

EPA/600/R-09/027
May 2009

Summary of U.S. EPA Dioxin Workshop February 18–20, 2009

Cincinnati, Ohio

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

DISCLAIMER

This document summarizes the discussions presented at the Dioxin Workshop in February 2009, in Cincinnati, OH, as documented by the Session Co-Chairs. This document is not all inclusive or binding. Conclusions and recommendations to the U.S. EPA may not represent full consensus. The views expressed in this document are those of the Dioxin Workshop Panelists and do not necessarily reflect the views and policies of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Preferred Citation:

U.S. Environmental Protection Agency (U.S. EPA). (2009) Summary of U.S. EPA Dioxin Workshop: February 18–20, 2009. U.S. Environmental Protection Agency, National Center for Environmental Assessment, Cincinnati, OH. EPA/600/R-09/027.

TABLE OF CONTENTS

DIOXIN WORKSHOP TEAM	iv
ACKNOWLEDGMENTS	iv
INTRODUCTION	1
REFERENCES	2
SCIENTIFIC WORKSHOP TO INFORM THE TECHNICAL WORK PLAN FOR U.S. EPA’S RESPONSE TO NAS COMMENTS ON THE HEALTH EFFECTS OF DIOXIN PRESENTED IN U.S. EPA’S DIOXIN REASSESSMENT	3
SESSION 1: QUANTITATIVE DOSE-RESPONSE MODELING ISSUES	3
SESSION 2: IMMUNOTOXICITY	6
SESSION 3A: DOSE-RESPONSE FOR NEUROTOXICITY AND NONREPRODUCTIVE ENDOCRINE EFFECTS.....	8
SESSION 3B: DOSE-RESPONSE FOR CARDIOVASCULAR TOXICITY AND HEPATOTOXICITY	11
SESSION 4A: DOSE-RESPONSE FOR CANCER	13
SESSION 4B: DOSE-RESPONSE FOR REPRODUCTIVE/DEVELOPMENTAL TOXICITY.....	16
SESSION 5: QUANTITATIVE UNCERTAINTY ANALYSIS OF DOSE- RESPONSE.....	20
APPENDIX A: 2009 U.S. EPA DIOXIN WORKSHOP AGENDA.....	24
APPENDIX B: 2009 U.S. EPA DIOXIN WORKSHOP QUESTIONS TO GUIDE PANEL DISCUSSIONS.....	31
APPENDIX C: 2009 U.S. EPA DIOXIN WORKSHOP DRAFT SELECTION CRITERIA TO IDENTIFY KEY <i>IN VIVO</i> MAMMALIAN STUDIES THAT INFORM DOSE-RESPONSE MODELING FOR 2,3,7,8-TETRACHLORODIBENZO- <i>p</i> -DIOXIN (TCDD)	34

DIOXIN WORKSHOP TEAM

The Dioxin Workshop Team, under the leadership of Peter W. Preuss, Director, NCEA, comprised the following members:

National Center for Environmental Assessment, Office of Research and Development,
U.S. Environmental Protection Agency, Cincinnati, OH 45268

Belinda S. Hawkins
Janet Hess-Wilson
Glenn Rice
Jeff Swartout
Linda K. Teuschler
Bette Zwayer

Argonne National Laboratory, Argonne, IL 60439

Maryka H. Bhattacharyya
Andrew Davidson
Mary E. Finster
Margaret M. MacDonell
David P. Peterson

ACKNOWLEDGMENTS

The Track Group, Alexandria, VA 22312

Kara Hennigan
Alan Minton
Brandy Quinn

ECFlex, Inc., Fairborn, OH 45324

Dan Heing
Heidi Glick
Amy Prues
Lana Wood

IntelliTech Systems, Inc., Fairborn, OH 45324

Cris Broyles
Luella Kessler
Stacey Lewis
Linda Tackett

INTRODUCTION

This document provides a summary of the Scientific Workshop to Inform EPA's Response to National Academy of Science Comments on the Health Effects of Dioxin in EPA's 2003 Dioxin Reassessment. The U.S. Environmental Protection Agency (U.S. EPA) and Argonne National Laboratories (ANL), through an inter-Agency agreement with the U.S. Department of Energy, convened this scientific workshop ("Dioxin Workshop") on February 18–20, 2009, in Cincinnati, Ohio. The goals of the Dioxin Workshop were to identify and address issues related to the dose-response assessment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). This report summarizes the discussions and conclusions from this workshop. Previously, at the request of the U.S. EPA, the National Academy of Sciences (NAS) prepared a report, *Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment* (NAS, 2006), which made a number of recommendations to improve the U.S. EPA's risk assessment for TCDD (U.S. EPA, 2003). The 3-day Dioxin Workshop was convened specifically to ensure that the U.S. EPA's response to the NAS recommendations focuses on the key issues and reflects the most meaningful science.

The Dioxin Workshop included seven scientific sessions:

- (1) Session 1: Quantitative Dose-Response Modeling Issues
- (2) Session 2: Immunotoxicity
- (3) Session 3A: Dose-Response for Neurotoxicity and Nonreproductive Endocrine Effects
- (4) Session 3B: Dose-Response for Cardiovascular Toxicity and Hepatotoxicity
- (5) Session 4A: Dose-Response for Cancer
- (6) Session 4B: Dose-Response for Reproductive/Developmental Toxicity
- (7) Session 5: Quantitative Uncertainty Analysis of Dose-Response

During each session, the U.S. EPA asked a panel of expert scientists to:

- identify and discuss the technical challenges involved in addressing the key NAS comments on the TCDD dose-response assessment in the U.S. EPA Reassessment (U.S. EPA, 2003);
- discuss approaches for addressing the key NAS comments; and
- identify important published, independently peer-reviewed literature, particularly studies describing epidemiologic and *in vivo* mammalian bioassays, which are expected to be most useful for informing the U.S. EPA's response.

The sessions were followed by open comment periods during which members of the audience were invited to address the Panels. At the conclusion of the open comment periods, the Panel Co-Chairs were asked to summarize and present the results of the panel discussions. The summaries could include minority opinions stated by panelists. The main points derived from the session summaries were used to prepare this document. Additionally, this document includes a list of the session panelists and their affiliations and three appendices. Appendix A presents the Dioxin Workshop Agenda. Appendix B identifies the charge questions presented to the Panel. Appendix C describes draft study selection criteria proposed by the Dioxin Workshop Team for consideration by the workshop panelists.

REFERENCES

NAS (National Academy of Sciences). 2006. Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment. National Academies Press, Washington, DC (July). Available at http://www.nap.edu/catalog.php?record_id=11688.

U.S. EPA (U.S. Environmental Protection Agency). 2003. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds. NAS review draft, Volumes 1–3 (EPA/600/P-00/001Cb, Volume 1). U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC (December). Available at <http://www.epa.gov/nceawww1/pdfs/dioxin/nas-review/>.

SCIENTIFIC WORKSHOP TO INFORM THE TECHNICAL WORK PLAN FOR U.S. EPA'S RESPONSE TO NAS COMMENTS ON THE HEALTH EFFECTS OF DIOXIN PRESENTED IN U.S. EPA'S DIOXIN REASSESSMENT

Dioxin Workshop Co-Chairs: Peter W. Preuss and Glenn Rice

The Dioxin Workshop session summaries were prepared by the session panel Co-Chairs with input from the panelists, as requested by the U.S. EPA prior to the workshop. The Co-Chairs subsequently presented these summaries to all of the workshop participants during designated periods at the workshop. In these summaries, the U.S. EPA asked that the Co-Chairs summarize the key issues from the panel discussions. Because the sessions were not designed to achieve consensus among the panelists, the summaries do not necessarily represent consensus opinions; rather, they reflect the essence of the panel discussions. Some of the specific points may represent the views of multiple panelists, while others only the views of a single panelist. Prior to the summarizations, there were opportunities for public comments on the discussion topics. Some Co-Chairs met with their sessions' panelists after their sessions ended to develop these summaries, while others developed reports based on their personal notes. Because Session 5 was the last session of the workshop—with little time provided to develop the summary—the Co-Chairs circulated a draft for comment by the Session 5 panelists after the workshop, prior to finalizing the session summary. The U.S. EPA collected the session summaries and then prepared this document. A draft of this document was distributed to all of the session Co-Chairs to provide them with a final opportunity to comment and make revisions. Finally, it should be noted that U.S. EPA was not prescriptive to the session Co-Chairs with respect to the format of the presentation materials and provided no specific instructions, resulting in unique formats among the session summaries.

SESSION 1: QUANTITATIVE DOSE-RESPONSE MODELING ISSUES

This session discussed the general dose-response modeling issues related to TCDD. Many of these issues were highlighted by NAS (2006). There was a general introductory presentation on TCDD kinetics, including information and uncertainties pertaining to the conversion of administered doses in animals to human body burden (BB) and additivity to background issues. This presentation was followed by a Panel discussion on the state of the science regarding dioxin dose-response modeling issues.

Session 1 Panelists (Session Co-Chairs are identified by asterisk)

- Bruce Allen, Bruce Allen Consulting
- Lesa Aylward, Summit Toxicology
- Roger Cooke, Resources for the Future
- Kenny Crump, Louisiana Tech University
- Mike DeVito, U.S. EPA
- Dale Hattis, Clark University
- Rick Hertzberg, Biomath Consulting
- Rob McDowell, U.S. Department of Agriculture
- Jim Olson, State University of New York, University at Buffalo

- *Lorenz Rhomberg, Gradient
- Woody Setzer, U.S. EPA
- *Jeff Swartout, U.S. EPA

Please note that the use of the term “concluded” or “recommended” in this summary does not mean that a consensus was reached. Session Summaries were written from the material prepared by the non-EPA/ANL Co-Chair and represent a synopsis of the panel discussions.

Key Study Selection Criteria

The Panel discussed the advantages and disadvantages of using key study criteria (Appendix C). They concluded that *a priori* criteria foster transparency and consistency, and could deflect *a posteriori* criticism. However, the Panel also acknowledged that having *a priori* criteria could introduce the potential for excluding useful data. Although the key study criteria provided by the U.S. EPA listed studies using TCDD only as a criterion, the Panel posed the possibility of using closely related dioxin-like compounds (DLCs) as surrogates for TCDD. The criterion for use of data from mammalian studies only was one criterion that received generalized support due to the lack of extrapolation protocols for nonmammalian species. The Panel also discussed the specific exposure-duration criterion and asked if there should be a preference for longer-term rather than acute studies. The Panel made three suggestions to modify U.S. EPA’s key study selection criteria:

- (1) Define more relevant exposure-level (i.e., dose) cut points using tissue concentrations.
- (2) Reword statistical criteria to include do-it-yourself analysis.
- (3) Reword the response criteria to clarify “outside of normal range.”

Dose Metrics

The Panel discussed the relative merits of various measures of dose for modeling TCDD dose response. One general conclusion was that tissue concentration (TC) is the preferred metric, especially lipid-adjusted TC, because this measure more closely approximates exposures close to the target tissue when compared to administered doses. However, the Panel acknowledged that these data are often unavailable. They further noted that BB, which is defined as the concentration of TCDD in the body (ng/kg body weight) (U.S. EPA, 2003), might be useful as a surrogate for TC provided the two measures were proportional.

The Panel suggested that a linear approach to BB estimation, which was utilized by U.S. EPA (2003), is too simplistic because this approach does not take into account toxicokinetic issues related to TCDD—e.g., sequestration in the liver and fat, age-dependent elimination, and changing elimination rates over time. The Panel recommended the use of kinetic/mechanistic modeling to the extent possible to quantify tissue-based metrics.

The Panel raised the issue of whether the preferred dose metric would be different for different endpoints and exposure durations. This led to the Panel’s comment that the peak exposure might be a more important metric than average BB for variable exposure scenarios. Given this discussion about different exposure durations being relevant to a specific endpoint, the Panel suggested that the U.S. EPA also consider peak measures in dose-response modeling.

The last point raised in this part of the discussion centered on the possibility of dose errors in experimental studies. The Panel highlighted the need for the U.S. EPA to consider dose error (i.e., uncertainty in the x-axis of the dose-response curve) when using dose surrogates.

Dose-Response Modeling of Mammalian Bioassays

The Panel considered several issues related to dose-response modeling of mammalian bioassay data for TCDD: supralinearity and incomplete response data (“anchoring”), defining the benchmark response (BMR) level with respect to establishing the point of departure (POD), and the use of threshold modeling—as further explained below.

The Panel discussed the specific issues of supralinearity and anchoring raised by the U.S. EPA with respect to modeling noncancer endpoints. The panel recognized that, for many of the most sensitive endpoints, the response at the lowest dose is high (e.g., quantal responses above 25% and continuous endpoints differ substantially from the mean, often implying 100% incidence in the treated animals). This lack of response anchoring at the low end of the dose-response curve (near the BMR) results in the higher responses determining the shape of the curve.

The Panel asked whether new tools might be needed or whether the current tools could be applied differently. In the context of developing new tools, the Panel emphasized the need for collaboration between biologists and mathematicians. When discussing application, the Panel suggested that the problem with supralinearity might be overcome by simply dropping the requirement for using the lower bound on the Benchmark Dose. In addition, the Panel posed several more approaches for further consideration in dose-response modeling by the U.S. EPA:

- (1) Combine similar data sets to fill in data gaps.
- (2) Use mechanistic approaches to model the data gaps.
- (3) Dichotomize continuous data.

Finally, the Panel acknowledged that, in certain situations, there simply may not be enough information to provide meaningful answers.

The Panel discussed the BMR level for establishing a POD in the context of deriving a Reference Dose (RfD). The Panel generally agreed that, while the effective dose level (ED_{01}) used in the 2003 Reassessment may be useful for comparative analysis across endpoints, the ED_{01} estimates developed for all endpoints considered in the Reassessment were not appropriate for deriving an RfD because they were not based on the effect’s adversity. The panel noted that ED_{01} also is much lower than typical EPA BMR levels. The Panel recommended that the U.S. EPA work to define endpoint-specific BMRs based on the consideration of adversity. Given that the same uncertainty factor framework is applied to all PODs, the Panel emphasized the need for consistency in BMRs; numerical consistency is needed for quantal BMRs and consistency in the choice of biological relevance should be applied for continuous BMRs.

The Panel generally discouraged threshold modeling by stating that thresholds are very difficult to pin down and suggested that the lower bound may always be zero.

Dose-Response Modeling of Epidemiological Studies

The Panel noted that many studies have been published with measured concentrations of TCDD that could be used for dose reconstruction. In this discussion, the Panel acknowledged that use of these data would entail dealing with toxicity equivalence (TEQ) issues and pharmacokinetic (PK) modeling. Pertaining to the use of these data for quantitative risk assessment by the U.S. EPA, the Panel posed the question, “At what point does indirect or confounded human data supersede controlled animal bioassay data?”, or alternatively, “How much human data uncertainty can we tolerate?” The Panel suggested, at the least, that the epidemiologic data could be used to “ground-truth” the animal bioassay modeling results.

Supporting Information

The Panel acknowledged that Ah receptor (AhR) binding affinities are not necessarily tied to endpoint sensitivity, but they reiterated the need to consider mechanistic modeling to aid in developing appropriate dose metrics or filling in data gaps in the existing dose-response data.

References

NAS (National Academy of Sciences). 2006. Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment. National Academies Press, Washington, DC (July). Available at http://www.nap.edu/catalog.php?record_id=11688.

U.S. EPA (U.S. Environmental Protection Agency). 2003. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds. NAS Review Draft (EPA/600/P-00/001Cb). U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC. Available at <http://www.epa.gov/nceawww1/pdfs/dioxin/nas-review/>.

SESSION 2: IMMUNOTOXICITY

The U.S. EPA plans to consider development of a quantitative dose-response assessment for the immunologic effects associated with TCDD exposure. Such an assessment would be based on information in U.S. EPA (2003), NAS (2006) and key studies identified in this workshop. The purpose of this session was to identify and discuss key issues pertaining to dose-response assessment for dioxin-induced immunologic effects.

Session 2 Panelists (Session Co-Chairs are identified by asterisk)

- Roger Cooke, Resources for the Future
- Rob Goble, Clark University
- *Belinda Hawkins, U.S. EPA
- Nancy Kerkvliet, Oregon State University
- Manolis Kogevinas, Centre for Research in Environmental Epidemiology
- Robert Luebke, U.S. EPA
- Paolo Mocarelli, University of Milan
- *Allen Silverstone, State University of New York, Upstate Medical University

- Courtney Sulentic, Wright State University
- Nigel Walker, National Institute of Environmental Health Sciences

Please note that the use of the term “concluded” or “recommended” in this summary does not mean that a consensus was reached. Session Summaries were written from the material prepared by the non-EPA/ANL Co-Chair and represent a synopsis of the panel discussions.

Key Study Selection Criteria

The Panel first addressed the Key Study Selection Criteria proposed by the U.S. EPA (Appendix C). The Panel raised the issue that the key study criteria do not apply to most studies designed to investigate immunotoxicity, including those used to calculate ED_{01s} (U.S. EPA, 2003). The Panel observed that most dioxin immunotoxicity studies are relatively high dose (>200 ng/kg-d) acute studies and/or use parenteral rather than oral administration.

The Panel discussed several studies often considered important for assessing the immunotoxic effects of TCDD exposure. The Oughton et al. (1995) mouse bioassay was discussed and, although the study does meet the proposed criteria, it could not be considered a key study; specifically, the Panel contended that since there were no functional alterations observed or measured in this bioassay, the changes in cellular phenotypes are only “suggestive” of immune alterations and cannot be regarded as having immunopathologic significance.

The Panel discussed two additional studies for further consideration by the U.S. EPA:

- Baccarelli et al. (2002). The Panel discussed this as a potentially key human epidemiological study that should be reviewed and considered further by the U.S. EPA. It measured the level of IgG, demonstrating a significant decline relative to dioxin body burdens.
- Smialowicz et al. (2008). The Panel noted that this study identified the antibody response to sheep red blood cells (SRBCs) as the critical effect, labeling this protocol as a functional assay. The Panel stated that if modeled, the U.S. EPA could calculate the BMR for this endpoint as 1 standard deviation from the control mean.

References

Baccarelli, A., P. Mocarelli, D.G. Patterson et al. 2002. Immunologic effects of dioxin: New results from Seveso and comparison with other studies. *Environ. Health Perspect.* 110(12):1169-1173.

NAS (National Academy of Sciences). 2006. Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment. National Academies Press, Washington, DC (July). Available at http://www.nap.edu/catalog.php?record_id=11688.

Oughton, J.A., C.B. Pereira, G.K. Dekrey, J.M. Collier, A.A. Frank and N.I. Kerkvliet. 1995. Phenotypic analysis of spleen, thymus, and peripheral blood cells in aged C57BI/6 mice following long-term exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Sci.* 25(1):60-69.

Smialowicz, R.J., M.J. DeVito, W.C. Williams and L.S. Birnbaum. 2008. Relative potency based on hepatic enzyme induction predicts immunosuppressive effects of a mixture of PCDDS/PCDFS and PCBS. *Toxicol. Appl. Pharmacol.* 227(3):477-484.

U.S. EPA (U.S. Environmental Protection Agency). 2003. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds. NAS Review Draft (EPA/600/P-00/001Cb). U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC. Available at <http://www.epa.gov/nceawww1/pdfs/dioxin/nas-review/>.

SESSION 3A: DOSE-RESPONSE FOR NEUROTOXICITY AND NONREPRODUCTIVE ENDOCRINE EFFECTS

The U.S. EPA plans to consider development of a quantitative dose-response assessment for neurological and/or nonreproductive endocrine effects associated with TCDD exposure. Such an assessment would be based on information in U.S. EPA (2003), NAS (2006) and key studies identified in this workshop. The purpose of this session was to identify and discuss key issues pertaining to dose-response assessment for dioxin-induced neurological and/or nonreproductive endocrine effects.

Session 3A Panelists (Session Co-Chairs are identified by asterisk)

- *Maryka Bhattacharyya, Argonne National Laboratory
- Mike DeVito, U.S. EPA
- Mary Gilbert, U.S. EPA
- Rob Goble, Clark University
- Nancy Kerkvliet, Oregon State University
- Fumio Matsumura, University of California-Davis
- Paolo Mocarelli, University of Milan
- Chris Portier, National Institute of Environmental Health Sciences
- Lorenz Rhomberg, Gradient
- Allen Silverstone, State University of New York, Upstate Medical University
- Marie Sweeney, National Institute of Occupational Safety and Health
- *Bernie Weiss, University of Rochester

Please note that the use of the term “concluded” or “recommended” in this summary does not mean that a consensus was reached. Session Summaries were written from the material prepared by the non-EPA/ANL Co-Chair and represent a synopsis of the panel discussions.

What Are the Key Questions Regarding These Endpoints?

The Panel used the following question to initiate discussion: “*Are there identifiable indices of neurotoxicity and nonreproductive endocrine effects in animal studies and human populations?*” Under this discussion topic, the Panel discussed three endpoints: neurotoxicity (with focus on developmental exposures), thyroid dysfunction (e.g., thyroid hormone deficits), and diabetes. The Panel also addressed the relevance of windows of vulnerability to each

endpoint. The Panel acknowledged that, in some cases, the window of exposure may precede the window of expression of toxicity.

Epidemiological Study Selection

Developmental Neurotoxicity

The Panel recognized that an unusual feature for this endpoint is that there are sufficient human data for dose-response modeling (e.g., Dutch children [Huisman et al., 1995; Patandin et al., 1999] and U.S. children [Jacobson and Jacobson, 1996]) and there is an internal dose metric (serum concentrations). Additionally, the Panel discussed recent studies that address this endpoint in humans (from Japan [reference not provided] and Holland [e.g., Koopman-Esseboom et al., 1996; Vreugdenhil et al., 2002]). For continued investigation into this endpoint, the Panel raised two issues to the U.S. EPA:

- Conduct an evaluation of whether a modeled effect can be attributed to TCDD and not some other persistent organic pollutant (POP), although the Panel recognized that it is unlikely U.S. EPA will be able to distinguish among these exposures because other POPs are intrinsic confounders in the Dutch study.
- Allow animal data to inform the dose-response modeling of epidemiological data.

Thyroid Dysfunction

The Panel identified the availability of human data for this endpoint (e.g., Calvert et al., 1999; Koopman-Esseboom et al., 1994). Much of the thyroid dysfunction literature has been published since the 2003 Reassessment (e.g., Wang et al., 2005; Baccarelli et al., 2008). The Panel also noted the availability of an internal dose metric (serum concentrations). Additionally, the Panel discussed the mechanistic studies in animals that link TCDD to thyroid dysfunction. For continued investigation into this endpoint, the Panel raised three issues for the U.S. EPA to consider:

- Consider the newly available human data since the Reassessment.
- Investigate and clarify of the role of TCDD-induced thyroid dysfunction in developmental neurotoxicity.
- Evaluate and determine whether an effect can be attributed to TCDD or other contaminants.

Diabetes

The Panel discussed that data suggest that diabetes incidence in those under 55 years old may be associated with exposure to PCBs. They acknowledged that whether this is a dioxin-like compound (DLC) mediated effect or whether other POPs are responsible is still undetermined. The Panel also acknowledged that no animal model exists for the investigation of xenobiotic-induced diabetes, and that separating the injury dose level from the current body burdens would depend on good pharmacokinetics in humans. For continued investigation into this endpoint, the Panel listed two issues for the U.S. EPA to consider:

- Results from the Anniston study and the Great Lakes Fishermen study (references not provided) should be examined for dose metrics (both studies examine human PCB exposures).

- Changes of adipose tissue status need to be considered, given that dieting can cause release of lipid-soluble contaminants.

References

Baccarelli, A., S.M. Giacomini, C. Corbetta et al. 2008. Neonatal thyroid function in Seveso 25 years after maternal exposure to dDioxin. *PLoS Med.* 5(7):e161. doi:10.1371/journal.pmed.0050161.

Calvert, G.M., M.H. Sweeney, J. Deddens and D.K. Wall. 1999. Evaluation of diabetes mellitus, serum glucose, and thyroid function among United States workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Occ. Env. Med.* 56:270-276.

Huisman, M., C. Koopman-Esseboom, V. Fidler et al. 1995. Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. *Early Hum. Devel.* 41(2):111-127.

Jacobson, J.L. and S.W. Jacobson. 1996. Intellectual impairment in children exposed to polychlorinated biphenyls *in utero*. *N. Engl. J. Med.* 335:783–789.

Koopman-Esseboom, C., N. Weisglas-Kuperus, M.A.J. de Ridder, C.G. Van der Paauw, L.G.M.Th. Tuinstra and P.J.J. Sauer. 1996. Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. *J. Pediatr.* 97(5):700-706.

Koopman-Esseboom, C., D.-C. Morse, N. Weisglas-Kuperus et al. 1994. Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. *Pediatr. Res.* 36:468–473.

Patandin, S., C.I. Lanting, P.G.H. Mulder, E.R. Boersma, P.J.J. Sauer and N. Weisglas-Kuperus. 1999. Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. *J. Pediatr.* 134:33–41.

NAS (National Academy of Sciences). 2006. Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment. National Academies Press, Washington, DC (July). Available at http://www.nap.edu/catalog.php?record_id=11688.

U.S. EPA (U.S. Environmental Protection Agency). 2003. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds. NAS Review Draft (EPA/600/P-00/001Cb). U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC. Available at <http://www.epa.gov/nceawww1/pdfs/dioxin/nas-review/>.

Vreugdenhil, H.J., C.I. Lanting, P.G. Mulder, E.R. Boersma and N. Weisglas-Kuperus. 2002. Effects of prenatal PCB and dioxin background exposure on cognitive and motor abilities in Dutch children at school age. *J. Pediatr.* 140:48–56.

Wang S.L., P.H. Su, S.B. Jong, Y.L. Guo, W.L. Chou and O. Päpke. 2005. *In utero* exposure to dioxins and polychlorinated biphenyls and its relations to thyroid function and growth hormone in newborns. *Environ. Health Perspect.* 113:1645–1650.

SESSION 3B: DOSE-RESPONSE FOR CARDIOVASCULAR TOXICITY AND HEPATOTOXICITY

The U.S. EPA plans to consider development of a quantitative dose-response assessment for cardiovascular and/or hepatic effects associated with TCDD exposure. Such an assessment would be based on information in U.S. EPA (2003), NAS (2006) and key studies identified in this workshop. The purpose of this session was to identify and discuss key issues pertaining to dose-response assessment for dioxin-induced cardiovascular and/or hepatic effects.

Session 3B Panelists (Session Co-Chairs are identified by asterisk)

- Bob Budinsky, Dow Chemical
- Manolis Kogevinas, Centre for Research in Environmental Epidemiology
- Rob McDowell, U.S. Department of Agriculture
- Jim Olson, State University of New York, University at Buffalo
- Marian Pavuk, Agency for Toxic Substances and Disease Registry
- *Jeff Swartout, U.S. EPA
- *Mary Walker, University of New Mexico
- Nigel Walker, National Institute of Environmental Health Sciences

Please note that the use of the term “concluded” or “recommended” in this summary does not mean that a consensus was reached. Session Summaries were written from the material prepared by the non-EPA/ANL Co-chair and represents a synopsis of the panel discussions.

Key Study Selection Criteria

The Panel initially focused on the draft key study selection criteria offered by the U.S. EPA (Appendix C). The panel recommended that for cardiovascular effects, which are not usually observed in rodents, the use of knockout mouse models (ApoE KO and LDLR KO) be moved to the “primary” column because only these studies establish the cardiovascular toxicity model in mice.

The panel also was concerned that the gavage procedure can increase mouse blood pressure. Consequently, the panel recommended that gavage studies not be used for the blood pressure endpoint (i.e., only dietary dosing studies should be considered).

Human Health Endpoints

In relation to the hepatic endpoint, the Panel acknowledged the large body of dose response information on hepatic effects in rodents and that enzyme (mostly CYP1A1) induction was a sensitive effect. However, the Panel cited the lack of linkage of CYP1A1 to downstream events, which complicates the toxicological interpretation of this endpoint, and concluded that

the more important liver effects in rodents are probably on the “road to cancer.” The Panel noted that hepatic effects were not seen in the epidemiological studies, but acknowledged that these studies were not designed to detect them.

In relation to the cardiovascular endpoint, the Panel identified hypertension and ischemic heart disease (IHD) as two key endpoints from the epidemiological studies. The Panel recommended that the U.S. EPA perform a meta-analysis of these data. The Panel also commented that recent animal studies support the observations linking TCDD exposure to IHD and hypertension. In particular, the National Toxicology Program (NTP) study shows inflammatory and structural effects on resistant vascular arterioles (NTP, 2006). Additional evidence from the study suggests that the vascular effects may be CYP1A1-dependent. The Panel suggested that the NTP study data might be used as a surrogate for dose-response modeling of hypertension and that such an approach would be supported by data on the role of AhR in vascular function and remodeling.

POD Issues

The Panel was not supportive of 1% of maximal response (ED_{01}), which was utilized in the 2003 Reassessment. The Panel concluded that the POD should depend on the specific endpoint and recommended the following to the U.S. EPA:

- For continuous measures, base the BMR on difference from control. Consider the adversity level—at what point does the endpoint become adverse?
- For incidence data, set the BMR to a fixed-risk level.

Supporting Information

The Panel posed several suggestions to the U.S. EPA for reducing uncertainty and improving the knowledge base for TCDD toxicity.

- Use in vitro data to define uncertainties, such as the relative sensitivity between rodents and humans and around the definition of a POD.
- Consider studies on dioxin-like compounds (DLCs).
- Use PK modeling to define the dose metric for hepatic effects.
- Use body burden or serum concentrations for cardiovascular endpoints.

Finally, the Panel recommended that U.S. EPA finish the reassessment quickly and establish a definitive plan to review and incorporate new data as they become available.

References

NAS (National Academy of Sciences). 2006. Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment. National Academies Press, Washington, DC (July). Available at http://www.nap.edu/catalog.php?record_id=11688.

NTP (National Toxicology Program). 2006. Toxicology and Carcinogenesis Studies of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) (CAS No. 1746-01-6) in Female Harlan Sprague-Dawley Rats (Gavage Studies). U.S. Department of Health and Human Services. NTP TR 521. Research Triangle Park, NC (April).

U.S. EPA (U.S. Environmental Protection Agency). 2003. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds. NAS Review Draft (EPA/600/P-00/001Cb). U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC. Available at <http://www.epa.gov/nceawww1/pdfs/dioxin/nas-review/>.

SESSION 4A: DOSE-RESPONSE FOR CANCER

The U.S. EPA plans to consider development of a quantitative dose-response assessment for cancer associated with TCDD exposure. Such an assessment would be based on information in U.S. EPA (2003), NAS (2006) and key studies identified in this workshop. The purpose of this session was to identify and discuss key issues pertaining to dose-response assessment for dioxin-induced cancer.

Session 4A Panelists (Session Co-Chairs are identified by asterisk)

- Lesa Aylward, Summit Toxicology
- Kenny Crump, Louisiana Tech University
- Dale Hattis, Clark University
- *Janet Hess-Wilson, U.S. EPA
- Karen Hogan, U.S. EPA
- Manolis Kogevinas, Centre for Research in Environmental Epidemiology
- Marian Pavuk, Agency for Toxic Substances and Disease Registry
- Chris Portier, National Institute of Environmental Health Sciences
- Lorenz Rhomberg, Gradient
- Jay Silkworth, General Electric
- *Nigel Walker, National Institute of Environmental Health Sciences

Please note that the use of the term “concluded” or “recommended” in this summary does not mean that a consensus was reached. Session Summaries were written from the material prepared by the non-EPA/ANL Co-chair and represent a synopsis of the panel discussions.

Key Study Selection

The Panel discussed both human and rodent studies. In reviewing the epidemiological data, the Panel agreed the EPA should focus on four cohort studies (Dutch cohort, NIOSH cohort, BASF accident cohort, and Hamburg cohort) and pointed out that there are numerous updates and reevaluations of data now in the literature and others will be published soon. The Panel stated that it is appropriate for the U.S. EPA to consider the increase in total cancers for modeling human cancer data, however, Non-Hodgkin's lymphoma, and lung tumors are the main TCDD-related cancer types seen in humans exposed to TCDD. The Panel suggested the U.S. EPA focus the quantitative dose-response modeling on the human data.

In reviewing the rat data, the Panel identified four new NTP rodent cancer bioassays with liver and lungs as the main target organs. However, they suggested that dose-response modeling efforts should model “all cancers” from these NTP data sets as well and use tumor incidence—not individual rats as measures.

Key Study Selection Criteria

The Panel discussed whether data for TCDD only should be used or if PCB126 could be used to develop a dose-response curve. From this discussion, the Panel reached a general agreement that limiting the dose-response modeling and cancer assessment to TCDD only would be the best approach.

Regarding the oral dosing regimens, the Panel discussed the differences in results from different bioassays. They concluded that there were insufficient data to pick between oral feed (Kociba et al., 1978) and oral gavage (NTP, 2006) studies, but stated “If all aspects of studies were equal, an oral feed study is preferred.” However, given that current data sets are not equal, they agreed that U.S. EPA should consider both feed and gavage studies.

The Panel put forth the recommendation that studies that include initiation-promotion model data and TgAC transgenic model data from oral exposure studies should be excluded from the primary category in the key study selection criteria (Appendix C lists the draft study selection criteria distributed prior to the meeting). Studies from both classifications should be moved to the second tier.

The Panel was also unsupportive of the “response magnitude outside the range of normal variability” criterion, as they did not believe it was applicable to a cancer endpoint.

Critical Endpoints to Consider

The Panel recognized that the MOA for TCDD includes cell growth/differentiation dysregulation, that different endpoints (tumor types) across species may be expected, and that there are differences in tumor sites across species. The Panel further acknowledged that there is insufficient information to determine if rodent tumor types observed are relevant to humans. Thus, the Panel suggests the following:

- U.S. EPA should consider all the observed cancer endpoints in its evaluation.

Nonlinear (aka threshold) Versus Linear Dose-Response Modeling

The Panel agreed that NTP bioassays appear to demonstrate nonlinear dose response, but they expressed concern about using animal data to infer slope and dose response for humans. The Panel pointed out that there are differences in slopes across different bioassays, and specifically, that some appear linear while others appear nonlinear. Given the observation of both nonlinear vs. linear, the Panel concluded that neither could be ruled out for extrapolation below the POD simply based on the available data. One panelist noted that U.S. EPA Cancer Guidelines (U.S. EPA, 2005) state that only if one can demonstrate that the MOA has a threshold dose-response shape, and can exclude all other potential linear MOAs, can one use a nonlinear model. Lastly, the Panel noted that there are data and rationales to support use of both linear and

nonlinear response below POD. From this discussion, the Panel raised one possibility to the U.S. EPA:

- Both linear and nonlinear model functions should be considered in the dose-response analysis.

Dose Metrics

In considering human data, the Panel expressed a preference for lipid-adjusted serum levels over body burden (BB), and they expressed concerns over the assumptions used in the back calculation of the BB in the epidemiologic cohorts. In considering the rat data, the Panel supported the use of BB—especially lipid-adjusted BB. The Panel, however, did express concern over the sequestering of TCDD in liver and then the use of liver levels in BB calculations.

Supporting Information—Biologically-Based Dose-Response (BBDR) Models and MOA

The Panel discussed BBDR. Though once considered an attractive proposition, BBDR models may mask uncertainty within the models, necessitating them to be used with greater caution. The Panel suggested two issues for the U.S. EPA to consider:

- If there is a published model, use it if it is valid—do not generate a new model.
- Focus on the actual experimental data to drive the analysis.

References

Kociba, R.J., D.G. Keyes, J.E. Beyer et al. 1978. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats. *Toxicol. Appl. Pharmacol.* 46:279-303.

NAS (National Academy of Sciences). 2006. Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment. National Academies Press, Washington, DC (July). Available at http://www.nap.edu/catalog.php?record_id=11688.

NTP (National Toxicology Program). 2006. Toxicology and Carcinogenesis Studies of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) (CAS No. 1746-01-6) in Female Harlan Sprague-Dawley Rats (Gavage Studies). U.S. Department of Health and Human Services. NTP TR 521. Research Triangle Park, NC (April).

U.S. EPA (U.S. Environmental Protection Agency). 2003. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) and Related Compounds. NAS Review Draft (EPA/600/P-00/001Cb). U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC. Available at <http://www.epa.gov/nceawww1/pdfs/dioxin/nas-review/>.

US EPA (U.S. Environmental Protection Agency). 2005. Guidelines for Carcinogen Risk Assessment. U.S. Environmental Protection Agency Risk Assessment Forum. EPA/630/P-03/001F.

SESSION 4B: DOSE-RESPONSE FOR REPRODUCTIVE/DEVELOPMENTAL TOXICITY

The U.S. EPA plans to consider development of a quantitative dose-response assessment for reproductive and developmental effects associated with TCDD exposure. Such an assessment would be based on information in U.S. EPA (2003), NAS (2006) and key studies identified in this workshop. The purpose of this session was to identify and discuss key issues pertaining to dose-response assessment for dioxin-induced reproductive and developmental effects.

Session 4B Panelists (Session Co-Chairs are identified by asterisk)

- Barbara Abbott, U.S. EPA
- Bruce Allen, Bruce Allen Consulting
- Roger Cooke, Resources for the Future
- George Daston, Procter & Gamble
- Mike DeVito, U.S. EPA
- Rob Goble, Clark University
- *Fumio Matsumura, University of California-Davis
- Paolo Mocarelli, University of Milan
- Brian Petroff, University of Kansas
- *Glenn Rice, U.S. EPA
- Marie Sweeney, National Institute of Occupational Safety and Health
- Mary Walker, University of New Mexico
- Bernie Weiss, University of Rochester

Please note that the use of the term “concluded” or “recommended” in this summary does not mean that a consensus was reached. Session Summaries were written from the material prepared by the non-EPA/ANL Co-Chair and represent a synopsis of the panel discussions.

A Major Question Posed During this Workshop Session was “Are Human Embryos and Infants Less Sensitive to Dioxin Exposures Than Some Experimental Animals?”

The Panel recognized that animal data show a wide range of species sensitivity to dioxin for a given developmental or reproductive endpoint. Presently, there are data for some endpoints that show that human sensitivity is comparable to experimental animals (e.g., semen quality), and for other endpoints the data demonstrate that humans are insensitive compared to other species (e.g., cleft palate). Lastly, the Panel recognized that there are some endpoints for which relative human sensitivity remains uncertain.

Key Study Selection

The Panel reviewed the charge questions (Appendix B), discussed them, and listed two issues for the U.S. EPA to consider:

- Concerning key study determination, use a stepwise approach that is dependent upon the information available and needed to address the question.

- Concerning the key studies informing the POD and the POD endpoint choice, use the POD to depart from what is certain and use a high-confidence study that has found effects at a low enough level at which other effects are protected.

The Panel also developed Table 1, based on the information presented in this session. Table 1 identifies specific reproductive and developmental effects of concern, listing whether an effect has been observed in test animals and epidemiologic cohorts. It also identifies the ED₁₀ estimated by the U.S. EPA (2003) for health effects observed in rodent bioassays. If the U.S. EPA did not report an ED₁₀ for an effect, the table identifies a study where the effect was reported and the lowest study dose where the effect was observed. Table 1 also identifies the epidemiologic cohort where the specific reproductive and developmental effects were observed.

Epidemiological Study Utility

The Panel reviewed the charge questions (Appendix B), discussed them, and made two suggestions to the U.S. EPA:

- Concerning the ability of epidemiological studies to inform critical effects, start with concordance across species (including humans) for the spectrum of effects.
- Concerning the ability of epidemiological studies to inform dose-response modeling, start with the epidemiology and then go to animal data if the dose response has not been well characterized for an endpoint of interest and compare to animal data as a reality check.

Animal Model Utility

The Panel reviewed and discussed the charge questions (Appendix B). Table 1, which identifies the effects that occur in animals and also have relevance to humans, summarizes much of this discussion. Regarding the influence of mode of action (MOA) on animal model choice, the Panel concluded that by evaluating concordance among health effects reported in epidemiologic and animal bioassay data, the U.S. EPA could identify a set of plausible reproductive and developmental effects to consider. Actual animal and human MOA information is helpful in that it creates comfort with the animal models and in defining the boundaries of possible effects.

TABLE 1			
Reproductive/Developmental Effects of Concern for Human Health			
Endpoint	Rodent (ED ₁₀ ng/kg-d)	Human	Notes
Sperm Count/Motility	Yes (6.2–28; 66–200)	Yes	ED ₁₀ bases Mabley et al. (1992a,b) caudal sperm count and daily sperm production range from 6.2–28; Gray et al. (1997) epididymal sperm count and total testis sperm counts range from 66–200.
Sex Ratio	No	Yes, Seveso	
Delayed Puberty Males	Yes (94)	Yu-cheng	ED ₁₀ basis rat male puberty delay Gray et al. (1997). Need to qualify epidemiology data because of cohort PCDD/PCDFs exposures.
Delayed Puberty in Females	Yes	No in Seveso	Gray and Ostby (2002) report delayed puberty in female offspring of pregnant rats receiving a single dose of 1 µg TCDD/kg on GD 15.
Cleft Palate	Yes (6300–6400)	No	ED ₁₀ basis Birnbaum et al. (1989).
Premature Senescence	Yes	No, Seveso	Franczac et al. (2006) report that rats prematurely entered reproductive senescence, after receiving cumulative TCDD doses as low as 1.7 µg TCDD/kg. They considered first occurrence of prolonged interestrus interval (>6 d) as evidence of onset of reproductive senescence.
Hormones E2	Yes	Yes, Males— Seveso	Li et al (1995) report serum estradiol-17β (E2) concentrations induced by equine Chorionic Gonadotropin injection were significantly elevated in female rats orally administered 10 µg/kg TCDD on PND 22. While E2 decreased dramatically in control animals during the preovulatory LH surge, it did not in TCDD-treated rats.
Low Birth Weight	Yes (190)	Suggestive effect in Seveso in first 8 years after exposure	ED ₁₀ basis Gray et al. (1997).
Reproductive Cycling (prolongation)	Yes	Yes, Seveso Prepubertal exposure	Franczac et al (2006) report loss of normal cyclicity in female rats at 8 months of age following a cumulative dose of 1.7 µg TCDD/kg.

Supporting Information

The Panel reviewed the charge questions (Appendix B), discussed them, and made two suggestions to the U.S. EPA:

- Concerning deviation from default approaches for noncancer endpoints, there needs to be a careful assessment of the POD and the application of uncertainty factors in light of PK/pharmacodynamics (PD), population characteristics and variability, and MOA information.
- Concerning the MOA's ability to clarify endpoint and the incorporation of a cascade of cellular event into dose-response for noncancer endpoint, any study that helps inform the dose response should be considered—including studies not specific to dioxins. Complicated mechanistic models need not be developed. Standard dose-response models can be applied. One can look at the cascade of events in a stepwise, simple way.

References

- Birnbaum, L.S., M.W. Harris, L.M. Stocking et al. 1989. Retinoic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin selectively enhance teratogenesis in C57BL/6N mice. *Toxicol. Appl. Pharmacol.* 98:487-500.
- Franczak, A., A. Nynca, K.E. Valdez, K.M. Mizinga and B.K. Petroff. 2006. Effects of acute and chronic exposure to the aryl hydrocarbon receptor agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin on the transition to reproductive senescence in female Sprague-Dawley rats. *Biol. Reprod.* 74:125-130.
- Gray, L.E. and J.S. Ostby. 2002. *In utero* 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alters reproductive morphology and function in female rat offspring. *Toxicol. Appl. Pharmacol.* 133(2):285-294.
- Gray, L.E., J.S. Ostby and W.R. Kelce. 1997. A dose-response analysis of the reproductive effects of a single gestational dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin in male Long Evans Hooded rat offspring. *Toxicol. Appl. Pharmacol.* 146:11-20.
- Li, X., D.C. Johnson and K.K. Rozman. 1995. Reproductive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in female rats: ovulation, hormonal regulation, and possible mechanism(s). *Toxicol. Appl. Pharmacol.* 133:321-327.
- Mably, T.A., D.L. Bjerke, R.W. Moore et al. 1992a. *In utero* and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin. 3. Effects on spermatogenesis and reproductive capability. *Toxicol. Appl. Pharmacol.* 114:118-126.
- Mably, T.A., R.W. Moore, R.W. Goy et al. 1992b. *In utero* and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin. 2. Effects on sexual behavior and the regulation of luteinizing hormone secretion in adulthood. *Toxicol. Appl. Pharmacol.* 114:108-117.

NAS (National Academy of Sciences). 2006. Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment. National Academies Press, Washington, DC (July). Available at http://www.nap.edu/catalog.php?record_id=11688.

U.S. EPA (U.S. Environmental Protection Agency). 2003. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds. NAS Review Draft (EPA/600/P-00/001Cb). U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC. Available at <http://www.epa.gov/nceawww1/pdfs/dioxin/nas-review/>.

SESSION 5: QUANTITATIVE UNCERTAINTY ANALYSIS OF DOSE-RESPONSE

This session addressed the uncertainty analysis to be considered for the dose-response assessments. The session opened with a presentation on current estimates of dioxin exposure levels. Then it focused on the factors to include in the scope of an uncertainty analysis including dioxin kinetics.

Session 5 Panelists (Session Co-Chairs are identified by asterisk)

- Bruce Allen, Bruce Allen Consulting
- Lesa Aylward, Summit Toxicology
- Roger Cooke, Resources for the Future
- Kenny Crump, Louisiana Tech University
- Mike DeVito, U.S. EPA
- Dale Hattis, Clark University
- *Rick Hertzberg, Biomath Consulting
- Nancy Kerkvliet, Oregon State University
- Leonid Kopylev, U.S. EPA
- Rob McDowell, U.S. Department of Agriculture
- Lorenz Rhomberg, Gradient
- Woody Setzer, U.S. EPA
- Marie Sweeney, National Institute of Occupational Safety and Health
- *Linda Teuschler, U.S. EPA

Please note that the use of the term “concluded” or “recommended” in this summary does not mean that a consensus was reached. Session Summaries were written from the material prepared by the non-EPA/ANL Co-Chair and represent a synopsis of the panel discussions.

The Panel summarized the NAS comments regarding uncertainty. Areas for improvement include:

- Ensure “transparency, thoroughness, and clarity in quantitative uncertainty analysis.”
- Describe and define (quantitatively to the extent possible) the variability and uncertainty for key assumptions used for each key endpoint-specific risk assessment, including choices of data set, point of departure, dose-response model, and dose metric.
- Incorporate probabilistic models to represent the range of plausible values.

- Assess goodness-of-fit of dose-response models.
- Provide upper and lower bounds on central tendency estimates for all statistical estimates.
- When quantification is not possible, clearly state it, and explain what would be required to achieve quantification.

Identification of Important Uncertainties

The Panel reviewed the charge questions (Appendix B), discussed them, and listed eight issues for consideration by the U.S. EPA:

- Concerning species and strain differences in the U.S. EPA's Response to NAS, current U.S. EPA procedures do not take this into account when selecting one data set for risk assessment. Issues include "Where are humans in the distribution of potencies that can be generated? How likely is it that human response is similar to the selected data? Can we infer inter-individual variability from these differences?"
- Concerning the use of animal data for cross species extrapolation to humans (PK and PD uncertainties), issues to consider include differences in distribution and responses following bolus doses from those of subchronic and chronic protocols; uncertainty in liver doses due to sequestration; differences in receptor binding affinity among congeners; and age factors (e.g., assumption of a lifetime constant daily dose for a cancer extrapolation).
- Concerning the description of AhR response, biochemical changes occur at lower doses than toxicological changes. There should be an effort to identify the biochemical changes that would mark Ah receptor binding to inform the BMR, and, thus, prevent toxicity.
- Concerning model uncertainty, the mathematical model choice depends on endpoint. There should be an effort towards determining what is the most sensitive endpoint(s) for humans and conducting animal studies to model that endpoint(s).
- Concerning exposure and dose response in human studies, ensure enough similarity to current human exposure profiles (mixture composition) so that a dose-response assessment can be done. Incorporate new epidemiological studies. Evaluate concordance with animal data and consistency across studies. Panel-acknowledged uncertainties include exposure estimates from person to person, shape of human dose-response curve, healthy worker effect, and age dependence.
- Concerning POD determination, uncertainty factors are inherently mathematically inconsistent and that should be conveyed in the discussion of uncertainties when interpreting the POD.
- Concerning dose metric, tissue concentration is preferred. It should be evaluated against a background of variability in AhR-binding expression. There is uncertainty in what level of binding should be considered, in different cell types, tissues, life stage (development). The relationship between dose metric and causation of adverse effects should be examined.

Low-Dose Extrapolation

The Panel reviewed the charge questions and discussed them (Appendix B). The Panel concluded that curve-fitting uncertainty (for a given dataset, dose metric, and model) can be characterized and is useful, but, by itself, it is an incomplete characterization of uncertainty. The Panel acknowledged the difficulty of fully characterizing uncertainty, especially quantitatively. Some panelists argued that the problem is insurmountable and that no meaningful uncertainty analysis is likely to be performable. Other panelists contended that, the difficulties notwithstanding, “good-faith” efforts to do something practical and forthright to characterize uncertainty in low-dose extrapolation would be useful and important. The Panel clarified “good faith” as meaning a characterization that is useful and not misleading to decision makers and is inclusive of approaches that have meaningful support in the scientific community as a whole. Being in “good faith” is more important than being complete (i.e., addressing every uncertain element), especially since completeness is not a realistic goal. From this discussion, the Panel listed four issues for consideration by the U.S. EPA:

- Review alternative data sets, dose metrics, and models to see where consequential uncertainties and impacts on low-dose implications arise.
- Consider the impacts of choices among plausible alternative data sets, dose metrics, models, and other more qualitative choices—issues include how much difference the choices make and also how much relative credence should be put to each alternative as a way of gauging and describing the landscape of imperfect knowledge regarding possibilities for the true dose-response.
 - Hard to do quantitatively, since the factors are not readily expressed as statistical distributions, but can describe the rationale for believing/doubting each alternative in terms of available supporting evidence, contrary evidence, and needed assumptions.
 - Expert judgment methods may be helpful in characterizing the relative weights of scientific credibility among alternatives. The expert judgment process, when conducted systematically, can be thought of as adding data to the assessment of credibility of alternatives, rather than as just an opinion poll.
 - Information on plausibility of alternative low-dose extrapolation approaches can come from external considerations of mode of action, and not just from statistical success at fitting particular (high-dose) data sets.
- Characterizing uncertainty through a variety of approaches could be tried, and their relative merits and shortcomings discussed, as a way forward.
- Consider the sources of potential error, particularly in epidemiological data (e.g., TEF uncertainty and variation in congener mixtures) and if possible quantify their impact on the dose-response assessment.

Considerations for Conducting Uncertainty Analysis

Overall, the Panel was split on whether U.S. EPA should do quantitative uncertainty analyses. The Panel noted that if done on only some of the uncertainties, then results would be misleading and could be misused. Ultimately, the Panel listed seven issues for consideration by the U.S. EPA:

- The Panel recapped what some consider as being the first integrated risk assessment, with structured expert judgment and uncertainty analysis, i.e., the Rasmussen Report (WASH-1400; U.S. Nuclear Regulatory Commission, 1975). In their discussion of the report, the Panel noted that in addition to standard event tree/fault tree modeling, this report also tackled difficult model uncertainty issues involved in accident progression, dispersion of released pollutants in the atmosphere, environmental transport, exposure, health, and economic impacts. And though the Panel also recognized that this method was no longer state-of-the-art, the Panel contended that it represents a good example of a structured approach and methodology that could be built upon.
- The Panel also discussed TEQs used in epidemiological studies, based on intake, and recognized that the key uncertainty in what was measured was not just intake but also involved PK/PD issues. The Panel acknowledged that the TEQ system is regularly used on a concentration basis, but they expressed concern that the qualification becomes lost. TEQs ignore pharmacokinetics and the common practice of rounding to orders of magnitude introduces more error.
- Structure the risk assessment along MOA steps—identify key biochemical measures (~5–10) common across toxic endpoints and identify the degree of meaningful change in effect or effect variance. Make a table with all options for data set, model, etc.; make best estimates/choices and determine which of these choices matter the most to the answer.
- Use expert panels—expert judgment can be collected scientifically (procedures are published). But there are known biases; central tendency estimates work much better than extremes.
- Use supporting studies to fill in critical data gaps—Info filling methods do exist (e.g., PK modeling). Put short-term studies into the “supporting info” category (unless, of course, the risk assessment is for acute exposures, such as chemical spills).
- Be creative in the analysis of uncertainty. Intermediate steps between AhR binding and the end processes can be hypothesized based on data, experiences, and analogies related to other chemicals.
- The 2003 Reassessment presented potency estimates on wide variety of endpoints/models; needed to be more transparent in that discussion. Statistical graphics can be used to convey uncertainties.

Reference

U.S. Nuclear Regulatory Commission. 1975. Reactor Safety Study: An Assessment of Accident Risks in U.S. Commercial Nuclear Power Plants. WASH-1400 (NUREG-75-014). Washington, DC.

APPENDIX A: 2009 U.S. EPA DIOXIN WORKSHOP AGENDA

SCIENTIFIC WORKSHOP TO INFORM THE TECHNICAL WORK PLAN FOR U.S. EPA'S RESPONSE TO NAS COMMENTS ON THE HEALTH EFFECTS OF DIOXIN PRESENTED IN U.S. EPA'S DIOXIN REASSESSMENT

Cincinnati, OH

Date: February 18–20, 2009

BACKGROUND/WORKSHOP OBJECTIVE

At the request of the U.S. Environmental Protection Agency (U.S. EPA), the National Academy of Sciences (NAS) prepared a report, *Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment* (NAS, 2006), that made a number of recommendations to improve the U.S. EPA's risk assessment for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). In response, the U.S. EPA will prepare a technical report that addresses key comments on the dose-response assessment for TCDD. The U.S. EPA intends to develop its response through a transparent process that provides multiple opportunities for input.

To assist in this effort, a Workshop will be held to inform the U.S. EPA's evaluation of the NAS recommendations. The Workshop will be open to the public. At the Workshop, the U.S. EPA will solicit input from expert scientists and the public.

The goal of the Workshop is to ensure that the U.S. EPA's response to the NAS comments focuses on the key issues and reflects the most meaningful science. The three main objectives of the Workshop are to (1) identify and discuss the technical challenges involved in addressing the NAS key comments on the TCDD dose-response assessment in the U.S. EPA Reassessment (U.S. EPA, 2003), (2) discuss approaches for addressing these comments, and (3) identify key published, independently peer-reviewed literature, particularly studies describing epidemiologic and *in vivo* mammalian bioassays, which are expected to be most useful for informing the U.S. EPA response.

Workshop participants will be encouraged to think broadly about the body of scientific information that can be used to inform the U.S. EPA's response and to participate in open dialogue regarding ways in which the science can best be used to address the key dose-response issues. This Workshop is similar to scientific workshops being conducted under the new review process for the National Ambient Air Quality Standards (NAAQS)¹ that assess health-related information for criteria pollutants.

¹ Please see <http://www.epa.gov/ttn/naaqs/> for more information on the new NAAQS review process.

The Workshop discussions are expected to build upon two prior publications:

1. *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds* (U.S. EPA, 2003). This external review draft provides a comprehensive reassessment of dioxin exposure and human health effects. This “dioxin reassessment” was submitted in October 2004 to the National Academy of Sciences (NAS) for review.
2. *Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment* (NAS, 2006).

Workshop participants are encouraged to review both of these documents and other relevant materials (e.g., the National Toxicology Program report on TCDD [NTP, 2006]) before the meeting because they provide important insights into the key questions and challenges. There are a number of open comment periods that are intended to facilitate a broad discussion of the issues.

Scientists with significant expertise and experience relevant to the health effects of TCDD or dioxin-like compounds and associated topics will be asked to serve on “expert panels” for discussions throughout the Workshop. Workshop panelists will include a wide range of experts representing many scientific areas needed to assess TCDD dose-response (e.g., epidemiology, human and animal toxicology, nuclear receptor biology, dose-response modeling, risk assessment, and uncertainty analysis). The Workshop panelists will be asked to highlight significant and emerging research and to make recommendations to the U.S. EPA regarding the design and scope of the technical response to NAS comments on the dose-response analysis for TCDD—including, but not limited to, recommendations for evaluating associated uncertainty. Open comment periods will follow each panel discussion session. Public participation will be encouraged by way of these designated open comment periods and, also, by participation in the scientific poster session planned for the second evening (February 19).

U.S. EPA will use the input received during this Workshop as the foundation for its development of a technical work plan for responding to the NAS comments on the TCDD dose-response analysis. The work plan will outline the schedule, process, and approaches for evaluating the relevant scientific information and addressing the key issues. The work plan also will identify the key literature to be utilized in U.S. EPA’s response.

As a follow-on activity to this Workshop, a panel is being established under the Federal Advisory Committee Act (FACA) to guide and review the U.S. EPA’s response to NAS comments. The FACA panel will be asked to conduct a consultation with the Agency on the draft technical work plan. At the same time, the public will also have the opportunity to provide comments to the FACA panel on the work plan. The final technical work plan will guide the development of the technical report that will constitute the U.S. EPA’s response to NAS comments. During the development of this response, the U.S. EPA will seek advice from the FACA panel and the public several times. Finally, the FACA panel will be asked to review the technical report in a public forum.

The preliminary Agenda presented on the following pages may be revised prior to the Workshop following review by the session Co-Chairs; the dates and general timing of the

sessions, however, will not change. A final Agenda and a set of charge questions, intended to provide general direction for the Workshop discussions, will be posted on the Workshop Internet site (<http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=199923>) prior to the meeting.

A poster session will be held on the evening of the second day (February 19). The purpose of this poster session is to provide a forum for scientists to present recent studies relevant to TCDD dose-response assessment and to encourage open discussion about these presentations.

REFERENCES

NAS (National Academy of Sciences). 2006. Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment. National Academies Press, Washington, DC (July). Available at http://www.nap.edu/catalog.php?record_id=11688.

NTP (National Toxicology Program). 2006. Toxicology and Carcinogenesis Studies of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) (CAS No. 1746-01-6) in Female Harlan Sprague-Dawley Rats (Gavage Studies). U.S. Department of Health and Human Services. NTP TR 521. Research Triangle Park, NC (April).

U.S. EPA (U.S. Environmental Protection Agency). 2003. Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds, NAS review draft, Volumes 1-3 (EPA/600/P-00/001Cb, Volume 1). U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC (December). Available at <http://www.epa.gov/nceawww1/pdfs/dioxin/nas-review/>.

WORKSHOP AGENDA

Day 1

- 8:00–9:00 **Registration**
- 9:00–9:30 **Welcome/Purpose of Meeting/Document Development Process**
- 9:30–9:45 **Panel Comments/Questions on Charge**
- 9:45–2:45** **Session 1: Quantitative Dose-Response Modeling Issues**
(Hall of Mirrors)
- 9:45–10:10 **Background/Introductory Remarks**
- 10:10–10:35 **TCDD Kinetics: Converting Administered Doses in Animals to**
 Human Body Burdens
 Presenter: Michael Devito
- 10:35–11:30 **Panel Discussion**
- 11:30–1:00 **Lunch**
- 1:00–2:00 **Panel Discussion cont.**
- 2:00–2:45 **Open Comment Period**
- 2:45–3:05** **Break**
- 3:05–5:15** **Session 2: Immunotoxicity (Hall of Mirrors)**
- 3:05–3:15 **Background/Introductory Remarks**
- 3:15–4:45 **Panel Discussion**
- 4:45–5:15 **Open Comment Period**

Day 2

<u>8:00–8:30</u>	<u>Report-Outs for Sessions 1 and 2 (Hall of Mirrors)</u>
8:00–8:15	Report-Out for 1: Quantitative Dose-Response Modeling Issues
8:15–8:30	Report-Out for 2: Immunotoxicity
<u>8:30–11:30</u>	<u>Sessions 3A and 3B (concurrent sessions)</u>
<u>8:30–11:30</u>	<u>Session 3A: Dose-Response for Neurotoxicity and Nonreproductive Endocrine Effects (Hall of Mirrors)</u>
8:30–8:45	Background/Introductory Remarks
8:45–11:00	Panel Discussion
11:00–11:30	Open Comment Period
<u>8:30–11:30</u>	<u>Session 3B: Dose-Response for Cardiovascular Toxicity and Hepatotoxicity (Rookwood Room)</u>
8:30–8:45	Background/Introductory Remarks
8:45–11:00	Panel Discussion
11:00–11:30	Open Comment Period
<u>11:30–1:00</u>	Lunch
<u>1:00–2:00</u>	<u>Report-Outs for Sessions 3A and 3B (Hall of Mirrors)</u>

The structure of the session report-outs will include the following:

- Summary of session presentation including minority opinion
- Public comments
- Discussion

1:00–1:15	Report-Out for 3A: Dose-Response for Neurotoxicity and Nonreproductive Endocrine Effects
1:15–1:30	Open Comment Period

1:30–1:45 **Report-Out for 3B: Dose-Response for Cardiovascular Toxicity and Hepatotoxicity**

1:45–2:00 **Open Comment Period**

2:00–5:15 **Sessions 4A and 4B (concurrent sessions)**

2:00–5:15 **Session 4A: Dose-Response for Cancer (Hall of Mirrors)**

2:00–2:15 **Background/Introductory Remarks**

2:15–4:45 **Panel Discussion**

4:45–5:15 **Open Comment Period**

2:00–5:15 **Session 4B: Dose-Response for Reproductive/Developmental Toxicity (Rookwood Room)**

2:00–2:15 **Background/Introductory Remarks**

2:15–4:45 **Panel Discussion**

4:45–5:15 **Open Comment Period**

6:45–8:15 **Poster Session (Rosewood Room)**

Day 3

8:30–9:30 **Report-Outs for Sessions 4A and 4B (Hall of Mirrors)**

8:30–8:45 **Report-Out for 4A: Dose-Response for Cancer**

8:45–9:00 **Open Comment Period**

9:00–9:15 **Report-Out for 4B: Dose-Response for Reproductive/Developmental Toxicity**

9:15–9:30 **Open Comment Period**

9:30–3:30

Session 5: Quantitative Uncertainty Analysis of Dose-Response (Hall of Mirrors)

9:30–9:40

Background/Introductory Remarks

9:40–10:10

Evidence of a Decline in Background Dioxin Exposures in Americans Between the 1990s and 2000s
Presenter: Matt Lorber

10:10–10:30

Break

10:30–11:30

Panel Discussion

11:30–1:00

Lunch

1:00–2:15

Panel Discussion cont.

2:15–2:30

Break

2:30–3:00

Open Comment Period

3:00–3:15

Report-Out for 5: Quantitative Uncertainty Analysis of Dose-Response

3:15–3:30

Closing Remarks

3:30

Adjourn

APPENDIX B: 2009 U.S. EPA DIOXIN WORKSHOP QUESTIONS TO GUIDE PANEL DISCUSSIONS

SESSION 1

Dose Metric

Considering all of the endpoints or target tissues, and species that U.S. Environmental Protection Agency (U.S. EPA)'s dose-response modeling might evaluate, what are the best measures of dose (e.g., ingested, tissue concentrations, body burden, receptor occupancy, other surrogate) and why?

Developing Dose-Response Models from Mammalian Bioassays

How best can the point of departure (POD) be determined when the response range is incompletely characterized (i.e., high response at the lowest dose or low response at the highest dose; observed in several key 2,3,7,8-Tetrachlorodibenzo-p-Dioxin [TCDD] studies)?

If considered to be biologically plausible, how can a threshold be incorporated into a dose-response function (e.g., for TCDD cancer data)?

How can nonmonotonic responses be incorporated into the dose-response function?

Developing Dose-Response Models from Epidemiological Studies

How can the epidemiological data be utilized best to inform the TCDD exposure-response modeling? Which epidemiological studies are most relevant?

Supporting Information

For those toxicological endpoints that are Ah receptor-mediated, how would the receptor kinetics influence the shape of the dose-response curve? How would downstream cellular events affect the shape of the dose-response curve? How can this cascade of cellular events be incorporated into a quantitative model of dose-response?

SESSIONS 2, 3A, 3B, 4A, AND 4B

Key Study Selection

For this endpoint, what refinements should be made to the draft criteria for selection of key studies?

What are the specific effects of concern for human health for this endpoint?

Based on the draft criteria for the selection of key studies, what are the key studies informing the shape of the dose-response curve above the POD and the choice of the POD for this endpoint?

Epidemiological Study Utility

How and to what extent do the epidemiological data inform the choice of critical effect?

How can the epidemiological data inform the quantitative dose-response modeling?

Animal Model Utility

Are there types of effects observed in animal models that are more relevant to humans than others? To what extent does information on mode of action (MOA) influence the choice of animal model (species, strain, sex)?

Supporting Information

Are there studies that establish a sufficient justification for departure from the default procedures that address the shape of the dose-response curve below the POD under the cancer guidelines?

Are there studies that establish a sufficient justification for departing from U.S. EPA's default approaches for noncancer endpoints?

To what extent can MOA information clarify the identification of endpoints of concern and dose-response metric for this endpoint? How can the cascade of cellular events for this endpoint be incorporated into a quantitative model of dose response?

SESSION 5

For cancer and noncancer TCDD dose-response assessments, U.S. EPA is interested in developing a quantitative uncertainty analysis addressing both parameter and model uncertainty, if feasible. Uncertainties will include, among others, choice of endpoint; underlying study uncertainties; choice of dose metric; interspecies extrapolations such as kinetic uncertainties; and choice of dose-response model, including threshold models. The U.S. EPA is currently examining techniques and tools for uncertainty analysis—including Bayesian and frequentist approaches.

Identification of Important Uncertainties

What are the major uncertainties pertaining to modeling the animal data?

Consider the dose metric (species or tissue specificity), vehicle of administration, exposure frequency, exposure duration, and POD determination (e.g., benchmark response selection or no-observed-adverse-effect level/lowest-observed-adverse-effect level identification).

What are the major uncertainties pertaining to dose-response modeling below the POD?

Consider how receptor kinetics and downstream cellular event information might be used to bound the uncertainties associated with dose-response modeling below the POD.

What are the major uncertainties in cross-species extrapolation (e.g., half-lives, tissue distribution, and toxicodynamics)?

Consider the primary species dosed with TCDD: mice, hamsters, rats, guinea pigs, and monkeys.

What are the major uncertainties pertaining to intrahuman variability?

Consider what data sets would be useful to represent sensitive subpopulations.

What are other significant sources of uncertainty for the cancer and noncancer assessments?

Considerations for Conducting Uncertainty Analysis

What data sets could be used to quantify uncertainties in cancer and noncancer TCDD dose-response assessments?

Consider dioxin-like compound dose-response data.
Consider MOA information.

What are the appropriate techniques for the TCDD dose-response uncertainty analysis, and what are their respective strengths and weaknesses of these approaches as applied to TCDD?

APPENDIX C: 2009 U.S. EPA DIOXIN WORKSHOP DRAFT SELECTION CRITERIA TO IDENTIFY KEY *IN VIVO* MAMMALIAN STUDIES THAT INFORM DOSE-RESPONSE MODELING FOR 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD)^a

Study Feature	Selection Rationale		
	<i>Primary^b</i>	<i>Secondary^c</i>	<i>Currently Excluded</i>
Chemical, purity, matrix/medium	TCDD-only doses included, purity specified, matrix in which TCDD is administered is identified	TCDD purity or matrix not clearly identified	Studies of dioxin-like compounds (DLCs) or mixtures
Peer review	Independently peer-reviewed, publicly available	Supplementary materials accompanying peer-reviewed publication	Not formally peer-reviewed; literature not publicly available
Study design, execution, and reporting	Clearly documented and consistent with standard toxicological principles, testing protocols, and practice (i.e., endpoint-appropriate, particularly for negative findings)	Testing protocol provides incomplete coverage of relevant endpoint-specific measures, particularly for negative findings	Studies not meeting standard principles and practices
Study subject: species, strain, and sensitivity for given endpoint; litter; life stage; gender	Mammalian species Strain and gender identified Animal age at beginning of treatment identified Litter confounders (within/between) accounted for	Mammalian species, <i>in vivo</i> , but only studying an artificially sensitive subject (e.g., knockout mouse)	Non-mammalian or not <i>in vivo</i>
Exposure route	Oral	Parenteral (e.g., intravenous, intramuscular, intraperitoneal, subcutaneous)	Inhalation, dermal, ocular
Dose level	Lowest dose ≤200 ng/kg-d for noncancer endpoints and ≤1 µg/kg-d for cancer	Lowest dose >200 ng/kg-d for noncancer endpoints, or >1.0 µg/kg-d for cancer	
Exposure frequency, duration, and timing	Dosing regimen characterized and explained		Characterization/explanation missing or cannot be determined
Controls	Appropriate and well characterized	Effect reported, but with no negative control	
Response	Effect relevant to human health Magnitude outside range of normal variability	Precursor effects, or adaptive responses potentially relevant to human health	Lethality
Statistical evaluation	Clearly described and appropriate to the endpoint and study design (e.g., per error variance, magnitude of effect)	Limited statistical context	

^a NAS (2006) commented that the selection of data sets for quantitative dose-response modeling needed to be more transparent. These draft criteria are offered for consideration at the kickoff workshop. These criteria would be used to identify candidate studies of non-human mammals that would be used to define the point-of-departure (POD). These criteria are not designed for hazard identification or weight-of-evidence determinations. Studies addressing data other than direct TCDD dose-response in mammals (including toxicokinetic data on absorption, distribution, metabolism, or elimination; information on physiologically-based pharmacokinetic [PBPK] modeling, and mode of action data) will be evaluated separately.

^b Presents preliminary draft criteria for evaluating a study being considered for estimating a POD in a TCDD dose-response model.

^c Presents preliminary draft criteria that could qualify a study as primary with support from other lines of evidence (e.g., PBPK modeling), when no study for an endpoint meets the “primary” criteria.