always", the phrase "as a joint distribution" should be "as some characterization of a joint distribution" to be correct.

Page 6-4, lines 9-12: This text is strange and off-putting. A reader might ask who wrote this and why. It seems opinionated and unnecessary.

Page 6-4, line 9: The tone is too pedagogical ("This is not the place . . .").

Footnote 54: The discussion of alternatives to strict, single-measure probability theory is ham-handed. Neither interval probabilities nor imprecise probabilities (sensu Walley, 1991) depart from probability theory; they follow the Kolmogorov axioms. They are motivationally and essentially equivalent to sensitivity analyses, except they do not make use of sampling strategies and can be more comprehensive.

Lines 29-30: It is simply untrue that sensitivity analyses have to be systematic. The word "systematic" might better be "comprehensive" and the word "essential" should be weakened, perhaps to "advantageous".

Page 6-5, lines 4-7 and footnote 55: There seem to be only two axioms mentioned in the text, but Kolmogorov needs three to make probability theory.

Page 6-5: The meaning of the phrase "epistemic uncertainty" given on this page is plainly incorrect. Epistemic uncertainty is the uncertainty that arises from imperfect knowledge such as from limitations on the amount or quality of data available or deficiencies in our causal understanding about a system. It

is not true that a quantity about which there is epistemic uncertainty is necessarily fixed. Although it is perhaps clear how one might come to this mistaken impression, no researchers use the phrase to imply that the underlying quantity has no variability (although all would admit that this could be the case given our ignorance about it). Indeed, a variable can have both forms of uncertainty. For example, when body weight varies across a population, but with a distribution that is unknown, the variable has both aleatory and epistemic uncertainty. This mistake echoes in a couple of other places throughout this section.

It is not clear what the authors take to be the difference between epistemic uncertainty and what they call "cognitive uncertainty." It seems that the latter phrase was introduced because the meaning of "epistemic uncertainty" had been misunderstood. Normally, the phrase "cognitive uncertainty" would refer to an individual person's uncertainty about the validity of the results of his or her own information processing. The assertion that cognitive uncertainty may be represented by probability (i.e., by precise probability measures) is unnecessary and may be misleading. In fact, researchers in human cognition and neuroscience have shown that humans process this kind of uncertainty (which they often call "ambiguity") separately and differently from what we think of as probability or frequentist risk (Glimcher, 2003; Hsu et al., 2005). The section can omit the phrase "cognitive uncertainty" altogether and use in its place "epistemic uncertainty." There are slight differences between the two ideas (e.g., epistemic uncertainty could be shared by members in a group, whereas cognitive uncertainty is always personal), but these appear to be unimportant in this context.

Page 6-5: The words "aleatoric" and "aleatory" are both used on this page as (synonymous) adjectives of uncertainty. Actually, in the engineering literature, only "aleatory" is preferred for this use. In any case, please pick one to use.

Page 6-5, line 10: The assertion that the frequentist and Bayesian interpretations are not mutually exclusive may be misleading. They are mutually exclusive in the sense that it would be improper to mix and match components of each into an analysis. It would be appropriate to omit the clause with the phrase "mutually exclusive," although it is surely fair to say that subjective probabilities can and do track relative frequencies.

Page 6-5, lines 30-32: The text on the subject of dependence is strange here, and also in section 6.1.3.3. It is incorrect that the "[i]ssues involving…epistemic and aleatory uncertainty translate into issues of dependence." This is just wrong (even under their unusual definition of "epistemic").

Page 6-6: Section 6.1.3.2 starting on this page discusses a way to address uncertainty for sample data. This Spartan treatment does not mention that sampling uncertainty is not the only kind of uncertainty that can be associated with data, nor that it may not even be the largest kind of uncertainty. Mensurational uncertainty (including the plus-minus part of a measurement, and censoring) may be more important. In some cases, the family or shape of the marginal distribution may be unknown, which is a kind of model uncertainty. As suggested on page 6-35, such uncertainties can be significant. The section suggests only a resampling approach to expressing the uncertainty, but fails to mention the often severe limitations of such approaches, and says nothing about what one might do if there is no relevant sample data.

Page 6-6, line 20: Maybe the last word of the header should be plural.

Line 21: Modern practice has replaced "error" with "uncertainty" in this context.

Footnote 56: EPA could add “or subtracting” after “adding.”

Page 6-7, line 14: “The role of dependence modeling” should be replaced with “Dependence among variables.”

Page 6-7. More examples of use of expert judgment for health assessment are available and should be cited.

Page 6-7, last paragraph: This paragraph extending onto the next page should be rewritten. The example is reasonable and important, but the discussion about it is confused. The first sentence is incorrect. The uncertainty mentioned in the second sentence may be epistemic, but the sentence is erroneous in its claim. In the following sentences, the words “variable” and “fixed” (or “constant”) should be used rather than “aleatoric” and “epistemic.” It is nonsense to say that a kinetic constant is “completely correlated across individuals.” It’s not correlated; it is invariant. This case is not an example of a dependence issue. There is no correlation between a distribution and a fixed quantity (even if it’s uncertain). Correlation is defined between *varying* quantities. If the number is fixed, whether or not we know what it is, then one cannot say it is correlated with anything. The authors may have come to this twisted language because they’re thinking of the uncertainties in terms of how they might plan to quantitatively characterize them in a Monte Carlo simulation (repeatedly selecting a random deviate for the kinetic constant but assigning it to every individual). Of course, variables such as body fat, age, and smoking, on the other hand, can and do exhibit correlations that definitely should be accounted for in the quantitative assessments. Likewise, it is also important to keep track of the constancy of particular quantities about which we may not know the precise value. These two issues should be untangled and discussed in a less confusing way.

Page 6-8, line 12: The first paragraph of section 6.1.3.4 seems to be saying that one can sometimes express model uncertainty as parametric uncertainty, which simplifies its handling. This could be said more plainly. It would be helpful to mention that this trick cannot always be used (as when the possible models cannot be listed). It might also be especially helpful to mention that this trick is not so much a way to propagate model uncertainty as a way to sweep it under the rug. Model averaging, including Bayesian model averaging (which is mentioned in several places, including 6-36, lines 3ff), erases model uncertainty in the same way that averaging variable quantities erases their variation.

Page 6-8. line 13: Omit the unnecessary fancy after the semicolon.

Lines 15-17: This sentence is nonsense, if we understand what a linear low-dose model is. Parsing the sentence, it seems to say “uncertainty over a…slope…may be quantified, but uncertainty…in slope…cannot be captured” which is self-contradictory. Perhaps what the authors mean to say is that the linearity assumption is not itself subject to uncertainty quantification.

Page 6-9, line 1: The mathematical symbol *x* should be italicized, as should all Roman letters throughout the document that represent unknown quantities, i.e., are symbols representing something else rather than names like “e” the base of the natural logarithms.

Lines 14 and 16: The prefixes “pseudo” and “quasi” are not words. Hyphens are needed.

Page 6-9, line 18: Provide citations for dependence modeling.

Page 6-9: Section 6.1.3.6 might also mention *graphs*, and other traditional communication tools other than correlation indices.

Page 6-10, line 4: Add mention of methods that identify uncertain assumptions or parameters that are *important* for determining whether the model is consistent with observed data (Hornberger and Spear, 1983) and for affecting a decision that is made as a result of the model (Merz et al., 2009).

Page 6-10, lines 29-30: Do the authors mean "*this* probabilistic language," referring to the word "likely" in the quoted text?

Page 6-11, line 19: Of course there is no guarantee that linear will be protective.

Page 6-13, line 18: Of course it isn't really apodictic knowledge at all, but rather only an opinion or an assumption. We see the authors' point and agree with it entirely, but perhaps they should use a word other than "apodictic" here since it's not technically correct.

Page 6-14, lines 33-34: The parenthetical phrase "volitional uncertainty" should be expanded into a sentence that says what the authors mean to express. The phrase "cognitive uncertainty" does not mean anything in this context. Perhaps if the authors expanded it into a sentence too, maybe making it "epistemic uncertainty" along the way, it would be possible to understand what they are trying to say here.

Footnote 62: "Effective" is misspelled, as is "cancer."

Page 6-16, line 5: We note that it's not really a guarantee of course.

Line 8: The word "common" should be "predominant".

Page 6-16, line 20: Perhaps we can say that variability (and uncertainty) in the factors that are used to determine a particular UF can be considered in choosing the particular value of the UF.

Page 6-17, lines 3-14: This problem can be addressed using a Bayesian analysis with a beta conjugate for the uncertain response probability, p, with uniformative (uniform) prior for p. The probability that "an experiment with a null response might have yielded a positive response" can be estimated from the predictive distribution (which will depend on the number of test animals in the original study that yielded zero responses) for the next experiment (with any number of exposed animals).

Page 6-17, line 28: The word "band" should be "limit".

Page 6-20, footnote 66: The text starting "each have an error factor" should be followed by "of' rather than "or".

Page 6-21, line 6: It would be helpful to say something about what the concerns are.

Page 6-21, lines12-14: NAS was not suggesting that EPA use the *uncertainty factors* approach to mount an uncertainty analysis, but rather a more modern approach.

Page 6-22, line 19: Would it be appropriate to note "and establishes a concomitant reduction in some UFs?"

Line 29: The word "invokes" should perhaps be "would require".

Page 6-23, line 33 and passim: The word "exotic" is a poor choice that is unnecessarily and transparently loaded.

Page 6-25, line 29: This sentence is ungrammatical.

Page 6-26, line 24 and Figure 6-1: Would it be helpful to draw the 45-degree line on the graph?

Page 6-27, line 10: The word "epistemic" here is acceptable.

Line 14: The word "epistemic" here should be replaced by "fixed across individuals," and " is estimated from" should be replace by "varies with." How does half life's estimability from data imply that it is variable?

Page 6-28, lines 1-2: One would need the dependence between the variables to proceed.

Line 9: We suggest that "and" should be "although."

Page 6-29, line 1-2: There are bounding techniques based on the classical Fréchet inequality that do not require any knowledge of or any assumptions about dependencies.

Line 32: Omit "to."

Page 6-31, line 24: The pessimistic conclusion is a bit strong. Any *estimate* made from data is amenable to a quantitative uncertainty analysis so, if one is measuring anything, one can propagate uncertainties such as mensurational uncertainty, sampling uncertainty, and perhaps even surrogacy uncertainty. It's not quite as hard to get quantitative models as the text here seems to suggest.

Page 6-32, lines 13-14: The dour conclusion is confusing. One could do a sensitivity analysis in this case, couldn't one? If so, it seems that some kind of uncertainty analysis is clearly possible.

Page 6-33: The example in the text box is great, but the second table seems to say the log-likelihood for LLD is 2.46 and for Hill is 2.16, which would make LLD's larger than Hill's, which contradicts what's said in the text.

Page 6-34, line 4: Shouldn't "*Delivered dose*" be a new bullet?

Line 8: We don't think this statement is true. Perhaps "statistically more powerful" should be "typically yield more sensitive".

Lines 24-25: We don't think it is necessary or helpful to persist with Box's platitude. Model uncertainty is the uncertainty about a model's predictions that arises from doubt about the relevance of that model for making such predictions.

Page 6-37, line 29: The caveat is overwrought. Exploring relevant alternative values in a sensitivity analysis could constitute a quantitative uncertainty analysis, even if the exploration is limited.

Page 6-37, line 30: This sentence is false. Analytical methods of propagation (convolution) don't "sample" anything, and analyses based on intervals or imprecise probabilities don't depend on uncertainty "distributions" (i.e., precise probability distributions).

It is important to keep in mind that, in general, we are not necessarily limited to identifying precise probability distributions for everything that is to be characterized as uncertain (as seems to be suggested here). Simple intervals about uncertain quantities can support a straightforward, albeit crude, interval analysis that propagates uncertainty about parameters and other model choices to statements about the range of possible results. Similarly, an approach based on interval probabilities, probability boxes, or general imprecise probabilities (Walley, 1991) can combine such intervals with precise distributions if they are known for some other inputs, and with structures that are intermediate between coarse intervals and delicate probability distributions when some but incomplete knowledge is available. If the inputs are profoundly uncertain, the results from such analyses are likely to be wide in reflection of these uncertainties. In almost all cases, it is possible to be entirely rigorous without necessarily being precise and without completely specifying each probability distribution.

Page 6-37, line 31: There does not need to be a specified "underlying distribution from which to sample" in order to conduct a quantitative uncertainty analysis. It is facile to shrug off a call to characterize and account for important uncertainties in the assessment process on these grounds alone. Even when the uncertainty is volitional, there can be relevant ranges that are interesting to decision makers and stakeholders. In such cases, the analysis may be formally closer to a sensitivity analysis, but some appropriate response is usually possible, if not always practicable. To their credit, EPA has acknowledged the legitimacy of the call and undertaken some efforts in this direction, notably Tables 5-18 and 5-19 (although some kind of graphical summary of the results might have been better).

Page 6-38, line 30 and passim: The adjective "data driven" needs a hyphen, as it has elsewhere in the document.

Line 23-24: We think this sentence is true, but, again, sampling from a distribution is not the only way to conduct a quantitative uncertainty analysis.

Line 26: What is "(2.a)?"

Page 6-41, line 23: Omitting the word "extra" would make the sentence more easily understandable.

Line 31: What does "How Forward?" mean? Is this idiomatic?

Section 6.5.2: The assertions in this section are rather surprising and questionable. EPA says that uncertainty quantification is an "emerging area in science" and that it is "an area where research could be focused" because "the requisite knowledge does not yet exist" to apply quantitative uncertainty analysis in assessments such as this one for dioxin. The document peremptorily dismisses the utility of "convening a blue-ribbon panel" to identify the proper approach and suggests instead that "multiple approaches should be encouraged." Is the inference that the present review panel shouldn't try to say what the proper approaches to uncertainty quantification are, even if we think the area is more mature than emerging? It is hard to understand what these statements are suggesting. Will the Agency support

intramural and extramural research efforts in this direction? If not, what do the statements mean? Is it impossible that EPA could benefit from some tech transfer efforts as well as basic research on uncertainty quantification? The paragraph beginning on page 6-42 (line 3) mentions a European idea of bench-test exercises to compare different approaches. It may be worth mentioning that this idea has been implemented in the United States as well (Ferson et al., 2004; Oberkampf et al., 2004).

The document's reference list is alphabetically arranged, but seems to go from Z back to A again on page R-33.

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