Peer Review Workshop of Dioxin Reassessment-Chapter 9: Toxicity Equivalency Factors for Dioxin and Related Compounds and the Revised Integrated Summary and Risk Characterization Document

Premeeting Comments

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CHARGE TO THE REVIEWERS

Introduction and Background

In April 1991, the U.S. Environmental Protection Agency (EPA) announced it would conduct a scientific reassessment of the health risks of exposure to dioxins. This reassessment was initiated in response to emerging scientific knowledge of the biological, human health, and environmental effects of dioxin. Significant advances have occurred in the scientific understanding of mechanisms of dioxin toxicity, of the carcinogenic and other adverse health effects of dioxin in people, of the pathways to human exposure, and of the toxic effects of dioxin to the environment.

EPA's reassessment activity led to the publication of a 1994 draft multi-volume document titled Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds. This 1994 draft was reviewed by the agency's Science Advisory Board (SAB) in May 1995. Their review and subsequent Fall 1995 report had four key recommendations:

- The review provided substantive comments on two sections in the reassessment documents-the chapter on Dose Response Modeling (chapter 8) and the Risk Characterization document (identified as chapter 9 in a previous draft).

- The review recommended development of a new chapter on toxicity equivalent factors (TEF) for the purpose of gathering in one place the discussion and scientific information on the complex issue and use of TEFs for dioxin and dioxin-like compounds.

- The review approved the health and exposure sections (chapters 1-7) without the need for further SAB review, provided EPA updated these sections with any relevant new information before finalizing.

- The review recommended that the revised chapters on Dose Response Modeling and Risk Characterization and the new chapter on TEFs should undergo external peer review prior to the SAB's re-review.

To date, EPA has addressed the first three SAB recommendations listed above and conducted an external peer review on the revised Dose-Response Modeling analysis (chapter 8), but the agency has not yet conducted an external peer review of the updated Integrated Summary and Risk Characterization or the new Chapter 9-Toxicity Equivalency Factors (TEF) for Dioxin And Related Compounds. The scientific rigor of these documents is the subject of this peer review.

During this peer review, EPA seeks expert opinions on several key questions that pertain to the content of the documents, Integrated Summary and Risk Characterization and the Toxicity Equivalence Factors (TEF) for Dioxin and Related Compounds. The questions are classified into twelve general topics, listed on the following pages. Further, EPA welcomes insights on additional topics relevant to these documents, but not explicitly addressed in the other questions. Following the workshop, ERG will prepare a summary report that documents the reviewers' responses to these questions. The reviewers will then be asked to review the ERG report for accuracy, after which ERG will submit the final peer review meeting report to the agency.

General Instructions When Preparing Written Comments

When addressing the questions to which you have been assigned, please make sure that you have addressed the following general questions in your responses: Are the assumptions and uncertainties clearly and adequately expressed?

- Are the key issues, statements, and conclusions clearly stated?
- Are the conclusions in the Integrated Summary and Risk Characterization supported with sufficient data, information, arguments, and references?
- Are the discussion points clear? How would you suggest improving the clarity of the text?
- Please make specific recommendations on improvements that can be made to the document to improve it.

REVIEWER ASSIGNMENTS

ASSIGNED CHARGE QUESTIONS

TOPIC 1: BODY BURDENS

In Section 5.1 of the Integrated Summary and Risk Characterization report, EPA concluded that body burden is a better metric for assessing inter-species scaling (animal to human) than are other methods (e.g., daily dose information combined with an uncertainty factor for pharmacokinetics, or 3/4 power body weight scaling).

Question 1:
Primary Reviewer-Brent Kerger
Secondary Reviewer-Richard Dickerson

Did EPA adequately justify its use of body burden as a dose metric for inter-species scaling? Should the document present conclusions based upon daily dose?

TOPIC 2: USE OF A "MARGIN-OF-EXPOSURE APPROACH" TO EVALUATE RISKS

EPA has recommended the use of margin-of-exposure (MOE) to evaluate the potential for health effects from dioxin. This approach expresses exposure as a percent additivity to background and recommends that it is a policy decision as to whether such increments reach significance for decision making. EPA decided not to apply the RfD/RfC methods to dioxin because of the relatively high background compared to effect levels and because these methods are most useful for evaluating increments of exposure from specific sources when background exposures are low and insignificant. EPA's decision to use an MOE approach differs from the approaches taken by the Agency for Toxic Substances and Disease Registry (ATSDR 1999) and WHO (1998), who calculate a minimal risk level (MRL) of 1 pg/kg/day and tolerable daily intake (TDI) of 1 - 4 pg/kg/day, respectively.

Question 2
Primary Reviewer-Colin Park
Secondary Reviewer-Richard Dickerson, Lorenz Rhomberg

How might the rationale be improved for EPA's decision not to calculate an RfD/RfC, and for the recommended MOE approach for conveying risk information? Is an MOE approach appropriate, as compared to the traditional RfD/RfC? Should the document present an RfD/RfC?

Question 3
Primary Reviewer-Lorenz Rhomberg
Secondary Reviewer-Mark Harris

The SAB commented that previous dose-response modeling was too limited to biochemical endpoints (CYPIA1, IA2, ...), Are the calculations of a range of ED01 body burden calculations for non-cancer effects in rodents responsive and clearly presented? Please comment on the weight-of-evidence interpretation of the body burden data associated with a 1% response rate for non-cancer effects that is presented in Chapter 8, Appendix I and Figure 8-1 (where EPA considers that the data best support a range estimate for ED01 body burdens between 10 ng/kg to 50 ng/kg).

TOPIC #3: MECHANISMS AND MODE OF ACTION

The scientific community has identified and described a series of common biological steps that play a role in most, if not all, observed dioxin-related effects in vertebrates, including humans. Biochemical, cellular, and organ-level endpoints have been shown to be affected by TCDD, but specific data on many of these endpoints do not generally exist for other congeners. The discussion in Part III indicates that our understanding of mechanisms of toxic action of TCDD is limited, but that a generalized mode-of-action can be discussed in light of these uncertainties.

Question 4
Primary Reviewer-Mark Harris
Secondary Reviewer-Matti Viluksela

How might the discussion of mode-of-action of dioxin and related compounds be improved?

Question 5
Primary Reviewer-Mark Harris
Secondary Reviewer-Matti Viluksela

Despite the lack of congener-specific data, does the discussion in the Integrated Summary and Risk Characterization support EPA's inference that these effects may occur for all dioxin-like compounds, based on the concept of toxicity equivalence?

TOPIC #4: TOXICITY EQUIVALENCY FACTORS

Dioxin and related compounds exist in nature as complex mixtures, the biological activity of which can be estimated using relative potency values and an assumption of dose additivity. Such an approach has evolved over time and has been characterized as a useful interim procedure to assess complex mixtures. The TEF approach has been accepted by numerous countries and several international organizations. In 1995, the Science Advisory Board supported "... EPA's use of Toxic Equivalencies for exposure analysis ...", but suggested that the Agency describe the history and application of the TEF process more explicitly.

Question 6
Primary Reviewer-Peter deFur
Secondary Reviewer-Mark Harris

Is the history, rationale and support for the TEQ concept, including its limitations and caveats, laid out by EPA in a clear and balanced way in Chapter 9? Did EPA clearly describe its rationale for recommending adoption of the 1998 WHO TEFs?

Question 7
Primary Reviewer-Brent Kerger
Secondary Reviewer-Matti Viluksela

Does EPA establish clear procedures for using, calculating, and interpreting toxicity equivalence factors?

TOPIC #5: NON-CANCER EFFECTS

Based on the information presented in Part II of the reassessment, Health Assessment for 2,3,7,8-TCDD and Related Compounds, EPA believes that adequate evidence supports the inference that humans are likely to respond with a broad spectrum of non-cancer effects from exposure to dioxin and related compounds. These effects will likely range from biochemical changes at or near background levels of exposure to adverse effects with increasing severity as body burdens increase above background levels.

Question 8
Primary Reviewer-Richard Dickerson
Secondary Reviewer-Allan Smith

Have the available human data been adequately integrated with animal information in evaluating likely effect levels for the non-cancer endpoints discussed in the reassessment?

Question 9
Primary Reviewer-Richard Dickerson
Secondary Reviewer-Peter deFur

Do reviewers agree with the characterization of human developmental, reproductive, immunological, and endocrinological hazard? What, if any, additional assumptions and uncertainties should EPA embody in these characterizations to make them more explicit?

TOPIC #6: CANCER EFFECTS

A weight-of-the-evidence evaluation suggests that mixtures of dioxin and related compounds are strong cancer promoters and weak direct or indirect initiators, and thus are likely to present a cancer hazard to humans. Although uncertainties remain regarding quantitative estimates of upper bound cancer risk from dioxin and related compounds, the reassessment uses various data sources to evaluate the slope of the dose-response curve at the low end of the observed range (using the LED01). This approach uses a simple proportional (linear) model and a calculation of both upper bound risk and margin of exposure (MOE) based on human equivalent background exposures and associated body burdens.

Question 10
Primary Reviewer-Allan Smith
Secondary Reviewer-Brent Kerger, Curtis Travis

Do you agree with the characterization in this document that dioxin and related compounds are carcinogenic hazards for humans?

Question 11
Primary Reviewer-Allan Smith
Secondary Reviewer-Brent Kerger

Does the document clearly present the evolving approaches to estimating cancer risk (e.g.,

margin of exposure and the LED01 as a point of departure), as described in the EPA "Proposed Guidelines for Carcingenic Risk Assessment" (EPA/600/P-92/003C; April 1996)? Is this approach equally as valid for dioxin-like compounds.

Question 12
Primary Reviewer-Holly Hattemer-Fry
Secondary Reviewer-Colin Park

Please comment on the presentation of the range of upper bound risks for the general population based on this reassessment. What alternative approaches should be explored to better characterize quantitative aspects of potential cancer risk? Is the range that is given sufficient, or should more weight be given to specific data sources?

TOPIC #7: BACKGROUND AND POPULATION EXPOSURES

The term "background exposures" is used to describe dioxin exposures for the general population (i.e., individuals who are not exposed to readily identifiable point sources of dioxin-like compounds). Current adult daily intakes of CDD/CDFs and dioxin-like PCBs are estimated to average 45 and 25 pg TEQDFP-WHO98/day, respectively, for a daily total intake of 70 pg TEQDFP-WHO98/day (~1 pg/kg/day). The estimated current average adult body burden of 5 ng TEQDFP-WHO98/kg is substantially less than levels measured in the late 1980s/early 1990s (~14 ng TEQDFP-WHO98/kg), yet still reflects intakes from past exposure levels which are thought to be higher than current levels. Considerable variability around these means exists due to both the quantity and types of foods consumed. For instance, EPA has estimated that background exposures to dioxin-like compounds may extend to levels at least three times higher than the mean, based on normal variability in human diet and behavior. Beyond this variability, EPA notes the existence of special populations that may be exposed to higher levels, such as individuals living near discrete local sources, subsistence or recreational fishers consuming more highly contaminated species, and nursing infants.

Question 13
Primary Reviewer-Myrto Petreas
Secondary Reviewer-Holly Hattemer-Fry, Christopher Rappe

Have the estimates of background exposure been clearly and reasonably characterized?

Question 14
Primary Reviewer-Myro Petreas
Secondary Reviewer-Holly Hattemer-Fry, Christopher Rappe

Has the relationship between estimating exposure from dietary intake and estimating exposure from body burden been clearly explained and adequately supported?

Question 15
Primary Reviewer-Myrto Petreas
Secondary Reviewer-Holly Hattemer-Fry, Christopher Rappe

Have important "special populations" and age specific exposures been identified and appropriately characterized?

TOPIC #8: CHILDREN'S RISK

Federal agencies are obliged to consider risks to children in their regulatory decisions, with risks that differentially impact children being particularly important. Based on the weight of evidence, EPA considers that risks to children from dioxin and related compounds may be increased compared to the general population, but acknowledges that more data are needed to fully address this issue. EPA's conclusion is based on the spectrum of higher dose toxicity evident in the Yusho/Yu-Cheng and Seveso incidents and on the contemporary epidemiological literature which has shown structural and developmental effects associated with low dose/background exposure to dioxin TEQ levels in various children's cohorts. EPA has concluded that these human developmental effects are consistent with those seen in animal bioassays and in in vitro studies, as well as with dioxin's mechanism of action on cellular differentiation. However, the relative paucity of data has prevented EPA from determining if children are differentially sensitive compared to adults, and the extent to which such differential sensitivity occurs.

Question 16
Primary Reviewer-Matti Viluksela
Secondary Reviewer-Richard Dickerson, Allan Smith

Is the characterization on increased or decreased childhood sensitivity to possible cancer and non cancer outcomes scientifically supported and reasonable? Is the weight of evidence approach appropriate?

TOPIC #9: RELATIVE RISKS OF BREAST FEEDING

Based on estimates that human breast milk contains 35 ppt TEQDFP-WHO98 and a six month nursing scenario, the average daily dioxin intake (on a TEQ basis) for an infant is about 100 times higher than an adult-a notable finding given that infants' exposures occur during sensitive developmental stages. However, the differences in body burden between infants and adults are expected to be much less than differences in the daily intakes, primarily because (1) the long half-life and cumulative nature of the body burden, (2) equilibration throughout the infant's body, and (3) rapid growth in size of the infant.

Question 17
Primary Reviewer-Curtis Travis
Secondary Reviewer-Lorenz Rhomberg

Has EPA adequately characterized how nursing affects short-term and long-term body burdens of dioxins and related compounds?

TOPIC #10 RISK CHARACTERIZATION SUMMARY STATEMENT

Based on the data reviewed in this reassessment and on scientific inference, a picture emerges of TCDD and related compounds as potent toxicants in animals with the potential to produce a spectrum of effects. Some of these effects may be occurring in humans at general population background levels, particularly among more highly exposed groups or special populations, with the spectrum of effects, and their potential to be adverse, increasing as body burdens rise.

Question 18
Primary Reviewer-Peter deFur
Secondary Reviewer-All

Does the summary and analysis support the conclusion that enzyme induction, changes in hormone levels, and indicators of altered cellular function seen in humans and laboratory animals, represent effects of unknown clinical significance, but that may be early indicators of toxic response? (Refer to pages 84-86.)

Question 19
Primary Reviewer-Colin Park
Secondary Reviewer-All

Has the short summary statement in the risk and hazard characterization on page 107 adequately captured the important conclusions, and the areas where further evaluation is needed? What additional points should be made in this short statement?

TOPIC # 11: SOURCES

Many Dioxin sources have been identified and emissions to the environment are being reduced. EPA's detailed inventory of dioxin emission sources quantifies, to the extent possible,the emissions for 1987 and 1995 from the majority of known sources in the United States, and provides preliminary estimates of emission factors for other sources where the data are too preliminary to be used to provide national averages. This inventory is presented in Part I of the dioxin reassessment, and has undergone peer review by the SAB. The inventory and other exposure information provide evidence that environmental levels of dioxin-like substances are being reduced through direct and indirect emission control methods. The present information also suggests that reservoir sources in the environment may be important factors to evaluate human exposures.

Question 20
Primary Reviewer-Christopher Rappe
Secondary Reviewer-Curtis Travis

Are these sources adequately described and are the relationships to exposure adequately explained?

Question 21
Primary Reviewer-Peter deFur, Colin Park
Secondary Reviewer-All

Please provide any other comments or suggestions relevant to the two review documents, as interest and time allow.

REVIEWER ASSIGNMENTS: PRIMARY, SECONDARY, AND ALL

REVIEWER	PRIMARY REVIEWER FOR QUESTIONS:	SECONDARY REVIEWER FOR QUESTIONS:	ALL REVIEWERS ADDRESS QUESTIONS:
DeFur	6, 18, 21	9	19
Dickerson	8, 9	1, 2, 16	18, 19 & 21
Harris	4, 5	3, 6	18, 19 & 21
Hattemer-Frey	12	13, 14, 15	18, 19 & 21
Kerger	1, 7	10, 11	18, 19 & 21
Park	2, 19, 21	12	18
Petreas	13, 14, 15		18, 19 & 21
Rappe	20	13, 14, 15	18, 19 & 21
Rhomberg	3	2, 17	18, 19 & 21
Smith	10, 11	8, 16	18, 19 & 21
Travis	17	10, 20	18, 19 & 21
Viluksela	16	4, 5, 7	18, 19 & 21

Peter deFur
Environmental Stewardship Concepts

Primary Reviewer for Questions 6, 18, 21
Secondary Reviewer for Question 9
General Reviewer for Question 19

Peter deFur

President
Environmental Stewardship Concepts
11223 Fox Meadow Drive
Richmond, VA 23233
804-360-4213
Fax: 801-360-7935
E-mail: pldefur@igc.org

B.S., Biology & M.A, Biology, College of William and Mary
Ph.D. , Biology, University of Calgary

With more than 25 years of research experience, Dr. deFur is a recognized expert in biology, risk assessment, ecology, physiology, and toxicology. Recent research projects of Dr. deFur's include a review (as an outside consultant) of the EPA reassessment of the health and environmental risks of dioxin and related compounds, and development of policy recommendations and examination of federal policies and practice of risk assessment. In March 1999, he served as the technical workshop chair for an EPA peer review of the Draft Risk Characterization Guidance and Case Studies. He was a member of the Steering Committee for the Society for Environmental Toxicology and Chemistry (SETAC) workshop on multiple stressors in ecological risk assessment, the SETAC workshop Ecological Risk Assessment Modeling System, and the National Research Council Committee on Risk Characterization.

Richard Dickerson
Texas Tech University

Primary Reviewer for Questions 8 and 9
Secondary Reviewer for Questions 1, 2, 16
General Reviewer for Questions 18, 19, 21

Richard Dickerson

Associate Professor
Department of Biological Sciences & Department of Pharmacology
Institute of Environmental & Human Health
Texas Tech University
3601 4th Street
Lubbock, TX 79430
806-743-2425
Fax: 806-743-2744
E-mail: richard.dickerson@ttmc.ttuhsc.edu

Richard Lee Dickerson is an associate professor with joint appointments in the Department of Pharmacology at Texas Tech University Health Sciences Center and the Department of Biological Sciences at Texas Tech University. He is a member of the core faculty of the Institute of Environmental and Human Health (TIEHH) shared between TTU and TTUHSC. He received a Bachelor of Science degree in chemistry from Midwestern State University (Wichita Falls, Texas) in 1974. He worked as a technician in the Department of Pharmacology at the University of Texas Health Science Center at San Antonio for several years before obtaining a Master of Science in chemical engineering from the University of Arkansas (Fayetteville) in 1980. Dr. Dickerson worked for Dow Chemical Texas Division as an environmental engineer until 1988. He obtained a doctor of philosophy in toxicology from Texas A&M University in 1992. Dr. Dickerson worked at Clemson University until 1997, achieving the rank of associate professor. In 1997, his department moved to Texas Tech University. In 1995, Dr. Dickerson became a diplomate of the American Board of Toxicology. Dr. Dickerson's current research projects involve the effects of TCDD on circadian rhythm in rodents as well as endocrine dysfunction caused by a number of halogenated aromatic hydrocarbons. In addition, he is a member of a multidisciplinary team examining the effects of jet fuel on military personnel.

Mark Harris
Risk Management, Inc.

Primary Reviewer for Questions 4, 5
Secondary Reviewer for Questions 3, 6
General Reviewer for Questions 18, 19, 21

Mark Harris

President
Harris Environmental Risk Management, Inc.
1900 Bluffview Court
Flower Mound, TX 75022
972-691-8338
Fax: 972-691-8099
E-mail: mark-harris@home.com

Dr. Mark A. Harris is President of Harris Environmental Risk Management, Inc. of Flower Mound, Texas. His expertise is in human health risk assessment of environmental contaminants with expertise in addressing halogenated aromatic hydrocarbons (HAH) and chromium. His educational background includes receiving a Bachelor of Science degree in Biochemisty from Texas A&M University, a Masters degree in Business Administration from Southern Methodist University and a Ph.D. in Toxicology from Texas A&M University. He has published over 25 peer reviewed articles addressing various aspects of HAH and chromium toxicology and human health risk assessment and has co-edited a book addressing various aspects of chromium risk assessment. His employment experience includes working in both the consulting arena preparing human health risk assessments for the regulated community for submission to various state and federal agencies, and for a multinational integrated oil company addressing numerous environmental issues on their behalf. In 1999, he co-founded Harris Environmental Risk Management, Inc., a consulting firm that assists the regulated community in addressing complex environmental issues. He is a member of the Socity of Environmental Toxicology and Chemistry (SETAC), the Society for Risk Analysis (SRA) and the American Society of Testing and Materials (ASTM).

Holly Hattemer-Frey
SAF*Risk

Primary Reviewer for Question 12
Secondary Reviewer for Questions 13, 14, 15
General Reviewer for Questions 18, 19, 21

Holly Hattemer-Frey

Senior Risk Assessment Scientist
SAF*Risk
1100 Sanders Road
Knoxville, TN 37923
865-531-9050
Fax: 865-691-9652
E-mail: safrisk_tn@earthlink.net

Holly A. Hattemer-Frey is a Senior Risk Assessment Scientist with 15 years of risk assessment experience who has authored more than 40 open literature publications and technical reports and given numerous oral presentations on diverse risk assessment topics. She has authored 15 papers on various dioxin-related topics including human exposure to, environmental sources of, and fate and transport of dioxin. She edited *Health Effects of Municipal Waste Incineration* and served as a consultant to the National Academy of Sciences Committee on Animals as Monitors of Environmental Hazards. While with SAF*Risk, she prepared a full-scale Ecological Risk Assessment for a Boiler and Industrial Furnce in Louisianna and prepared Risk-Based Corrective Action (RBCA) analyses for various UST sites. Previously with Dames & Moore, she performed many Human Health and Ecological Risk Assessments for RCRA, CERCLA, and other types of hazardous waste sites for private and government clients. She also coordinated human health and ecological risk efforts at DOE's Oak Ridge Reservation for Radian Corporation. As an Environmental Scientist with Oak Ridge National Laboratory's Office of Risk Analysis, Ms. Hattemer-Frey gained experience in assessing the extent of human exposure to organics through the food chain, evaluating human health effects associated with municipal waste incineration, evaluating the potential impacts of releasing genetically-altered oprganisms into the environment, and using pharmacokinetics to improve the risk assessment process. She obtained a Master of Environmental Sciences degree from Miami University (Ohio) and a Bachelor of Arts degree, *cum laude*, from Ohio Wesleyan University.

Brent Kerger
Health Science Resource Integration, Inc.

Primary Reviewer for Questions 1, 7
Secondary Reviewer for Questions 10, 11
General Reviewer for Questions 18, 19, 21

Brent Kerger

Principal Scientist and Director
Health Science Resource Integration, Inc.
2976 Wellington Circle, W
Tallahassee, FL 32308
850-894-4800
Fax: 850-906-9777
E-mail: brentkerger@worldnet.att.net

B.S., Chemistry, Florida State University
Ph.D., Toxicology, University of Arkansas for Medical Sciences

Dr. Kerger, a Principal Health Scientist and Director of Health Science Resource Integration, Inc., has over 18 years experience in conducting, managing, and publishing studies involving toxicology, environmental chemistry, and the investigation of environmental problems. A board-certified toxicologist, he has thoroughly researched key toxicological issues surrounding human exposures to a wide range of chemicals, particularly chlorinated compounds, heavy metals, and benzene. He is very familiar with the clinical and epidemiological studies of dioxin-exposed cohorts, as well as the animal and epidemiological literature on chlorinated dibenzofurans and PCBs. His work related to dioxin has included authoring many summary and interpretation documents regarding weight-of-evidence for disease causation; performing dioxin exposure assessments and risk assessment calculations in dozens of site-specific risk assessments and occupational exposure evaluations; evaluating epidemiological dose-response date in relation to animal dose-response data for cancer; examining human versus animal susceptibility to cancer potentially cause by dioxins; researching the available scientific literature to develop plausible, refined estimates of dioxin uptake resulting from specific work activities; developing dermal uptake models that were integrated with PBPK models for total dioxin uptake to estimate change in tissue concentration of dioxin over time; and comparison of quantitative estimates of daily and total dioxin dose to dietary uptake. He has published numerous peer-reviewed articles on chemical toxicity, PBPK modeling, and innovative exposure and risk assessment techniques, including *Validating Dermal Exposure Assessment Techniques for Dioxin Using Body Burden Data and Pharmacokinetic Modeling; The Use of Health Risk Assessment in Toxic Tort Litigation: A Case Study Evaluating Off-Site Impact of Dioxin-Contaminated Soils; Multipathway Assessment of Dioxin Uptake and Resulting Body Burden for Selected Occupational Exposure Scenarios; Exposure Modeling and Validation Studies for Aerosols: A Case Study of Dioxin Exposure During Roadside Weed Abatement with 2,4,5-T; and Risk Communication Regarding Dioxin Exposures to Infants from Mother's Milk.*

Colin Park

Primary Reviewer for Questions 2, 19, 21
Secondary Reviewer for Question 12
General Reviewer for Question 18

Colin Park

Private Consultant
1878 Hicks Road
Midland, MI 48642
517-835-5249
Fax: 517-837-8704
E-mail: crewcolin@aol.com

Dr. Park worked for The Dow Chemical Company for 29 years and retired in 1998 as a senior associate environmental consultant and issues manager in the environmental and health area. His main interest was in the use of risk assessment in regulation, legislation and communications.

He received a B.S. degree in mathematics in 1965 from the University of British Columbia and earned a M.S. and Ph.D. in applied statistics from Purdue University in 1970. He joined Dow in 1970 as a statistical consultant and held a number of statistics, computer, and risk assessment positions in Dow.

Dr. Park has served on the Science Advisory Board of the National Center for Toxicology Research, and on the Risk Assessment Committee of the Council on Environmental Quality for the State of Michigan, as well as on numerous EPA review committees.

Myrto Petreas
California Environmental Protection Agency

Primary Reviewer for Questions 13, 14, 15
General Reviewer for Questions 18, 19, 21

Myrto Petreas

Scientist
Hazardous Materials Lab
Department of Toxic Substances Control
California Environmental Protection Agency
2151 Berkeley Way
Berkeley, CA 94704
510-540-3624
Fax: 510-540-2305
E-mail: mpetreas@dtsc.ca.gov

B.Sc., Chemistry, University of Thessaloniki
M.S., Environmental Health Sciences; M.P.H., Epidemiology, and Ph.D., Environmental Health Sciences, University of California, Berkeley

Myrto Petreas is an environmental scientist managing the Special Studies section within the Hazardous Materials Laboratory of the California Environmental Protection Agency (Cal/EPA). She is a chemist and an epidemiologist focusing on exposure assessment. She designs, co-ordinates and manages studies to assess exposures to environmental contaminants posing health effects to humans and wildlife, and oversees the development of analytical methods for ultra trace chemicals (dioxins, PCBs, organochlorine pesticides, and new, emerging chemicals with endocrine disrupting properties) in environmental and biological samples. She is currently directing a systematic effort to characterize dioxin body burdens, as well as baseline dioxin levels in soils and biota in California.

Christopher Rappe
Umeå University

Primary Reviewer for Question 20
Secondary Reviewer for Questions 13, 14, 15
General Reviewer for Questions 18, 19, 21

Christopher Rappe

Professor Emeritus
Department of Chemistry
Umeå University
Umeå 901 87
Sweden
011-46-90-786-5266
Fax: 011-46-90-128-133
E-mail: christpher.rappe@chem.umu.se

B.S., Chemistry and Geology
M.S., Organic Chemistry, Chemistry, and Biochemistry
Ph.D., Organic Chemistry, all from Uppsala University, Sweden

Dr. Christoffer Rappe is a leading international expert on dioxin, with a particular focus on dioxin exposure assessment. He has worked and taught in academia for over 40 years and is currently Professor Emeritus at Umea University. Dr. Rappe has published hundreds of papers in environmental chemistry and has served as an invited expert, member, advisor, or speaker for innumerable committees and events concerning dioxin and related compounds. These professional assignments have included serving as an author for the World Health Organization's Environmental Health Criteria Document on PCDDs and PCDFs; as a peer reviewer for EPA documents on polychlorinated dibenzofurans in 1980 and 1986 and polychorinated dioxins in 1983; as an invited expert to the EPA workshop on dioxins in 1996; as an invited panel member for the IEHR review panel on EPA's Dioxin Draft Document; and as a member of the organizing committee for four American Chemical Society meetings on dioxin and for all DIOXIN meetings from 1984 to the present.

Lorenz Rhomberg
Gradient Corporation

Primary Reviewer for Question 3
Secondary Reviewer for Questions 2, 17
General Reviewer for Questions 18, 19, 21

Lorenz Rhomberg

Principal Scientist
Gradient Corporation
238 Main Street
Cambridge, MA 02142-1016
617-395-5000
Fax: 617-395-5001
E-mail: lrhomber@gradientcorp.com

B.Sc., Biology, Queen's University
Ph.D., Biology, State University of New York at Stony Brook

Prior to joining Gradient Corp. Dr. Rhomberg was an Assistant Professor of Risk Analysis at the Harvard School of Public Health, Harvard University. He has extensive experience with risk analysis had has written numerous peer reviewed articles including *Trichloroethylene Health Risk Assessment: A New and Improved Process; A Survey of Methods for Chemical Health Risk Assessment among Federal Regulatory Agencies; Physiological Parameter Values for Physiologically Based Pharmacokinetic Models; Use of Quantitative Modeling Methylene Chloride Risk Assessment;* and *Risk Assessment of Formaldehyde.* Dr. Rhomberg was a consultant to the Presidential/Congressional Commission on Risk Assessment and Management. Dr. Rhomberg served as the Chair of the Pharmacokinetics Focus Group for the Office of Health and Environmental Assessment from 1990-1994. Dr. Rhomberg is a member of the Society for Risk Analysis, Risk Assessment and Policy Association, and Society of Sigma Xi.

Allan Smith
University of California - Berkeley

Primary Reviewer for Questions 10, 11

(Secondary and General comments are forthcoming)
Secondary Reviewer for Questions 8, 16
General Reviewer for Questions 18, 19, 21

Allan Smith

Professor Epidemiology
School of Public Health
University of California - Berkeley
140 Warren Hall
Berkeley, CA 94720-7360
510-843-1736
Fax: 510-843-5539
E-mail: ahsmith@uclink4.berkeley.edu

B.Sc., Mathematics, University of Wellington, New Zealand, B.Med.Sc., M.B., Ch.B., (M.D. equivalent), Ph.D., Epidemiology, University of Otago, New Zealand, and MCCM, Foundation Member of the NZ College of Community Medicine

Dr. Smith is a dean at the University of California- Berkeley and a professor in the School of Public Health, He is a past-president of the International Society for Environmental Epidemiology, and the author of hundreds of publications on environmental and occupational health issues, including several focusing on dioxin. His experience encompasses epidemiology studies (occupational, environmental), cancer risk assessment, and exposure assessment.

Curtis Travis

Primary Reviewer for Question 17
Secondary Reviewer for Questions 10, 20
General Reviewer for Questions 18, 19, 21

Curtis Travis

8112 Bennington
Knoxville, TN 37909
423-693-5970
Fax: 423-539-9287
E-mail: traviscc@icx.net

Dr. Travis has worked in the field of risk management and risk analysis for over 20 years. Prior to joining Project Performance Corporation, Dr. Travis was the Director of the Center for Risk Management and the Head of the Risk Analysis Section at Oak Ridge National Laboratory. He is Editor-in-Chief of *Risk Analysis: An International Journal,* and he is on the editorial board of *Health and Environmental Toxicology, Toxicological and Environmental Chemistry, Toxicology and Industrial Health, Journal of Hazardous Materials,* and *Critical Reviews in Environmental Science and Technology*. Dr. Travis has authored over 250 articles including *Human Exposure to Dioxin from Municipal Solid Waste Incineration; Dioxin, Dioxin, Everywhere; Multimedia Partitioning of Dioxin; Dioxin: Research Needs for Risk Assessment;* and *A Perspective on Municipal Waste Combustors as a Source of Environmental Dioxin.* Dr. Travis has given the plenary address "Dioxin Research Needs for Risk Assessment," at the Dioxin 89 Conference, as well as the plenary address on "Human Exposure to Dioxin" at the Dioxins and Dibenzofurans Symposium.

Matti Viluksela
National Public Health Institute

Primary Reviewer for Question 16
Secondary Reviewer for Questions 4, 5, 7
General Reviewer for Questions 18, 19, 21

Matti Viluksela

Department of Environmental Medicine
National Public Health Institute
Neulaniementie 4
Kuopio, Finland FIN-70701
011-358-17-201-329
Fax: 011-358-17-201-265
E-mail: matti.viluksela@kti.fi

Dr. Matti J. Viluksela, Ph.D., is a senior toxicologist and researcher at the National Public Health Institute, Department of Environmental Medicine, Kuopio, Finland. Following 6 years as a toxicologist and laboratory manager in pharmaceutical industry he has worked in the Department of Pharmacology and Toxicology, University of Helsinki, for 4 years, and in the Department of Pharmacology, Toxicology and Therapeutics at the University of Kansas Medical Center for 2 years. He is a Diplomate of the American Board of Toxicology. He has carried out toxicological research mainly on pharmaceuticals, chlorophenols and dioxins, and his current research interest is in the mechanisms of dioxin toxicity. He is currently serving on the Expert Advisory Committee on Toxicology (the Finnish National Product Control Agency for Welfare and Health), the Finnish Scientific Committee on Health Effects of Chemicals, the Advisory Committee on Chemicals (the Ministry of Social Affairs and Health), the Novel Food Board (Ministry of Trade and Industry) and the Scientific Advisory Committee of the European Centre for the Validation of Alternative Methods (ECVAM; Commission of the European Communities). He has also served on the Executive Board of the Finnish Society of Toxicology, and on several Nordic working groups in toxicology.

Peter L. deFur

Dioxin Reassessment Peer Review

July 25, 2000

Washington DC

General Comments:

Change the term "background" to present or ambient. EPA uses the term "background" to refer to the present ambient levels of dioxins and related in the environment. In using the term "background" in this way, EPA adopts its own definition and practical use of the term. "Background" already has a definition and connotation. The use of the term indicates "normal and acceptable", rather than ambient. EPA errs in using this term. The term implies a level that is natural, acceptable and does not cause a response. EPA in the text attempts to explain that such is not the case. Rather than try to convince the English language using public to adopt a new understanding of the word, EPA should use a more appropriate term that is already accurate, such as "ambient, existing or current." Using the term "background" will convey the message that these levels are acceptable. Such a message would be an error, based on EPA's own information in the reassessment, and on the literature.

The addition of cross-referencing to other chapters and sections in the document makes the product easier to use on a technical level.

Topic 1

Question 1

General Reviewer

Did EPA adequately justify its use of body burden as a dose metric for inter-species scaling? Should the document present conclusions based upon daily dose?

Yes, although the less scientific readers will not follow the explanation. The document should provide some comparisons with daily dose, but if daily doses are used along side body burdens, then some readers will equate them and use one interchangeably with the other.

Topic 2

Question 2

How might the rationale be improved for EPA's decision not to calculate an RfD/RfC, and for the recommended MOE approach for conveying risk information? Is an MOE approach appropriate, as compared to the traditional RfD/RfC? Should the document present an RfD/RfC?

No comment at this time.

Question 3

Are the calculations of a range of ED01 body burden for non-cancer effects in rodents responsive and clearly presented? Please comment on the weight-of-evidence interpretation of the body burden data associated with a 1% response rate for non-cancer effects that is presented in Chapter 8, Appendix I and Figure 8-1.

No comment at this time.

Topic 3

Question #4

How might the discussion of mode-of-action of dioxin and related compounds be improved?

No comment at this time.

Question #5

General Reviewer

Despite the lack of congener-specific data, does the discussion in the Integrated Summary and Risk Characterization support EPA's inference that these effects may occur for all dioxin-like compounds, based on the concept of toxicity equivalence?

Yes.

The data for wildlife and other non-human or non-experimental animals is most useful here. These data are principally for reproductive and developmental effects, less so for immune effects. EPA could insert more of these data into the discussion to strengthen the justification both in the RCh and in the chapters.

Topic 4

Question #6

Primary Reviewer

Is the history, rationale and support for the TEQ concept, including its limitations and caveats, laid out by EPA in a clear and balanced way in Chapter 9? Did EPA clearly describe its rationale for recommending adoption of the 1998 WHO TEFs?

The TEF concept is largely explained in Chapter 9, now a stand-alone chapter. This concept has been in the scientific literature for a number of years, and EPA has used the TEF concept for dioxin and dioxin-like compounds in many regulatory situations, especially at the national level. EPA has been using TEF's determined as a result of scientific deliberation and published in Agency documents (EPA, 1987).

The chapter presents history and scientific background for the idea of TEF's at conceptual and quantitative levels and of the application of TEF's in EPA. This history and background is intended to bring together in one chapter the scientific explanation and key literature. The chapter also is intended to present the framework for applying the TEF concept and comment on developments that are anticipated in the scientific background and application.

Generally, the chapter accomplishes the goals listed by EPA, charged by the SAB and that this reviewer expected in a stand-alone piece on the subject. This chapter is more detailed and explanatory than other review pieces on the subject in the open literature, largely owing to the lack of space(page) limits in the document.

This chapter is more technical than the Risk Characterization and the Integrated Summary. A non-expert would have a great deal of trouble understanding the chapter here, with the possible exception of some of the introductory sections.

Most of this reviewer's comments fall under the category of specific comments, including recommended changes in wording and style to improve clarity and understanding. Several comments are quite substantive and these are offered first.

Section 9.3.2 page 9-14, lines 18-30. This text seeks to explain a suggestion in the literature (Safe, 1995) regarding the relative significance of anthropogenic and "natural" chemicals that bind to the Ah receptor. The original suggestion (Safe, 1995) has not been substantiated experimentally or in theoretical analysis, and has not received, to this reviewer's knowledge, any support in the five years since first published. This reviewer finds the concept untenable and without merit on scientific grounds, based on what is known of the behavior of natural and anthropogenic compounds that bind to receptors in multiple animal systems. EPA should drastically reduce the treatment of this issue and merely note that the point was raised and that there is no evidence or argument

to support it in the face of a wealth of contradictory information and reasoning. This could be done in a single paragraph that lists the reasons with citations that refute the suggestion of Safe (1995).

Section 9.4.6: The text of the second paragraph does not convince me that TE functions are beyond the reach of experimental determination and estimation. If, in fact, all of the effects of TCDD-like chemicals are mediated through the Ah receptor (which I do not doubt), and the next level of response is DNA transcription, then it should be true that all of the subsequent cellular responses fall into one of three categories: cellular process, product or development changes. Such basic outcomes should be common among animals and amenable to experimentation. Furthermore, I do not agree that these are likely to be fundamentally different among species or among individuals. The inherent conservatism in these basic biological functions, and the universal nature of other similar basic functions (HSP, cytochromes, respiratory pigments, Na+ ATPases, etc.) in fact offers reason to pursue this line of research. Differences among animal species are likely to be modifications of common pathways, as seen now, rather than fundamentally different pathways. The difficulty faced at present is that none of the biochemical pathways for TCDD has been detailed experimentally. I recommend that EPA compare biochemical pathways of responses in animals from different taxonomic groups, e.g. fish and rats.

Section 9.7 Summary

This is a key section that needs a bit of expansion, and split it into two paragraphs. The opening sentence does not do full justice to the point made. And Lines 16 –17 could state that ignoring other dioxins and furans will greatly understate the threat, and treating all as equipotent to TCDD will overstate the true threats.

Specific comments:

P 9-2, l 21—does the document need to include TEFn =

Line 30- please give date for the NATO meeting.

Page 9-3, line 22- give citation for the WHO guidance

Line 32- clarify that this means birds and mammalian wildlife

Page 9-4, l 14-18—the text needs to clarify the date sequence with the conept development sequence; as is, it reads oddly

Page 9-5, line 2 et seq: suggest putting these criteria in bullets

Line 18 et seq.- include research by Cook et al on the synergism between PCB's and dioxins in wildlife, espceially fish

Line 32 – insert (EROD)

Line 35 – spell out REP here

Page 9-7, lines 11-17 – I understand that WHO has on the agenda this August to add brominated dioxins and furans to the WHO Tolerable Daily Intake for dioxins and furans.

Line 26-7—EPA needs to give a better citation than "In Review" for this important report on TEF's in wildlife that is now more than 2 years after the completion of the peer-review workshop.

Page 9-8, lines 7-14 – this section seems to understate the significance of the issue.

Line 35- should be "among" not between species

Page 9-9, lines 6-16—In addition, there is a literature on a growing number of genes, families and specific proteins that occur in phyla that cover the same range as listed here. This commonality is akin to other proteins and gene families, such as Na+ ATPAases, cytochromes, hemoglobins, etc. and the finding here in multiple phyla is a critically important point to EPA (and WHO) using experimental data as a (or the) key data for determinations that dioxins and furans should be classified as health risks to humans.

Lines 18-23 – reword, not clear enough here

Lines 35-6—this sentence is one of the most important (and the reference needs to be more clear) and should be highlighted somehow in the way it is presented. State it more emphatically as an important experimental result.

Page 9-18, line 24-28 is a run on sentence and needs fixing

Line 36—could EPA insert this figure in the document?

Page 9-23, lines 1-10 – suggest putting these numbers into a table, for clarity

Page 9-30, lines 11-15 – This point needs better explanation. One need is to elaborate on how TEQ is only one element of uncertainty.

Lines 32-24 – this is an awkward text, please rewrite.

Literature:

See papers by R. van Beneden on dioxin effects (including Ah receptor work) in *Mya* and *Mercenaria*.

Question #7

General Reviewer

Does EPA establish clear procedures for using, calculating, and interpreting toxicity equivalence factors?

The methods here are fairly straightforward, but this reviewer is familiar with the use, the concept etc. Applying TEF's also requires some understanding of the uncertainties and variability in the steps, and how to express both uncertainty and variability. I doubt that most practitioners and managers in EPA could do so from the reassessment.

EPA should consider an additional document for application in field situations such as superfund sites, emissions permits, discharge permits, food consumption advisories, and soil assessments.

Topic 5

Question #8

Have the available human data been adequately integrated with animal information in evaluating likely effect levels for the non-cancer endpoints discussed in the reassessment?

No comment at this time.

Question #9

Secondary Reviewer

Do reviewers agree with the characterization of human developmental, reproductive, immunological, and endocrinological hazard? What, if any, additional assumptions and uncertainties should EPA embody in these characterizations to make them more explicit?

The Risk Characterization refers to the major portions of the reassessment to support the conclusions that dioxin (and related compounds). In reaching these conclusions, EPA relies on the large body of evidence regarding dioxins, furans and PCB's actions and effects in a range of species. Much, but not all of this literature is in the health chapters.

This reviewer agrees with EPA's characterization on this point.

The explanation and justification for the conclusions here could be greatly enhanced. The literature unequivocally demonstrates the extent of basic biological functions that are conserved across vertebrate orders and families as well as across phyla. Some of these include structure and function of cytochromes, hemoglobin, Na+ ATPases, steroid hormones and their receptors, protein hormones, neurotransmitters, and the basic structure and function of all cells. The point of made in Chapter 9 that the Ah receptor gene is found in the nematode (*C. elegans*) genome and additional elements occur in the few other invertebrates that have been examined in detail. Thus, the basis for taking a comparative approach is much stronger than stated here; EPA is almost defensive on the point.

This reviewer predicts that most EPA managers will have difficulty when faced with interpreting the reassessment and basing decisions to protect human health on comparative data (when EPA has relied heavily on human epidemiological data for decades). Thus, EPA should make it easier on the users and provide the conceptual background.

EPA has done a better job of expressing the uncertainties in predicting human health effects. The wide range of quantitative Ah activities (20 fold) indicates the range of sensitivities that may be found in humans. EPA could add in the range of sensitivities that have been reported for some other common anthropogenic toxic compounds, such as lead, benzene, DES, pharmaceuticals, etc. The response of the human system to toxic chemicals is dependent on more factors than EPA could possibly elaborate in this report. Yet EPA should provide some additional text to explain this point for managers who will not be familiar with such issues.

Topic 6

Question #10

General Reviewer

Do you agree with the characterization in this document that dioxin and related compounds are carcinogenic hazards for humans?

Yes. The evidence of common mechanism of action in experimental animals, coupled with the large number (18) of positive animal studies and the solid epidemiological data from studies around the world support the conclusion of dioxin being a carcinogen. Furthermore, the common mode of action/mechanism of action of the dioxin like compounds supports the application of the conclusion to these compounds as well.

Page 11, lines 23 –30—Change text. The explanation is so filled with caveates that the reader is left with the impression that no one has the vaguest idea how TCDD causes cancer, when in fact, there is a general scheme presented here. Instead of the text that uses the word “may” in several places in each sentence, simply open with a statement that the specific means by which dioxin causes cancer is not yet fully explained. The current hypothesis is … and spell it out as theorized and written in Vol 2, Chapter 2. The present text is just too defensive.

Page 14, line 16-18—Insert the language that an increase in relative risk for all cancers is a rare outcome in epidemiology studies.

Page 19, lines 6-7 – Does EPA truly believe that "beneficial effects" can result from dioxin exposures? The data do not support this statement, and the text needs to reword this point. While it may be true that the cohort in Seveso exhibited reduced incidence of breast cancer, other cancers increased. Is that a beneficial effect on health of the exposed population, compared with a cohort not exposed to dioxin, clearly not. So do not say it was.

EPA can reword this to note the finding of reduced cancer incidence and the lab findings, but do NOT imply "beneficial effects" from dioxin exposure; the data are just not there.

Question #11

General Reviewer

Does the document clearly present the evolving approaches to estimating cancer risk (e.g., margin of exposure and the LED01 as a point of departure), as described in the EPA "Proposed Guidelines for Carcinogenic Risk Assessment" (EPA/600/P-92/003C; April 1996)? Is this approach equally as valid for dioxin-like compounds?

This approach is not as easy to explain and understand as the one known and used for years, so EPA has much work to do in explaining it to both the public and the EPA management staff around the country.

Question #12

Please comment on the presentation of the range of upper bound risks for the general population based on this reassessment. What alternative approaches should be explored to better characterize quantitative aspects of potential cancer risk? Is the range that is given sufficient, or should more weight be given to specific data sources?

No comment at this time.

Topic 7

Question #13

General Reviewer

Have the estimates of background exposure been clearly and reasonably characterized?

No comment at this time.

However, the point on use of the term “background” remains. EPA needs to use a different term.

Question #14

General Reviewer

Has the relationship between estimating exposure from dietary intake and estimating exposure from body burden been clearly explained and adequately supported?

Yes.

Question #15

General Reviewer

Have important "special populations" and age-specific exposures been identified and appropriately characterized?

Partially. This grouping depends on what EPA knows, and needs to comment more carefully on populations that are not specifically noted.

Topic 8

Question #16

Is the characterization on increased or decreased childhood sensitivity to possible cancer and non-cancer outcomes scientifically supported and reasonable? Is the weight of evidence approach appropriate?

No comment at this time.

Topic 9

Question #17

Has EPA adequately characterized how nursing affects short-term and long-term body burdens of dioxins and related compounds?

No comment at this time.

Topic 10

Question #18

Primary Reviewer

Does the summary and analysis support the conclusion that enzyme induction, changes in hormone levels, and indicators of altered cellular function seen in humans and laboratory animals, represent effects of unknown clinical significance, but that may be early indicators of toxic response? (Refer to pages 84-86.)

The document supports the conclusion, but the way it does so is awkward. First, the text is defensive in many places, and the data and literature from earlier parts of the report (Part II) do not reflect the level of uncertainty and hesitancy expressed in the Risk Characterization. Second, the language and style are more technical and complex than needed for the audience likely to need this material. Finally, the text should spell out more carefully the logical explanation why the early biochemical responses are inferred to present early signs of or manifestations of health risks.

Page 84, lines 19-22: run-on sentence; start with the last statement
Page 85, line 8: rephrase "The sensitivity of individual species to dioxin and related compounds varies considerably (state a range?)."
Page 86, lines 3-6—This run-on sentence is difficult to follow. Split into smaller sentences and simplify the language.

Question #19

General Reviewer

Has the short summary statement in the risk and hazard characterization on page 107 adequately captured the important conclusions, and the areas where further evaluation is needed? What additional points should be made in this short statement?

Not really. It is too brief and apologetic with too many caveates. EPA based much of the conclusions on the strong experimental evidence that is overwhelming. The fact that most scientists would like to see absolutely all the steps identified in detail, that is not yet the state of knowledge. Filling in all the steps will not change the relationship between initial stimulus and advesre outcomes. The additional piece of evidence comes from the epidemiology of human health effects that are perfectly in agreement with the experimental data. The imperfection of the epidemiological data is good, as EPA notes, and indicates some limitation of high dose exposures.

Page 107, lines 8-10 – move the "in animals" from line 9 to after the spectrum of effects AND move the phrase at the beginning of the sentence (up to "inference") to the end of the sentence.

Lines 22-26—list the criteria in a list of bulleted items. This sentence needs to end with some sort of expectation of purpose for the evaluation – what would EPA expect to gain from further evaluating called for in line 22?

Topic 11

Question # 20

General Reviewer

Are these sources adequately described and are the relationships to exposure adequately explained?

Page 49, lines23-25—Insert the explanation that this procedure is necessary because it is a sequence of events all of which are necessary, thus, like probabilities that must be multiplied, the lowest one determines the overall sequence.

Page 52, line 28-31 et seq. – This explanation gives the reader the impression that there is absolutely no relationship between chlorine input and dioxin formation and release from incinerators. This reviewer's read of the literature is that the relationship is not linear and dependent only on chlorine concentration AND that there is a dependence of dioxin formation on chlorine presence. Thus, it seems to be the case that chlorine content in waste feed into incinerators is necessary for dioxin formation. The text needs to reflect that relationship. As it is worded now, the reader is left believing that waste containing high levels of chlorinated waste have no more dioxin formation and emission rates than waste with no chlorinated waste as feed for an incinerator.

This text also must include the conclusion about the process that would be necessary to eliminate dioxin formation and emission from incinerator emissions. If it is not possible to eliminate formation and emissions, say so; if eliminating chlorinated waste from the feed will eliminate dioxin formation and emission, so that. If EPA has no idea whether dioxin formation and emission can be eliminated from incinerator emissions (the largest source of emissions), then say that. But the text must not remain silent on the topic.

Page 61, line 10 et seq. – The great advantage of using site specific data on such issues as fish consumption is that the real data make the case in a convincing way. The limitation is that other studies not conducted are omitted. This reviewer suggests that the area of the county where fish consumption is highest is the Gulf coast where consumption of fish and shellfish is year round and shifts with the fishery. Crabs, clams, oysters, finfish, shrimp and crawfish are consumed constantly in the Gulf states. Have any data been collected on this population?

Question #21

Primary Reviewer

Please provide any other comments or suggestions relevant to the two review documents, as interest and time allow.

The data on wildlife and non-human, non-experimental animals gives a great deal of support to the conclusions and should be included in more of the chapters.

July 13, 2000

Meg Vrablik
Eastern Research Group, Inc (ERG)
110 Hartwell Avenue
Lexington, MA 02421-3136

Dear Ms. Vrablik:

Enclosed are my comments on the dioxin reassessment. I will be out of town during the next week (July 16-22). I will be leaving Sunday at 550 AM but will check my office email on Saturday. I can be reached at the Fairfield Inn in Fayetteville, North Carolina beginning Saturday PM. The phone number is (910) 487-1400. I will also be checking my email using my home email address (richarddickerson@earthlink.net). Please feel free to contact me by phone, fax or email regarding my comments and to forward me the comments of others.

Sincerely,

Richard L. Dickerson, Ph.D., DABT
Associate Professor

Biographical Sketch

Richard Lee Dickerson is an associate professor with joint appointments in the Department of Pharmacology at Texas Tech University Health Sciences Center and the Department of Biological Sciences at Texas Tech University. He is a member of the core faculty of the Institute of Environmental and Human Health (TIEHH) shared between TTU and TTUHSC. He received a Bachelor of Science degree in chemistry from Midwestern State University (Wichita Falls, Texas) in 1974. He worked as a technician in the Department of Pharmacology at the University of Texas Health Science Center at San Antonio for several years before obtaining a Master of Science in chemical engineering from the University of Arkansas (Fayetteville) in 1980. Dr. Dickerson worked for Dow Chemical Texas Division as an environmental engineer until 1988. He obtained a doctor of philosophy in toxicology from Texas A&M University in 1992. Dr. Dickerson worked at Clemson University until 1997, achieving the rank of associate professor. In 1997, his department moved to Texas Tech University. In 1995, Dr. Dickerson became a diplomate of the American Board of Toxicology. Dr. Dickerson's current research projects involve the effects of TCDD on circadian rhythm in rodents as well as endocrine dysfunction caused by a number of halogenated aromatic hydrocarbons. In addition, he is a member of a multidisciplinary team examining the effects of jet fuel on military personnel.

Topic # 1

Question # 1

Role: Secondary Reviewer

Question: Did EPA adequately justify its use of body burden as a dose metric for interspecies scaling? Should the document present conclusions based upon daily dose?

Response:

Based upon the information contained within the document, and in the supporting documents, the use of body burden as a dose metric is supported for the average adult. However, it is not supported for a number of at risk populations where daily exposure is a significant fraction of body burden. These include infants nursing on breast milk (particularly first-born infants), young children, individuals losing weight rapidly, and individuals that receive occupational or accidental exposure to dioxin-like compounds. In addition, body burden may not be an acceptable dose metric for individuals whose life style results in above average daily intake of dioxin-like compounds (subsistence fishermen and other individuals exposed to dietary fat with high levels of dioxin-like compounds). My comments are based upon the following reasons related to the achievement of steady state levels of TCDD-TEQ.

Infants nursing on breast milk receive a hefty percentage of their eventual body burden within a few months. This is particularly true for the first child born who is breast-fed. Succeeding children have the benefit that the mother has less time to re-approach steady state concentrations of TCDD TEQs in her adipose tissue. It has been estimated that it requires some 15-30 years of dietary exposure (at current levels) to reach steady state. Thus, daily exposure rates of TCDD affect plasma concentrations of infants and children to a greater extent than individuals who have already reached steady state levels.

Individuals that are losing weight rapidly will have greater levels of TCDD-TEQ in their plasma than would be predicted by body burden, as will individuals that are accidentally or occupationally exposed to TCDD and its congeners. However, the use of body burden as a dose metric is appropriate for the majority of the population (excluding infants and children).

Topic # 2
Question # 2
Role: Secondary Reviewer

Question: How might the rationale be improved for EPA's decision not to calculate a RfD/RfC, and for the recommended MOE approach for conveying risk information? Is a MOE approach appropriate, as compared to the traditional RfD/RfC? Should the document present a RfD/RfC?

Response:

In my opinion, it should be explicitly stated that the plasma concentration of TCDD-TEQ is affected by the body burden as well as by the daily intake and excretion rate of the congeners. Second, the relative contributions of the liver/adipose depots to that plasma concentration should be made clear. This will clearly show that for an adult (one that has presumably reached a steady state body burden), that the contribution from body burden greatly exceeds that from daily intake. In addition, a plot of body burden versus lethality would underscore the point that body burden, not daily dose or acute dose, best predicts toxic effects across species. This will help justify the decision not to calculate a RfD/RfC. The part of the document that justifies the MOE approach using the body burden was acceptable and the literature supports the logic.

I would support a RfD/RfC approach only for infants and small children because they have not reached significant body burdens. For this subset of the population, the minimal risk level of 1 pg/kg/day suggested by ASTDR is reasonable, as is the tolerable daily intake of 1-4 pg/kg/day suggested by WHO.

Topic # 5
Question # 8
Role: Primary Reviewer

Question: Have the available human data been adequately integrated with animal information in evaluating likely effect levels for the non-cancer endpoints discussed in the reassessment?

Response:

Both the integrated summary and the previous documents (Part II, Chapters 4 Iimmunotoxicity, 5 Developmental and Reproductive Toxicity, and 7B Effects Other than Cancer) contain excellent reviews of the literature that appear to contain many of the most recent reports dealing with both animal and human studies. There are no significant omissions that would affect this risk assessment. The authors performed well in correlating the available human data to the more rigorous animal data. However, certain aspects appear to require clarification. The human endocrinological effects are listed in Table 2-1 as +/-. Based upon the effects upon testosterone levels seen some of the studies as well as the effects on male development from the Yu-Cheng studies, I feel this should be a +.

In terms of likely effect levels, it was stated that insufficient data was available to model non-cancer endpoints in humans although sufficient data was available for noncancer endpoints in animals. I feel that the authors were correct in stating that the uncertainties

were great enough to prevent accurate interspecies and animal-to-human comparisons. Among these are uncertainties as to critical gestational periods in many species, differences in the sensitivity of endpoints between species and strains, exposure routes, and study design. In my opinion, these uncertainties prevent the determination of likely effect levels for reproductive and developmental endpoints in humans to any degree of confidence, and thus require further research. However, the Dutch studies do suggest that PCB and other dioxin-like compounds have the potential to retard growth and certain developmental milestones at levels approaching current background.

In terms of immunotoxicity, there is a wealth of animal research that clearly defines TCCD and similar compounds as immunotoxicants. In these studies, there is clear evidence that TCDD can suppress host resistance to a number of pathogens and transplanted tumors. However, the human epidemiological data is contradictory and demonstrates uncertainties in regards to choice of sensitive endpoints and critical times of exposure. In particular, more studies need to be initiated in order to evaluate the sensitivity of the human immune system, focusing on neonates and children, to TCDD exposure. Until these uncertainties are resolved, the selection of likely effect levels for immunotoxicity likewise can not be determined.

The data for human chloracne is adequate. However, there are uncertainties as to what exposure level and frequency of exposure is required to initiate the condition. In terms of diabetes mellitus, human epidemiological data suggests a correlation between this condition and serum TCDD level. However, at the current time, none of the available animal models for diabetes have been used to study the effects of TCDD exposure on this disease condition. More research needs to be done in order to elucidate the mechanism by which TCDD could cause diabetes.

TCDD may cause an increase in T4 at low doses and a decrease in T4 with elevated TSH at higher doses. Human epidemiological data is scarce and inconsistent in regards to

thyroid function. There is sufficient animal data to consider TCDD as a hazard to the thyroid in humans but uncertainties prevent the suggestion of likely human effect levels.

Human studies have suggested TCDD-induced effects on the incidence of cardiovascular disease. Animal studies have demonstrated effects on cardiac rhythm and on the development of the heart and vasculature (primarily in avian species). There is an apparent data gap on the effects of TCDD and related compounds on mammalian heart development. This suggests the possibility that gestational exposure could cause developmental abnormalities in the heart that might increase the chances for later cardiovascular disease.

Topic # 5
Question # 9
Role: Primary Reviewer

Question: Do reviewers agree with the characterization of human developmental, reproductive, immunological and endocrinological hazard? What, if any, additional assumptions and uncertainties should EPA embody in these characterizations to make them more explicit?

Response:

There is, in my opinion, sufficient evidence to indicate that TCDD can cause adverse effects to human development and reproduction. I agree with the document's characterization of these hazards. The assumption that human sensitivity to TCDD and similar compounds is in the midrange as compared to other species for these endpoints appears to be well founded. The authors make the point that these substances occur in complex mixtures and that uncertainties lie in the response that humans and animals may have to such mixtures. In my opinion, this is the major stumbling block in applying the

current animal data to the determination of potential human risk. This is an area that needs consorted research to clarify. Another area of uncertainty lies in whether TCDD can affect the developing mammalian heart in the way it does the developing avian heart.

Whether TCDD is an immunotoxicant in humans is less clear. Certainly the animal data indicates that TCDD and similar compounds are potent immunotoxicants. However, the human epidemiological data is inconsistent and sparse. I agree that more research needs to be done in order to determine human risk, particularly to sensitive subsets of the population. However, the data from subhuman primates coupled with the data derived from laboratory species is sufficient to consider TCDD and other dioxin-like compounds to be probable human immunotoxicants capable of decreasing host resistance to a number of pathogens.

The document needs to address in more detail the uncertainties in the area of endocrine effects. The limited human data coupled with some intriguing studies in rodent models suggests a role for TCDD in the development of thyroid disease and diabetes.

Topic # 8 Children's Risk

Question # 16

Role: Secondary Reviewer

Question: Is the characterization on increased or decreased childhood sensitivity to possible cancer or non-cancer outcomes scientifically supported and reasonable? Is the weight of the evidence approach appropriate?

Response:

The document states that children's risk of adverse effects resulting from exposure to TCDD and related compounds may be increased as compared to adults but that more data

are needed to fully address this issue. The available human data coupled with animal data suggesting that the young are more susceptible support that statement. It is both scientifically valid and logical to suggest that children's risk may be increased relative to adults. It is not clear that all endpoints will be more sensitive in children than adults, but the data suggest that a number of endpoints will be. It is also clear that the relative exposure of children is higher than that of an adult, often three-fold and higher. Even is one assumes equal sensitivity, the increased dose is indicative of increased risk. In my opinion, the weight of the evidence is in favor of increased risk but not necessarily increased sensitivity.

Topic # 10 Risk Characterization Summary Statement

Question # 18

Role: General Reviewer

Question: Does the summary and analysis support the conclusion that enzyme induction, changes in hormone levels, and indicators of altered cellular function seen in humans and laboratory animals, represent effects of unknown clinical significance but that may be early indicators of toxic responses? (Refer to pages 84-86)

Response:

These statements are made in several places in this section. Enzyme induction is supported by the statement that this effect may increase metabolism and elimination of other toxic compounds or may increase reactive intermediates. This support could be strengthened by the brief discussion of adaptive responses as well as discussing the effects on endogenous compounds such as estrogen. Changes in circulating reproductive hormones in men exposed to TCDD are rightfully characterized as adverse. Altered cellular function may serve as a biomarker of exposure and possibly effect but might also be indicative of an

adaptive, non-adverse response. This statement merits a more thorough discussion in this section.

Topic # 10 Risk Characterization Summary Statement

Question # 19

Role: General Reviewer

Question: Has the short summary statement in the risk and hazard characterization on page 107 adequately captured the important conclusions, and the areas where further evaluation is needed? What additional points should be made in this short statement?

Response:

In my opinion, this statement needs to be expanded by approximately 50% to convey clearly the important conclusions. Specifically, it should state that TCDD is a known human carcinogen by all known criteria. It explicitly should state that TCDD and related compounds are developmental, reproductive and endocrinological hazards to humans and animals. It should also state that TCDD and similar compounds are known immunotoxicants in animal models. The document should specify that children are at risk, not because of increased sensitivity, but because of increased exposure at critical developmental times.

July 13, 2000

Meg Vrablik
Eastern Research Group, Inc (ERG)
110 Hartwell Avenue
Lexington, MA 02421-3136

Dear Ms. Vrablik:

Enclosed are my comments on the dioxin reassessment. I will be out of town during the next week (July 16-22). I will be leaving Sunday at 550 AM but will check my office email on Saturday. I can be reached at the Fairfield Inn in Fayetteville, North Carolina beginning Saturday PM. The phone number is (910) 487-1400. I will also be checking my email using my home email address (richarddickerson@earthlink.net). Please feel free to contact me by phone, fax or email regarding my comments and to forward me the comments of others.

Sincerely,

Richard L. Dickerson, Ph.D., DABT
Associate Professor

Biographical Sketch

Richard Lee Dickerson is an associate professor with joint appointments in the Department of Pharmacology at Texas Tech University Health Sciences Center and the Department of Biological Sciences at Texas Tech University. He is a member of the core faculty of the Institute of Environmental and Human Health (TIEHH) shared between TTU and TTUHSC. He received a Bachelor of Science degree in chemistry from Midwestern State University (Wichita Falls, Texas) in 1974. He worked as a technician in the Department of Pharmacology at the University of Texas Health Science Center at San Antonio for several years before obtaining a Master of Science in chemical engineering from the University of Arkansas (Fayetteville) in 1980. Dr. Dickerson worked for Dow Chemical Texas Division as an environmental engineer until 1988. He obtained a doctor of philosophy in toxicology from Texas A&M University in 1992. Dr. Dickerson worked at Clemson University until 1997, achieving the rank of associate professor. In 1997, his department moved to Texas Tech University. In 1995, Dr. Dickerson became a diplomate of the American Board of Toxicology. Dr. Dickerson's current research projects involve the effects of TCDD on circadian rhythm in rodents as well as endocrine dysfunction caused by a number of halogenated aromatic hydrocarbons. In addition, he is a member of a multidisciplinary team examining the effects of jet fuel on military personnel.

Topic # 1

Question # 1

Role: Secondary Reviewer

Question: Did EPA adequately justify its use of body burden as a dose metric for interspecies scaling? Should the document present conclusions based upon daily dose?

Response:

Based upon the information contained within the document, and in the supporting documents, the use of body burden as a dose metric is supported for the average adult. However, it is not supported for a number of at risk populations where daily exposure is a significant fraction of body burden. These include infants nursing on breast milk (particularly first-born infants), young children, individuals losing weight rapidly, and individuals that receive occupational or accidental exposure to dioxin-like compounds. In addition, body burden may not be an acceptable dose metric for individuals whose life style results in above average daily intake of dioxin-like compounds (subsistence fishermen and other individuals exposed to dietary fat with high levels of dioxin-like compounds). My comments are based upon the following reasons related to the achievement of steady state levels of TCDD-TEQ.

Infants nursing on breast milk receive a hefty percentage of their eventual body burden within a few months. This is particularly true for the first child born who is breast-fed. Succeeding children have the benefit that the mother has less time to re-approach steady state concentrations of TCDD TEQs in her adipose tissue. It has been estimated that it requires some 15-30 years of dietary exposure (at current levels) to reach steady state. Thus, daily exposure rates of TCDD affect plasma concentrations of infants and children to a greater extent than individuals who have already reached steady state levels.

Individuals that are losing weight rapidly will have greater levels of TCDD-TEQ in their plasma than would be predicted by body burden, as will individuals that are accidentally or occupationally exposed to TCDD and its congeners. However, the use of body burden as a dose metric is appropriate for the majority of the population (excluding infants and children).

Topic # 2
Question # 2
Role: Secondary Reviewer

Question: How might the rationale be improved for EPA's decision not to calculate a RfD/RfC, and for the recommended MOE approach for conveying risk information? Is a MOE approach appropriate, as compared to the traditional RfD/RfC? Should the document present a RfD/RfC?

Response:

In my opinion, it should be explicitly stated that the plasma concentration of TCDD-TEQ is affected by the body burden as well as by the daily intake and excretion rate of the congeners. Second, the relative contributions of the liver/adipose depots to that plasma concentration should be made clear. This will clearly show that for an adult (one that has presumably reached a steady state body burden), that the contribution from body burden greatly exceeds that from daily intake. In addition, a plot of body burden versus lethality would underscore the point that body burden, not daily dose or acute dose, best predicts toxic effects across species. This will help justify the decision not to calculate a RfD/RfC. The part of the document that justifies the MOE approach using the body burden was acceptable and the literature supports the logic.

I would support a RfD/RfC approach only for infants and small children because they have not reached significant body burdens. For this subset of the population, the minimal risk level of 1 pg/kg/day suggested by ASTDR is reasonable, as is the tolerable daily intake of 1-4 pg/kg/day suggested by WHO.

Topic # 5
Question # 8
Role: Primary Reviewer

Question: Have the available human data been adequately integrated with animal information in evaluating likely effect levels for the non-cancer endpoints discussed in the reassessment?

Response:

Both the integrated summary and the previous documents (Part II, Chapters 4 Iimmunotoxicity, 5 Developmental and Reproductive Toxicity, and 7B Effects Other than Cancer) contain excellent reviews of the literature that appear to contain many of the most recent reports dealing with both animal and human studies. There are no significant omissions that would affect this risk assessment. The authors performed well in correlating the available human data to the more rigorous animal data. However, certain aspects appear to require clarification. The human endocrinological effects are listed in Table 2-1 as +/-. Based upon the effects upon testosterone levels seen some of the studies as well as the effects on male development from the Yu-Cheng studies, I feel this should be a +.

In terms of likely effect levels, it was stated that insufficient data was available to model non-cancer endpoints in humans although sufficient data was available for noncancer endpoints in animals. I feel that the authors were correct in stating that the uncertainties

were great enough to prevent accurate interspecies and animal-to-human comparisons. Among these are uncertainties as to critical gestational periods in many species, differences in the sensitivity of endpoints between species and strains, exposure routes, and study design. In my opinion, these uncertainties prevent the determination of likely effect levels for reproductive and developmental endpoints in humans to any degree of confidence, and thus require further research. However, the Dutch studies do suggest that PCB and other dioxin-like compounds have the potential to retard growth and certain developmental milestones at levels approaching current background.

In terms of immunotoxicity, there is a wealth of animal research that clearly defines TCCD and similar compounds as immunotoxicants. In these studies, there is clear evidence that TCDD can suppress host resistance to a number of pathogens and transplanted tumors. However, the human epidemiological data is contradictory and demonstrates uncertainties in regards to choice of sensitive endpoints and critical times of exposure. In particular, more studies need to be initiated in order to evaluate the sensitivity of the human immune system, focusing on neonates and children, to TCDD exposure. Until these uncertainties are resolved, the selection of likely effect levels for immunotoxicity likewise can not be determined.

The data for human chloracne is adequate. However, there are uncertainties as to what exposure level and frequency of exposure is required to initiate the condition. In terms of diabetes mellitus, human epidemiological data suggests a correlation between this condition and serum TCDD level. However, at the current time, none of the available animal models for diabetes have been used to study the effects of TCDD exposure on this disease condition. More research needs to be done in order to elucidate the mechanism by which TCDD could cause diabetes.

TCDD may cause an increase in T4 at low doses and a decrease in T4 with elevated TSH at higher doses. Human epidemiological data is scarce and inconsistent in regards to

thyroid function. There is sufficient animal data to consider TCDD as a hazard to the thyroid in humans but uncertainties prevent the suggestion of likely human effect levels.

Human studies have suggested TCDD-induced effects on the incidence of cardiovascular disease. Animal studies have demonstrated effects on cardiac rhythm and on the development of the heart and vasculature (primarily in avian species). There is an apparent data gap on the effects of TCDD and related compounds on mammalian heart development. This suggests the possibility that gestational exposure could cause developmental abnormalities in the heart that might increase the chances for later cardiovascular disease.

Topic # 5
Question # 9
Role: Primary Reviewer

Question: Do reviewers agree with the characterization of human developmental, reproductive, immunological and endocrinological hazard? What, if any, additional assumptions and uncertainties should EPA embody in these characterizations to make them more explicit?

Response:

There is, in my opinion, sufficient evidence to indicate that TCDD can cause adverse effects to human development and reproduction. I agree with the document's characterization of these hazards. The assumption that human sensitivity to TCDD and similar compounds is in the midrange as compared to other species for these endpoints appears to be well founded. The authors make the point that these substances occur in complex mixtures and that uncertainties lie in the response that humans and animals may have to such mixtures. In my opinion, this is the major stumbling block in applying the

current animal data to the determination of potential human risk. This is an area that needs consorted research to clarify. Another area of uncertainty lies in whether TCDD can affect the developing mammalian heart in the way it does the developing avian heart.

Whether TCDD is an immunotoxicant in humans is less clear. Certainly the animal data indicates that TCDD and similar compounds are potent immunotoxicants. However, the human epidemiological data is inconsistent and sparse. I agree that more research needs to be done in order to determine human risk, particularly to sensitive subsets of the population. However, the data from subhuman primates coupled with the data derived from laboratory species is sufficient to consider TCDD and other dioxin-like compounds to be probable human immunotoxicants capable of decreasing host resistance to a number of pathogens.

The document needs to address in more detail the uncertainties in the area of endocrine effects. The limited human data coupled with some intriguing studies in rodent models suggests a role for TCDD in the development of thyroid disease and diabetes.

Topic # 8 Children's Risk

Question # 16

Role: Secondary Reviewer

Question: Is the characterization on increased or decreased childhood sensitivity to possible cancer or non-cancer outcomes scientifically supported and reasonable? Is the weight of the evidence approach appropriate?

Response:

The document states that children's risk of adverse effects resulting from exposure to TCDD and related compounds may be increased as compared to adults but that more data

are needed to fully address this issue. The available human data coupled with animal data suggesting that the young are more susceptible support that statement. It is both scientifically valid and logical to suggest that children's risk may be increased relative to adults. It is not clear that all endpoints will be more sensitive in children than adults, but the data suggest that a number of endpoints will be. It is also clear that the relative exposure of children is higher than that of an adult, often three-fold and higher. Even is one assumes equal sensitivity, the increased dose is indicative of increased risk. In my opinion, the weight of the evidence is in favor of increased risk but not necessarily increased sensitivity.

Topic # 10 Risk Characterization Summary Statement

Question # 18

Role: General Reviewer

Question: Does the summary and analysis support the conclusion that enzyme induction, changes in hormone levels, and indicators of altered cellular function seen in humans and laboratory animals, represent effects of unknown clinical significance but that may be early indicators of toxic responses? (Refer to pages 84-86)

Response:

These statements are made in several places in this section. Enzyme induction is supported by the statement that this effect may increase metabolism and elimination of other toxic compounds or may increase reactive intermediates. This support could be strengthened by the brief discussion of adaptive responses as well as discussing the effects on endogenous compounds such as estrogen. Changes in circulating reproductive hormones in men exposed to TCDD are rightfully characterized as adverse. Altered cellular function may serve as a biomarker of exposure and possibly effect but might also be indicative of an

adaptive, non-adverse response. This statement merits a more thorough discussion in this section.

Topic # 10 Risk Characterization Summary Statement

Question # 19

Role: General Reviewer

Question: Has the short summary statement in the risk and hazard characterization on page 107 adequately captured the important conclusions, and the areas where further evaluation is needed? What additional points should be made in this short statement?

Response:

In my opinion, this statement needs to be expanded by approximately 50% to convey clearly the important conclusions. Specifically, it should state that TCDD is a known human carcinogen by all known criteria. It explicitly should state that TCDD and related compounds are developmental, reproductive and endocrinological hazards to humans and animals. It should also state that TCDD and similar compounds are known immunotoxicants in animal models. The document should specify that children are at risk, not because of increased sensitivity, but because of increased exposure at critical developmental times.

HARRIS ENVIRONMENTAL RISK MANAGEMENT, INC.

1900 BLUFFVIEW COURT
FLOWER MOUND, TX 75022
972-691-8338
972-691-8099 (FAX)

Federal Express
July 13, 2000

Ms. Kate Schalk
Peer Review Workshop Manager
Eastern Research Group, Inc.
110 Hartwell Avenue
Lexington, MA 02421-3136

RE: Comments on Chapters 8 and 9 and the Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds of the USEPA Dioxin Reassessment

Dear Ms. Schalk:

Please find attached comments on the above referenced documents. I have enclosed a disk with the comments and this letter on it in a Microsoft Word file as well as an Adobe PDF file. If you have any questions about these comments, please feel free to contact me at (972) 691-8838.

Sincerely,

Mark Harris, Ph.D.
President

Comments on Chapters 8 and 9 and the Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds of the USEPA Dioxin Reassessment

Topic 2

Question 3

Secondary Reviewer

EPA Question: The SAB commented that previous dose-response modeling was too limited to biochemical endpoints (CYP1A1, CYP1A2 etc.). Are the calculations of a range of EDO1 body burden for non-cancer effects in rodents responsive and clearly presented? Please comment on the weight-of-evidence interpretation of the body burden data associated with a 1% response rate for non-cancer effects that is presented in Chapter 8, Appendix I and Figure 8-1 (where EPA considers that the data best support a range estimate of ED01 body burdens between 10 ng/kg to 50 ng/kg).

Comments

To address this question, pages 8-11 through 8-14, pages 8-28 through 8-66, Figure 8-1, Figure 8-2, Table 8-4, Table 8-5, Table 8-6, Table 8-7 and Appendix I were reviewed.

Comment 3-1: The assessment does not provide an adequate basis for the selection of the body burden dose metric over other methods such as area under the curve (AUC) for use in determining EDO1's. A previous paper has suggested that the use of AUC is the appropriate dose metric (Aylward, Hayes et al. 1996). Additionally, in its review of the original reassessment document, the Science Advisory Board (SAB) stated the following:

> *"The document repeatedly referred to "average body burden" as a biologically meaningful dose metric, even though other measurements of dose associated with peak intake may be more important for some effects, and that "area under the curve" is the preferred dosimetric for dealing with agents with long biologic half-lives."*

This comment by the SAB has not been addressed in the assessment. Additionally, the selection of a dose metric appears to be key to the conclusions made in the reassessment document. With regard to non-carcinogenic ED01's developed in the assessment, the use of the body burden dose metric was used to compare across species and studies. Thus, it is important from a clarity perspective to

document why this particular dose metric was used as opposed to AUC or others (Cpeak etc.). Does the body burden metric used in the assessment account for duration of exposure? All of this being said, the assessment is correct to attempt to utilize a dose metric other than administered dose. However, it seems apparent that the assessment needs to take the next step and utilize a more sophisticated dose metric probably including the use of a PBPK model (even a simple one). These models can be used on animal data and also scaled to predict human concentrations. Thus, while it appears that the current dose metric is not sufficient for the assessment, the assessment is clearly headed in the right direction.

Comment 3-2: The information in Table 8-5 needs to be better identified to assist the reader in understanding the data. Specifically, the values in parenthesis need to be identified via a footnote. The text of Chapter 8 describes the meaning of these values in parenthesis. However, tables should be able to stand on there own in a document such as this.

Comment 3-3: In its review of the previous reassessment, the SAB commented that the document should provide enough information such that the calculations performed in the assessment could be reproduced by others. However, it would be difficult for anyone to reproduce the calculated EDO1's in Chapter 8 given the information presented in Appendix I. Given that the assessment document is so large, it does not seem to be out of place to add an additional 50 pages to provide the actual model inputs for each of the endpoints/studies. This would allow others to easily verify the calculated EDO1's.

Comment 3-4: The current version of BMDS is 1.2. However, version 1.1 was used to calculate body burden EDO1's for the non-carcinogenic endpoints. The assessment should confirm that the results presented will not change significantly due to the updating of the model.

Comment 3-5: Has the BMDS modeling software been published or subjected to scrutiny outside of the USEPA? How do the results with this software package compare to other similar software packages using the same input data? The assessment should provide some documentation about this model and probably should refer readers to the EPA Web-Site where there is significant information about this model.

Comment 3-6: The range of body burden EDO1's is large as illustrated in Figure 8-1 and Appendix I. The agency discusses this briefly in the text and in Table 8-5. However, is it possible to expand

this discussion as to why the ranges are so large (species differences, sex differences etc.)? The large ranges make it difficult to arrive at any particular lower range (e.g., the 10 ng/kg to 50 ng/kg range as suggested in the assessment). Is the range unusually large due to the body burden dose metric? Another dose metric that better describes the exposure might work to "tighten" the range of EDO1's for the various endpoints and make a decision about a point of departure somewhat easier. It is suggested the assessment team consider the use of other dose metrics such as area under the curve (AUC) and the use of some simple PBPK models.

The impact of the suggested range (10 ng/kg to 50 ng/kg) is significant as it is a key reason why the agency concludes that it is not appropriate to determine a non-carcinogenic toxicity criteria value (RfD) for dioxin. However, the large range of body burden EDO1's make the selection on any particular range difficult. Currently, it is not possible to select a range given the variance in the data set.

Comment 3-7: Utilizing other dose metrics (AUC, Cpeak etc.), how do the conclusions regarding the EDO1 change for non-carcinogenic effects?

Comment 3-8: Is the Body Burden EDO1 for body weight in the study by Fox et al. (1993) (page 8-79 of the assessment) correct? It seems very low compared to the other body burdens for this particular endpoint.

Topic 3
Question 4
Primary Reviewer
EPA Question: How might the discussion of mode-of-action of dioxin and related compounds be improved?

To address this question, Section 3 of the Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds was reviewed (pages 39-47).

Comment 4-1: Section 3.2.3 (lines 17-23) list four lines of research that substantiate the role of the Ah receptor in dioxin toxicity. However, appropriate references are provided for only one of the four lines of research. Please insert appropriate references for structure-activity relationships, responsive versus non-responsive mouse strains and mutant cell lines.

Comment 4-2: This section differentiates between mode-of-action and mechanism-of-action and references both the 1996 USEPA Proposed Guidelines for Carcinogen Risk Assessment (USEPA 1996) and the 1999 USEPA Revised Proposed Guidelines for Carcinogen Risk Assessment (USEPA 1999) as the basis for this distinction. What is the status of the 1999 document? Has it been approved for use? If so, there is no need to reference the 1996 document. If not, why is the assessment referencing an unapproved document?

Comment 4-3: There is no discussion regarding the role of naturally occurring Ah receptor agonists and their potential impact on the proposed mode-of-action. Additionally, there should be a description of the potential antagonistic actions of certain compounds with regard to dioxin like activity and the mode of action description. This need not be more than a few sentences acknowledging that these have been identified.

Comment 4-4: Page 47 (lines 1-11) provides possible mechanisms by which TCDD (and related compounds?) might act as a tumor promoter and developmental toxicant. Both of these endpoints as well as others discussed in previous sections are not genotoxic events. Thus, it is recommended that at least some words be included that mention the possibility of a threshold in this mode-of-action (i.e., below some minimum number of occupied receptors, some minimum body burden etc that no relevant biological effect will occur).

Comment 4-5: Section 3.2.1 provides a list of criteria that describe receptor mediated activities. A reference for these criteria (page 42 lines 3-11) should be provided.

Comment 4-6: On page 45 (lines 30-33), the following statement is made:

> *"However, it cannot be assumed that an increase in receptor occupancy will necessarily elicit a proportional increase in all biological response(s) because numerous molecular events (e.g., cofactors, other transcription factors, genes) contributing to the biological endpoint are integrated into the overall response"*

The statement above is correct. However, the meaning of such could be improved with the use of a figure to illustrate the point. An example of an appropriate figure to illustrate this point that could be

adapted for this assessment can be found in the textbook entitled "Textbook of Endocrinology" page 113 (Roth and Grunfield 1981).

Topic 3
Question 5
Primary Reviewer

EPA Question: Despite the lack of congener-specific data, does the discussion in the Integrated Summary and Risk Characterization support EPA's inference that these effects may occur for all dioxin-like compounds, based on the concept of toxicity equivalence?

The section entitled "Part III: Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds" was reviewed to address to this question.

Comment 5-1: The referenced section of the assessment was reviewed and found to not adequately address this subject. Specifically, Section 1.2 and Pages 87-89 of Section 6 of Part III touch on this subject. However, the discussion in these two sections is limited and does not suffice to support the inference that the effects associated with TCDD may occur for all dioxin-like compounds based on the toxicity equivalence concept. There simply is not enough discussion of this concept in Part III.

Comment 5-2: In light of comment 1 above, this portion of the assessment needs to include a section that directly addresses this subject (toxic equivalency).

Comment 5-3: When developing the text for Part III to support the concept that other dioxin-like compounds behave toxicologically similar to that of TCDD, the assessment should address the significant lack of data regarding half-life values for other dioxin-like compounds, the lack of human epidemiology data for these compounds etc. and how this impacts our ability to make definite conclusions about these compounds. Indeed, the recent study by Kimbrough calls into the question the carcinogenic potential of PCBs (Kimbrough, Doemland et al. 1999) some of which are clearly dioxin-like. Why was this study not mentioned in the assessment? It seems to be clearly relevant to the matter at hand.

Topic 4

Question 6

Secondary Reviewer

EPA Question: Is the history, rationale and support for the TEQ concept, including its limitations and caveats, laid out by the EPA in a clear and balanced way in Chapter 9? Did EPA clearly describe its rationale for recommending adoption of the 1998 WHO TEFs?

Comment 6-1: The history, rationale and support for the TEQ concept is adequately developed and discussed in Chapter 9.

Comment 6-2: This Chapter does not adequately address the emerging issue of naturally occurring dioxin-like compounds. The assessment argues that due to the short half-life of these compounds the resulting body burden of these compounds is low and not significant compared to that of PCDDs, PCDFs, etc. However, an alternative explanation could be that the use of the body burden dose metric is incorrect for these compounds given that they do not bioaccumulate. Table 1 below presents data that is relevant to this discussion.

Table 1. Dietary Intakes and Toxic Equivalent (TEQ) Values for Exodioxins and Some Natural AhR Agonists*

Compounds	Dietary Intake (mass, pg/day)	Relative Potency	TEQs (pg/day)
Antiestrogenicity			
PCDDs/PCDFs	1000-2000	-	120
I3C**	735,000,000	0.0001	73,500
ICZ (derived in gut from I3C)	73,500	0.01	735
PAHs	$1.2 – 5 \times 10^6$	0.001	1200-5000
Immunotoxicity			
PCDDs/PCDFs	1,000-2,000	-	120
I3C	735,000,000	0.0001	73,500
PAHs	$1.2 – 5 \times 10^6$	0.0001	120-500

*Table adapted from (Safe 1997/1998)

**I3C: indole-3-carbinol;

The naturally occurring dioxin-like compounds contribute the bulk of the TEQ in this example (table reproduced from Safe, 1997/1998). This paper and the conclusions of such should be addressed in Chapter 9 in order to have a complete discussion. Currently, this paper is not cited in the assessment. Additionally, one can infer from the SAB 1995 report (Section 4.12.1) that the use of body burdens or AUC dose metrics is not appropriate for short lived compounds in the body. The SAB states in this section the following:

> ".…….and that "area under the curve" is the preferred dosimetric for dealing with agents with long biologic half-lives."

At a minimum the assessment should address what is the appropriate dose metric for the short-lived naturally occurring dioxin-like compounds. It seems likely that in this situation, administered dose would be sufficient metric.

Comment 6-3: Page 9-16 (lines 14-22) states the following:

> *"Although Safe has suggested that exposure to natural AhR ligands is 100 times that of TCDD and other dioxin-like chemicals (Safe, 1995), the impact of the natural AhR ligand is uncertain. Epidemiological studies suggest that human exposures to TCDD and related chemicals are associated with adverse effects such as development impacts and cancer. In many of these studies, the exposed populations have approximately 100 times more TCDD exposure than background populations (Chapter 7). If the exposure to natural AhR ligands is included in these comparisons, then exposed populations should have only about 2 times higher total TEQ exposures than the background population. It seems unlikely that epidemiological studies could discriminate between such exposures."*

In reality, the epidemiology associated with dioxin has not provided conclusive evidence regarding cancer effects even when exposures have been very large (e.g., NIOSH cohorts). An alternative explanation is that because the dioxin exposure at the phenoxy herbicide plants made up only a small fraction of the total TEQ received by an individual, the epidemiology studies are unable to discern any significant differences in health outcomes.

Comment 6-4: A recent paper (published after the release of the draft assessment) provides a good example of unknown compounds eliciting dioxin like activity. In this particular study, sediments from a river known to contain elevated concentrations of PAHs, PCBs, PCDDs and PCDFs were divided into dioxin containing fractions, PCB containing fractions and PAH containing fractions through an elaborate fractionation procedure (Gale, Long et al. 2000). The various fractions of the sediment were introduced into the H4IIE bioassay and ethoxyresorufin-O-deethylase (EROD) induction measured. Interestingly, the whole sediment extract contained significantly more dioxin TEQ than the various subfractions of the sediment summed together suggesting the presence of other dioxin like compounds. This is an important finding that should be discussed in Chapter 9. Furthermore, the results of this paper suggest that Chapter 9 needs to include TEFs for PAHs as 80% of the attributed to PCDDs, PCDFs, PCBs and PAHs was associated with PAHs in the sediment. A second example of this is provided in (Jones, Giesy et al. 1993). In this case, analytically determined TEQ underestimated the bioassay determined TEQ by an average of 57%. Given the conclusions of the assessment, it will be important that the entire universe of dioxin-like compounds be identified and quantified for potency. Clearly, the compounds identified to date as "dioxin-like" do not account for all of the TEQ in the environment. In some cases, such as the two examples above, the underestimation is great.

Comment 6-5: Why are there no TEFs for polynuclear aromatic hydrocarbons (PAHs)? These compounds clearly possess dioxin-like activity and are present in the environment. A recent paper (Gale, Long et al. 2000), evaluated dioxin-like activity in sediment known to contain PCDDs, PCDFs, PCBs and PAHs. This group (USGS and NOAA staff) found that PAHs contributed more than 80% of the TEQ contributed by PCDDs, PCDFs, PCBs and PAHs as a group. This group calculated total TEQ using toxic equivalency factors for each of these group of chemicals. For PAHs, TEFs were used as suggested by (Willet, Gardinali et al. 1997). It is suggested that TEFs for PAHs be included in the assessment. Given the conclusions of the assessment, it will be important to accurately account for all of the TEQ in the environment and to determine exposure to such.

Topic 10

Question 18

General Reviewers – All

EPA Question: Does the summary and analysis support the conclusion that enzyme induction, changes in hormone levels, and indicators of altered cellular function seen in humans and

laboratory animals, represent effects of unknown clinical significance, but that may be early indicators of toxic response (pages 84-86)?

Comment 18-1: Page 84 (lines 22-24) state the following:

> *"These effects will likely range from biochemical changes at or near background levels of exposure to adverse effects with increasing severity as body burdens increase above background levels"*

What is the basis for suggesting that effects will begin to occur at or near background? The data presented in Part II Section 7b generally involve human study groups with elevated exposure to PCDDs, PCDFs or PCBs with effects that appear to be due to random chance in studies (i.e., not consistent among multiple studies) or probably due to confounding exposures (TCP production workers). Additionally, the conclusion regarding background exposure and toxic responses is strongly influenced by the selection of body burden as the appropriate dose metric. The continued use of the body burden dose metric needs to be justified. Why was AUC not used as previously suggested by the SAB? Additionally, the range of 10 ng/kg to 50 ng/kg body burden in animals as a point of departure may be playing a role in supporting such a statement. It needs to be noted that the range of EDO1 body burdens for various toxic endpoints in animals varied by orders of magnitude and thus the selection of the 10 ng/kg to 50 ng/kg may not be appropriate and introduces a large amount of uncertainty into the statement.

Comment 18-22: Based on the data in this assessment and including the mode-of-action concept, it seems likely that two conclusions that could be made are as follows:

1. The mode-of-action of TCDD and related compounds are likely to be similar in humans and animals
2. Given the appropriate dose of TCDD or related compounds, similar toxic responses would be expected in humans as in seen in animals.

Of course, the key to the second statement is what is a dose that will elicit a toxic response in humans.

Comment 18-3: Regarding the unknown clinical significance of these various biochemical endpoints, it should be noted that not very many altered biochemical endpoints have been observed

in exposed populations that can be linked to exposure to TCDD or related compounds. Thus, it seems premature to make this statement.

Comment 18-4: I note that for some of the Dutch studies cited in Section 7b, a dissertation was cited (Koopman-Esseboom, Huisman et al. 1995; Koopman-Esseboom, Weisglas-Kuperus et al. 1995). Are these appropriate citations or references to use in the assessment? The reference information indicates that these documents are chapters from a "dissertation". Have these documents undergone critical, unbiased peer review? These references are somewhat confusing as there is more than one author suggesting that this is not a Ph.D. dissertation. Please clarify.

Comment 18-5: On Page 85 of the Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds (lines 11 –12) it states the following:

> *"In other words, evaluation of the available data suggests that humans, in general, are neither extremely sensitive nor insensitive to the individual effects of dioxin-like compounds"*

The basis for this statement is unclear. The NIOSH cohort experienced significant exposure to TCDD and related compounds yet little if any adverse health events have been noted. Moreover, except for chloracne, there is not a lot of data suggesting any adverse impacts in the human population. Thus, the basis for this statement is unclear.

Comment 18-6: On Page 85 of the Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds (lines 16-21) it states the following:

> *"In TCDD-exposed men, subtle changes in biochemistry and physiology such as enzyme induction, altered levels of circulating reproductive hormones, or reduced glucose tolerance and, perhaps, diabetes, have been detected in a limited number of epidemiologic studies. These findings coupled with knowledge derived from animal experiments, suggest the potential for adverse impacts on human metabolism, and developmental and/or reproductive biology, and perhaps, other effects in the range of current human exposure."*

This text overstates what is known about TCDD and related compounds. The TCDD-exposed men (NIOSH cohort, German cohorts etc.) were exposed to numerous chemicals during their employment which could have impacted a host of biochemical and toxicological endpoints. Presented below is a list of chemicals known to be present at the Newark, New Jersey Phenoxy Herbicide Plant during operation (data from Remedial Investigation report on the site):

1. monochloroacetic acid
2. hexachlorobenzene
3. 2,4,6-trichlorophenol
4. 2,4-dichlorophenol
5. dichlorodiphenyltrichloroethane
6. p-chlorophenyl-p-chlorobenzene sulfonate (ovex)
7. 1,1,1-trichloroacetaldehyde
8. benzenesulfonyl chloride
9. p-chlorobenzenesulfonyl chloride
10. p-chlorobenzenesulfonamide
11. 4,4'-dichlorodiphenylsulfone
12. p-acetylaminobenzene sulfonyl chloride
13. p-methoxybenzene sulfonyl chloride
14. 1,2,4,5-tetrachlorobenzene
15. amine salts of N-oleyl-1,3-propylenediamine
16. Nicotine Sulfates
17. Muriatic Acid
18. 2,5-dichlorophenyl-p-chlorobenzene sulfonate
19. Lindane
20. Sodium 2,4,5-trichlorophenate
21. 2,4,5-trichlorophenoxyacetic acid
22. 2,4,5-T ester
23. 2,4,5-T amine
24. acetic acid
25. acetic anhydride
26. acetaldehyde
27. benzene
28. monochlorobenzene
29. tetrachlorobenzene
30. chlorosulfonic acid
31. methanol
32. oleum (20%)
33. phenol
34. sulfuric acid
35. dimethylamine
36. triethylamine
37. chlorine
38. 2-ethylhexanol
39. butyl alcohol
40. isopropyl alcohol
41. butoxyethoxypropanol
42. nicotine

43. sodium hydroxide

It is likely that similar lists could be generated for other plants in the NIOSH study. These data suggest numerous opportunities for confounding factors to be introduced into any analysis of the biochemical and/or toxicological impacts on these workers. These confounding factors should be considered when making conclusions about the epidemiology studies of phenoxy herbicide workers.

Additionally, this conclusion is based on the use of the body burden dose metric. The assessment needs to justify the use of this dose metric as opposed to others (e.g., AUC as suggested by the SAB).

Comment 18-7: Page 85 (lines 34-35) reference the Dutch studies where MDI and PDI indices were determined for breastfed and formula fed infants. Total TEQ was determined in the breast milk and formula etc. How much weight is this series of studies given in the assessment? I have only two of the papers published from this series of experiments. However, a review of Koopman-Esseboom, Weisglas et al (1996) suggests that interpretation of this paper is difficult at best (Koopman-Esseboom, Weisglas-Kuperus et al. 1996). Finally, the even the text in Part II Section 7b discussing these studies in detail casts doubt on the merit of these studies. It is suggested that the reference to the Dutch studies be removed or at a minimum not used to justify the concept of adverse effects at background exposure concentrations.

Comment 18-8: It seems likely that the total TEQ exposure by the general population is underestimated due to the fact the entire universe of dioxin-like compounds in the environment has not been identified yet. Thus, the underlying analyses supporting this statement have likely underestimated total TEQ exposure. How does this impact the conclusions of this section?

Topic 10
Question 19
General Reviewers - All
EPA Question: Has the short summary statement in the risk and hazard characterization on page 107 adequately captured the important conclusions, and the areas where further evaluation is needed? What additional points should be made in this short statement?

Comment 19-1: Page 85 (lines 10-11) state the following:

> *"Some of these effects may be occurring in humans at general population background levels and may be resulting in adverse impacts on humans."*

This statement should be removed from the text. The assessment has not provided adequate support for such a statement. The assessment utilizes a new dose metric for risk assessment to evaluate exposure (body burden) without any sort of justification for such. Why was other dose metrics not evaluated? Clearly the SAB and others (Aylward, Hayes et al. 1996) have suggested other appropriate metrics. The comparison of background levels and adverse effects is based on very little information (e.g., use of body burden EDO1 from animal studies in which the ranges of such varied by orders of magnitude and several studies in the Netherlands of questionable utility).

Finally, there was recently two papers published that described an acute exposure to 2,3,7,8-TCDD in Vienna (Abraham, Geusau et al. 1999; Geusau, Mexinar et al. 1999) by two individuals. These authors report up to 144,000 ppt TCDD in blood lipid and a lack of biochemical effects. Chloracne in these individuals was very severe. However, the lack of biochemical responses is significant given the conclusions of the assessment. These papers should be discussed and evaluated in the assessment (probably Chapter 7B and in Part III). Results such as these make it difficult to believe that the general population is suffering adverse effects from TCDD and related compound exposure.

Comment 19-2: If background exposures are indeed causing effects in the general population, then it seems reasonable that the NIOSH cohort would have experienced significant adverse responses. However, the bulk of these studies have failed show any significant adverse responses in the study populations. Additionally, general population background exposures have been decreasing for a long period of time. Should not adverse responses attributable to TCDD and related compounds been observable 20 to 30 years ago in the general population if the current population exposure is borderline (i.e., body burden was much greater 20 years ago than it is today in the general population).

Comment 19-3: Somewhere in this statement, the great uncertainty associated with this whole exercise needs to be communicated. We do not know the actual exposures at the phenoxy herbicide plants (we can only make estimates), we are unsure about the mechanism of action, we are unsure about the significance of naturally occurring dioxin-like compounds in the diet, we are unsure about the appropriate dose metric, the epidemiology studies cannot demonstrate an effect etc.

Comment 19-4: It is recommended that the statement be revised to reflect the uncertainty in this analysis and the text regarding impacts to the general population be removed.

Topic 11
Question 21
General Reviewers - All

EPA Question: Please provide any other comments or suggestions relevant to the two review documents, as interest and time allow.

Comments on the Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachloro-p-dioxin (TCDD and Related Compounds

General Comment: Throughout the assessment the issue of statistical significance is not clear. If the response or measurement is not statistically significant then it is not different than the control measurements. The assessment needs to be very clear what is statistically significant and what is not. For example, on Page 15 (Line 21-22), the assessment states the following:

> *"…..which had a borderline statistically significant increase in breast cancer….."*

Use of this language is not appropriate in a scientific document and the meaning is unclear as well. Was the effect just barely significant or was it not significant but close to being such?

Specific Comments

Comment 21-1: The assessment needs to further discuss the confounding factors associated with the epidemiology studies used for dose response modeling in Section 8. For example, the assessment needs to point out that these workers were exposed to a hosts of substances (a list of some are provided above).

Comment 21-2: This section (Part III) needs to point out that TCDD is really the first multi-site carcinogen ever identified (except maybe radiation) and discuss the significance of this. I find this concept to be a bit odd for a chemical.

Comment 21-3: The dose response modeling only utilized TCDD serum concentrations (converted to body burdens). What is the effect of ignoring likely exposure to other dioxin-like compounds at these facilities? Does not this approach result in an overestimation of the slope?

Comment 21-4: The SAB commented that all of the calculations used in the dose response calculations should be provided such that they could easily be reproduced. However, I was unable to find the details regarding the modeling in Chapter 8 that could be used to reproduce the EDO1's in animals. In the final document, these details need to be provided.

Comment 21-5: Page 7 (Lines 18-19): The assessment provides no justification for the selection of the body burden dose metric. Why not the AUC method? The AUC method has been published previously on this very subject (using data from the NIOSH cohort) and reached different conclusions about TCDD and related compounds.

Comment 21-6: Page 7 (Line 19): The text at this location mentions the following:

> *"recent data demonstrating that either allometric scaling or uncertainty factors underestimate the species differences….."*

References should be provided to support this assertion.

Comment 21-7: Page 8 (Line 2): The text at this location states the following:

> *"Evidence supports the assumption that TCDD and related compounds……"*

References should be provided to support this statement.

Comment 21-8: Page 8 (Lines 2-6): Has the body burden method been validated for humans and low dose exposures? Additionally, how does this method differ from the AUC method recommended by the SAB in 1995?

Comment 21-9: Page 8 (Lines 14-15): The text states the following:

"In addition, the sequestration is dose dependent, and at human background exposures, hepatic sequestration should not be significant."

References should be provided to support this statement.

Comment 21-10: Page 8 (Lines 16-35): Is this description of the body burden method complete? Should not a description of how the daily doses were calculated be provided as well?

Comment 21-11: Page 9 (Lines 11-16): The text states the following:

"The use of body burden, for many effects within species and, particularly, for cross-species scaling, appear to provide a better dose metric than daily dose."

The assessment needs to provide references to justify this statement. Furthermore, the SAB recommended that AUC be used (Section 4.12.1 of the SAB report). What if AUC had been used? How would this have impacted the conclusions of the assessment?

Comment 21-12: Page 14 (Lines 22-23): The text states the following:

"…….dioxin-contaminated phenoxy herbicides and increased cancer risk involved an increase in soft tissue sarcoma……"

Soft tissue refers to tissues that connect, support, or surround other structures and organs of the body. Soft tissue includes muscles, tendons (bands of fiber that connect muscles to bones), fibrous tissues, fat, blood vessels, nerves, and synovial tissues (tissues around joints) (National Cancer Institute, 2000). Is the Ah receptor present in these tissues? If so, a reference should be provided.

Comment 21-13: Page 15 (lines 19-27): Given the anti-estrogenicity of TCDD, why would one expect increases in breast cancer?

Comment 21-14: Page 16 (Lines 3-9): The assessment should point out that TCDD is the first multi-site carcinogen ever discovered (except maybe radiation). No where in the assessment is the reason for this unusual finding discussed? A discussion of why this is the case should be provided.

Comment 21-15: Page 17 (Line 18): The assessment cites Walker, Tritscher et al., 2000 which was in press when the assessment was published. However, the paper has now been published (Walker, Tritscher et al. 2000) and several key findings in this paper should be discussed including:

1. The liver half-lives for female Sprague-Dawley rats was reported to vary with dose. The larger the dose the longer the liver half-life. Does this observation have any significance in the dose response modeling of rat body burdens?

2. Female Sprague-Dawley rats (non-DEN initiated) treated with 125 ng/kg/day TCDD for 60 weeks did not develop adenomas or carcinomas in the liver. This appears to be significant as this daily dose is in excess of the Kociba, Keyes et al., 1978 study where a daily dose 100 ng/kg/day clearly caused an increase in cancer in hepatic tissue of the female Sprague-Dawley rats. Using the body burden approach suggested in the assessment, the Walker, Tritscher et al. 2000 study would be expected to produce a much greater body burden than that of Kociba, Keyes et al., 1978. However, while Walker, Tritscher et al., 2000 produces a much higher body burden it also produces no cancer. In contrast, the Kociba, Keyes et al., 1978 study has a lower body burden (per the assessment calculation) and elevated hepatocellular cancer (11 out 49 rats). The key difference in the two studies was the duration of exposure. In the Kociba, Keyes et al., 1978 study, the exposure was for a lifetime (two years for a rat) and in the Walker, Tritscher et al, 2000 study, exposure was about 60 weeks. The assessment should address these apparent discrepancies. Thus, it appears that the body burden dose metric fails to differentiate the two studies.

3. Given the comment on Walker, Tritscher et al, 2000 above (item 2), would another dose metric provide a different perspective on the two doses (i.e., AUC)?

4. The daily dose of 125 ng/kg/day is 162 times greater than the "animal intake for 1% excess risk" for liver cancer in female Sprague-Dawley rates (Table 5-2) yet no cancer was observed in non-DEN initiated rats. How does the Walker, Tritscher et al., 2000 study impact the validity of this modeled value?

5. Is the lack of a time component in the dose metric used in the assessment a serious shortcoming of the method?

Comment 21-16: Page 20 (Lines 10-14): The text of this bullet is unclear.

Comment 21-17: Page 23 (Line 9): The text states the following:

> *"…..concentrations were slightly, but not significantly, higher in Ranch Hands…"*

This is not an appropriate statement. Either they were statistically different or they were not. The fact that one measurement is slightly greater than the other in absolute value is meaningless.

Comment 21-18: Page 24 (Line 21): Who is Peterson?

Comment 21-19: Page 36 (Line 32): The text states the following:

> *"…..borderline significance, among Vietnam…."*

Again, the meaning is unclear. Either it is significant or it is not. The word borderline confuses the meaning of the sentence.

Comment 21-20: Page 37 (Lines 1-2): Why is the data of Suskind and Hertzberg, 1984 not included in Table 7-23 (Chapter 7)?

Comment 21-21: (Page 37 (Lines 3-11): How does exposure to so many other chemicals at phenoxy herbicide plants impact the conclusions about GGT (see comment 18-6 for a list of chemicals at the Newark, New Jersey plant)? Additionally, why did the workers at the plant in Great Britain not exhibit elevated GGT while workers at other plants did? The authors of the studies that describe the acute exposure in Vienna should be contacted to determine if these enzymes were elevated in their patients (Abraham, Geusau et al. 1999; Geusau, Mexinar et al. 1999).

Comment 21-22: Page 65 (Lines 27-29): The text states that the following:

> *"At this time, the knowledge of the mechanism of action of dioxin, receptor theory, and the available dose response data do not firmly establish a scientific basis for replacing a linear procedure for estimating cancer potency."*

This statement is not responsive to the SAB comments in 1995. Clearly, the SAB indicated that threshold models be evaluated in the reassessment. At a minimum, the revised assessment should provide some mathematical basis for this conclusion.

Comment 21-23: Page 66 (Lines 21-22): The text states the following:

> *"……multitude of metrics (DeVito et al., 1995) such as daily intake (ng/kg/d), current body burden (ng/kg), average body burden over a given period of time, plasma concentrations etc…."*

Why was average body burden over a given period of time not selected, or plasma concentrations etc.? A rationale needs to be provided.

Comment 21-24: Page 67 (Lines 7-8): The text states the following:

> *"….species and humans (Table 8-2), this dose metric appears to be the most practical for this class of compounds…."*

The use of AUC was suggested by the SAB in 1995 (Section 4.12.1). Furthermore, the SAB states the following:

> *"…and that "area under the curve" is the preferred dosimetric for dealing with agents with long biologic half-lives."*

Why was this dose metric not used or at least compared and contrasted with body burden? This would at least make the document responsive to the SAB comment.

Comment 21-25: Page 71 (Lines 33-34): The text states the following:

> *"……suggests that exposure may be associated with increases in all cancers combined, in respiratory tumors and, perhaps, in soft tissue sarcoma."*

Was smoking controlled for in the studies that found increased respiratory tumors? If not, how does this effect the assessment and this particular statement? Additionally, at least in the NIOSH cohort, confounding factors (other chemicals) were clearly present - how is this accounted for in the assessment? In the NIOSH cohort, all cancers combined were elevated. However, how much of the elevation in total cancer can be accounted for by an elevation of respiratory tumors? Specifically, is the elevation of all cancers combined a function of respiratory tumors? If so, how does this impact the analysis given the lack of information about smoking. Finally, is the Ah receptor present in soft tissue sarcomas? If not, how can this be a target tissue. If so, a reference should be noted somewhere in the document illustrating the presence of such. Have other chemicals been evaluated by looking at "total cancer increase" as opposed to specific tissues?

Comment 21-26: Page 73 (Line 27): The text references a Table 8.3.2? No such table is present in the Integrated Summary Document or in Chapter 8 of Part II?

Comment 21-27: Page 74 (Lines 21-32): What is the conclusion utilizing other dose metrics such as AUC?

Comment 21-28: Page 75 (Lines 2-3): The text states the following:

> *"At least for this comparison, if cancer is a function of average levels in the body, the most appropriate metric for comparison is the average or steady-state body burden."*

This statement seems to contradict the request of the SAB in 1995 where they suggested the use of AUC. Furthermore, the SAB seemed somewhat critical of the dose metric used in this assessment. Additionally, what is the scientific basis for the use of average body burden levels in the body as a dose metric? Clearly, Kociba, et al., 1978 and Walker, Tritscher et al., 2000 provide conflicting results using this dose metric.

Comment 21-29: Page 76 (Line 18): The text references a Table 8.3.1. There is no such table present in the Integrated Summary (Part III) or in Part II Chapter 8.

Comment 21-30: Page 76 (Lines 32-35): Failure to account for exposure to other dioxin-like compounds can result in a overestimation of the slope factor. A doubling of the slope factor results

in acceptable levels in the environment being decreased by a factor two. Such matters should not be trivialized.

Comment 21-31: Page 89 (Lines 9-13): This bullet is unclear.

Comments on Chapter 8 Dose Response Modeling

Comment 21-32: Page 8-12 (Bottom Paragraph continuing onto Page 8-13): The assessment should discuss the lack of a time component in the body burden calculation. Specifically, the assessment should discuss how the body burden metric can or cannot be used to compare the differences between the Kociba, Keyes et al., 1978 studies and the recently published Walker, Tritscher et. al., 2000 study. Both had approximately equivalent daily doses but the results of the studies were exactly opposite. The Kociba, Keyes et al., 1978 study administered TCDD for a lifetime in female rats whereas Walker, Tritscher et al., 2000 only administered TCDD for 60 weeks. However, using the body burden method, Walker, Tritscher et al., 2000 would have a greater body burden than that of Kociba, Keyes et al., 1978? Is this a shortcoming of the body burden dose metric? How does this impact the dose response modeling for TCDD?

Comment 21-33: page 8-13 (Equation in Footnote): Where is the "f" in this equation?

Comment 21-34: Page 8-15 (Section 8.2.3.): Walker, Tritscher et. al., 2000 suggests that the liver half-life for TCDD varies with dose. The article suggests that the larger the dose the longer the half-life in the liver. Does this new finding have any bearing on the dose response modeling? The last sentence of this section indicates that various measures of body burden will be used? Is this a correct statement? What other methods were used?

Comment 21-35: Page 8-17 (Section 8.3.2.1.1): In its 1995 report, the SAB suggested that the assessment document provide all of the necessary information in the report so that others could reproduce the calculations (Section 4.9.1 of the SAB report). However, the discussion on pages 8-16 through 8-19 and Table 8-2 do not provide sufficient information for others to reproduce the calculations. It should not be difficult to add additional tables providing the exact model input data, assumptions etc. so that others can reproduce the calculations.

Comment 21-36: Page 8-22 (Section 8.3.2.4): This is an excellent discussion of the uncertainties associated with the dose response modeling. However, much of this uncertainty discussion never makes it into the Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds. Much of this uncertainty text needs to be inserted into Part III.

Comment 21-37: Page 8-30 (Last sentence on page carrying over to the top of page 8-31): The text states that

> *"In order to compare multiple-dose studies using different routes of exposure, the average daily dose was estimated for each study by calculating the total dose administered to the animal over the course of the study and dividing by the length of the study in days. In addition, for the multiple-dose studies, average steady-state body burden at the ED01 was calculated using the equation in Section 8.2.2 and the percentage of dose absorbed and the half-lives for TCDD in Table 8-1."*

The formula in Section 8.2.1 is as follows:

Body Burden (ng/kg) = daily dose(ng/kg/day) * half-life/ln(2) *f

And the formulas in Section 8.2.2 is as follows:

ED01 (ng/kg body burden) = ED01(ng/kg/day) * half-life/ln(2) *f

Where,

F = fraction absorbed

Half-life = see Table 8-1 (days)

What is unclear about this procedure is the concept of time. If one study administered 100 ng/kg/day of TCDD to rats for one year and another study administered 100 ng/kg/day to rats for two years, this particular dose metric (assessment body burden dose metric) would not capture the fact that one group of animals received twice the administered dose as the other. An extreme example of this issue is the comparison between (Kociba, Keyes et al. 1978) and Walker, Tritscher et al. 2000 and ignoring what the EDO1 dose might be. Using the largest daily dose of Kociba, Keyes et al., 1978 and the daily dose reported by Walker, Tritscher et al., 2000 one arrives at the following body burdens:

Kociba Body Burden = 100 ng/kg/day * [25 days/ln(2)] * 0.50 = 1804 ng/kg

Walker et al., 2000 Body Burden = 125 ng/kg/day * [25 days/ln(2)] * 1 = 4509 ng/kg

Where f =1 for TCDD administered via corn oil and 0.5 for TCDD administered via feed

Yet, in the Walker, Tritscher et al., 2000 no hepatocellular cancer was observed whereas in Kociba, Keyes et al., 1978 11 out of 49 rats developed hepatocellular carcinoma. The only difference between these two studies was the length of time of exposure (Walker, Tritscher et al., 2000 was for 60 weeks and Kociba, Keyes et al., 1978 was for 2 years) and one study administered the TCDD in corn oil and another via the feed. However, the body burden in the Walker, Tritscher et al., 2000 study is calculated to be 2.5 times greater than that in the Kociba, Keyes et al., 1978 study. This seems to verify that the appropriate dose metric has not been utilized in the assessment as it fails to provide an adequate comparison among two important studies.

Comment 21-38 (Page 8-31 Section 8.3.4.2): The assessment needs to provide the data inputs for the BMDS model for these multiple dose studies so that others can reproduce the results. This would be consistent with the SAB comments in 1995. The BMDS model has numerous options, parameters etc. that can be adjusted by the user. Unless, the assessment provides all of this information, it will be difficult for others to duplicate the results.

Comment 21-39 (Page 8-59, first full paragraph): The comment about PBPK models and the stating that:

> *"it is unlikely that predictions from such a model would be any less uncertain that current methodologies used for estimating body burdens"*

is interesting. Would even the less sophisticated PBPK models not account for differences in the length of exposure to TCDD in animal and human studies? Finally, the word "that" (end of first line) in the above quote should be the word "than".

Comment 21-40 (Table 8-1): Does Walker, Tritscher et al., 2000 and the finding of different TCDD liver half-lives depending on dose impact the TCDD rat half-life estimates in this table?

Comment 21-41 (Table 8-2): footnote "b": Was the Aylward, Hayes et al., 1996 study also used for this cohort in this modeling exercise? If so, it should be noted.

Comparison with SAB 1995 Comments

Comment 21-42 (Page 47) Section 4.1.1.: The SAB commented that

> *"Thus, the Committee recommends that EPA provide either additional discussions of alternative approaches and their implications for risk assessment in Chapter 8, or present a clear justification for choosing this particular dose-response approach over others…."*

The assessment has not provided an extensive discussion about various low dose extrapolation models and which should be used and why. In fact, the modeling discussion for the human studies is almost non-existent except for a reference to Breslow and Day, 1987. Much more detail including modeling input parameters need to be provided so that the results of such can be reproduced.

Comment 21-43 (Page 78, Section 4.9.1): The SAB states the following:

> *"EPA should describe its analysis in sufficient detail that it can be fully understood by the reader, to the point of reproducing the analysis if desirable."*

The assessment has not provided enough detail for the human or animals studies for the reader to reproduce the analyses.

Comment 21-44 (Page 80, Section 4.9.1): The SAB states that

> *"EPA's preferred dose response model is linear, it seems clear that a threshold model would provide an equivalent or nearly equivalent description of the data. This is the most important issue in the dose response-modeling and should be thoroughly explored in EPA's analysis."*

It is clear the assessment has not responded adequately to this comment. The concept of a threshold model is given very little coverage in Chapter 8. Chapter 8 of the assessment should explore the threshold type models in detail. If they do not work for some reason, then assessment should explain why they do not work (lack of power, lack of good fit etc.).

Comment 21-45 (Page 81, Section 4.9.2): The SAB made two key comments:

> *"Chapter 8 of the assessment document needs to describe and evaluate this alternative dose response relationship, discuss the approaches and findings of the other relevant agencies, and justify the basis for selecting another approach."*
>
> *"Thus, the document cannot ignore a possible threshold dose-response relationship and claim to be comprehensive in its presentation."*

These comments were offered by the SAB in the context of an RfD for TCDD discussion. However, it is noted that a discussion about how other agencies use threshold and safety factor methodology for their health risk evaluations is missing from the revised assessment. Thus, the assessment has not responded adequately to this SAB comment. Furthermore, the assessment has not adequately investigated the use of possible threshold models to describe the action of TCDD and related compounds.

Comments from a Risk Management Perspective

Comment 21-46: (Page 90 of Part III, Lines 8-12): The text states the following:

> *"Consequently, the Agency, although fully recognizing this range and the public health conservative nature of the slope factors that make up the range, suggests that the use of 5 x10^{-3} per pg TEQ/kgBW/day as an estimator of upper bound cancer risk for both background intakes and incremental intakes above background."*

The impact of such a slope factor on environmental activities is expected to be great if such a slope factor is utilized. Most states utilize USEPA toxicity criteria and many states set acceptable risk at 1 x 10^{-6}. The following calculation demonstrates the potential impact of such a slope factor on a risk specific soil cleanup standard calculation:

Cancer Risk	=	Slope Factor x Dose	**(Equation 1)**
1 x 10^{-6}	=	5,000,000 (mg TEQ/kg/day)$^{-1}$ x Dose (mg TEQ/kg/day)	
Dose (mg TEQ/kg/day)	=	(1 x 10^{-6}) / 5,000,000 (mg TEQ/kg/day)$^{-1}$	

Dose (mg TEQ/kg/day) = 2×10^{-13} mg/kg/day

Dose (mg TEQ/kg/day) = (CS x IR x CF x FI x EF x ED)/(BW x AT) **(Equation 2)**

Where, CS = chemical concentration in soil (mg TEQ/kg)

IR = Ingestion rate (mg soil/day) (Assume 114 mg/day)

CF = Conversion Factor (10^{-6} kg/mg)

FI = Fraction ingested from contaminated source (unitless) (Generally set at 1)

EF = Exposure frequency (days/year) (350 days/year)

ED = Exposure duration (years) (Assume 30 years)

BW = Body weight (kg) (Assume 70 kg)

AT = Averaging Time (period over which exposure is averaged - days) (25,550 days)

Substituting into the equation above we have the following:

$$2 \times 10^{-13} \text{ mg TEQ/kg/day} = \frac{(\text{CS} \times 114 \text{ mg soil/day} \times 10^{-6} \text{ kg/mg} \times 1 \times 350 \text{ days/year} \times 30 \text{ years})}{(70\text{kg} \times 25{,}550 \text{ days})}$$

Thus, solving for CS, the equation looks like this:

CS (mg TEQ/kg) = $(2 \times 10^{-13}$ mg/kg/day)/$(6.693 \times 10^{-7}$/day)

CS (mg TEQ/kg) = 2.99×10^{-7} mg/kg

Or CS can be expressed as follows:

CS (pg TEQ/kg) = 299 pg TEQ/kg soil

Thus, acceptable soil concentrations using generic risk assessment equations and assumptions results in an acceptable total TEQ in soil of 299 pg TEQ/kg soil at a one in one million risk level. If a risk level of one in one hundred thousand (1×10^{-5}) is used, the acceptable value in soil would increase to 2,990 pg TEQ /kg soil etc. These are extremely small concentrations of total TEQ and are significantly smaller than the typical urban background concentrations reported in Table 4-5 of Part III (Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds). In Table 4-5, typical urban total TEQ is reported to range from 7,000 to

20,000 pg/kg. The risk based cleanup standard is 23 times lower than the lowest urban background value reported in Table 4-5.

There are a host of risk management issues that arise from such a large change in the slope factor. Given the significant uncertainty associated with the derivation of such, the uncertainty associated with the underlying toxicology and the use of a dose metric that may not adequately describe exposure, it seems premature to put the country through such an ordeal. Moreover, given the significant risk management issues associated with the assessment, it is very important the agency consider the comments by the SAB submitted in 1995, review the comments of the expert panel and to carefully review the science behind the assessment in order to produce a state of the art risk assessment for TCDD and related compounds.

References

Abraham, K., A. Geusau, et al. (1999). "Biomarkers in three subjects from Vienna highly exposed to 2,3,7,8-TCDD: CYP induction and immune function." Organohalogen Compounds **44**: 47-50.

Aylward, L., L., S. M. Hayes, et al. (1996). "Relative susceptibility of animals and humans to the cancer hazard posed by 2,3,7,8-tetrachlorodibenzo-p-dioxin using internal measures of dose." Env. Science Tech. **30**(12): 3534-3543.

Gale, R. W., E. R. Long, et al. (2000). "Evaluation of planar halogenated and polycyclic aromatic hydrocarbons in estuarine sediments using ethoxyresorufin-O-deethylase induction of H4IIE cells." Environ. Toxicol. Chem. **19**: 1348-1359.

Geusau, A., M. Mexinar, et al. (1999). "Chloracne and its clinical and laboratory implications in two patients with 2,3,7,8-tetrachlorodibenzo-p-dioxin intoxication in Vienna." Organohalogen Compounds **44**: 45-46.

National Cancer Institute (2000). CancerNet**:** Internet Site.

Jones, P. D., J. P. Giesy, et al. (1993). "2,3,7,8-Tetrachlorodibenzo-p-dioxin equivalents in tissue of birds at Green Bay, Wisconsin, USA." Arch. Environ. Contam. Toxicol. **24**: 345-354.

Kimbrough, R. D., M. L. Doemland, et al. (1999). "Mortality in male and female capacitor workers exposed to polychlorinated biphenyls." J. Occ. Env. Med. **41**(3): 161-171.

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

Koopman-Esseboom, C., M. Huisman, et al. (1995). Effects of PCB/dioxin exposure and feeding type on the infant's visual recognition memory. Dissertation entitled: Effects of perinatal exposure to PCBs and dioxins on early human development. Rotterdam, Erasmus Universiteit**:** 75-86.

Koopman-Esseboom, C., N. Weisglas-Kuperus, et al. (1995). Effects of PCB/dioxin exposure and feeding type on the infant's visual recognition memory. Dissertation entitled: Effects of perinatal exposure to PCBs and dioxins on early human development. Rotterdam, Erasmus Universiteit**:** 107-212.

Koopman-Esseboom, C., N. Weisglas-Kuperus, et al. (1996). "Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and pyschomotor development." Pediatrics **97**: 700-706.

Roth, J. and C. Grunfield (1981). Mechanism of action of peptide hormones and catecholamines. Textbook of Endocrinology. J. D. Wilson and D. W. Foster. Philadelphia, W.B. Saunders Company**:** 113.

Safe, S. (1997/1998). "Limitations of the toxic equivalency factor approach for risk assessment of TCDD and related compounds." Teratog. Carcinog. Mut. **17**: 285-304.

USEPA (1996). Proposed guidelines for carcinogen risk assessment. Federal Register. **61:** 17960-18011.

USEPA (1999). Revised proposed guidelines for carcinogen risk assessment.

Walker, N. J., A. M. Tritscher, et al. (2000). "Hepatocarcinogenesis in female Sprague-Dawley rats following discontinuous treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin." Toxicol. Sciences **54**: 330-337.

Willet, K. L., P. R. Gardinali, et al. (1997). "Characterization of the H4IIE rate hepatoma cell bioassay for evaluation of environmental samples containing polynuclear aromatic hydrocarbons (PAHs)." Arch. Environ. Contam. Toxicol. **32**: 442-448.

HARRIS ENVIRONMENTAL RISK MANAGEMENT, INC.

1900 BLUFFVIEW COURT
FLOWER MOUND, TX 75022
972-691-8338
972-691-8099 (FAX)

Federal Express
July 13, 2000

Ms. Kate Schalk
Peer Review Workshop Manager
Eastern Research Group, Inc.
110 Hartwell Avenue
Lexington, MA 02421-3136

RE: Comments on Chapters 8 and 9 and the Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds of the USEPA Dioxin Reassessment

Dear Ms. Schalk:

Please find attached comments on the above referenced documents. I have enclosed a disk with the comments and this letter on it in a Microsoft Word file as well as an Adobe PDF file. If you have any questions about these comments, please feel free to contact me at (972) 691-8838.

Sincerely,

Mark Harris, Ph.D.
President

Comments on Chapters 8 and 9 and the Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds of the USEPA Dioxin Reassessment

Topic 2

Question 3

Secondary Reviewer

EPA Question: The SAB commented that previous dose-response modeling was too limited to biochemical endpoints (CYP1A1, CYP1A2 etc.). Are the calculations of a range of EDO1 body burden for non-cancer effects in rodents responsive and clearly presented? Please comment on the weight-of-evidence interpretation of the body burden data associated with a 1% response rate for non-cancer effects that is presented in Chapter 8, Appendix I and Figure 8-1 (where EPA considers that the data best support a range estimate of ED01 body burdens between 10 ng/kg to 50 ng/kg).

Comments

To address this question, pages 8-11 through 8-14, pages 8-28 through 8-66, Figure 8-1, Figure 8-2, Table 8-4, Table 8-5, Table 8-6, Table 8-7 and Appendix I were reviewed.

Comment 3-1: The assessment does not provide an adequate basis for the selection of the body burden dose metric over other methods such as area under the curve (AUC) for use in determining EDO1's. A previous paper has suggested that the use of AUC is the appropriate dose metric (Aylward, Hayes et al. 1996). Additionally, in its review of the original reassessment document, the Science Advisory Board (SAB) stated the following:

> *"The document repeatedly referred to "average body burden" as a biologically meaningful dose metric, even though other measurements of dose associated with peak intake may be more important for some effects, and that "area under the curve" is the preferred dosimetric for dealing with agents with long biologic half-lives."*

This comment by the SAB has not been addressed in the assessment. Additionally, the selection of a dose metric appears to be key to the conclusions made in the reassessment document. With regard to non-carcinogenic ED01's developed in the assessment, the use of the body burden dose metric was used to compare across species and studies. Thus, it is important from a clarity perspective to

document why this particular dose metric was used as opposed to AUC or others (Cpeak etc.). Does the body burden metric used in the assessment account for duration of exposure? All of this being said, the assessment is correct to attempt to utilize a dose metric other than administered dose. However, it seems apparent that the assessment needs to take the next step and utilize a more sophisticated dose metric probably including the use of a PBPK model (even a simple one). These models can be used on animal data and also scaled to predict human concentrations. Thus, while it appears that the current dose metric is not sufficient for the assessment, the assessment is clearly headed in the right direction.

Comment 3-2: The information in Table 8-5 needs to be better identified to assist the reader in understanding the data. Specifically, the values in parenthesis need to be identified via a footnote. The text of Chapter 8 describes the meaning of these values in parenthesis. However, tables should be able to stand on there own in a document such as this.

Comment 3-3: In its review of the previous reassessment, the SAB commented that the document should provide enough information such that the calculations performed in the assessment could be reproduced by others. However, it would be difficult for anyone to reproduce the calculated EDO1's in Chapter 8 given the information presented in Appendix I. Given that the assessment document is so large, it does not seem to be out of place to add an additional 50 pages to provide the actual model inputs for each of the endpoints/studies. This would allow others to easily verify the calculated EDO1's.

Comment 3-4: The current version of BMDS is 1.2. However, version 1.1 was used to calculate body burden EDO1's for the non-carcinogenic endpoints. The assessment should confirm that the results presented will not change significantly due to the updating of the model.

Comment 3-5: Has the BMDS modeling software been published or subjected to scrutiny outside of the USEPA? How do the results with this software package compare to other similar software packages using the same input data? The assessment should provide some documentation about this model and probably should refer readers to the EPA Web-Site where there is significant information about this model.

Comment 3-6: The range of body burden EDO1's is large as illustrated in Figure 8-1 and Appendix I. The agency discusses this briefly in the text and in Table 8-5. However, is it possible to expand

this discussion as to why the ranges are so large (species differences, sex differences etc.)? The large ranges make it difficult to arrive at any particular lower range (e.g., the 10 ng/kg to 50 ng/kg range as suggested in the assessment). Is the range unusually large due to the body burden dose metric? Another dose metric that better describes the exposure might work to "tighten" the range of EDO1's for the various endpoints and make a decision about a point of departure somewhat easier. It is suggested the assessment team consider the use of other dose metrics such as area under the curve (AUC) and the use of some simple PBPK models.

The impact of the suggested range (10 ng/kg to 50 ng/kg) is significant as it is a key reason why the agency concludes that it is not appropriate to determine a non-carcinogenic toxicity criteria value (RfD) for dioxin. However, the large range of body burden EDO1's make the selection on any particular range difficult. Currently, it is not possible to select a range given the variance in the data set.

Comment 3-7: Utilizing other dose metrics (AUC, Cpeak etc.), how do the conclusions regarding the EDO1 change for non-carcinogenic effects?

Comment 3-8: Is the Body Burden EDO1 for body weight in the study by Fox et al. (1993) (page 8-79 of the assessment) correct? It seems very low compared to the other body burdens for this particular endpoint.

Topic 3
Question 4
Primary Reviewer
EPA Question: How might the discussion of mode-of-action of dioxin and related compounds be improved?

To address this question, Section 3 of the Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds was reviewed (pages 39-47).

Comment 4-1: Section 3.2.3 (lines 17-23) list four lines of research that substantiate the role of the Ah receptor in dioxin toxicity. However, appropriate references are provided for only one of the four lines of research. Please insert appropriate references for structure-activity relationships, responsive versus non-responsive mouse strains and mutant cell lines.

Comment 4-2: This section differentiates between mode-of-action and mechanism-of-action and references both the 1996 USEPA Proposed Guidelines for Carcinogen Risk Assessment (USEPA 1996) and the 1999 USEPA Revised Proposed Guidelines for Carcinogen Risk Assessment (USEPA 1999) as the basis for this distinction. What is the status of the 1999 document? Has it been approved for use? If so, there is no need to reference the 1996 document. If not, why is the assessment referencing an unapproved document?

Comment 4-3: There is no discussion regarding the role of naturally occurring Ah receptor agonists and their potential impact on the proposed mode-of-action. Additionally, there should be a description of the potential antagonistic actions of certain compounds with regard to dioxin like activity and the mode of action description. This need not be more than a few sentences acknowledging that these have been identified.

Comment 4-4: Page 47 (lines 1-11) provides possible mechanisms by which TCDD (and related compounds?) might act as a tumor promoter and developmental toxicant. Both of these endpoints as well as others discussed in previous sections are not genotoxic events. Thus, it is recommended that at least some words be included that mention the possibility of a threshold in this mode-of-action (i.e., below some minimum number of occupied receptors, some minimum body burden etc that no relevant biological effect will occur).

Comment 4-5: Section 3.2.1 provides a list of criteria that describe receptor mediated activities. A reference for these criteria (page 42 lines 3-11) should be provided.

Comment 4-6: On page 45 (lines 30-33), the following statement is made:

> *"However, it cannot be assumed that an increase in receptor occupancy will necessarily elicit a proportional increase in all biological response(s) because numerous molecular events (e.g., cofactors, other transcription factors, genes) contributing to the biological endpoint are integrated into the overall response"*

The statement above is correct. However, the meaning of such could be improved with the use of a figure to illustrate the point. An example of an appropriate figure to illustrate this point that could be

adapted for this assessment can be found in the textbook entitled "Textbook of Endocrinology" page 113 (Roth and Grunfield 1981).

Topic 3
Question 5
Primary Reviewer

EPA Question: Despite the lack of congener-specific data, does the discussion in the Integrated Summary and Risk Characterization support EPA's inference that these effects may occur for all dioxin-like compounds, based on the concept of toxicity equivalence?

The section entitled "Part III: Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds" was reviewed to address to this question.

Comment 5-1: The referenced section of the assessment was reviewed and found to not adequately address this subject. Specifically, Section 1.2 and Pages 87-89 of Section 6 of Part III touch on this subject. However, the discussion in these two sections is limited and does not suffice to support the inference that the effects associated with TCDD may occur for all dioxin-like compounds based on the toxicity equivalence concept. There simply is not enough discussion of this concept in Part III.

Comment 5-2: In light of comment 1 above, this portion of the assessment needs to include a section that directly addresses this subject (toxic equivalency).

Comment 5-3: When developing the text for Part III to support the concept that other dioxin-like compounds behave toxicologically similar to that of TCDD, the assessment should address the significant lack of data regarding half-life values for other dioxin-like compounds, the lack of human epidemiology data for these compounds etc. and how this impacts our ability to make definite conclusions about these compounds. Indeed, the recent study by Kimbrough calls into the question the carcinogenic potential of PCBs (Kimbrough, Doemland et al. 1999) some of which are clearly dioxin-like. Why was this study not mentioned in the assessment? It seems to be clearly relevant to the matter at hand.

Topic 4

Question 6

Secondary Reviewer

EPA Question: Is the history, rationale and support for the TEQ concept, including its limitations and caveats, laid out by the EPA in a clear and balanced way in Chapter 9? Did EPA clearly describe its rationale for recommending adoption of the 1998 WHO TEFs?

Comment 6-1: The history, rationale and support for the TEQ concept is adequately developed and discussed in Chapter 9.

Comment 6-2: This Chapter does not adequately address the emerging issue of naturally occurring dioxin-like compounds. The assessment argues that due to the short half-life of these compounds the resulting body burden of these compounds is low and not significant compared to that of PCDDs, PCDFs, etc. However, an alternative explanation could be that the use of the body burden dose metric is incorrect for these compounds given that they do not bioaccumulate. Table 1 below presents data that is relevant to this discussion.

Table 1. Dietary Intakes and Toxic Equivalent (TEQ) Values for Exodioxins and Some Natural AhR Agonists*

Compounds	Dietary Intake (mass, pg/day)	Relative Potency	TEQs (pg/day)
Antiestrogenicity			
PCDDs/PCDFs	1000-2000	-	120
I3C**	735,000,000	0.0001	73,500
ICZ (derived in gut from I3C)	73,500	0.01	735
PAHs	$1.2 – 5 \times 10^6$	0.001	1200-5000
Immunotoxicity			
PCDDs/PCDFs	1,000-2,000	-	120
I3C	735,000,000	0.0001	73,500
PAHs	$1.2 – 5 \times 10^6$	0.0001	120-500

*Table adapted from (Safe 1997/1998)

**I3C: indole-3-carbinol;

The naturally occurring dioxin-like compounds contribute the bulk of the TEQ in this example (table reproduced from Safe, 1997/1998). This paper and the conclusions of such should be addressed in Chapter 9 in order to have a complete discussion. Currently, this paper is not cited in the assessment. Additionally, one can infer from the SAB 1995 report (Section 4.12.1) that the use of body burdens or AUC dose metrics is not appropriate for short lived compounds in the body. The SAB states in this section the following:

> ".…….and that "area under the curve" is the preferred dosimetric for dealing with agents with long biologic half-lives."

At a minimum the assessment should address what is the appropriate dose metric for the short-lived naturally occurring dioxin-like compounds. It seems likely that in this situation, administered dose would be sufficient metric.

Comment 6-3: Page 9-16 (lines 14-22) states the following:

> *"Although Safe has suggested that exposure to natural AhR ligands is 100 times that of TCDD and other dioxin-like chemicals (Safe, 1995), the impact of the natural AhR ligand is uncertain. Epidemiological studies suggest that human exposures to TCDD and related chemicals are associated with adverse effects such as development impacts and cancer. In many of these studies, the exposed populations have approximately 100 times more TCDD exposure than background populations (Chapter 7). If the exposure to natural AhR ligands is included in these comparisons, then exposed populations should have only about 2 times higher total TEQ exposures than the background population. It seems unlikely that epidemiological studies could discriminate between such exposures."*

In reality, the epidemiology associated with dioxin has not provided conclusive evidence regarding cancer effects even when exposures have been very large (e.g., NIOSH cohorts). An alternative explanation is that because the dioxin exposure at the phenoxy herbicide plants made up only a small fraction of the total TEQ received by an individual, the epidemiology studies are unable to discern any significant differences in health outcomes.

Comment 6-4: A recent paper (published after the release of the draft assessment) provides a good example of unknown compounds eliciting dioxin like activity. In this particular study, sediments from a river known to contain elevated concentrations of PAHs, PCBs, PCDDs and PCDFs were divided into dioxin containing fractions, PCB containing fractions and PAH containing fractions through an elaborate fractionation procedure (Gale, Long et al. 2000). The various fractions of the sediment were introduced into the H4IIE bioassay and ethoxyresorufin-O-deethylase (EROD) induction measured. Interestingly, the whole sediment extract contained significantly more dioxin TEQ than the various subfractions of the sediment summed together suggesting the presence of other dioxin like compounds. This is an important finding that should be discussed in Chapter 9. Furthermore, the results of this paper suggest that Chapter 9 needs to include TEFs for PAHs as 80% of the attributed to PCDDs, PCDFs, PCBs and PAHs was associated with PAHs in the sediment. A second example of this is provided in (Jones, Giesy et al. 1993). In this case, analytically determined TEQ underestimated the bioassay determined TEQ by an average of 57%. Given the conclusions of the assessment, it will be important that the entire universe of dioxin-like compounds be identified and quantified for potency. Clearly, the compounds identified to date as "dioxin-like" do not account for all of the TEQ in the environment. In some cases, such as the two examples above, the underestimation is great.

Comment 6-5: Why are there no TEFs for polynuclear aromatic hydrocarbons (PAHs)? These compounds clearly possess dioxin-like activity and are present in the environment. A recent paper (Gale, Long et al. 2000), evaluated dioxin-like activity in sediment known to contain PCDDs, PCDFs, PCBs and PAHs. This group (USGS and NOAA staff) found that PAHs contributed more than 80% of the TEQ contributed by PCDDs, PCDFs, PCBs and PAHs as a group. This group calculated total TEQ using toxic equivalency factors for each of these group of chemicals. For PAHs, TEFs were used as suggested by (Willet, Gardinali et al. 1997). It is suggested that TEFs for PAHs be included in the assessment. Given the conclusions of the assessment, it will be important to accurately account for all of the TEQ in the environment and to determine exposure to such.

Topic 10

Question 18

General Reviewers – All

EPA Question: Does the summary and analysis support the conclusion that enzyme induction, changes in hormone levels, and indicators of altered cellular function seen in humans and

laboratory animals, represent effects of unknown clinical significance, but that may be early indicators of toxic response (pages 84-86)?

Comment 18-1: Page 84 (lines 22-24) state the following:

> *"These effects will likely range from biochemical changes at or near background levels of exposure to adverse effects with increasing severity as body burdens increase above background levels"*

What is the basis for suggesting that effects will begin to occur at or near background? The data presented in Part II Section 7b generally involve human study groups with elevated exposure to PCDDs, PCDFs or PCBs with effects that appear to be due to random chance in studies (i.e., not consistent among multiple studies) or probably due to confounding exposures (TCP production workers). Additionally, the conclusion regarding background exposure and toxic responses is strongly influenced by the selection of body burden as the appropriate dose metric. The continued use of the body burden dose metric needs to be justified. Why was AUC not used as previously suggested by the SAB? Additionally, the range of 10 ng/kg to 50 ng/kg body burden in animals as a point of departure may be playing a role in supporting such a statement. It needs to be noted that the range of EDO1 body burdens for various toxic endpoints in animals varied by orders of magnitude and thus the selection of the 10 ng/kg to 50 ng/kg may not be appropriate and introduces a large amount of uncertainty into the statement.

Comment 18-22: Based on the data in this assessment and including the mode-of-action concept, it seems likely that two conclusions that could be made are as follows:

1. The mode-of-action of TCDD and related compounds are likely to be similar in humans and animals
2. Given the appropriate dose of TCDD or related compounds, similar toxic responses would be expected in humans as in seen in animals.

Of course, the key to the second statement is what is a dose that will elicit a toxic response in humans.

Comment 18-3: Regarding the unknown clinical significance of these various biochemical endpoints, it should be noted that not very many altered biochemical endpoints have been observed

in exposed populations that can be linked to exposure to TCDD or related compounds. Thus, it seems premature to make this statement.

Comment 18-4: I note that for some of the Dutch studies cited in Section 7b, a dissertation was cited (Koopman-Esseboom, Huisman et al. 1995; Koopman-Esseboom, Weisglas-Kuperus et al. 1995). Are these appropriate citations or references to use in the assessment? The reference information indicates that these documents are chapters from a "dissertation". Have these documents undergone critical, unbiased peer review? These references are somewhat confusing as there is more than one author suggesting that this is not a Ph.D. dissertation. Please clarify.

Comment 18-5: On Page 85 of the Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds (lines 11 –12) it states the following:

> *"In other words, evaluation of the available data suggests that humans, in general, are neither extremely sensitive nor insensitive to the individual effects of dioxin-like compounds"*

The basis for this statement is unclear. The NIOSH cohort experienced significant exposure to TCDD and related compounds yet little if any adverse health events have been noted. Moreover, except for chloracne, there is not a lot of data suggesting any adverse impacts in the human population. Thus, the basis for this statement is unclear.

Comment 18-6: On Page 85 of the Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds (lines 16-21) it states the following:

> *"In TCDD-exposed men, subtle changes in biochemistry and physiology such as enzyme induction, altered levels of circulating reproductive hormones, or reduced glucose tolerance and, perhaps, diabetes, have been detected in a limited number of epidemiologic studies. These findings coupled with knowledge derived from animal experiments, suggest the potential for adverse impacts on human metabolism, and developmental and/or reproductive biology, and perhaps, other effects in the range of current human exposure."*

This text overstates what is known about TCDD and related compounds. The TCDD-exposed men (NIOSH cohort, German cohorts etc.) were exposed to numerous chemicals during their employment which could have impacted a host of biochemical and toxicological endpoints. Presented below is a list of chemicals known to be present at the Newark, New Jersey Phenoxy Herbicide Plant during operation (data from Remedial Investigation report on the site):

1. monochloroacetic acid
2. hexachlorobenzene
3. 2,4,6-trichlorophenol
4. 2,4-dichlorophenol
5. dichlorodiphenyltrichloroethane
6. p-chlorophenyl-p-chlorobenzene sulfonate (ovex)
7. 1,1,1-trichloroacetaldehyde
8. benzenesulfonyl chloride
9. p-chlorobenzenesulfonyl chloride
10. p-chlorobenzenesulfonamide
11. 4,4'-dichlorodiphenylsulfone
12. p-acetylaminobenzene sulfonyl chloride
13. p-methoxybenzene sulfonyl chloride
14. 1,2,4,5-tetrachlorobenzene
15. amine salts of N-oleyl-1,3-propylenediamine
16. Nicotine Sulfates
17. Muriatic Acid
18. 2,5-dichlorophenyl-p-chlorobenzene sulfonate
19. Lindane
20. Sodium 2,4,5-trichlorophenate
21. 2,4,5-trichlorophenoxyacetic acid
22. 2,4,5-T ester
23. 2,4,5-T amine
24. acetic acid
25. acetic anhydride
26. acetaldehyde
27. benzene
28. monochlorobenzene
29. tetrachlorobenzene
30. chlorosulfonic acid
31. methanol
32. oleum (20%)
33. phenol
34. sulfuric acid
35. dimethylamine
36. triethylamine
37. chlorine
38. 2-ethylhexanol
39. butyl alcohol
40. isopropyl alcohol
41. butoxyethoxypropanol
42. nicotine

43. sodium hydroxide

It is likely that similar lists could be generated for other plants in the NIOSH study. These data suggest numerous opportunities for confounding factors to be introduced into any analysis of the biochemical and/or toxicological impacts on these workers. These confounding factors should be considered when making conclusions about the epidemiology studies of phenoxy herbicide workers.

Additionally, this conclusion is based on the use of the body burden dose metric. The assessment needs to justify the use of this dose metric as opposed to others (e.g., AUC as suggested by the SAB).

Comment 18-7: Page 85 (lines 34-35) reference the Dutch studies where MDI and PDI indices were determined for breastfed and formula fed infants. Total TEQ was determined in the breast milk and formula etc. How much weight is this series of studies given in the assessment? I have only two of the papers published from this series of experiments. However, a review of Koopman-Esseboom, Weisglas et al (1996) suggests that interpretation of this paper is difficult at best (Koopman-Esseboom, Weisglas-Kuperus et al. 1996). Finally, the even the text in Part II Section 7b discussing these studies in detail casts doubt on the merit of these studies. It is suggested that the reference to the Dutch studies be removed or at a minimum not used to justify the concept of adverse effects at background exposure concentrations.

Comment 18-8: It seems likely that the total TEQ exposure by the general population is underestimated due to the fact the entire universe of dioxin-like compounds in the environment has not been identified yet. Thus, the underlying analyses supporting this statement have likely underestimated total TEQ exposure. How does this impact the conclusions of this section?

Topic 10
Question 19
General Reviewers - All
EPA Question: Has the short summary statement in the risk and hazard characterization on page 107 adequately captured the important conclusions, and the areas where further evaluation is needed? What additional points should be made in this short statement?

Comment 19-1: Page 85 (lines 10-11) state the following:

> *"Some of these effects may be occurring in humans at general population background levels and may be resulting in adverse impacts on humans."*

This statement should be removed from the text. The assessment has not provided adequate support for such a statement. The assessment utilizes a new dose metric for risk assessment to evaluate exposure (body burden) without any sort of justification for such. Why was other dose metrics not evaluated? Clearly the SAB and others (Aylward, Hayes et al. 1996) have suggested other appropriate metrics. The comparison of background levels and adverse effects is based on very little information (e.g., use of body burden EDO1 from animal studies in which the ranges of such varied by orders of magnitude and several studies in the Netherlands of questionable utility).

Finally, there was recently two papers published that described an acute exposure to 2,3,7,8-TCDD in Vienna (Abraham, Geusau et al. 1999; Geusau, Mexinar et al. 1999) by two individuals. These authors report up to 144,000 ppt TCDD in blood lipid and a lack of biochemical effects. Chloracne in these individuals was very severe. However, the lack of biochemical responses is significant given the conclusions of the assessment. These papers should be discussed and evaluated in the assessment (probably Chapter 7B and in Part III). Results such as these make it difficult to believe that the general population is suffering adverse effects from TCDD and related compound exposure.

Comment 19-2: If background exposures are indeed causing effects in the general population, then it seems reasonable that the NIOSH cohort would have experienced significant adverse responses. However, the bulk of these studies have failed show any significant adverse responses in the study populations. Additionally, general population background exposures have been decreasing for a long period of time. Should not adverse responses attributable to TCDD and related compounds been observable 20 to 30 years ago in the general population if the current population exposure is borderline (i.e., body burden was much greater 20 years ago than it is today in the general population).

Comment 19-3: Somewhere in this statement, the great uncertainty associated with this whole exercise needs to be communicated. We do not know the actual exposures at the phenoxy herbicide plants (we can only make estimates), we are unsure about the mechanism of action, we are unsure about the significance of naturally occurring dioxin-like compounds in the diet, we are unsure about the appropriate dose metric, the epidemiology studies cannot demonstrate an effect etc.

Comment 19-4: It is recommended that the statement be revised to reflect the uncertainty in this analysis and the text regarding impacts to the general population be removed.

Topic 11
Question 21
General Reviewers - All

EPA Question: Please provide any other comments or suggestions relevant to the two review documents, as interest and time allow.

Comments on the Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachloro-p-dioxin (TCDD and Related Compounds

General Comment: Throughout the assessment the issue of statistical significance is not clear. If the response or measurement is not statistically significant then it is not different than the control measurements. The assessment needs to be very clear what is statistically significant and what is not. For example, on Page 15 (Line 21-22), the assessment states the following:

> *"…..which had a borderline statistically significant increase in breast cancer….."*

Use of this language is not appropriate in a scientific document and the meaning is unclear as well. Was the effect just barely significant or was it not significant but close to being such?

Specific Comments

Comment 21-1: The assessment needs to further discuss the confounding factors associated with the epidemiology studies used for dose response modeling in Section 8. For example, the assessment needs to point out that these workers were exposed to a hosts of substances (a list of some are provided above).

Comment 21-2: This section (Part III) needs to point out that TCDD is really the first multi-site carcinogen ever identified (except maybe radiation) and discuss the significance of this. I find this concept to be a bit odd for a chemical.

Comment 21-3: The dose response modeling only utilized TCDD serum concentrations (converted to body burdens). What is the effect of ignoring likely exposure to other dioxin-like compounds at these facilities? Does not this approach result in an overestimation of the slope?

Comment 21-4: The SAB commented that all of the calculations used in the dose response calculations should be provided such that they could easily be reproduced. However, I was unable to find the details regarding the modeling in Chapter 8 that could be used to reproduce the EDO1's in animals. In the final document, these details need to be provided.

Comment 21-5: Page 7 (Lines 18-19): The assessment provides no justification for the selection of the body burden dose metric. Why not the AUC method? The AUC method has been published previously on this very subject (using data from the NIOSH cohort) and reached different conclusions about TCDD and related compounds.

Comment 21-6: Page 7 (Line 19): The text at this location mentions the following:

> *"recent data demonstrating that either allometric scaling or uncertainty factors underestimate the species differences….."*

References should be provided to support this assertion.

Comment 21-7: Page 8 (Line 2): The text at this location states the following:

> *"Evidence supports the assumption that TCDD and related compounds……"*

References should be provided to support this statement.

Comment 21-8: Page 8 (Lines 2-6): Has the body burden method been validated for humans and low dose exposures? Additionally, how does this method differ from the AUC method recommended by the SAB in 1995?

Comment 21-9: Page 8 (Lines 14-15): The text states the following:

"In addition, the sequestration is dose dependent, and at human background exposures, hepatic sequestration should not be significant."

References should be provided to support this statement.

Comment 21-10: Page 8 (Lines 16-35): Is this description of the body burden method complete? Should not a description of how the daily doses were calculated be provided as well?

Comment 21-11: Page 9 (Lines 11-16): The text states the following:

"The use of body burden, for many effects within species and, particularly, for cross-species scaling, appear to provide a better dose metric than daily dose."

The assessment needs to provide references to justify this statement. Furthermore, the SAB recommended that AUC be used (Section 4.12.1 of the SAB report). What if AUC had been used? How would this have impacted the conclusions of the assessment?

Comment 21-12: Page 14 (Lines 22-23): The text states the following:

"…….dioxin-contaminated phenoxy herbicides and increased cancer risk involved an increase in soft tissue sarcoma……"

Soft tissue refers to tissues that connect, support, or surround other structures and organs of the body. Soft tissue includes muscles, tendons (bands of fiber that connect muscles to bones), fibrous tissues, fat, blood vessels, nerves, and synovial tissues (tissues around joints) (National Cancer Institute, 2000). Is the Ah receptor present in these tissues? If so, a reference should be provided.

Comment 21-13: Page 15 (lines 19-27): Given the anti-estrogenicity of TCDD, why would one expect increases in breast cancer?

Comment 21-14: Page 16 (Lines 3-9): The assessment should point out that TCDD is the first multi-site carcinogen ever discovered (except maybe radiation). No where in the assessment is the reason for this unusual finding discussed? A discussion of why this is the case should be provided.

Comment 21-15: Page 17 (Line 18): The assessment cites Walker, Tritscher et al., 2000 which was in press when the assessment was published. However, the paper has now been published (Walker, Tritscher et al. 2000) and several key findings in this paper should be discussed including:

1. The liver half-lives for female Sprague-Dawley rats was reported to vary with dose. The larger the dose the longer the liver half-life. Does this observation have any significance in the dose response modeling of rat body burdens?

2. Female Sprague-Dawley rats (non-DEN initiated) treated with 125 ng/kg/day TCDD for 60 weeks did not develop adenomas or carcinomas in the liver. This appears to be significant as this daily dose is in excess of the Kociba, Keyes et al., 1978 study where a daily dose 100 ng/kg/day clearly caused an increase in cancer in hepatic tissue of the female Sprague-Dawley rats. Using the body burden approach suggested in the assessment, the Walker, Tritscher et al. 2000 study would be expected to produce a much greater body burden than that of Kociba, Keyes et al., 1978. However, while Walker, Tritscher et al., 2000 produces a much higher body burden it also produces no cancer. In contrast, the Kociba, Keyes et al., 1978 study has a lower body burden (per the assessment calculation) and elevated hepatocellular cancer (11 out 49 rats). The key difference in the two studies was the duration of exposure. In the Kociba, Keyes et al., 1978 study, the exposure was for a lifetime (two years for a rat) and in the Walker, Tritscher et al, 2000 study, exposure was about 60 weeks. The assessment should address these apparent discrepancies. Thus, it appears that the body burden dose metric fails to differentiate the two studies.

3. Given the comment on Walker, Tritscher et al, 2000 above (item 2), would another dose metric provide a different perspective on the two doses (i.e., AUC)?

4. The daily dose of 125 ng/kg/day is 162 times greater than the "animal intake for 1% excess risk" for liver cancer in female Sprague-Dawley rates (Table 5-2) yet no cancer was observed in non-DEN initiated rats. How does the Walker, Tritscher et al., 2000 study impact the validity of this modeled value?

5. Is the lack of a time component in the dose metric used in the assessment a serious shortcoming of the method?

Comment 21-16: Page 20 (Lines 10-14): The text of this bullet is unclear.

Comment 21-17: Page 23 (Line 9): The text states the following:

> *"…..concentrations were slightly, but not significantly, higher in Ranch Hands…"*

This is not an appropriate statement. Either they were statistically different or they were not. The fact that one measurement is slightly greater than the other in absolute value is meaningless.

Comment 21-18: Page 24 (Line 21): Who is Peterson?

Comment 21-19: Page 36 (Line 32): The text states the following:

> *"…..borderline significance, among Vietnam…."*

Again, the meaning is unclear. Either it is significant or it is not. The word borderline confuses the meaning of the sentence.

Comment 21-20: Page 37 (Lines 1-2): Why is the data of Suskind and Hertzberg, 1984 not included in Table 7-23 (Chapter 7)?

Comment 21-21: (Page 37 (Lines 3-11): How does exposure to so many other chemicals at phenoxy herbicide plants impact the conclusions about GGT (see comment 18-6 for a list of chemicals at the Newark, New Jersey plant)? Additionally, why did the workers at the plant in Great Britain not exhibit elevated GGT while workers at other plants did? The authors of the studies that describe the acute exposure in Vienna should be contacted to determine if these enzymes were elevated in their patients (Abraham, Geusau et al. 1999; Geusau, Mexinar et al. 1999).

Comment 21-22: Page 65 (Lines 27-29): The text states that the following:

> *"At this time, the knowledge of the mechanism of action of dioxin, receptor theory, and the available dose response data do not firmly establish a scientific basis for replacing a linear procedure for estimating cancer potency."*

This statement is not responsive to the SAB comments in 1995. Clearly, the SAB indicated that threshold models be evaluated in the reassessment. At a minimum, the revised assessment should provide some mathematical basis for this conclusion.

Comment 21-23: Page 66 (Lines 21-22): The text states the following:

> *"……multitude of metrics (DeVito et al., 1995) such as daily intake (ng/kg/d), current body burden (ng/kg), average body burden over a given period of time, plasma concentrations etc…."*

Why was average body burden over a given period of time not selected, or plasma concentrations etc.? A rationale needs to be provided.

Comment 21-24: Page 67 (Lines 7-8): The text states the following:

> *"….species and humans (Table 8-2), this dose metric appears to be the most practical for this class of compounds…."*

The use of AUC was suggested by the SAB in 1995 (Section 4.12.1). Furthermore, the SAB states the following:

> *"…and that "area under the curve" is the preferred dosimetric for dealing with agents with long biologic half-lives."*

Why was this dose metric not used or at least compared and contrasted with body burden? This would at least make the document responsive to the SAB comment.

Comment 21-25: Page 71 (Lines 33-34): The text states the following:

> *"……suggests that exposure may be associated with increases in all cancers combined, in respiratory tumors and, perhaps, in soft tissue sarcoma."*

Was smoking controlled for in the studies that found increased respiratory tumors? If not, how does this effect the assessment and this particular statement? Additionally, at least in the NIOSH cohort, confounding factors (other chemicals) were clearly present - how is this accounted for in the assessment? In the NIOSH cohort, all cancers combined were elevated. However, how much of the elevation in total cancer can be accounted for by an elevation of respiratory tumors? Specifically, is the elevation of all cancers combined a function of respiratory tumors? If so, how does this impact the analysis given the lack of information about smoking. Finally, is the Ah receptor present in soft tissue sarcomas? If not, how can this be a target tissue. If so, a reference should be noted somewhere in the document illustrating the presence of such. Have other chemicals been evaluated by looking at "total cancer increase" as opposed to specific tissues?

Comment 21-26: Page 73 (Line 27): The text references a Table 8.3.2? No such table is present in the Integrated Summary Document or in Chapter 8 of Part II?

Comment 21-27: Page 74 (Lines 21-32): What is the conclusion utilizing other dose metrics such as AUC?

Comment 21-28: Page 75 (Lines 2-3): The text states the following:

> *"At least for this comparison, if cancer is a function of average levels in the body, the most appropriate metric for comparison is the average or steady-state body burden."*

This statement seems to contradict the request of the SAB in 1995 where they suggested the use of AUC. Furthermore, the SAB seemed somewhat critical of the dose metric used in this assessment. Additionally, what is the scientific basis for the use of average body burden levels in the body as a dose metric? Clearly, Kociba, et al., 1978 and Walker, Tritscher et al., 2000 provide conflicting results using this dose metric.

Comment 21-29: Page 76 (Line 18): The text references a Table 8.3.1. There is no such table present in the Integrated Summary (Part III) or in Part II Chapter 8.

Comment 21-30: Page 76 (Lines 32-35): Failure to account for exposure to other dioxin-like compounds can result in a overestimation of the slope factor. A doubling of the slope factor results

in acceptable levels in the environment being decreased by a factor two. Such matters should not be trivialized.

Comment 21-31: Page 89 (Lines 9-13): This bullet is unclear.

Comments on Chapter 8 Dose Response Modeling

Comment 21-32: Page 8-12 (Bottom Paragraph continuing onto Page 8-13): The assessment should discuss the lack of a time component in the body burden calculation. Specifically, the assessment should discuss how the body burden metric can or cannot be used to compare the differences between the Kociba, Keyes et al., 1978 studies and the recently published Walker, Tritscher et. al., 2000 study. Both had approximately equivalent daily doses but the results of the studies were exactly opposite. The Kociba, Keyes et al., 1978 study administered TCDD for a lifetime in female rats whereas Walker, Tritscher et al., 2000 only administered TCDD for 60 weeks. However, using the body burden method, Walker, Tritscher et al., 2000 would have a greater body burden than that of Kociba, Keyes et al., 1978? Is this a shortcoming of the body burden dose metric? How does this impact the dose response modeling for TCDD?

Comment 21-33: page 8-13 (Equation in Footnote): Where is the "f" in this equation?

Comment 21-34: Page 8-15 (Section 8.2.3.): Walker, Tritscher et. al., 2000 suggests that the liver half-life for TCDD varies with dose. The article suggests that the larger the dose the longer the half-life in the liver. Does this new finding have any bearing on the dose response modeling? The last sentence of this section indicates that various measures of body burden will be used? Is this a correct statement? What other methods were used?

Comment 21-35: Page 8-17 (Section 8.3.2.1.1): In its 1995 report, the SAB suggested that the assessment document provide all of the necessary information in the report so that others could reproduce the calculations (Section 4.9.1 of the SAB report). However, the discussion on pages 8-16 through 8-19 and Table 8-2 do not provide sufficient information for others to reproduce the calculations. It should not be difficult to add additional tables providing the exact model input data, assumptions etc. so that others can reproduce the calculations.

Comment 21-36: Page 8-22 (Section 8.3.2.4): This is an excellent discussion of the uncertainties associated with the dose response modeling. However, much of this uncertainty discussion never makes it into the Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds. Much of this uncertainty text needs to be inserted into Part III.

Comment 21-37: Page 8-30 (Last sentence on page carrying over to the top of page 8-31): The text states that

> *"In order to compare multiple-dose studies using different routes of exposure, the average daily dose was estimated for each study by calculating the total dose administered to the animal over the course of the study and dividing by the length of the study in days. In addition, for the multiple-dose studies, average steady-state body burden at the ED01 was calculated using the equation in Section 8.2.2 and the percentage of dose absorbed and the half-lives for TCDD in Table 8-1."*

The formula in Section 8.2.1 is as follows:

Body Burden (ng/kg) = daily dose(ng/kg/day) * half-life/ln(2) *f

And the formulas in Section 8.2.2 is as follows:

ED01 (ng/kg body burden) = ED01(ng/kg/day) * half-life/ln(2) *f

Where,

F = fraction absorbed

Half-life = see Table 8-1 (days)

What is unclear about this procedure is the concept of time. If one study administered 100 ng/kg/day of TCDD to rats for one year and another study administered 100 ng/kg/day to rats for two years, this particular dose metric (assessment body burden dose metric) would not capture the fact that one group of animals received twice the administered dose as the other. An extreme example of this issue is the comparison between (Kociba, Keyes et al. 1978) and Walker, Tritscher et al. 2000 and ignoring what the EDO1 dose might be. Using the largest daily dose of Kociba, Keyes et al., 1978 and the daily dose reported by Walker, Tritscher et al., 2000 one arrives at the following body burdens:

Kociba Body Burden = 100 ng/kg/day * [25 days/ln(2)] * 0.50 = 1804 ng/kg

Walker et al., 2000 Body Burden = 125 ng/kg/day * [25 days/ln(2)] * 1 = 4509 ng/kg

Where f =1 for TCDD administered via corn oil and 0.5 for TCDD administered via feed

Yet, in the Walker, Tritscher et al., 2000 no hepatocellular cancer was observed whereas in Kociba, Keyes et al., 1978 11 out of 49 rats developed hepatocellular carcinoma. The only difference between these two studies was the length of time of exposure (Walker, Tritscher et al., 2000 was for 60 weeks and Kociba, Keyes et al., 1978 was for 2 years) and one study administered the TCDD in corn oil and another via the feed. However, the body burden in the Walker, Tritscher et al., 2000 study is calculated to be 2.5 times greater than that in the Kociba, Keyes et al., 1978 study. This seems to verify that the appropriate dose metric has not been utilized in the assessment as it fails to provide an adequate comparison among two important studies.

Comment 21-38 (Page 8-31 Section 8.3.4.2): The assessment needs to provide the data inputs for the BMDS model for these multiple dose studies so that others can reproduce the results. This would be consistent with the SAB comments in 1995. The BMDS model has numerous options, parameters etc. that can be adjusted by the user. Unless, the assessment provides all of this information, it will be difficult for others to duplicate the results.

Comment 21-39 (Page 8-59, first full paragraph): The comment about PBPK models and the stating that:

> *"it is unlikely that predictions from such a model would be any less uncertain that current methodologies used for estimating body burdens"*

is interesting. Would even the less sophisticated PBPK models not account for differences in the length of exposure to TCDD in animal and human studies? Finally, the word "that" (end of first line) in the above quote should be the word "than".

Comment 21-40 (Table 8-1): Does Walker, Tritscher et al., 2000 and the finding of different TCDD liver half-lives depending on dose impact the TCDD rat half-life estimates in this table?

Comment 21-41 (Table 8-2): footnote "b": Was the Aylward, Hayes et al., 1996 study also used for this cohort in this modeling exercise? If so, it should be noted.

Comparison with SAB 1995 Comments

Comment 21-42 (Page 47) Section 4.1.1.: The SAB commented that

> *"Thus, the Committee recommends that EPA provide either additional discussions of alternative approaches and their implications for risk assessment in Chapter 8, or present a clear justification for choosing this particular dose-response approach over others…."*

The assessment has not provided an extensive discussion about various low dose extrapolation models and which should be used and why. In fact, the modeling discussion for the human studies is almost non-existent except for a reference to Breslow and Day, 1987. Much more detail including modeling input parameters need to be provided so that the results of such can be reproduced.

Comment 21-43 (Page 78, Section 4.9.1): The SAB states the following:

> *"EPA should describe its analysis in sufficient detail that it can be fully understood by the reader, to the point of reproducing the analysis if desirable."*

The assessment has not provided enough detail for the human or animals studies for the reader to reproduce the analyses.

Comment 21-44 (Page 80, Section 4.9.1): The SAB states that

> *"EPA's preferred dose response model is linear, it seems clear that a threshold model would provide an equivalent or nearly equivalent description of the data. This is the most important issue in the dose response-modeling and should be thoroughly explored in EPA's analysis."*

It is clear the assessment has not responded adequately to this comment. The concept of a threshold model is given very little coverage in Chapter 8. Chapter 8 of the assessment should explore the threshold type models in detail. If they do not work for some reason, then assessment should explain why they do not work (lack of power, lack of good fit etc.).

Comment 21-45 (Page 81, Section 4.9.2): The SAB made two key comments:

> *"Chapter 8 of the assessment document needs to describe and evaluate this alternative dose response relationship, discuss the approaches and findings of the other relevant agencies, and justify the basis for selecting another approach."*
>
> *"Thus, the document cannot ignore a possible threshold dose-response relationship and claim to be comprehensive in its presentation."*

These comments were offered by the SAB in the context of an RfD for TCDD discussion. However, it is noted that a discussion about how other agencies use threshold and safety factor methodology for their health risk evaluations is missing from the revised assessment. Thus, the assessment has not responded adequately to this SAB comment. Furthermore, the assessment has not adequately investigated the use of possible threshold models to describe the action of TCDD and related compounds.

Comments from a Risk Management Perspective

Comment 21-46: (Page 90 of Part III, Lines 8-12): The text states the following:

> *"Consequently, the Agency, although fully recognizing this range and the public health conservative nature of the slope factors that make up the range, suggests that the use of 5 x10^{-3} per pg TEQ/kgBW/day as an estimator of upper bound cancer risk for both background intakes and incremental intakes above background."*

The impact of such a slope factor on environmental activities is expected to be great if such a slope factor is utilized. Most states utilize USEPA toxicity criteria and many states set acceptable risk at 1 x 10^{-6}. The following calculation demonstrates the potential impact of such a slope factor on a risk specific soil cleanup standard calculation:

Cancer Risk	=	Slope Factor x Dose	**(Equation 1)**
1 x 10^{-6}	=	5,000,000 (mg TEQ/kg/day)$^{-1}$ x Dose (mg TEQ/kg/day)	
Dose (mg TEQ/kg/day)	=	(1 x 10^{-6}) / 5,000,000 (mg TEQ/kg/day)$^{-1}$	

Dose (mg TEQ/kg/day) = 2×10^{-13} mg/kg/day

Dose (mg TEQ/kg/day) = (CS x IR x CF x FI x EF x ED)/(BW x AT) **(Equation 2)**

Where, CS = chemical concentration in soil (mg TEQ/kg)

IR = Ingestion rate (mg soil/day) (Assume 114 mg/day)

CF = Conversion Factor (10^{-6} kg/mg)

FI = Fraction ingested from contaminated source (unitless) (Generally set at 1)

EF = Exposure frequency (days/year) (350 days/year)

ED = Exposure duration (years) (Assume 30 years)

BW = Body weight (kg) (Assume 70 kg)

AT = Averaging Time (period over which exposure is averaged - days) (25,550 days)

Substituting into the equation above we have the following:

$$2 \times 10^{-13} \text{ mg TEQ/kg/day} = \frac{(\text{CS} \times 114 \text{ mg soil/day} \times 10^{-6} \text{ kg/mg} \times 1 \times 350 \text{ days/year} \times 30 \text{ years})}{(70\text{kg} \times 25{,}550 \text{ days})}$$

Thus, solving for CS, the equation looks like this:

CS (mg TEQ/kg) = $(2 \times 10^{-13}$ mg/kg/day)/$(6.693 \times 10^{-7}$/day)

CS (mg TEQ/kg) = 2.99×10^{-7} mg/kg

Or CS can be expressed as follows:

CS (pg TEQ/kg) = 299 pg TEQ/kg soil

Thus, acceptable soil concentrations using generic risk assessment equations and assumptions results in an acceptable total TEQ in soil of 299 pg TEQ/kg soil at a one in one million risk level. If a risk level of one in one hundred thousand (1×10^{-5}) is used, the acceptable value in soil would increase to 2,990 pg TEQ /kg soil etc. These are extremely small concentrations of total TEQ and are significantly smaller than the typical urban background concentrations reported in Table 4-5 of Part III (Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds). In Table 4-5, typical urban total TEQ is reported to range from 7,000 to

20,000 pg/kg. The risk based cleanup standard is 23 times lower than the lowest urban background value reported in Table 4-5.

There are a host of risk management issues that arise from such a large change in the slope factor. Given the significant uncertainty associated with the derivation of such, the uncertainty associated with the underlying toxicology and the use of a dose metric that may not adequately describe exposure, it seems premature to put the country through such an ordeal. Moreover, given the significant risk management issues associated with the assessment, it is very important the agency consider the comments by the SAB submitted in 1995, review the comments of the expert panel and to carefully review the science behind the assessment in order to produce a state of the art risk assessment for TCDD and related compounds.

References

Abraham, K., A. Geusau, et al. (1999). "Biomarkers in three subjects from Vienna highly exposed to 2,3,7,8-TCDD: CYP induction and immune function." Organohalogen Compounds **44**: 47-50.

Aylward, L., L., S. M. Hayes, et al. (1996). "Relative susceptibility of animals and humans to the cancer hazard posed by 2,3,7,8-tetrachlorodibenzo-p-dioxin using internal measures of dose." Env. Science Tech. **30**(12): 3534-3543.

Gale, R. W., E. R. Long, et al. (2000). "Evaluation of planar halogenated and polycyclic aromatic hydrocarbons in estuarine sediments using ethoxyresorufin-O-deethylase induction of H4IIE cells." Environ. Toxicol. Chem. **19**: 1348-1359.

Geusau, A., M. Mexinar, et al. (1999). "Chloracne and its clinical and laboratory implications in two patients with 2,3,7,8-tetrachlorodibenzo-p-dioxin intoxication in Vienna." Organohalogen Compounds **44**: 45-46.

National Cancer Institute (2000). CancerNet**:** Internet Site.

Jones, P. D., J. P. Giesy, et al. (1993). "2,3,7,8-Tetrachlorodibenzo-p-dioxin equivalents in tissue of birds at Green Bay, Wisconsin, USA." Arch. Environ. Contam. Toxicol. **24**: 345-354.

Kimbrough, R. D., M. L. Doemland, et al. (1999). "Mortality in male and female capacitor workers exposed to polychlorinated biphenyls." J. Occ. Env. Med. **41**(3): 161-171.

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

Koopman-Esseboom, C., M. Huisman, et al. (1995). Effects of PCB/dioxin exposure and feeding type on the infant's visual recognition memory. Dissertation entitled: Effects of perinatal exposure to PCBs and dioxins on early human development. Rotterdam, Erasmus Universiteit**:** 75-86.

Koopman-Esseboom, C., N. Weisglas-Kuperus, et al. (1995). Effects of PCB/dioxin exposure and feeding type on the infant's visual recognition memory. Dissertation entitled: Effects of perinatal exposure to PCBs and dioxins on early human development. Rotterdam, Erasmus Universiteit**:** 107-212.

Koopman-Esseboom, C., N. Weisglas-Kuperus, et al. (1996). "Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and pyschomotor development." Pediatrics **97**: 700-706.

Roth, J. and C. Grunfield (1981). Mechanism of action of peptide hormones and catecholamines. Textbook of Endocrinology. J. D. Wilson and D. W. Foster. Philadelphia, W.B. Saunders Company**:** 113.

Safe, S. (1997/1998). "Limitations of the toxic equivalency factor approach for risk assessment of TCDD and related compounds." Teratog. Carcinog. Mut. **17**: 285-304.

USEPA (1996). Proposed guidelines for carcinogen risk assessment. Federal Register. **61:** 17960-18011.

USEPA (1999). Revised proposed guidelines for carcinogen risk assessment.

Walker, N. J., A. M. Tritscher, et al. (2000). "Hepatocarcinogenesis in female Sprague-Dawley rats following discontinuous treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin." Toxicol. Sciences **54**: 330-337.

Willet, K. L., P. R. Gardinali, et al. (1997). "Characterization of the H4IIE rate hepatoma cell bioassay for evaluation of environmental samples containing polynuclear aromatic hydrocarbons (PAHs)." Arch. Environ. Contam. Toxicol. **32**: 442-448.

REVIEW OF EXPOSURE AND HUMAN HEALTH REASSESSMENT OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) AND RELATED COMPOUNDS, EPA/600/P-00/001Ag

Part III: Integrated Summary and Risk Characterization for 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds

Holly A. Hattemer-Frey
SAF*Risk
1100 Sanders Road
Knoxville, TN 37923
(865) 531-9050
(865) 691-9652 (FAX)
e-mail: safrisk_tn@earthlink.net

TOPIC 1: BODY BURDENS

Question 1

General Reviewer

On page 8, para 1, the text notes that one limitation associated with estimating body burdens is the fact that rodents sequester more dioxins in the liver than humans and that this accumulation may lead to an under prediction of body burdens. The text claims the under prediction to be "relatively small" but says it could be as high as 50%. A 50% under prediction seems potentially significant to me. The discussion on dioxin accumulation in the liver, its role in predicting body burdens, and the differences between how rodents and humans sequester dioxin in the liver needs to be expanded.

TOPIC 2: USE OF MARGIN-OF-EXPOSURE APPROACH TO EVALUATE RISKS

Question 3

General Reviewer

I agree that less weight should be given to biochemical endpoints and more weight given to endpoints that are clinically-significant in humans. The text noted that many of the biochemical changes observed in animals and humans "represent effects of unknown clinical significance." Without compelling evidence that these changes are indicators of toxic response, such changes do

not represent a firm basis for risk evaluation.

TOPIC 6: CANCER EFFECTS
Question 10
General Reviewer

See response to Question 12. I agree with other scientists who contend that the cancer epidemiology data alone remain inadequate to label TCDD as a "known" human carcinogen.

TOPIC 6: CANCER EFFECTS
Question 12
Primary Reviewer

Revised upper-bound cancer slope factors (SFs) for dioxin based on current background body burdens and human toxicological data range from $5.3x10^{6}$ $(mg/kg\text{-}day)^{-1}$ to $2.4x10^{5}$ $(mg/kg\text{-}day)^{-1}$. These revised SFs are 2 to 33 times higher (more stringent) than EPA's previous slope factor of $1.6x10^{5}$ $(mg/kg\text{-}day)^{-1}$. Using current human body burden of 5 ng TEQ/kg and the range of upper-bound SFs noted above, revised upper-bound risk estimates range from $1.4x10^{-2}$ to $1.3x10^{-3}$, which is an increase of an order of magnitude at background exposure levels (pg 77). Upper-bound SFs based on animal cancer data range from $1.9x10^{6}$ $(mg/kg\text{-}day)^{-1}$ to $8.4x10^{4}$ $(mg/kg\text{-}day)^{-1}$, which are 2 times lower (less stringent) to 12 times higher than the previous SF. Using the body burden values based on animal data from the Kociba and Portier rodent studies adjusted for early mortality yields revised upper-bound SFs of $3.1x10^{6}$ $(mg/kg\text{-}day)^{-1}$ to $1.9x10^{6}$ $(mg/kg\text{-}day)^{-1}$, which are 12 to 19 times higher than the original SF. Derivation of a SF based on a new count of tumors observed in the Kociba study results in a revised SF of $8.3x10^{5}$ $(mg/kg\text{-}day)^{-1}$, which is fives times higher than the original SF. Hence, in general, all revised SFs are more stringent than the current value.

It is not clear from the sections reviewed exactly how these cancer SFs for TCDD were derived. It is not clear if a new mathematical calculation of the existing toxicological was done, or if the existing toxicological data were reevaluated and a higher incidence a cancer was

determined.

On page 90, para 1, the text recommends a new SF of $5x10^{6}$ $(mg/kg\text{-}day)^{-1}$ for dioxin, which is 32 times higher than the previous SF and yields an order of magnitude increase in cancer risk estimates (pg. 82, para 1). An order of magnitude increase in cancer risk estimates for background exposures seems unjustified, particularly given that “few statistically-significant effects" have been observed in affected cohorts (top pg. 86). The "doom and gloom" tone of the Risk Characterization section does not seem justified given that a causal relationship between dioxin and cancer incidence (despite high past exposures in some cohorts) has not been established. While I agree that the "compelling" data on the carcinogenic effects of TCDD in laboratory animals cannot be ignored, I disagree that laboratory results indicating that TCDD "probably" increases cancer mortality in animals and humans should dominate policy. More weight needs to be given to the available epidemiological data. Further the text on pg. 90, lines 1-5 states that the revised SF was based on the most sensitive cancer response in animals and humans. It would be useful if the text clarified whether the SF is actually based on animal or human data. If it is based on animal data, has a cancer response been observed in humans from exposure to similar levels of TCDD?

On pg. 14, lines 28-32, the text states that "the incidence of soft tissue sarcoma is elevated in several of the most recent studies." Elevated compared to what? Background incidence? Also, please note if these increases were statistically significant?

TOPIC 7: BACKGROUND AND POPULATION EXPOSURES
Question 13
Secondary Reviewer

I disagree that background exposures to dioxin-like compounds have been adequately characterized. Expansion/clarification of Table 4-2 would enhance the reader's understanding of background exposures. Recommend clarifying if the concentration values listed in Columns 3 and 5 and the contact rates in Column 2 are upper-bound or mean values. Recommend that Columns

2, 3, and 5 include mean and upper-bound values and that a range of intake estimates be presented in Columns 4 and 6 to provide a broader perspective on the range of possible background exposures. Also see response to Question 15.

TOPIC 7: BACKGROUND AND POPULATION EXPOSURES
Question 14
Secondary Reviewer

Section 4.4.1 does a good job outlining the decline in human tissue levels over the last two decades due to declining environmental concentrations. The method used to back calculate average daily intakes from tissue levels, however, is vague (pg 57, lines 19-26). The specific equation used to back calculate intakes should be included here or at least the section in Part II where it occurs referenced. Given that the text argues that toxicological effects are better linked to body burden that intakes, the quantitative relationship between body burdens and intakes should be clarified here.

TOPIC 7: BACKGROUND AND POPULATION EXPOSURES
Question 15
Secondary Reviewer

A adequate discussion of potential exposures by children, infants, and individuals who consumed TCDD-contaminated fish is lacking.

Children: A quantitative discussion of potential exposures to children is lacking. A table similar to Table 4-8 outlining child contact rates and background daily intake by children should be developed. Table 4-9 does not provide sufficient information on how child intakes were derived. Mean and upper-bound values for contact rates, concentrations, and background intakes should be included to provide a broader perspective on the range of background exposures likely to be experienced by children. Then a more detailed discussion of the differences and similarities

between child and adult exposures should be included in Section 4.4.

Individuals Who Consume Fish: Intake of dioxin-like compounds can vary dramatically due to variation in fish consumption rates and dioxin levels in fish. The text on page 61, lines 14-17 is confusing. It states that individuals who consume large quantities of TCDD-contaminated fish may have "elevated exposures," but these exposures are not "considered to result in highly exposed individuals." These two statements seem contradictory. I disagree with characterizing subsistence and recreational anglers' exposures as "in addition to background" (p. 59, lines 19-22). Background exposures are correctly defined on pg. 96, lines 29-31 as exposures by individuals "who are not exposed to readily-identifiable point sources."

The conclusions outlined on page 61, lines 10-25 that recreational anglers may have "elevated exposures," but these exposures are not "considered to result in highly exposed individuals"seem to be based on average fish concentrations and average ingestion rates. Individuals who consume fish from specific areas (e.g., Great Lakes, near pulp and paper mills, etc) and/or individuals who consume large quantities of fish (e.g., subsistence fishermen) may be substantially exposed. To calculate a background daily intake from fish ingestion, Table 4-8 uses an ingestion rate of 6 g/day and a mean concentration of 1.2 pg/g for freshwater fish. As part of the National Dioxin Study, EPA reported TCDD concentrations in fish fillets (WW basis) taken from urban sites, the Great Lakes area, and from sites near pulp and paper mills. The 95th percentile value for these three groups was 70 pg/g WW, 110 pg/g WW, and 110 pg/g WW, respectively (Travis and Hattemer-Frey, 1990), or 58 to 90 times higher than the 1.2 pg/g value EPA used to calculate background daily intake from fish consumption. While I agree that 6 g/day is representative of average fish consumption, it is possible that some individuals could consume much higher amounts of fish. If, hypothetically, an angler consumed average (6 g/day) amounts of Great Lakes fish contaminated at the upper-bound concentration (110 pg/g), their intake of TCDD from fish ingestion alone would be 660 pg/day, which is 15 times the average daily intake from all sources. It is reasonable to assume that some U.S. anglers could consume fish contaminated with higher than average levels of dioxin and/or consume fish at a higher ingestion rate than those reported in Table 4-8,

and these exposures need to be evaluated.

I disagree with that statement on p. 61, lines 19-20 that there is no supporting evidence of individuals highly exposed to CDDs/CDFs via fish consumption. It is premature to conclude based on one study (that isn't even referenced in this section) that high-end fish consumers are not being highly exposed to CDDs/CDFs. Line 21-22 state that TCDD blood levels in Great Lakes sport anglers were "elevated over mean background but within the range of normal variability." The text needs to specify how many Great Lakes sport anglers were evaluated, what their TCDD blood levels were, and provide a definition of what is meant by the "range of normal variability" before conclusions about exposures by this cohort can be reasonably made. The report also notes that elevated blood TCDD levels were measured in Baltic fishermen. Specific TCDD blood levels in these fishermen and a comparison to background TCDD blood levels should be included. Finally, the studies cited to support the conclusions drawn in lines 20-25 should be referenced.

Infant and *In Utero* Exposures: Page 60-61 presents a comprehensive discussion of exposures to nursing infants outlining differences to infants that are breast fed versus bottle fed as well as putting infant exposures into perspective relative to lifetime exposures. Similar information on *in utero* exposures should be added. For example, what evidence is available, if any, to indicate that dioxin crosses the placenta? If it does cross the placenta, what doses are individuals likely to receive *in utero*? I acknowledge that the information on this topic may be limited, but it still should be addressed.

TOPIC 8: CHILDREN'S RISK
Question 16:
General Reviewer

While body burden may be a more useful metric for evaluating adult exposures, it is not clear if this is the most appropriate metric for evaluating exposures to nursing infants, *in utero* exposures,

and small children. Page 67, para 1 states that difficulties arise when trying to use body burden as the dose metric for acute (e.g., short-term) exposures. Page 101, lines 1-8 note that while the infant dose is 77 times higher than the adult dose, infant body burden will not be 77 times higher than adult body burdens. Similarly, breast fed infants were exposed to four times as much CDDs/CDFs as bottle fed infants. Given that these two statements are true, there is little discussion of how these relatively high but short-term doses may affect infants and children and if infants and children are more sensitive than adults.

TOPIC 10: RISK CHARACTERIZATION SUMMARY STATEMENT
Question 18:
General Reviewer

There are a few issues associated with the information presented on pages 84 through 86 that should be clarified. First, in which cases have effects been observed in animals but not in humans (i.e., the effects are only hypothesized to occur in humans, since an effect was observed in animals). Substantial weight is given to scientific inference (i.e., inferring that because some adverse effect occurs in other species, that same effect is likely to occur at some levels in humans. While I understand that in many cases the potential for adverse effects must be extrapolated from animal data, I do not agree that scientific inference should necessarily be given more weight than the existing human epidemiological data. In the case of dioxin, I agree that "to deduce that a spectrum of noncancer effects will occur in humans ..." overstates the science. Figure 5-1 depicts that while several cohorts have received dioxin exposures high enough to increase body burdens significantly over background levels, "relatively few clinically significant effects" have been observed. Secondly, in which cases were the same effect been observed in animals and humans and how did dose levels vary? Thirdly, on page 85, lines 34-35 and page 86, lines 6-8, the text needs to be more explicit about what effects "may occur at or near background levels." Have any of the effects referred to actually been observed in humans exposed to

background levels and, if so, were the effects observed of clinical significance?

TOPIC 10: RISK CHARACTERIZATION SUMMARY STATEMENT
Question 19
General Reviewer

See response to Question 18. The tone of this section seems biased. Lines 16-21 infer that adverse effects in humans are probably occurring, scientists just haven't figured out how to measure them. The possibility that humans are not actually experiencing clinically-significant effects from exposure to background levels of dioxin doesn't seem to be an option.

TOPIC 11: SOURCES
Question 20
General Reviewer

Section 4.1 makes many sweeping statements about the reduction of CDDs/CDFs between 1987 and 1995 and yet not a single reference is cited. Sources and references for the conclusions summarized in this section need to be included to lend credence to the claims made. For example, bullet two at the bottom of p. 50 states that the "decrease in estimated releases of CDDs/CDFs between 1987 and 1995 was primarily due to reductions in air emission from municipal and medical waste incinerators..." I saw no proof supporting this conclusion. EPA seems convinced that incineration is the major source of CDDs/CDFs despite the fact that as shown in Table 4-1 (p. 129), EPA has very limited knowledge about the mass of CDD/CDF release from other potentially significant sources, including medical waste incinerators, forest fires, cement kilns, residential wood burning, smelting operations, etc. The text appears to conclude that incineration is the primary source of CDD/CDF release into the environment simply because it is the best characterized.

EPA's method for identifying major sources was to multiply estimated emissions for a given

source by estimated total activity level for that source. Of the 29 source categories listed in Table 4-2 that release CDDs/CDFs into air, none are given an "A" rating (i.e., for no source category does EPA have high confidence in the emissions estimate). Ten (34%) were given a "B" rating (emissions estimate was derived from testing conducted at a limited number of facilities but results are "believed to be representative of the source category"). And 19 (66%) were given a "C" rating (emissions estimate was derived from testing conducted at only a few "possibly non representative" facilities and results "may not be representative of the source category"). In Tables 4-3 and 4-4, EPA identifies several additional source categories whose CDD/CDF emissions cannot be reliably quantified at all. While emissions from various sources have been measured or estimated, information on the relative contribution of various sources to total CDD/CDF load remain highly uncertain. Given this uncertainty, it is premature to conclude that municipal and medical waste incinerators are the major source of CDD/CDF input into the environment. It could be that there are multiple sources of CDDs/CDFs, and no one source category dominates total input.

A comparison of the isomeric patterns of CDDs/CDFs observed in air, water, and soil with the pattern of CDDs/CDFs emitted from various sources could also be included as a means of identifying sources.

On page 51 (lines 25-28), EPA dismisses the use of mass balance and other similar analyses as a reliable means of estimating total CDD/CDF releases. While I agree that such methods may also be uncertain, I do not agree that they are *a priori* less uncertain the EPA's method. Therefore, I recommend that results from these types of analyses (and their inherent uncertainties) be included.

TOPIC GENERAL
Question 21
General Reviewer

While reading the report, I developed the feeling that it was trying vigorously to convince the scientific community and the public of three things: a) that adverse health effects from exposure to background levels of dioxin-like compounds ARE occurring (versus could be possible), b) that dioxin should be characterized as a human carcinogen, and c) that municipal waste and medical incineration are the primary sources of environmental dioxins. I was convinced of none of these and found the presentation lacking objectivity.

Throughout much of the document, conclusions stated are not adequately supported with sufficient references.

A list of acronyms included at the beginning of the document would be useful.

REFERENCES

Travis, C.C. and H.A. Hattemer-Frey, 1991. Concenrtation of TCDD in Fish and the Potential for Human Exposure, *Environ. Inter*., 16: 155-162.

Topic # 1

Question # 1

Role: Primary Reviewer

Question: Did EPA adequately justify its use of body burden as a dose metric for inter-species scaling? Should the document present conclusions based upon daily dose?

Response/Comments:

EPA does not adequately justify its use of body burden as a dose metric for interspecies scaling, and the document should present the historic daily dose-based calculations and weigh the potential relevance and uncertainties that can be assigned to risk extrapolations based on the daily dose versus the mechanism-based dose metric.

The term "body burden" as used in this document and in the general scientific literature is a relatively nonspecific term that is not commonly used in pharmacokinetic analysis of drug or chemical effects, and is not a relevant dose metric for examining dose-response relationships for chemicals or drugs with a receptor-based mechanism of action. Body burden is most often used as an alternate term for the estimated quantity of the chemical present in the body, which for dioxins is typically expressed in several ways. The dose metrics commonly included under the term "body burden" include the adipose tissue or blood lipid concentration of the chemical, the estimated total mass of chemical in the body (calculated by lipid weight times dioxin content in lipid), and the estimated "steady state" adipose tissue or blood lipid concentration of the chemical. The estimated steady state "body burden" is the apparent focal point of the effective dose calculations for cancer responses as described in the Reassessment document. This dose metric is inappropriate because it does not include the necessary time element that allows one to assess the duration of time that the body experiences a sufficient blood level of the chemical to induce the receptor binding and related cascade effects that must be sustained in order to cause more than a transient perturbation (e.g., a blood level that is consistently high enough to cause elevated enzyme activity in the liver for a period of months or years). See detailed discussions of this in Aylward et al. (1996, ES&T 30(12):3534-3543).

In pharmacokinetic terminology, one would refer to the appropriate dose metric as the area-under-the-curve (AUC) for the blood concentration of the chemical vs. time relationship, and might be referred to in units such as ppt-years for dioxins. For acute and chronic drug

administration regarding receptor-activated responses, there is a “loading dose” phase and then a “maintenance dose” phase, both of which target a specific blood concentration range that is known to effect the desired therapeutic response. In the absence of a continuous maintenance of the blood concentrations within the “therapeutic window”, the efficacy of a drug with a receptor-based mode of action is lost. Exceeding the blood concentration range of the therapeutic window may result in undesired or toxic effects. Thus, in a graphical chart that examines the blood concentration versus time relationship, predictions of the likelihood of undesired or toxic effects can be most accurately related to the duration of time at which the blood concentration exceeded the therapeutic window. See Aylward et al. (1996) for a graphical representation of how this concept likely relates to TCDD effects and dose-response relationships.

The loading dose and maintenance dose of a given drug or chemical that relates to a given receptor-activated response may vary by orders of magnitude from one species to the next, but generally does not vary dramatically from one individual to the next. There are many potential explanations for this being the case with respect to extrapolation of the effects of TCDD and other dioxin-like compounds from rodents to humans. Each of these issues must be discussed and accounted for in any valid extrapolation of dose-response relationships using the appropriate dose metric (AUC) for TCDD and other dioxin-like compounds:

1. The AUC dose metric most certainly relates to the differences in effective blood concentrations and the pharmacokinetic parameters that relate to blood concentration over the lifespan of the test species and humans. The predicted steady state “body burden” calculated by EPA is not a scientifically appropriate equivalent nor is it a proportional surrogate for deriving a dose metric relevant to human exposures at or within a factor of 10-to 100-fold of current background levels. In the case of extrapolating the Kociba et al. (1976) rat data to humans, there are at least three important distinctions from a pharmacokinetic perspective.
2. The one factor examined and accounted for by EPA is the difference in TCDD half-life between rats and humans (e.g., 25 days versus 7.5 years). While this is an important and appropriate consideration in the extrapolation process, correction for metabolic half life differences is only one of at least three important aspects that we know must be involved if the assumption of receptor-based mode of action is to be extrapolated in a scientifically correct manner.

3. The second factor is the estimated blood concentration and/or the affected target organ concentration in the rats as compared to humans. EPA does not adequately account for the obvious species differences in liver accumulation of TCDD and the significant influences of higher P-450 enzyme activity on the susceptibility of rats to the tumor response observed in the Kociba study. It is clear that liver enzyme induction has very different dose-response characteristics and implications in rats versus humans, based on several lines of evidence – all of which seem to indicate that rats will be more sensitive to TCDD-induced tumors. The likely greater sensitivity of rats to liver tumor induction is predicted based on clear species-related differences in TCDD metabolism, effective receptor binding in the target tissue, lipid depot distribution, and demonstrated differentials in liver tissue dose and effects at high doses in rats as compared to humans. There is also an important element of dose mischaracterization with respect to the back-calculated TCDD body burdens for humans. Based on the Yusho studies (Yoshimura et al., 1996 book on Yusho, Kyushu University Press), it is apparent that the half life of the penta and hexa chlorinated dibenzofurans was short (i.e., faster, on the order of 2 to 5 years) during the high dose elimination phase of Yusho patients soon after the rice oil ingestion (loading dose), while the half life measured in these same people 20 years later (at background body burden levels) was similar to those never exposed (e.g., 10 to 12 years). This dose-dependent increase in half-life has a number of implications with respect to the attempted EPA extrapolations. First, it seems likely that in the EPA extrapolations the humans in occupational studies were likely assigned TCDD body burdens (based on a uniform assumption of 7.5 years half life) that were considerably lower at peak and on average than they likely were in reality. This consideration makes the likely peak and average body burdens of exposed humans far higher, indicating a lower susceptibility to cancer as compared to the measured rat body burdens in the Kociba study. Second, it is clear from the Kociba study that the half-life estimate in rats is probably biased by the dose-related continuous state of liver enzyme induction for essentially the entire lifespan of the animals (which influences half life). Based on the Yusho study results, even a very high, acute exposure does not lead to lifetime induction of liver enzymes in exposed humans, and the TCDD occupational study cohorts certainly did not have a lifetime exposure to TCDD at levels that would cause continuous liver enzyme induction as occurred in the high dose rats of the Kociba study. Moreover, liver enzyme induction does not appear to be relevant to background body burdens in humans. Since increased liver enzyme induction and higher rates of receptor stimulation are

related to tumor induction and to the greater metabolic capacity (re: P-450) in rodents as compared to humans, these issues must be dealt with appropriately in order for a scientifically accurate and appropriate extrapolation to be made.

4. The third factor is the duration of sustained elevation of the blood and/or target tissue TCDD concentration with respect to the lifespan of rats versus humans. As mentioned above, there are various aspects of rat physiology and metabolism that suggest a greater susceptibility to liver tumors. These same factors inherently relate to any extrapolation that is conceptually based on Ah receptor binding and enzyme induction generally. The AUC dose metric (or any other scientifically correct dose metric) must involve an appropriate adjustment that accounts for species differences in lifespan. Conceptually based on AUC, the duration of time that the blood concentration is in the "toxic effect" range, and the magnitude of that elevation over time, relates to the occurrence, frequency, severity, and/or risk of toxic effects. In the Kociba study, the high dose rats likely had sufficient blood and liver concentrations of TCDD to place them in a state of continuous liver enzyme induction for more than 95% of their lifespan, with blood and/or liver concentrations that were likely in the "toxic effect" range for most of their lifespan based on the interim sacrifice liver pathology findings. In contrast, we have no similar pathology results in the occupational cohort studies to suggest a parallel expectation of sustained TCDD blood levels in the "toxic effect" range, though their extrapolated body burdens were presumably similar to or higher than the rats, and they were exposed to TCDD at levels that might have caused such sustained high blood levels for only a relatively small fraction of their lifespan. Since cancer risk most definitely relates to the aging process, differences in the aging process (i.e., a relative lifespan correction) must be accounted for in examining the dose-response relationships between species for chemicals like TCDD and related compounds that are presumed to have predominantly receptor-based mechanism of action.

With respect to the inclusion of data, calculations, and conclusions based on daily dose of TCDD, these considerations are certainly relevant to the overall discussion. A review of the current approach (LMS model of Kociba data) and the various criticisms and changes that have been documented since its inception would be instructive. In particular, more detailed discussions of the re-evaluation of the Kociba study liver tumor pathology in 1990-91 and the wide range of plausible cancer slope factors indicated by differing applications of these revised pathology guidelines would provide a more balanced view of the plausible range of cancer potency based on

the average daily dose/LMS extrapolation concept (see Keenan et al., 1991). Further, discussion of the use of alternative slope factors for TCDD adopted by FDA and the Consumer Product Safety Commission would be helpful in illustrating that significant digressions from the original EPA approach have been adopted by other responsible agency scientists in the U.S.

At very high daily TCDD doses, as in the Kociba rat study, it is likely that the daily dose contribution to blood and/or target tissue concentrations of TCDD may predominate for a substantial portion of the animal's lifespan, especially given the relatively rapid half life (25 days) estimated for high dose rats in a continuous state of liver enzyme induction. When attempting to estimate risks to humans from daily TCDD/TEQ doses that are up to 10-fold higher than the typical background range, there is no scientific information indicating any definite increase in liver enzyme induction, which could be a baseline indicator of biological response for increased cancer risk and/or tumor promotion response in animals and humans. At higher daily dose levels (which are less relevant to the dose range generally of concern in the risk assessment process), the risk of toxicity must be correlated to the duration of time when blood or target tissue concentrations exceed a "toxic effect" level, for which statistically increased liver enzyme activity or increased prevalence of elevated liver enzyme activity might be considered as a conservative surrogate. In the lower, background range of body burdens, it is more likely that daily dose fluctuations could result in at least a temporary elevation in blood or target organ TCDD concentration in humans (i.e., to cause a brief spurt of enzyme induction), whereas the steady state body burden estimates would not reflect the magnitude or duration of such relevant responses.

Further, while the LMS extrapolation of the Kociba study based on daily dose seems less mechanism-based than some dose metric based on receptor pharmacokinetics of TCDD, the term to be used in risk assessment calculations (lifetime average daily dose) does incorporate a relative lifespan correction and is consistent with the EPA approach taken for other high molecular weight, relatively persistent compounds that activate Ah receptor, like the polycyclic aromatic hydrocarbons. After considering all the weight of evidence and uncertainties surrounding a new dose metric and a unique mechanism-based approach, it might be concluded that the original LADD-based approach has no less uncertainty or scientific validity than the newly proposed approach.

Topic # 4

Question # 7

Role: Primary Reviewer

Question: Does EPA establish clear procedures for using, calculating, and interpreting toxicity equivalence factors?

Response/Comments:

The discussion of procedures for using, calculating, and interpreting TCDD toxicity equivalence factors is greatly improved in the revised Chapter 9. The historical perspective and updated discussions on in vitro and in vivo correlation studies were an extremely valuable addition to this chapter, creating much more depth of discussion. The more detailed discussions of both agonistic and antagonistic influences of various congeners on Ah receptor binding characteristics, and of the inherent uncertainties involved in their application within the risk assessment process, are also important and helpful additions that help to balance the presentation with respect to the weight of scientific evidence on TEQs and dose response. However, the scope of the uncertainty section is quite limited and should be expanded to discuss the inherent problems with applying such a simplified approach in evaluating risks of such a toxicologically and pharmacokinetically diverse series of compounds. At the very least, a listing and brief overview should be provided of all the key points of uncertainty involved in the assumptions made in the main document, e.g., problems with species extrapolation, sufficiency of the data base for various congeners, and implications of the dose metric used when attempting to apply TEQ factors in risk assessment calculations.

The fact that the currently recommended TEQ approach derives directly from the international forum of scientists convened by the World Health Organization is critical, and it would be worthwhile providing a separate section that describes the issues and interim resolutions of this group in great detail. Such a section should include discussions of EPA's rationale for any digression in logic or application of the TEQ method from the World Health Organization methodology, discussion of uncertainties, and conclusions.

The discussion provided in Section 9.3.2 , Receptor Ligands, proposes a scientific rationale for special consideration of the long elimination half-life of dioxin-like compounds in terms of Ah receptor binding and the effects known (.e.g, enzyme induction) or suspected (e.g., cancer) to be related to the dose-response curves for binding affinity. The argument is posited in several

discussions that, because other potent AhR binding compounds may have relatively short residence time in the body, that those compounds are somehow not as likely to be important in the ultimate induction of responses that could be related to AhR binding. The significant uncertainties and flawed logic applicable to this relatively simple concept of "persistence equals importance" requires extensive discussion in Section 9.3.2, as well as in the Uncertainties discussion and the summary. The following considerations explain why this is necessary:

1. The conceptual dose-response evaluation based on AhR binding affinity assumes that, on an average daily dose perspective in which risk assessments are currently performed, the average daily dose of ANY compound with high affinity should be related in a linear manner to the cancer risks or other effects thought to be implicated by the TEQ factor designations. The shorter half-life of "nondioxin-like ligands" for AhR does not provide a meaningful basis for excluding them from this discussion. As discussed previously, differences in the pharmacokinetics of TCDD tend to favor humans in terms of indicating lesser susceptible than rodents with respect to both biochemical and pathological effects. Thus, the application of TEQ analysis for daily dose estimates should be conservative in human health risk assessment as long as the same methodology is applied to ANY given chemical with AhR affinity, expressed on a daily dose basis.
2. As discussed in the preceding comments, the appropriate dose metric for predicting TCDD toxicity is the duration of elevated blood or target tissue TCDD concentration in the "toxic effect" range (based on AUC), adjusted for species differences in lifespan and the known physiologic and pharmacokinetic factors that make rodent species more susceptible to enzyme induction and pathological responses to TCDD as compared to humans. At very high daily TCDD doses, as in the Kociba rat study, it is likely that the daily dose contribution to blood and/or target tissue concentrations of TCDD may predominate for a substantial portion of the animal's lifespan, especially given the relatively rapid half life (25 days) estimated for high dose rats in a continuous state of liver enzyme induction. In contrast, humans exposed to background body burdens that do not cause significant liver enzyme induction and likely will not sustain any substantial duration of blood or target organ TCDD levels in the toxic range unless a) their body burden is high enough to trigger the basal AhR response of enzyme induction, which will shorten the half-life and limit the duration of time above the no-effect blood level, or b) their acute daily dose causes a temporary elevation above the basal AhR response level for enzyme induction, which would likely result in a brief spurt of enzyme induction

before redistribution of TCDD from the blood to adipose tissue stores. In essence, the relevant exposure-effect profile for TCDD is no different than that for AhR-related processes triggered by short-lived species such as endogenous and exogenous hormones, PAHs, and other common substances with higher AhR binding affinity.

3. As discussed in the preceding comments, there are several physiologic and pharmacokinetic factors that significantly limit the relevance of TEQ factors based on animal responses to human exposure conditions and responses. The cross-species comparisons between rodent and human responses in vivo with respect to enzyme induction alone indicate that rodents are probably more susceptible to AhR mediated effects than are humans. In general, it is reasonable to state that such dose-response predictions are likely orders of magnitude more conservative than is likely to be observed in humans based on the available clinical and epidemiological studies.

Topic # 6

Question # 10

Role: Secondary Reviewer

Question: Do you agree with the characterization in this document that dioxin and related compounds are carcinogenic hazards for humans?

Response/Comments:

From a strict application of causation criteria (e.g., those of Hill (1965)), I cannot conclude that dioxin and related compounds as defined in this document are carcinogenic hazards for humans. I acknowledge that there is adequate animal evidence to implicate TCDD and certain other congeners discussed in the document as animal carcinogens. However, the epidemiological evidence to date does not demonstrate a strong, dose-related, consistent, or coherent relationship between relatively high exposures to TCDD and excess cancers in humans. This same conclusion extends to the evidence pertaining to other PCDDs and PCDFs, as well as to PCBs.

Using the EPA Guidelines for Carcinogen Risk Assessment (1996), I believe that for regulatory purposes an appropriate designation of the carcinogenic hazards of the compounds addressed in the Reassessment document would be as follows:

1. 2,3,7,8-TCDD is likely to produce cancer in humans due to the production or anticipated production of tumors by modes of action that are relevant or assumed

to be relevant to human carcinogenicity, but only at daily doses that exceed current background levels by at least two to three orders of magnitude for a number of years (e.g., 500 to 5000 ppt in lipid).

2. The carcinogenic potential of 2,3,7,8-tetra-, penta-, and hexa-chlorinated dibenzodioxins and dibenzofurans cannot be determined, but there is suggestive evidence that raises concern for carcinogenic effects.
3. The carcinogenic potential of 2,3,7,8-hepta- and octa-chlorinated dibenzodioxins and dibenzofurans, and for the designated "dioxin-like" PCB congeners, cannot be determined because the existing evidence is composed of conflicting data and/or there are inadequate data to perform an assessment.

Topic # 6

Question # 11

Role: Secondary Reviewer

Question: **Does the document clearly present the evolving approaches to estimating cancer risk (e.g., margin of exposure and the LED01 as point of departure) as described in the EPA "Proposed Guidelines for Carcinogenic Risk Assessment" (EPA./600/P-92/003C; April 1996)? Is this approach equally as valid for dioxin-like compounds?**

Response/Comments:

The Reassessment document does not present a balanced viewpoint on the evolving approaches to estimating cancer risk in accordance with EPA (1996), although such approaches could be applied to TCDD and dioxin-like compounds if appropriate scientific rigor is applied to the process.

The document clearly presents one newer approach (the LED01/ED01) that is an order of magnitude more conservative then that proposed by EPA (1996; i.e., the LED10) and which is based on flawed logic and calculations that are not consistent with the weight of scientific evidence for the mechanism of, or the human cancer dose response relationship for TCDD or other dioxin-like compounds. As discussed in my prior comments and in Aylward et al. (1996), EPA has been on notice that "body burden" estimates alone are not a scientifically appropriate dose metric for predicting human cancer risks from dioxin-like compounds, nor for extrapolating such risks based on an Ah receptor-triggered mode of action. While there are many caveats and

admissions in the document with respect to weaknesses of the available evidence and uncertainties involved in the assumptions they selected, there is a clear bias towards an assertion that is both undocumented and probably cannot be scientifically demonstrated, i.e., that background exposures to dioxin-like compounds are causing adverse effects including excess cancers in humans. Moreover, the statistical methods applied to examine the range of possible dose-response factors for TCDD are based on highly selective data sets and applications of these newer (LED01) and older (Kociba study/LMS model) methodologies that emulate a false consistency across the methods and a narrower range of uncertainty in the slope factor equivalent than is represented by a more objective assessment of the weight of scientific evidence. The end results (revised slope factors or "point of departure") are more contrived and uncertain than the original linearized multistage model, and the dose metric ("body burden") utilized for extrapolation is not demonstrably better than the lifetime average daily dose model..

The scientific basis underlying the omnipresent assertion in the document that it is reasonable to assume that background exposures to (and body burdens of) dioxin-like compounds might be associated with a cancer risk approaching one percent is severely flawed, and ignores many of the basic considerations called out in the proposed guidelines for cancer risk assessment (EPA, 1996). While their justification for mode of action for TCDD is reasonably explained with appropriate caveats, the depth of scientific consideration with respect to receptor-based pharmacokinetics is seriously lacking. Further, there is a lack of appropriate consideration of obvious species differences in target organ (e.g., liver enzyme induction and liver toxicity) and dose-response relationships that must be accounted for when attempting to extrapolate animal tumor data to humans. Inadequate consideration is given to the physiological and pharmacokinetic differences relevant to TCDD dose-response relationships for animals versus humans. EPA (1996) calls for appropriate consideration of the preceding issues in addition to providing justification for a proposed mode of action.

Importantly, the weight of human evidence evaluation for carcinogenicity is not sufficiently rigorous and relies heavily upon less robust epidemiological data (e.g., the German cohort/Becher et al. studies) that are not adequately focused on examining TCDD-related effects as are the two most robust cohort studies (IARC and NIOSH). Despite the reference to the Hill (1965) methodology for weight of evidence evaluation in the Reassessment document, there is no open acknowledgment that the available epidemiological studies show: a) no strong and consistent excess of any specific cancer type that can be distinguished from smoking-related cancers, b) no

strong or consistent demonstration of a TCDD dose-response relationship for general or specific cancers, and c) no demonstrable consistency with respect to target organ affected in animals, despite human exposures that equal or exceed those administered to animals.

Topic # 10

Question # 18

Role: General Reviewer

Question: Does the summary and analysis support the conclusion that enzyme induction, changes in hormone levels, and indicators of altered cellular function seen in humans and laboratory animals, represent effects of unknown clinical significance but that may be early indicators of toxic response?

Response/Comments:

The summary discussion asserts and attempts to support this conclusion, although it is based on inadequate scientific evidence and flawed logic with respect to receptor-mediated toxic responses. Lines of evidence for serious problems with the body burden dose metric, with extrapolation methods for interspecies tumor risk, and with the serious weaknesses and limited implications of the epidemiological data have been explained in my prior comments.

Topic # 10

Question # 19

Role: General Reviewer

Question: Has the short summary statement in the risk and hazard characterization on page 107 adequately captured the important conclusions, and the areas where further evaluation is needed? What additional points should be made in this short statement?

Response/Comments:

The summary statement, like the remainder of the document, is biased towards the unproven assertion that serious adverse health effects may be occurring within the background level of human exposures (and body burdens) to dioxin-like compounds. The summary statement would have to be completely revised to exclude this bias and to provide a more balanced and scientifically supported overview of the weight of scientific evidence regarding the potential risks

of TCDD and other dioxin-like compounds that are considered in the Reassessment document. Additional points to be made might include:

1. The current reassessment focuses primarily upon toxicological evidence pertaining to 2,3,7,8-TCDD, and there are significant data gaps and greater uncertainties attached to the generalization of effects of TCDD to other dioxins, dibenzofurans, and PCB congeners.
2. When considering the dose metric of the time duration when blood TCDD levels exceed a known effect level for any documented effect of TCDD, including liver enzyme induction, it is apparent that a threshold mechanism is likely relevant for AhR triggered effects of TCDD and that most or all background daily doses and body burdens in humans do not trigger the continuous AhR induction response seen in animal studies demonstrating excess tumorigenicity at relatively low doses.
3. Consideration of AUC as the appropriate dose metric wiould dramatically change the assertions and conclusions of the Reassessment document, reflecting a much reduced level of concern and a plausible threshold for excess cancer risk that is keyed, at a minimum, to doses and/or body burdens known to cause continuous induction of liver enzyme induction for a period of years. EPA should develop and examine the relative strengths and weaknesses of appropriate, scientifically-supported models that are both mechanism-based and threshold-based for TCDD and related compounds.
4. The old approach of extrapolation from the Kociba rat study tumor incidence to human cancer risk could support plausible risk estimates ranging from 10,000 to 156,000 per mg/kg-day, whereas appropriate scaling of dose and risk based on AhR induction doses indicates a lower susceptibility of humans to the acute and chronic effects possibly caused by TCDD and related compounds, including cancer.
5. Insert language on the objective weight of evidence that is consistent with EPA (1996).
6. Acknowledge that the noncancer dose-response evaluation has not been addressed in the current analysis.

Topic # 10

Question # 21

Role: General Reviewer

Question: Please provide any other comments or suggestions relevant to the review documents, as interest and time allow.

Response/Comments:

1. It seems that Chapter 9 gave inadequate consideration to the available PBPK models that were described in detail in Chapter 8. The physiologic and pharmacokinetic differences between species are probably the most critical considerations to address in arriving at a valid cancer slope factor or threshold that is relevant to humans. With proper focus and research, it seems likely that PBPK modeling can provide useful qualitative and quantitative answers to the dose-response questions in animals and in humans that cannot be answered based on the level of scientific knowledge available to date on the mechanism of TCDD carcinogenicity and of AhR-triggered responses in general. Moreover, straying from pharmacokinetic principles (like AUC analysis for receptor-based mechanisms) appears to be the main reason why the EPA-supported research group has adopted an unproven and unlikely theory as the basis for their regulatory program on dioxins.
2. A scientifically appropriate and balanced treatment of the available data, consistent with EPA (1996), would have included one or more threshold models that are consistent with the accepted nongenotoxic promotor role of TCDD and related compounds.
3. I have many other specific comments, but do not have sufficient time to elaborate in this document. I will be prepared to elaborate these additional points at the Dioxin Workshop.

Charge question number 2
Primary Reviewer

The rationale that EPA is using for not calculating an RfD/RfC is sound, given their assumptions, but the discrepancy between how EPA and WHO/ATSDR approach the data base should be explored further.

EPA's rationale, in brief, is that any RfD/RfC that they set would be below current background, so it has little use in a regulatory context. Instead of the traditional approach, EPA recommends that any specific source should be viewed in the context of what increment to background the source is adding.

The WHO and ATSDR, on the other hand, have set their equivalent of an RfD/RfC at 1 – 4 pg/kg/day and 1 pg/kg/day respectively, as compared with the current EPA estimate of exposure of 1 pg/kg/day. Considering the enormous health implications of this difference, there should be more discussion of why these differences exist. A significant component of this difference appears to be the relevant dose metric for interspecies comparison. EPA has recommended a dose metric of body burden for cross study and cross species comparison because of the unique persistence characteristics of dioxins. This metric may be more appropriate than daily dose but the difference between extrapolating from animals to humans on a body burden basis rather than daily dose is approximately 100-fold. That is, the exposure on a body burden basis is approximately 100-fold more than on a daily dose basis.

The EPA rationale for this approach appears to be that the half lives in laboratory rodents are much less than half life in humans, resulting in lower body burdens for the same dose, and that because of dioxin's persistence in the body, the relevant measure of toxicity is the body burden. While this may be correct, it could also be argued that, for most parent compounds, there will be a shorter half life in smaller animals because of their inherently greater rates of metabolic processes. Laboratory rodents have a shorter life time, and have higher metabolic rates, often resulting in shorter half lives, but they are assumed to be reasonable models for extrapolation to humans. Thus shorter half lives may reflect the compressed lifetime of rodents used as animal models. For the case of dioxins, however, standard differences in metabolic rates, e.g. body weight to the ¾ power, only account for a part of the uniquely longer half life in humans.

Recommendation

The document should more fully explore the difference between the EPA approach and the WHO/ATSDR approach, and identify what key assumptions result in the difference.

Question 12
Secondary Reviewer

The presentation of a range of risks is appropriate, but the key assumptions impacting this quantitative range should be described in much more detail. Differences between EPA and ATSDR/WHO assumptions also need to be spelled out, as described under question 2.

The overall conclusion on cancer risk (10^{-3} - 10^{-2}) at current body burdens (e.g. page 90) is also not sufficiently qualified as to its numerical uncertainty. There is no doubt that 2,3,7,8-TCDD and some of the other 2,3,7,8 substituted dioxins are highly toxic, and cause a wide range of effects in animals at very low doses. It is also clear that, based upon the animal data, there is no reason not to conclude that the same effects could occur in the human population at some exposure. There are also sufficient animal data to conclude that the margins of exposure for humans are sufficiently narrow to warrant regulatory actions and that sources of dioxins should be significantly reduced even further.

The human data base itself is less certain, although there are certainly indications that effects other than chloracne may have occurred in specific cohorts.

Cohorts of primarily industrial workers exposed in the past, with body burdens of up to thousands of times higher TCDD levels compared to today's background levels, tend to show patterns of increased tumor incidences, albeit inconsistently as to site and elevation. These human data are interpreted to demonstrate some correlation between exposure and risk, but are limited, by themselves, in ability to demonstrate cause and effect.

"……..the data are insufficient on their own to demonstrate a causal association" (page 20)

While these data can be used <u>qualitatively</u> to assess potential risk, I believe they should not be used <u>quantitatively</u> to calculate numerical risk. EPA acknowledges the uncertainty in the data and the fact that confounders are present,

"Although uncertainty remains in interpreting these studies because not all potential confounders have been ruled out and coincident exposures to other carcinogens are likely, …."(page 14)
"Again while smoking as a confounder cannot be totally eliminated as a potential explanation of the occupational results, analyses …. suggest that smoking is not likely to explain the entire increase in lung cancer……."(page 15),

"TCDD's biological effects likely reflect a complicated interplay between genetic and environmental factors. These issues complicate the risk assessment process for dioxin." (page 47, line 20)

yet these data are used to predict that there is an ongoing epidemic in the general population:

"based on these slope factor estimates, average current body burdens (5 ng/kgBW) that result from average intakes of approximately 3 pgTEQ/kgBW/day are in the range of 10^{-3} - 10^{-2}. A very small percentage of the population (<1%) may experience risks that are 2-3 times higher than this if they are among both the most vulnerable and the most highly exposed..... This range of upper bound risks for the general population has increased an order of magnitude ....."

Thus, based on data that are by themselves "limited", even qualitatively, and with acknowledged significant confounders, e.g. smoking, EPA is predicting on the order of 2,000 - 20,000 deaths per year ongoing currently in the USA. This is in contradiction to the World Health Organization conclusion made in June of 1998 that the Tolerable Daily Intake of dioxins is in the range of 1 - 4 pg/kg/day (EPA estimates current intake at 1 pg/kg/day), and the ATSDR minimal risk level of 1 pg/kg/day (1999).

I believe that the qualitative conclusions in this section, that dioxins should be treated as though they pose a potential carcinogenic risk to humans, is supportable, but the quantitative conclusion should either not be present or should be much more heavily qualified as to the assumptions being made.

For the human data it is assumed that all increased risk in the three primary studies (Manz, NIOSH, Zober) is attributable to dioxins. But it is also acknowledged that confounders have not been quantitatively removed in the three studies. In the Zober study, as I remember the data, 35 out of the 37 lung cancer cases were smokers. In the face of the overwhelming data base on risk of smoking, it does not seem to be logical to quantitatively attribute all the excess risk in a group of smokers to dioxin. Similarly, the chemical manufacturing workers in the NIOSH cohorts have had exposures to other chemicals, some of which have been high exposures to acknowledged carcinogens. Yet in the slope estimates, all the excess risk is quantitatively attributed to dioxin.

The animal data, when viewed in a metric of body burden, tend to quantitatively support the human potency estimates, albeit with somewhat lower potency estimates. Body burden may be the correct metric to extrapolate from animals to humans, but the difference between body burden and daily intake dose metrics results in an approximately 100-fold difference in risk. Thus 99% of the reported risk (or some other percentage for some other metric) is based quantitatively on an extrapolation assumption. The assumption may be correct, even though the document does acknowledge the various uncertainties, but the conclusions need to be qualified in light of the implication that thousands of Americans are dying yearly from dioxin exposure, and that our food supply, including eating fish and including breast feeding of infants, is the major source of risk.

Additionally, the quoted risk levels of 10^{-3} - 10^{-2} at current body burdens appear to be at odds with quoted body burdens of past highly exposed groups that are as much as 100 times current background and were higher previous to measurements being taken. If current risk is 1/1000 to 1/100, wouldn't this show up in populations with body burdens 100 times, or more, higher? I believe the reason for this apparent discrepancy is that the risk estimates are based upon sifting the data for the worst of the worst estimates, rather than doing more of a meta analysis for a particular tumor type, or all sites, across all the relevant studies.

Question 19
Primary Reviewer

The summary statement says that results could be occurring in the population, although there is no clear empirical evidence of that.

"Some of these effects may be occurring in humans at general population background levels and may be resulting in adverse impacts on human health."

The natural question to be asked, then, by someone seeing only the summary, is what is being done? I would recommend adding a statement in the summary that releases to the environment have been reduced by about 80% between 1987 and 1995 (page 50), and are expected to continue to be reduced as a result of recent EPA regulations (page 51).

Additionally, this statement appears to go beyond the risk characterization statements. For example in the risk characterization,

"These effects will likely range from biochemical changes at or near background levels of exposure to adverse effects with increasing severity as body burden increase above background levels."

The risk characterization makes the distinction between adverse effects and biochemical changes, relative to exposure at and above current background, but the summary statement appears to go beyond the risk characterization. These statements seem at odds with each other.

Question 20
General Reviewer

The sources, exposure and trend sections appear to be quite well done, given our knowledge and uncertainties. Pages 55, 59 and 99 refer to the contaminated ball clay issue, and there is a reference on page 102 to no known large natural reservoirs. It should be pointed out that the ball clay has no known industrial source, is likely to be a natural source, and likely involves kilograms of 2,3,7,8-TCDD

Question 16
General Reviewer

Infant exposures. The discussion on page 60 should reference LaKind et al (1999). The authors have simulated body burdens using ranges of data taken from the pediatric literature, rather than just the extremes, such as 200 pg/day. Their conclusions are that body burdens for nursing infants are not as high as often calculated. For example back calculating the 200 pg/kg/day on line 24, using the assumptions from line 28, and assuming 4% fat in milk results in a daily milk intake for the infant of 20% of it's body weight. This sounds high.

Question 21 – other comments
Primary Reviewer

- **Organization** – If I understand the objectives of the document, I think it is too long for a risk summary/characterization. Although the subject is clearly complex, the main conclusions get lost in some of the details. I would suggest reducing, in particular, sections 3.2 and 5.2.

- Page 9, line 30. Substitute "which" for "that".

- Seveso - In the altered sex ratio, what are the number of cases, and what is the ratio? Has this happened in other studies? Some of these results seem to be reported uncritically

- Page 9, line 14. Replace "is" with "are".
- Page 10, line 2. Replace "best" with "assumed to be best"
- Page 23, line 6. This is a very odd section. It appears to report that both increase and decreases in male testosterone have been found at presumed exposures that were not highly dissimilar, yet the next paragraph appears to argue that both these increases and decreases are plausible.
- Characterization of "low" exposures – pages 24, 25 and 29. On these pages there are references to a number of "low" single dose exposures.

"……as low as 0.064 ug/kg…." for rats.

This dose is numerically almost 100 pg/kg/day daily for the lifetime of a rat. I would not characterize a lifetime daily dose of 100 pg/kg/day, compressed into one single dose, as a "low" dose.

- The GGT data presentation should be more objectively reported.

The document states that Army Vietnam veterans were minimally exposed to dioxin (as opposed to the Ranch Hands), but that these veterans, as compared to non-veterans had "borderline significance" elevated GGT. The report then goes on to say that GGT is

clinically used as a marker for alcohol intake. Is it possible that alcohol intake is a confounder when comparing Vietnam Army veterans to non veterans?

- page 50, line 21.

"1987 was selected primarily because little empirical data existed for making source-specific emission estimates."

Is there a clause missing, e.g. "previous to this"?

- Reference is made on pages 52, 53 and 54 to rural soil erosion as a non point source of dioxin emissions to water. What is this referring to? What source to the soil?
- Page 54, line 11. Dioxins are classified as "semi-volatile"?
- Linearity. It is stated on page 71 that

"Therefore the same receptor occupancy assumption of the classic receptor theory is interpreted by different parties as support for and against the existence of a threshold."

And on page 91 that

"The current evidence suggests that both receptor binding and most early biochemical events such as enzyme induction are likely to demonstrate low dose linearity."

As before, the document should more carefully distinguish between science policy conclusions and conclusions based purely on the science.

- Page 92, line 19. Sentence should be rewritten for clarity.

Topic: Background and population exposures

Question #13 Have the estimates of background exposures been clearly and reasonably characterized?

Primary Reviewer

Comments

The main exposure pathways for "typical" or "background" US populations have been identified. Food intake is the major exposure pathway for background populations, dwarfing other pathways. Exposures through food intake are a function of TEQ levels in the food items and contact rates. Both need to be well characterized to minimize uncertainties. In this revised document, greatly improved, statistically-based estimates are used to determine dioxin concentrations for many food items, as well as contact rates with such items.

Levels in food. New estimates of food levels have been provided since the 1994 draft. Whereas the 1994 draft relied on limited and mostly unrepresentative samples, the 2000 document incorporates statistically designed surveys of beef, poultry, milk and dairy products. The distributions of TEQ and congener levels in these major food groups are adequately characterized. The flow of food items from the mostly centralized mega-production sites quite uniformly throughout the country has been well documented, implying little geographic variation. The current data base of contaminants n foods is based on more food items analysed than the one used in 1994. However, there are also less well-characterized sources of data mentioned in the document, such as eggs or marine fish. The Summary document Tables, e.g., Table 4-6, should have footnotes making a distinction between the well-characterized (beef, etc.) and the less well-characterized other food items. Number of samples used in the estimates, whether the data came from a statistical survey, etc., could appear in parentheses or a footnote to assist the reader.

Note In 1994 the "disappearance" rate was much higher than the intake rate and the average of these 2 estimates was used for the consumption rate. Disappearance rates were derived from USDA's 1993 report on Food Consumption, Prices and Expenditures from 1970-1992. It is not clear if the "disappearance" rate was used in the revised document. For example, for the 1994 Draft: pork consumption (47 g/day) was the average of intake (32 g/day and disappearance (62 g/day). In 2000: pork consumption is based on 15 g/day intake (NO DISAPPEARANCE?)

Cooking and fat trimming have been adequately reviewed in Vol1 Ch3 and their impact dismissed. A one-sentence statement on this topic could be placed in the Summary.

Levels in food are lower than in the 1994 Draft. Many reasons explain this drop:

- Overall downwards trends are documented in all environmental media
- Better analytical techniques allow uncensored measurements
- Use of WHO-98 TEFs reduces impact of OCDD/F (but raises PeCDD)?

Contact rates: Contact rates (1994) were based on 1-day diary data derived from the 1987/88 USDA National Food Consumption Survey (NFCS). The current document uses newer USDA data (CSFII) from a 3-day survey of 15,000 participants. Contact rates for certain food items (milk, dairy, and vegetable fats) were based on USDA studies covering many geographic areas, ages, seasons. Contact rates for beef, pork, poultry, fish, eggs, water, soil and air were based on USEPA revised Exposure Factors Handbook (1997). Meat mixtures were adjusted based on new information and are now lower.

Based on average TEQ content and contact rates, the average daily intake can be calculated (Table 4-8). Data are shown separately for PCDD/F and PCBs and the paucity of PCB data is discussed. In general, there is more uncertainty in the PCB estimates. The variability in these intake estimates is discussed in the main volumes and only the conclusions presented in the Summary, i.e., that intakes may be 3 times the tabulated means. The intake variability is attributed to food choices rather than to variability in contaminant levels. Consistent with this variability, is the well-established variability in fat intake (Tab 4-37 Block's data on fat intake consistent with Bogalusa, 3-sd) where, again a 3-fold difference from the mean is observed at the extremes of the distribution. Perhaps a public health campaign to reduce animal fat intake to minimize dioxin exposures may have more of a (short-term, probably) effect than the dated and worn out cardiovascular campaign.

Intakes were also estimated for different age groups based on age-specific food consumption and average contaminant levels. (Table 4-9)

Topic: Background and population exposures

Question #14 Estimate of exposure from dietary intake vs. estimate of exposure from body burden

Primary Reviewer

Estimate of exposure from dietary intake.

The estimate of dietary intake is based on the distribution of dioxin levels in food items and the distribution of contact rates. Exposure estimates from dietary intake may result in an underestimation, if not all pathways are considered. The data bases used for these estimates were discussed in Q-13 and were found adequately described and justified. The Summary document estimates a 1 pg/kg-d TEQ_{DFP} average intake or, for a 70 kg man, a 70 pg/d TEQ_{DFP} (45 DF +25 P). The large variability in background exposures (top 1% may be 3*mean) is due to inter-individual differences in dietary choices (frequency and amounts of various food items consumed). Inter-individual differences in absorption, metabolism and elimination are of smaller importance (?).

Estimate of exposure from body burden

In addition to the daily intake approach discussed in Q13, exposures can be estimated from body burden.

The term “body burden” needs to be defined. Throughout the text the term is used to refer to (mainly) tissue concentrations (milk, adipose, serum) and also to the total body burden, i.e., the lipid-based tissue concentration multiplied by the fat fraction of the whole body. In this discussion the latter definition will be used for the term, whereas tissue levels will be called “tissue levels”.

Body burden= tissue TEQ (pg/g fat) * total body fat (Kg)= ng TEQ

On p.8 of the Summary the derivation of body burden from tissue levels is introduced. It is not clear, however, what assumptions are used to estimate the fat content of the whole body. At some points, a value of 19L (p.56) while on p. 4-24 a fat volume of 14 L is used with no explanation. Which one should be used? Should it be age- and sex-specific?

Also, the absorption rate assumed varies from 50% (p.98), to 80% (p.57) to 100% (p.4-24) Which one should be used? Why

Hepatic sequestration may result in an underestimate of body burdens calculated from tissue, serum or milk levels. As the Summary states, however, sequestration will not be significant at background levels (dose-dependent).

Tissue levels have been dropping in Europe and the US (Liem, Furst, Noren, NHANES). New data from California confirm this trend (statistically significant changes between 1988 and 1998, in surgically sampled adipose from cancer-free women). Tissue levels, therefore, are not at steady state and a PK model that assumes a constant intake (at current levels) would underestimate the predicted tissue levels, as correctly pointed out.

In summary, the Summary document should state clearly what assumptions are used for absorption rate, lipid volume, and whether a steady-state or a non-steady-state is assumed in the PK model.

Assuming that the T 1/2 for TCDD can be applied to the T1/2 for the TEQ DFP can introduce errors, particularly because of the limited PCB data.

Use of body burden estimates allows comparison across species better than does the use of daily intake.

Relationship between the two estimates

The two approaches have been well described (at least conceptually, with some discrepancies and contradictions in the examples provided in the Summary and main chapters), and the strengths and limitations of each approach are discussed in the Summary and main chapters. Given these uncertainties and limitations, there is reasonable agreement between the two estimates of exposure.

Topic: Background and population exposures

Question #15 Special Populations

Primary Reviewer

Comments

The summary clearly states that the "background exposures" and the related variability, apply only to the typical, background adults with no known exposures to dioxin-like chemicals. Any exposures due to unusual circumstances (special diets, localized sources, accidents, occupation) would be in addition to the background exposures.

The document addresses special populations with higher exposures:
Nursing infants
Backyard subsistence farmers
High fish consumers
Occupational cohorts
Accident cohorts
Cigarette Smokers

Incidents of accidental contamination of the food supply have occurred in Europe, US, Japan and Taiwan. These incidents have led to well-documented increases in exposure to small populations (Yusho, Yusheng), but the impact on the general population was probably negligible. In the US, the ball clay episode involved less than 5% of the poultry production and was soon eliminated. It is not clear what mechanisms exist to quickly detect such potential threats to the national food supply, and whether other similar problems exist unnoticed. The national beef survey did not find any hot-spots, whereas elevated dioxin levels were found in dairy animals (Fries) and chickens (Harnly) associated with PCP contamination. Even though the national food supply may be safe, and most people consume a variety of items from various origins, the impact on small populations of subsistence farmers may be grave should a local source contaminate their food supply.

Similarly, subsistence fishers, or even sport fishers eating a lot of fish, have been shown to have elevated tissue levels.

Occupational cohorts have been identified, followed, and their exposures either measured directly or back-calculated from current tissue levels. In spite of certain limitations, these epidemiological studies have provided valuable information on exposures and effects (not discussed in this Summary).

A special population of concern is that of nursing infants. Since exposure to the fetus occurs transplacentally, non-nursing infants may not have escaped any dioxin-related effects, particularly since susceptibility may be significant during fetal development. On top of the in-utero exposure, nursing has been shown to increase infant tissue levels. Modeling of infant exposures assuming both constant and decreasing dioxin concentration in mothers' milk are presented and discussed in the Summary and Chapter 5 of vol.3.

Finally, limited data are presented on cigarette smoking. According to some estimates, cigarette smoking may contribute over 10% of the total intake. The limited data and layers of assumptions are discussed in Chapter 5 of vol.3, but the conclusions are not included in the Summary. If the data and estimates are considered less solid than the rest of the "special population" data, some statement should be placed in the Summary.

Topic: Summary Statement

Question #19

General Reviewer

Comments:

1. Key issues well summarized.
2. The fact that background human exposures are dropping should be mentioned. The "..need to further evaluate the impact… at or near current background levels…" may be an everlasting quest.
3. Language needs polishing, particularly the last sentence.

Topic: Overview

Question #21

General Reviewer

Comments:

Overall very good effort to summarize information. Tremendous amount of data with many assumptions, caveats, limitations clearly stated in the main volumes. The Integrated Summary, however, does not adequately capture the depth of the analysis included in the main volumes. It appears as many different individuals/groups have worked on separate sections of these documents. More work is needed to better blend the information, avoid repetitions and eliminate contradictions.

This review has been quite painful and frustrating, mainly because navigation across and among Parts, Volumes and Chapters was very difficult. An overall table of contents would have been very helpful.

A Summary should be a summary, nevertheless, there could be footnotes in Tables referring to assumptions, ranges etc. shown and discussed in the main volumes. For example, Table 4-6 of the Summary shows estimated (mean) levels without mentioning ranges or variability and the related analysis on mid- and upper percentiles. A footnote could refer to the assumptions etc.

Also, the documents need to use certain recurring terms consistently to minimize confusion. Specific examples:

TEQ_{DFP}-WHO_{98} and all other permutations. These are numerous points where this notation is not followed, e.g. Table 4.2, and p57, line 18, etc. Although this notation is cumbersome, it has its value, so let's set an example to be consistent in its use.

Early on, the term "coplanar PCBs" has been used in the literature to refer to PCBs 77, 126, 169, and later, 81. On p.3, line 28, the term is used to refer to all PCBs with assigned TEFs "…sometimes referred to as coplanar…". Also the term "coplanar" is used throughout the various volumes as synonymous to PCBs with assigned TEFs. Chapter 1.1 of the Summary should better define dioxin-like PCBs and establish consistent terms. Add Glossary.

The term “body burden” needs to be defined. Throughout the text the term is used to refer to (mainly) tissue concentrations (milk, adipose, serum) and also to the total body burden, i.e., the lipid-based tissue concentration multiplied by the fat fraction of the whole body.

P16, line 25. The term Agency is used to probably refer to USEPA. Given that IARC is mentioned in the same paragraph this can be confusing. Suggestion: Replace all “Agency” references to “USEPA” where appropriate.

p.20, line 23 and p89, line 24. Discussion of hazard potential and how an OCDD-dominated mixture would be treated “differently” from a TCDD-dominated mixture with the same TEQ. This statement contradicts the TEF approach. What guidance does this statement give to the regulated and regulating communities? This is a real problem for many regulatory bodies, who use e.g., effluent measurements to regulate discharges, and where frequently the TEQ is driven by OCDD to exceed the permitted level. Discharges claim that OCDD comes from background sources unrelated to their operation.

p.23, line 3. Identify Egeland et al. with the NIOSH study, as it is used afterwards.

p.88, line 3. Use “likely” in quotation marks.

Fig 5.1 and discussion on p.67 need clarification. Why is the US background population estimate for non-TCDD used with non-US cohorts? Why not use German or Dutch data with the respective cohorts? How do the latter vary from US estimates? Some additional discussion and justification is needed.

Lastly, some linguistic input. The word “anthropogenic” is wrong. The correct word should be “anthropogenous” or, simply, man-made. Anthropogenic means “causing or creating humans” as the analogous “carcinogenic” means “causing or creating cancer”, whereas “anthropogenous” means “created by humans”. Analogous examples are the words “endogenous”, etc. Although the word “anthropogenic” has appeared in the literature (with the wrong meaning), it is not too late to correct and this document and subsequent USEPA policies are the right vehicles to correct the error.

Primapara, plural=primaparae not primiparas

Similarly, medium, plural=media, not medias

Topic: Question 20

Primary reviewer

Comments: Yes I agree, but I also have some remarks, see also Table 4.2 and Table 4.3.

Emissions to air

Waste Incineration

The figures given here seems to be quite good. It seems to me very important to count the Backyard Barrel Burning in this category. It is evident that this burning is the second largest source with the figure 804g TEF_{DF}WHO per year, which is 65% of all the emissions from the Municipal Waste Incineration.

Together with Landfill Fires (1050g TEF_{DF}-WHO) the Backyard Barrel Burning is the most important source altogether 1850g TEF_{DF}WHO per year. I really hope that the database will allow a less pronounced activity.

The major thing here is the downgoing trend for the MWI, and a more strict guideline of Cl_4-Cl_8 total 30 ng/m^3 will even decrease these levels.

Power/Energy Generation

The figures given here seems to be appropriate and relevant.

Other High Temperature Sources

The figures given here seems to be representative and relevant.

Minimally Controlled or Uncontrolled Combustion

The figures given here seems to be representative and relevant.

Metallurgical Processes

Sintering plants. In Germany, Sweden, the Netherlands and United Kingdom the sintering plants are now considered to be a major contributor to the emissions of the PCDDs and PCDFs. In the United States this is not the case, 32.7 g TEQ_{DF}-WHO. Two plants were investigated.

It has recently been found that the chlorine content in the carbon and the temperature during the process are the major contributor see Weber et al, 1999. Since these parameters were not included in the EPA-tests I do not consider the values reported to have a ”high rating”.

Coke production. The amount of PCDDs and PCDFs is depending on the amount of chlorine in the coal. No estimation of this parameter was mad by by Bremmer et al. (1994). For this reason the estimated emission factor is very preliminary.

Primary copper, secondary aluminum, secondary copper, secondary lead and drum reclamation. These figures seem to be representative and relevant.

Primary magnesium. No figure is given for this source. Taking into consideration that two of the three US plants follow the same procedure for the preparation of magnesium as the plant in Norway, this seems to me to be a major drawback.

Chemical manufacturing /Process source

The ethylene dichloride and vinyl chloride production has been monitoring very extensive by the Vinyl Institute. The data reported here seems relevant and representative.

Releases to water and to land

The data given here seems to be relevant and representative.

Topic: Question 13 and 14

Secondary reviewer

Comments:

I have a feeling that 70 pg WHO_{DFP}-TEQ per day might be too low. The people in the US eat a lot of fried meat like Hamburgers and the content of the TEQ in the finished meat has not been estimated. In a recent study in the Netherlands these frying oils were analyzed and they contained quite a lot of PCDDs and PCDFs. They came from the fishing industry, see Liem and Theelen, 1997.

Part III, p.56 line 9. “For example, no farm-raised fish were sampled and they represent almost all of the commercial fish consumed.”

Part III, 1.59, line 29. “For example, in the United States contaminated ball clay was used as an anticaking agent in soybean meal and resulted in elevated dioxin levels in some poultry and catfish. This incident, which occurred in 1998 involved less than 5% of the national poultry production and has since then been eliminated.”

In 1995 we reported on a case where high concentrations of 2,3,7,8-substituted PCDDs have been observed in the commercial catfish samples. These samples were collected in supermarket in southern Mississippi. The values found in these samples were 1.19, 2.59 and 2.64 pg/g.

A second series of samples were collected in 1995 and they were reported in 1996. In this study it was found that the catfish from Arkansas had a higher value for the contamination of PCDDs, up to 43.4 pg TEQ_{DFP}-WHO per fat weight. It was also found that one single constituent, the soybean meal, produced this contamination. Later on in the chicken feed study, this observation was quite important.

It is my understanding that farm-raised catfish from this part of the US constitutes a major portion of the catfish on the US market. It is also my understanding that this contamination could be found during at least 5 years, perhaps a much longer time.

Question 15

Secondary reviewer

Comments:

Yes, in my opinion this is the case. It is a difference between this Great Lake group and the Swedish “high exposure” group concerning the risk scenario. The Swedish group consumed fish from the Baltic Sea several times per day, the Great Lake group only once a week.

Question 10

General reviewer

Comments:

I do not agree that the data in these documents support any other conclusion that only 2,3,7,8-tetraCDD is a known human carcinogen. At the IARC meeting in Lyon in February 1997 two other compounds were also discussed 2,3,4,7,8-penta CDF and a hexa-CDF. They were not accepted (inadequate, see p.91).

In this document I cannot see any other conclusion than that only 2,3,7,8-tetraCDD is a known human carcinogen, see also p.20 and p.89” would be characterized differently for a mixture where TEQ was dominated by OCDD as compared to one dominated by other PCDDs or (penta CDF).” I completely agree with such a statement.

Chapter 2 and 3 in Part III, Vol. 9.

General reviewer

Comments:

It is possible to have an idea about the toxic effects by 2,3,7,8-tetra CDD and the other PCDDs, and PCDFs and also PCBs. The dose in never given and that is very important here.

Lorenz Rhomberg

Topic No.: 2

Question No.: 3

Role: Lead

It is necessary to begin by parsing this complex question into parts that can individually be addressed. First, Appendix I and Figure 8-1 refer to Section 8.3.4, "Rodent Dose-Response Models: Noncancer Endpoints," of the Dose-Response chapter. As such, this is the place where the report summarizes the noncancer effects data that are to be used for quantitative analysis. One part of the present question, then, is to comment on the adequacy of this summary. Issues include comprehensiveness of coverage of datasets, organization, and presentation of data.

Second, because of the Agency's "continuum of responses" argument, effects are also included in Appendix I and Figure 8-1 that are not necessarily manifestations of toxicity per se, but rather candidates for consideration as precursors, intermediate endpoints, or biomarkers of effect. These effects might be considered as precursors or markers relevant to frank cancer or noncancer toxicity or to both. Thus, another part of the question invites comment on whether these results are adequately presented, whether they are (or should be) distinguished from frankly toxic effects, and whether their potential relation to particular toxic endpoints, cancer and/or noncancer, is (or should be) proposed. Related questions are whether the categorization of endpoints into groups (Table 8-7) is appropriate and whether the reporting of ED_{01} results as box-and-whisker plots (Figure 8-1) to denote the distribution within these categories is helpful.

Third, Appendix I and Figure 8-1 are where the quantitative modeling results are presented for all the multiple-dose study endpoints other than cancer. One cannot address their interpretation without addressing the adequacy of the modeling approaches taken to generate the tabulated results and the utility of the presentation and discussion of those results.

Finally, the charge question cites the SAB comment that previous dose-response modeling focused too much on biochemical endpoints and asks whether this has been redressed in the update. In reading the report of the SAB's September 29, 1995 review, it becomes apparent that they made several interrelated comments that are relevant. They asked for a more detailed and specific description of the modeling, finding the then existing descriptions inadequate to fully convey what was done and enable informed review; they asked for discussion and analysis of the sensitivity of the results to the particular assumptions and analytical methods chosen. They specifically questioned the lack of concurrent presentation of threshold models and call on the

Agency to justify the basis for selecting another approach. They ask for a characterization of noncancer risk that can facilitate meaningful analysis of incremental benefits of risk management alternatives (*i.e.*, that can estimate changes of health impact with changes in exposure). Importantly, the SAB found the "continuum of response" argument to be insufficiently supported, warning that "the statement is far too general and could be taken as implying that all (or any) early changes will necessarily lead to ultimate toxicity." In short, addressing this final aspect entails asking whether the "weight of evidence" interpretation of the suite of noncancer effects is well argued and supports the uses to which these data are put in the larger analysis.

The above parsing of the overall question into components is in approximate order of least to most important. The parts will be considered in turn.

Regarding the summarization of data on noncancer effects, the report clearly sets out the criteria for selection of datasets from the literature. These were chosen to focus on datasets amenable to dose-response analysis, and favor data on continuous endpoints measured at several dose levels. The criteria are well chosen for this purpose. Although the experimental literature on dioxins and related compounds is very large, the criteria led to reliance on fewer than 50 articles contributing somewhat more than 100 datasets on repeated-dosing experiments. Many of these are biochemical endpoints or effects at low levels of organization, while a number are on organ weights. No attempt is made to discuss these effects in terms of adversity, as manifestations of frank toxicity, or as indicators of more frankly toxic responses at higher levels of organization. Indeed, among the 106 repeated-dose datasets listed in Appendix I, none is put in the category of "toxicity" according to the classification of Table 8-7. (Of 77 single-dose studies listed in Appendix II, only 13 address "toxicity" endpoints, many of these reproductive and developmental effects.)

In fact, these data seem to have been assembled and analyzed solely for the purpose of an attempt to characterize the sweep of the "continuum of response." The report contains no attempt (here or elsewhere) at a comprehensive characterization of noncancer health risks that might arise from exposure to dioxins. Chapters 3-5 describe many noncancer effects and provide some dose-response information as well as some LD_{50}s, NOAELs, and LOAELs, chiefly from animal studies, but these are not carried through to an explicit noncancer quantitative analysis in either Chapters 8 or 9. Such a characterization would include endpoint-by-endpoint discussion of which data provide the most relevant basis for projection potential for human health impacts, adversity of different effects, dosimetry considerations, including questions of acute *versus*

ongoing exposures, susceptible human populations, and so on—in short, a real risk analysis, done either traditionally or otherwise, but taken up in a considered way to address the question of what impacts might be risked at what human exposure conditions. Instead, this whole aspect is handled implicitly by assuming that exposures protecting against earlier, minor effects in the continuum of responses will protect against actual noncancer toxicity as well. Given the risk management setting for dioxins and the questions the present document will be called upon to address, it is questionable whether this approach to noncancer risk assessment is tenable (see Charge Question 2).

It is noteworthy that almost all of the endpoints listed in Appendix I are continuous measures rather than the dichotomous outcomes (with/without an effect) on individuals that are the staple of most risk analyses. While continuous outcomes offer advantages of power and possibly of mechanistic interpretation, attempts to define methods for their use in noncancer risk assessment have experienced difficulty; the chief problem is defining how much of a quantitative change is to be judged an adverse response. A second issue is that a curve fitted to continuous measures on a population of subjects describes the mean quantitative change among individuals in the population, and description of variation in effects among individuals (leading to adverse impacts in the more sensitive among them) is not well defined. The document makes at best offhand reference to the first problem and fails to mention the second at all. Although analysis of continuous measures from the point of view of risk measures and health impacts is challenging, it is not unapproachable, and failure to conduct a real risk analysis of the results in this way (as opposed to simply describing the shape of the dose-effect curve in animals) sharply limits the applicability of the document to determination of the potential noncancer impacts of exposures to dioxins in human populations.

It is clear that the main thrust of the analysis of these datasets is not risk analysis of the endpoints as such, but rather the attempt to describe the "continuum of responses" idea as it is manifested in quantitative changes of a variety of effects (whatever their relation to ultimate adverse impacts might or might not be) with increasing exposure. It appears to be aimed at turning into empirical reality the diagrammatic sketch of the continuum idea that emerged from the 1988 Banbury Conference, in which taking a unified, receptor-mediated approach to dioxin risk was first proposed. In this diagram, a series of parallel sigmoid curves (representing different endpoints) was drawn, with the more severe endpoints shifted to the right along the dose axis to indicate that a common underlying, receptor-mediated mechanism was presumed to modulate all

the endpoints. The second aspect to the present Charge question, then, is to ask whether the analysis reported in Section 8.3.4, its summarization in Appendix I and Table 8-1, and the characterization of this information in the Integrated Summary support these notions.

The results in Appendix I are well presented from this point of view, and a large amount of information is effectively summarized. Hill equations have been fit to all the datasets. For each dataset, the estimated ED_{01} is shown in terms of daily administered dose and estimated steady-state body burden, along with lower bounds on these. The shape parameter is listed, the adequacy of fit characterized, and the degree of extrapolation from the data range to the ED_{01} estimate listed. What is lacking is presentation of the original data and description of the experimental design, numbers of animals, dose spacing, and so on (only the lowest dose level being named). Provision of such data would allow a reader to attempt alternative analyses and to see how aspects of experimental design (which the report's text admits could affect the analytical outcomes) interact with the curve fitting. This might best be accomplished by posting the original data on the internet and providing an address through which the interested reader could access them. It would also help to illustrate one or two curve fits graphically to give a feel for the ability of the Hill equation to describe the data and to show how a fitted curve is used to establish an ED_{01}.

The results evince a good deal of heterogeneity in curve shape; many of the curves are judged to be linear while some have Hill-equation shape parameters as high as 18.0. (The fact that many datasets are tied for highest value at exactly 18.0 leads one to suspect an artifact of some top-end cut-off in the fitting algorithm; this issue needs to be clarified.) There is some tendency for higher-level endpoints to have more nonlinear curves than lower-level "biochemical" endpoints such as enzyme induction, but it is clear that the notion of a common dose-response shape over endpoints as a reflection of a common underlying receptor-mediated modulation does not hold up empirically.

There is also a good deal of variation among endpoints in ED_{01}, as illustrated in Figure 8-1. This figure presents a useful breakdown of results according to the level of organization at which the endpoint is expressed, showing some tendency for higher-level endpoints to be affected only at higher body burdens compared to early biochemical responses. This is important for interpretation of potential adversity, which will be a function of such higher-level effects, although the implications of this are not very thoroughly followed up upon in the Integrated Summary. There is also considerable diversity of ED_{01} within categories of level of organization, about two orders of

magnitude. Overall, as noted in the Integrated Summary, these "noncancer" effects vary in ED_{01} over a range of 10 orders of magnitude of daily dose (or inferred body burden). In view of the fact that simple receptor-ligand kinetics of the sort described on p.42 of the Integrated Summary result in receptor-mediated modulation only over a ligand concentration range of about 80-fold (*i.e.*, receptor occupancy varies from near minimal to near maximal over this range), it is clear that much more complex modes are necessary to explain dioxin's range of effects over a range of doses a million-fold wider. Various explanations could be considered, including those referring to pharmacokinetic differences among tissues and species, variation in receptor concentrations in different tissues, induction of receptor synthesis, and complex cascades of events following receptor binding, including interactions with other cellular biochemical constituents. Invoking such effects has logical consequences, however, and one needs to be sure that the hypothetical explanations erected are compatible with known biological information about pharmacokinetics, tissue and species differences, and the nature of biochemical events subsequent to receptor binding. For example, in the same cells in the same organism, one cannot simultaneously have low Ah receptor occupancy for one endpoint and high occupancy for another.

Appendix I provides some opportunity to try to begin sorting out some of these issues by comparing curve shapes and dioxin potencies for similar endpoints in different species, mechanistically connected endpoints within species, similarities and differences among different endpoints within the same tissues, and potency ranges for different kinds of endpoints. Table 8-1 and comments in the text begin to examine these comparisons, but they are not done systematically or with clearly stated hypotheses in mind. A more thorough and systematic study of these issues is warranted.

As has been noted, there is no real attempt to link "early" endpoints with later consequences of frank toxicity nor to identify which dose-response data are most telling about the exposure levels at which the various noncancer effects of dioxin exposure (discussed in Chapters 3-5) might appear in humans. This means that little can be said about potential risks to environmentally exposed humans for endpoints that are not explicitly included in Appendix I. (Appendix II has information on developmental endpoints following single-dose exposures that may have more ready application, however. The issues of how to relate these to ongoing human environmental exposures have not been systematically discussed, however.)

The third aspect of the Charge's Question 2 is to comment on the modeling of these data. Use of the Hill equation is described as the "first choice" for modeling, but a power model is used

as well "for data with either no experimentally evident maximal response or with few dose groups." (It is not clear from Appendix I which endpoints have been described with which model; there is a footnote symbol to indicate use of the power model, but it does not seem to be used for any dataset.)

While most of the datasets appear to be for continuously measured endpoints, some would appear to be quantal and probably dichotomous (*e.g.*, eye opening, incisor eruption, spleen and thymus atrophy, cleft palate, hydronephrosis, stomach edema, testis descent). These should be distinguished. It is not clear that the same modeling approach is appropriate for both quantal and continuous endpoints, and certainly the interpretation of the result differs markedly for the two applications. In one case (the continuous endpoints), one is describing the changing mean value of the continuous variable for a population of exposed subjects as a function of the degree of exposure. That is, one is describing a dose-effect curve (as opposed to a dose-response curve) averaged over the whole population. In the other case (dichotomous endpoints) one is describing differences among individuals, with some individuals responding (according to a defined criterion for what constitutes having the effect) and others not. In this case, the curve describes the change in proportion responding as a function of dose.

Unfortunately, the same terminology of "ED_x", in this case ED_{01}, is often used to describe outcomes for both kinds of modeling, even though the meaning differs considerably. In the case of continuous endpoints, it refers to the dose at which the population mean measurement differs by 1% from controls. In the case of dichotomous outcomes, it refers to the dose at which 1% of the exposed population is expected to bear the response, however that response may be defined.

Chapter 8 defines its use of ED_{01} on p.8-15. Although the explanation is quite unclear, it appears that the failure to distinguish these two meanings of ED_{01} is intentional rather than inadvertent. Presumably, the meaning of this passage is that the ratio

$$\frac{R(ED_{01}) - R(0)}{R(\infty) - R(0)}$$

is to be equated to 1% and ED_{01} is defined as the dose that satisfies such an equation. For a dichotomous endpoint, the *R*'s are proportions responding, so $R(4) = 1$ and ED_{01} is defined according to the traditional definition of "extra" risk above background. For a continuous endpoint, the *R*'s are quantitative measures of the variable's mean value in the population, and $R(4)$ is the maximum quantitative value of the endpoint, so ED_{01} is defined as a change from the controls that is 1% as big as the largest possible change. It is not clear how the maximum possible response is

estimated, and no values are given for any endpoint. In the case of endpoints without an evident maximum (to which the power law was applied, according to p.8-29) it is not clear how ED_{01} can be defined using the above equation, and one must presume that in this case the 1% is a change from the control level.

Even if the two (or perhaps three) different definitions of ED_{01} can be cast in terms of one equation, doing so requires changing definitions of other terms (the *R*'s) and the resulting quantities still have conflicting meanings. When it is not apparent whether quantal or continuous data are being used, one has no idea which interpretation to follow. Since the different kinds of ED_{01} are not comparable, there is no advantage to squeezing them into false comparability.

The purpose of adopting this approach seems to be to express a variety of endpoints as fraction of maximum response over a range of doses to see if they are in some sense congruent and reflective of some underlying commonality of mechanism. It is not really made clear that this is the purpose, and the reader is easily misled into thinking that the calculations are aimed at a more usual kind of risk analysis, *e.g.*, a benchmark dose analysis or definition of a point of departure comparable to the ED_{01}s generated in the analysis of cancer risks.

Two additional points have to do with dosimetry. First, experimental animals live in the dioxin-laden world just as much as humans do, and they presumably have background levels of dioxin and related compounds in addition to the amounts specifically added by the experiment's dosing regime. The report briefly acknowledges the possibility, although the levels are generally not known. A consequence, however, is that I may be appropriate to fit models that are explicitly additive to this background, rather than assuming independent background, as the Hill equation on p.8-29 does. An additive-to-background version of the Hill equation would have the form

$$R(d) = \frac{v(d + d_0)^n}{k^n + (d + d_0)^n}$$

where d_0 is the background dose and d is the explicitly administered dose. The change of model form is important because such additive-to-background models tend toward linearity at low doses. Whether such an approach is appropriate depends on whether the background burden adds to the effective dose (as it seems it should do) and whether the response in controls is influenced by the fact that they have such a burden—*i.e.*, the control levels of response are already "on the curve" of changing effects with increased dose, as arguments elsewhere in the assessment suggest.

Another dosimetry issue is to note that many of the experiments listed in Appendix I are of limited duration. Although they have repeated dosing, the exposures continue for periods that

are short compared to a lifetime. It is questionable whether the simple calculation of steady-state body burden is appropriate in such cases. A steady state takes about 4-5 half-lives to achieve unless there is an extra large "loading dose" at the beginning of the experiment (and some, but not all, of the experiments have such a regime). For example, the experiment of Rhile *et al.* (1996) had daily dosing to mice for 11 days, or about one mouse TCDD half-life. Clearly, the body burden achieved in this experiment was far short of steady state. Thirteen week experiments in rats run about 3.6 half-lives, so steady state levels are being approached only at the end of the studies, and the average body burden during the experiment would be notably less than the steady state level.

Even though Chapter 8 states in several places that it is unlikely that a single dosimetry will be appropriate for all endpoints (and the Integrated Summary recapitulates this judgment), in practice the same dose measure is used for all repeated-dosing studies. The appropriate dose measure has to do both with the duration of the dosing regime (with a steady state assumption only being appropriate when most of the exposure is experienced during steady state, a condition that is only marginally true even for lifetime exposures in humans) and with the nature of the toxic effect, hinging on whether an integrated exposure over time or a peak tissue concentration is most predictive of the magnitude of effects engendered.

The discussion in Chapter 8 correctly notes that experimental design issues are critical and could influence interpretation. When the quantitative endpoints are measured vis-à-vis the dosing is a key element. For instance, many enzyme induction endpoints show high initial influence of dose, and then decline even in the face of ongoing exposure.

The final part of this question concerns the previous comments from the SAB about the over-reliance on biochemical endpoints. In the present document, a variety of endpoints comes under consideration. It can be argued that the available data have been examined. The question then becomes what is made of that examination and whether the continuum of responses idea is presented with more rigorous support than before. As noted above, there are still some questions about the explanation of the modeling and about whether the most appropriate approaches have been used. In particular, the earlier call by the SAB for analysis of sensitivity of particular results to particular analytical choices has yet to be addressed thoroughly. Although much evidence has been adduced through Chapters 3-5 and elsewhere that Ah receptor binding is involved in most endpoints and may have a critical role, the overall argument about how all responses represent a continuum and the sense in which they are or are not mechanistically linked still seems to lack

adequate discussion. The analyses of Section 8.3.4 and Appendix I show complex patterns of response over a wide range of doses in different systems. What is gained analytically by calling these a continuum is not clear—indeed, understanding how receptor binding can be a key part of understanding dioxin's modulation of effects ranging over 10 orders of magnitude seems problematic. Perhaps further comparison among dose-response relations that specifically examine hypotheses about comparisons among species, among tissues, and among endpoints can shed further light on this issue.

If it is accepted that receptor binding is a necessary precursor to each and every response analyzed in this section, then low levels of response of the most sensitive endpoints become an index of the exposure levels at which this "necessary but not sufficient" event begins to take place (at least in experimental animals). One could imagine using this as the basis for an RfD, and if all human exposures were comfortably below such a level, their safety could be assumed with confidence. Unfortunately, this is not the situation for dioxins—the human exposure levels are uncomfortably close to if not exceeding the levels at which minor biochemical effects begin to be seen. The question then becomes at what exposure levels such induced effects (and their consequences) come to have important impacts on health. That is, if the "necessary" part is a given, the risk managers need to know when the other "sufficient" factors kick in to lead to adverse health effects.

From this point of view, it is unfortunate that the document makes little effort to address the adversity of the effects modeled, and it does little to connect specific pre-toxicity responses to a characterization of exposure levels at which frankly toxic effects might be engendered. It is also unfortunate that the results are presented in terms of continuous-endpoint "ED_{01}" values that are easily confused with the 1% risk levels on which the cancer assessment has been based. The margin of exposure (MoE) below a risk-based ED_{01} (*i.e.*, below a dose leading to 1% elevation in incidence of a frankly adverse effect) can be judged based on understanding of the likely shape of the dose-response curve and the degree of assurance demanded that induced cases of disease will be substantially eliminated at doses deemed acceptable. On the other hand, the MoE below a dose leading to 1% of the possible degree of increase of induction of an enzyme (for which a substantial constitutive level already exists) has no clear interpretation in regard to risk or adverse effects.

The SAB's earlier call for methods that will allow risk managers to judge the potential health impacts of measures changing dioxin exposures shows that they deem an analysis of the

dose-response of actual toxic effects to be necessary. It is still necessary and the analysis in the present document does little to address the need.

Topic No.: 2

Question No.: 2

Role: Secondary

This question has two parts, the decision not to use an RfD/RfC and the decision to use an MOE in its place. The two aspects are best treated separately.

The reasons behind the decision not to use an RfD/RfC are clearly articulated. Much of the human population has a "background" exposure that is significant compared to levels that might be considered allowable under the traditional RfD/RfC approach. Because exposures above the RfD/RfC do not necessarily pose a risk, interpretation of impacts of exposures in the neighborhood of such levels has always been problematic. The RfD approach is most useful when actual exposures are well below it (or can easily be made to be so); in such settings, one can keep exposures well below those at which questions can be raised. In the case of dioxin, the management question is not how one can ensure safety but rather how one can manage exposures that appear to lead to body burdens in the general population that are already in the realm of concern, if not of potential health impacts. Because such measures are expensive and potentially intrusive, it is important to be able to estimate how much benefit is gained from efforts to limit exposures. It is for this reason that the SAB in its earlier comments called for characterization of noncancer risk that can "facilitate meaningful analysis of incremental benefits of risk management alternatives" (*i.e.*, that can estimate changes of health impact with changes in exposure).

Expressing specific sources of exposure as fractions of the overall burden is useful for perspectives in risk management decision-making, but it should not be used as a criterion in and of itself for acceptability of exposures. This is for two reasons. First, a small increment of exposure may nonetheless be associated with a large impact in absolute terms. Simply being part of a larger whole does not reduce the impact of that absolute amount. Second, it should not be overlooked that, for dioxins, the "background" burden is substantially anthropogenic, resulting from a variety of sources of exposure, all of which should be examined for means of reduction.

These things having been said, care must be taken in interpreting the document's findings regarding noncancer risks. The point of departure that has been chosen refers not to risks or

health impacts, but to the ability to detect small influences on biochemical endpoints of uncertain toxicological significance. For some major kinds of frank toxic effects (*e.g.*, chloracne) it is entirely possible that useful RfDs could be constructed that would show that the general population is at little risk at least of these endpoints, even when uncertainty factors are applied. Such calculations would differ from the usual RfDs in that they would be endpoint-specific rather than being based on the "most sensitive" effect. Such values could nonetheless be useful for certain risk-management problems. They would give important perspective regarding what established health effects might be expected or not expected at environmental exposure levels.

The second aspect for comment is the use of an MoE approach in place of the RfD/RfC. It is not clear that this makes for much improvement; it differs chiefly by foregoing making statements about the sufficiency of the smaller-than-usually-acceptable margin between exposures and the point of departure. It would seem that, in the setting of dioxins, it has become clear that we are out of the realm of making a simple safety assessment, and that the risk management tasks at hand require that assessments try to provide some estimates of actual potential impact (and thus of the benefits to be won by reducing exposures).

The MoE approach as implemented does little to help in this regard. First, as noted, the point of departure is defined not from a point of estimated impact (as is a risk-based quantal ED_{01} for cancer, for instance), but from biochemical effects of ill defined importance to health. Second, the diversity of exposure-effect curve shapes among endpoints and experiments (documented in Appendix I of Chapter 8) makes it difficult to judge the degree of concern that should be leveled at exposures of different margins below the PoD. Third, since frankly toxic effects seem to appear at exposures markedly higher than the initial biochemical responses on which the PoD is defined, and since these effects have not been separately characterized, it is not clear what endpoints are really being risked at what exposure levels.

Estimates of impacts of noncancer health effects at doses above those that can be deemed assuredly safe (*i.e.*, the "risk above the RfD" problem) are difficult to construct, but the problem has been approached in a number of contexts, such as in estimation of benefits from air toxics and water regulations. The case of dioxins is one in which such methods are especially relevant, and the Agency would be well advised to use this as a case for developing and advancing the methodology.

Lorenz Rhomberg

Topic No.: 9

Question No.: 17

Role: Secondary

I didn't really find very much on this question. What is presented on pp. 60-61 of the Integrated Summary seems quite clearly presented. In the short term, daily doses to nursing infants can be higher on a body weight basis than is typically received through other means to the more general population, but this elevated rate is short lived. What is important is that a high initial exposure can act as a "loading dose," accelerating the progress toward achieving steady state body burden in adults given ongoing low-level exposures.

The question of how to normalize this temporarily high dose rate (over its duration or prorated over lifetime) is more general than just about nursing. For endpoints that are expected to be responsive to peak concentrations, a temporarily high level may be of concern (although infants start out with their lowest lifetime accumulated burden). For endpoints more responsive to integrated concentrations over time (*e.g.*, cancer), the initially high exposure rate is relevant chiefly in its effect on average lifetime concentrations. Elsewhere, the issue of using lifetime average concentrations in temporal models of human cancer incidence has arisen. In models where the development of risks over time is modeled, it makes sense to base dose measures on cumulative risk up until the time of observation.

One caveat about nursing is that different dioxin-like compounds are likely to be differentially represented in breast milk, owing to their variation in lipophilicity. To the degree that milk production becomes a mode of excretion of such compounds for the mother, her profile of different compounds (in her body and in the milk) may shift over time as they are differentially lactationally excreted.

Topic No.: 1

Question No.: 1

Role: General

The use of estimated body burdens as a dose metric makes a great deal of difference to the projection of human risks, and thus reliance on it should not be undertaken hastily. There are several aspects that deserve further thought.

First, given the long half-life of TCDD in humans, it takes a good part of a lifetime to reach steady state given constant daily exposure. Conventionally, it is thought to require about 4-5 half-lives, which amounts to 28-35 years if one uses the 7-year half-life estimate. Thus, end-of-life steady-state body burden may make a poor surrogate for the lifetime average concentration, which is clearly the more relevant dose measure. It may be necessary to estimate these averages, rather than depend on the simple steady-state assumption. In experimental animals, many toxicity experiments are run for only fractions of a lifetime, and these animals may not reach steady state either.

Given the varying sources of dioxins, the assumption of constant daily exposure may also be too simple, and estimation of body burdens from exposure scenarios might be helpful.

The half-life of TCDD in humans is long not only in an absolute sense, but it is out of line with the allometric scaling of half-lives that usually prevails across species. It is expected that half-lives scale in proportion to the ¼-power of body mass, and this scaling actually does rather well at describing the differences among rats, mice, and hamsters as described in Chapter 8. Humans should have a half-life about 7-fold longer than mice and 4-fold longer than rats by this argument, and the prediction works out well for most chemicals. Thus, humans have an unexpected and unexplained increase in half-life of over ten-fold longer than expectations. This big difference (and its big impact on the assessment) demand some explanation. There is no obvious physiological reason for it, but potential causes need to be examined and discussed.

The question of how uncertain the half-life is deserves further discussion. A moderately different number could profoundly affect calculations. The evidence that rats may have different half-lives for different body burdens needs to be examined for possibilities of application to humans. If relative half-life is being compared on an inappropriate basis, large errors could occur.

The calculations are all based on estimates of TCDD half-life. To the extent that other dioxin-like compounds have different half-lives, their approach to steady state may be quite different. More to the point, if the rat:human or mouse:human ratios of half-lives are not as unusual for other congeners, their impact on toxicity may be markedly overestimated.

Lorenz Rhomberg

Topic No.: 4

Question No.: 7

Role: General

When TEFs are based on chronic toxicity endpoints, the factors depend not only on relative affinities for AhR and relative efficacies, but also on relative half-life. In long exposures, tissue concentrations build up gradually to steady-state levels (assuming constant daily exposure). For toxic endpoints that do not depend on integrated exposure over time, but rather on peak exposure over a shorter period, the TEF may mislead by building in an inappropriate correction for long term tendency to accumulate. Conversely, if a TEF is based on short term toxicity or simply on relative binding affinity, the factor may be inappropriate for application to long-term exposures, where different half-lives (in this case not factored in) lead to different long-term body burdens in relation to daily dose rates.

Care has to be taken to ensure that facile assumptions are not made about TEQs acting exactly like so much TCDD in all aspects. Some processes act differentially on different congeners, and over time the kinds of proportionality that TEFs assume may be skewed as these processes act differentially on different components.

Alan Smith

TOPIC#6: CANCER EFFECTS
Question 10
Primary Reviewer

Do you agree with the characterization in this document that dioxin and related computed are carcinogenic hazards for humans?

Comment:

In my opinion the characterizations that 2,3,7,8-TCDD is a human carcinogen, and that other dioxin-like compounds are "likely" human carcinogens, are appropriate. I participated in the IARC review and supported that conclusion there. More detailed consideration of the evidence, and some additional publications which have appeared since, have strengthened the basis for concluding that 2,3,7,8-TCDD is a human carcinogen.

However some of the causal inference material presented in the draft document we are reviewing is not appropriate. Indeed, based on the presentation of the epidemiological findings as given in the document, I would NOT conclude that 2,3,7,8-TCDD was a human carcinogen.

1. On page 14 it states that "not all potential confounders have been ruled out and coincident exposures to other carcinogens are likely". This statement is weak and rather negative, and not actually correct. What should be said is that " it is most unlikely that confounding factors could produce the findings reported in these studies" (a) with regard to occupational carcinogens, only heavily exposed asbestos cohort studies (e.g. U.S insulators, some asbestos factory workers) find increased risks of mortality from all cancers combined. It is inconceivable that the dioxin cohorts had comparable exposure to asbestos to these heavily exposed cohorts of asbestos workers. No other occupational carcinogen has come close to producing detectable increases in overall rates of cancer mortality. Hence, while it can be admitted that there may be some small amount of confounding due to other occupational carcinogens in this work place, they can be ruled out as the explanation for the overall increases in cancer mortality in the key dioxin cohort studies. (b) The only possible other explanation would be major confounding from smoking. It should be appreciated that although there are indeed increases in the overall rates of cancer in smokers compared to non-smokers, occupational cohorts include both smokers and non-smokers, and the general comparison population includes both smokers and non-smokers. For this reason, significant bias in the all cancer rate is unlikely. In addition, the cohort studies themselves provide data demonstrating that smoking does not explain the increased cancer rates found.

Based on the above one could say "known potential confounding factors can be ruled out as the explanation for the overall increased rate of cancer in these cohorts". Alternatively, it could be stated a little less strongly as indicated above: "it is most unlikely that confounding factors could produce the findings reported in these studies.

2. Equally important in my view is the fact that the document (as did the 1994 document) greatly weakens the evidence for 2,3,7,8-TCDD causing human cancer by including for consideration studies of phenoxyherbicide use. Phenoxyherbicide use does not result in increased body burdens of 2,3,7,8-TCDD unless there is *prolonged regular* use over *many years* (Smith AH et al, 1992). Studies which involve irregular occasional use over weeks and months, for example less than a year equivalent daily use for many hours, do not cause increases in body burdens beyond background. Even Ranch Hand type of exposure to phenoxyherbicides did not greatly increase body burdens of 2,3,7,8-TCDD (compared to the industrial cohorts). Finding increased cancer risks, including soft tissue sarcoma, in some herbicide use studies with minimal exposure therefore weakens the evidence that 2,3,7,8-TCDD itself is a cause of this and other cancers. On page 14 reference is made to the Hardell and Eriksson studies. Also Vineis (see spelling error). These studies have no relevance to 2,3,7,8-TCDD. You cannot use studies with major increase in body burdens of 2,3,7,8-TCDD, along with studies which have no increase (or trivial increase) in body burdens of 2,3,7,8-TCDD beyond background, and then say they provide evidence for 2,3,7,8-TCDD causing cancer. It is only the studies in which people have increased body burdens of 2,3,7,8-TCDD that are relevant. Each study compares "exposed" people to "non-exposed" in the general population. Surely it is obvious that if so called "exposed" persons have the same, or close to the same, body burden of 2,3,7,8-TCDD as the background in the comparison "unexposed" general population background, then any findings of such studies have nothing to do with 2,3,7,8-TCDD. I make this statement strongly because I also made it in my reviews of the 1994 document, and the comment was ignored. The studies of phenoxyherbicide use should be mentioned in passing, and it should then be pointed out that they are not relevant to 2,3,7,8-TCDD. In addition, it might be noted that herbicide use studies are inconsistent in themselves in any case.

In summary, the human evidence that 2,3,7,8-TCDD causes cancer in humans has been greatly weakened in this document by including evidence from studies in which there was no meaningful exposure to 2,3,7,8-TCDD, along with studies in which there were orders of magnitude increases in body burdens of 2,3,7,8-TCDD. I would NOT conclude from this type of presentation that 2,3,7,8-TCDD was a cause of human cancer.

However it is my view than when the evidence is properly considered then there is indeed good evidence from human studies that 2,3,7,8-TCDD is a human carcinogen. I recommend that the document be revised accordingly.

Alan Smith

TOPIC#6: CANCER EFFECTS
Question 11
Primary Reviewer

Does the document clearly present the evolving approaches to estimating cancer risk (e.g., margin of exposure and the LED01 as a point of departure), as described in the EPA "Proposed Guidelines for Carcinogenic Risk Assessment" (EPA/600/P-92/003C;April 1996)? Is this approach equally as valid for dioxin-like compounds?

On the whole the document does a good job on a complex topic. However there is no good summary, either in Chapter 8, nor in Part III that steps the reader through dose-response modeling and risk estimation. One has to read very carefully to see that the cancer risk potency estimate is derived form one human study, the Hamburg cohort (p. 90). Reference on page 90 is made to Section 5. Page 72 in Section 5 refers one to Part II Chapter 8. This sequence of searching has to be followed even just to get a rough idea of what was done.

Comment on derivation of cancer potency

I was very pleased to see the focus on the human studies. The human studies have much better diagnostic data (note the 3-fold change in tumor classification in the Kociba study!), just as good exposure data (the animal body burdens were estimated not measured) with direct measurements on at least members of each industrial cohort. Furthermore, any use of animal studies requires dealing with the two orders of magnitude difference in half life and all the uncertainties surrounding how to adjust for it. Finally, rat tumors are not human cancers. The evidence is overwhelming that the human studies should be used be used for this risk assessment.

However the bottom line here seems to be that cancer potency is now based on one epidemiological study. While the EPA has long had guidelines requiring selecting the most sensitive animal study, when animals are used for risk assessment, it is my opinion that this is an unfortunate policy to apply to epidemiological studies. There are three good cohort studies of industrial workers. In each of them, the most heavily exposed workers developed chloracne. It is not clear to me why the measured fat concentrations of TCDD were so low in the Hamburg cohort when measured in 1985. It is not clear if the measurements included workers who had developed chloracne. Mean concentrations in adipose tissue of 37 workers in the high-exposure group was 296 ng/kg. But the chloracne outbreak was in 1954. This is more than four half-lives later!

The industrial epidemiological studies are highly consistent with regard to overall increase in cancer, and they shared qualitative exposure similarities. I do not therefore think it appropriate to focus on the one study that apparently has the highest risks based on finding relatively low fat concentrations of TCDD between 4 and 5 half-lives (31 years) after the peak exposures. The problem may well lie in the assumption that averaged body burden (averaged over time) is the appropriate metric. In my opinion, the maximum body concentration achieved would relate to cancer relative risks, not spread

out averaged body concentrations. (By analogy, note that lung cancer *relative* risks for smokers depends on the cigarettes smoked per day, i.e. the dose-rate, and not to pack-years which is a cumulative dose measure).

(a) I strongly recommend that there be pooling of the human study findings in deriving a cancer potency estimate. This would involve the same studies given in Table 8-2.

(b) Furthermore, consideration should be given to using peak body burdens with an appropriate latency interval (e.g.20-30 years prior to cancer mortality), rather than average body burdens.

Comment on dealing with background exposures

Page 93 does a good job in describing a risk assessment method for dealing with background exposures. However such a method has been described in the literature, and I would appreciate that this be acknowledged. We have termed what is described on page 93 “Public Health Risk Assessment” (Smith AH et al. Consideration of background exposures in the management of hazardous waste sites: A new approach to risk assessment Risk Analysis 16: 619-625, 1996). TCDD is used as one of the two examples in this publication, and we discuss using 10% above background as an allowable point sources exposure to TCDD. This article should be cited.

Point of departure

The use of TD01 estimation based on human data originated with a publication of ours which should also have been cited in the Proposed Guidelines of Carcinogenic Risk Assessment (Smith AH, Sharp DS. A standardized benchmark approach to the used of cancer epidemiology data for risk assessment. Toxicology and Industrial Health 1:205-212, 1985). In this work we suggested that to avoid extrapolation beyond observed data, epidemiological studies should be used to work out doses necessary to cause 1% of exposed workers to get cancer. This is effectively what is now termed the “point-of departure” use of human data. This reference should be cited, e.g. on page 69.

Alan Smith

TOPIC #5: NON-CANCER EFFECTS
Question 8
Secondary Reviewer

Have the available human data been adequately integrated with animal information in evaluating likely effect levels for the non-cancer endpoints discussed in the reassessment?

It is not clear what the purpose of Section 5.2.2 is. There is no integration of information from animal and human studies. The last paragraph seems out of place.

My understanding is that the lowest dose effects in animal studies include endometriosis in monkeys and reduced sperm counts, immune suppression and other effects in offspring of rats. None of these effects are mentioned here. It seems to me that this section should identify the body burdens associated with these effects, and the equivalent human daily dose rates to achieve such body burdens. Some if this information is given in sections 2.2.2 and 2.2.3.

The LOAELs can be identified from these animal studies. It may also be appropriate to estimate ED10s. The studies are generally small, and I doubt that it is reasonable to identify ED01s from them. However consideration of margin of safety between background human body burdens and LOAELs and/or ED10s estimated from the animal studies seems to me to be critical in assessing non-cancer end-points. The lack of such information in the risk characterization section leaves me feeling that the focus is solely on cancer risks. In fact, my main concerns about general population body burdens, in particular those in the upper tail of the distribution, derive from the non-cancer effect studies in rats and monkeys mentioned above. The margin of safety for body burdens in the general population and body burdens associated with actual effects reported in the animal studies is less than 10.

In short, there are serious deficiencies in Part III in the assessment of non-cancer end-points and potential human population risks.

Alan Smith

TOPIC #8: CHILDREN'S RISK
Question 16
Secondary Reviewer

Is the characterization on increased or decreased childhood sensitivity to possible cancer and non-cancer outcomes scientifically supported and reasonable? Is the weight of evidence approach appropriate?

The discussion on this topic is lengthy. I did not see a clear concluding statement. This might be simply that children may or may not be more sensitive to effects, but the possibility that they might be more sensitive should add to caution in considering general population exposures.

Consideration of breast milk concentrations could also be improved by identifying baby body burden as they relate to maternal concentrations. On page 61, it is stated that "infant body burdens will not exceed adult body concentrations by 77 times". What should be presented is a direct comparison of estimated body burdens in infants with maternal concentrations according to the length of breast-feeding. This can be done simply using methods we presented in 1987 (Smith AH. Infant exposure assessment for breast milk dioxins and furans derived from waste incineration emissions. Risk analysis 7:347-353, 1987). Baby body concentrations of dioxins from breast milk sources will exceed that of the mother between 3 and 6 months of breast-feeding. After 12 months they will be approximately twice as high as the mother. Such information places the breast milk issue in perspective. In particular, it can be seen that baby body burdens only exceed the mother's with prolonged breast-feeding. (Note: At the time of our publication the half-life issue was not resolved so we used one year, and also five-year, half-lives. However it is a simple matter to insert 7 years as the half-life, and the results are much the same as when using the five-year half-life which is included in tables in the paper).

Question 19

It is extremely difficult to write good summary risk characterizations!

I think that the statement as its stands is rather vague and errs on the side of over-emphasizing potential risks. e.g. "Some of these effects may be occurring in humans at general population background levels and may be resulting in adverse impacts on human health". Although vague, this is an alarming statement. Also the third to last line "potential for *significant* risks to some portion of the general population".

I would prefer to see:

1. Presentation of a simple but specific risks estimate for potential cancer risks associated with background exposures (derived from the human studies), and

2. A summary statement noting that adverse effects in animals have been reported at body concentrations which are about a factor of ten above the general population average concentrations.

I also think it is important in risk characterization of population exposures to note that they have been reducing over time, and will continue to do so with policies currently in place. The risk characterization should raise concern consistent with wanting to see continuing reduction in general population body burdens.

In short, as it stands, I believe that the risk characterization summary would create more alarm than warranted by the scientific data. In part this is because the vagueness of it leaves the reader open to characterize the risks themselves. Incorporating specific key information in the summary statement is therefore important.

Curtis Travis

Review of the Dioxin Reassessment Chapter 9: Toxicity Equivalency Factors for Dioxin and Related Compounds and the Revised Integrated Summary and Risk Characterization Document.

By Curtis Travis
8112 Bennington Dr.
Knoxville, TN 37909
traviscc@icx.net

General Comments

The EPA and its staff are to be commended for the excellent job they have done in assembling, organizing, and analyzing a very large and complex set of data concerning human exposure and possible health effects resulting from the presence of dioxin and related compounds in the environment. The quantity of data that EPA needed to review and synthesize was monumental. Over 5,000 papers have been published on the toxicological properties of dioxin alone. In addition, significant advances that needed to be reviewed have occurred in recent years in our understanding of the mechanism of action of dioxin and related compounds and in the mathematical models that are being used to organize and understand this information.

In general, the documents are clear and well written. They should be easily understandable by the general scientific public. The key issues concerning dioxin health effects, discussion of the issues, and EPA conclusions regarding those issues are clearly stated. I have been particularly impressed and pleased by EPA's effort to clearly identify major uncertainties and limitations in the data and analysis presented. EPA has gone out of its way to present the uncertainties present in our current understanding of human exposure and resultant health effects from dioxin. This lends credibility to the documents. EPA cannot be expected to create understanding where no understanding exists. EPA can only summarize the current state of knowledge, clearly identify uncertainties, and make recommendation for further research. I believe that EPA has done an excellent job in summarizing the current state of knowledge concerning dioxin and related compounds.

My response to specific questions follows:

BODY BURDENS
Question 1
General Reviewer

Did EPA adequately justify its use of body burden as a dose metric for inter-species scaling? Should the document present conclusions based upon daily dose?

General Comment:
I support the use of body burden as appropriate interspecies scaling metric. However, I do not believe that EPA provides adequate justification for its use.

The EPA gives three reasons for this choice of dose metric:

- Data indicate that allometric scaling underestimates species differences in dioxin pharmacokinetics.
- Animal data support the use of dioxin tissue concentration as the appropriate dose metric for developmental, immunological, and biochemical effects.
- Tissue concentrations are directly related to body burden.

However, none of these reasons is sufficient justification of the choice of an interspecies scaling metric. One is not free to choose a scaling metric simply because allometric scaling underestimates species differences in dioxin pharmacokinetics (a fact that is not established in the documents). One must present justification based on empirical evidence (that is, demonstrate, either empirically or theoretically, that the use of the selected scaling metric produces approximately equal effects in different species).

The report is not clear as to whether the fact that the same tissue concentration produces the same developmental, immunological, and biochemical effects is true within a species or across species. The fact that are toxicological effects are proportional to tissue concentration within a single species, does not mean the tissue concentration can be used to extrapolate across species. Almost any measure of dose will be proportional to effect within a single species.

I also believe that the documents are very confusing in their presentation of the issue of interspecies scaling. Part II, Chapter 8 is full of statements that indicate that there are many different scaling metrics and that one if free to select the one that best suits the situation. This is simply not true. The only validated interspecies scaling metrics are mg/kg $^{0.75}$/day or body burden (mg/kg).

Scattered throughout the documents and the Summary (Part III, page 75; 78) are statements to the effect that the reason for the increase in the new EPA estimate of dioxin's cancer potency is that the analysis now accounts of the much longer half-life of dioxin in humans that in animals. This is not true. The increases come because EPA is now basing its analysis on body burdens rather than estimates of historical daily intake. The long half-life of dioxin in humans has nothing more to do with the increase in dioxin's cancer potency than any other of dioxin physiological parameters.

I believe that EPA should explicitly state that mg/kg $^{0.75}$ /day and body burden (mg/kg) are equivalent measures of dose that result in equal effects in all species. EPA can then list reasons that body burden (mg/kg) is the most convenient measure. These include (1) body burden integrates past exposures, (2) body burden integrates the different half-lives of different dioxin like compounds, and (3) body burden is convenient when comparing occupational exposure with background exposures.

Specific Comments.

The question of the appropriate dose metric for use in risk assessment arises because of the need to extrapolate toxicity data across species. Previous assessments of TCDD have used daily dose as the appropriate measure of dose and then applied either an allometric scaling factor (usually ¾ power of body weight) or an uncertainty factor (usually a factor of 10) for species extrapolation. The present assessment uses steady-state body burdens as the

dose metric of choice. I agree with this choice. However, I have the following comments regarding its justification.

- **Terminology.** EPA states that it will use steady-state body burden as the dose metric of choice for dioxin. General usage of the term "body burden" refers to the total quantity of a substance (mg) contained in the body. EPA does nto follow this convention and instead uses the term to refer to the total quantity of dioxin contained in the body divided by body weight (mg/kg). Technically, this is not body burden, but rather the steady- state average tissue concentration of dioxin in the body (averaged across the total mass (muscle, fat, bone, blood, etc.) of the body). Thus, it might be more appropriate to call this term "steady-state average tissue concentration". EPA correctly points out that specific tissue concentrations are proportional to this average tissue concentration, all-be-it, each tissue has a different constant of proportionality.

- **How does EPA estimate body burdens?** EPA computes body burden in one of two ways:

1) For humans, divide the dioxin lipid concentration (mg/kg) by four (assumes that 25% of body weight is lipid) to obtain mg/kg body weight.
2) Multiply the daily intake (mg/kg/day) by the half-life of dioxin/ln 2 times 50% for adsorption from food and 100% for other routes.

These formulas are approximately correct, although the first one is the most appropriate for everyday use. The point that EPA does not make sufficiently strongly is that the first approach (measured lipid concentrations) is preferred because it integrates past, and sometimes variable, exposures. The second approach can then be used to back calculate average historical daily intake (mg/kg/day) from the measured lipid concentrations. If average historical daily intake is estimated in this way, then risk estimates obtained using either body burden or daily intake should be the same. Thus, the two methods will produce the same results.

- **Dose Corrections for Species Differences in Half-life.** EPA uses the formula

  (1) ED_m (mg/kg body weight) = ED_m (mg/kg/day)*half-life/ln 2*f,

  where f is the fraction adsorbed, to make conversions from daily exposures to steady-state body burdens. This formula is correct and appropriate. In fact, implicit in this formula is the fact that both body weight (mg/kg) and daily intake (mg/kg/day) provide equivalent ways of measuring ED; that is, these two measures of dose produce equal effects after being adjusted for dioxin half-life and adsorbed fraction. It can be shown that ED_m (mg/kg) = ED_m (f*mg/$kg^{0.75}$/day), without the need for a half-life correction.

- **Half-life.** EPA repeated makes the statement that because of the longer half-life of dioxin in humans (100-fold difference in half-life between humans and animals), it is best to compare across species using body burden rather than daily intake (Part II, Chapter 8, page 8-15; Part III, page 67; Part III, page 78). The long half-life of dioxin in humans has nothing to do with that fact that body burden is a

better dose metric than daily intake. Humans have a longer half-life than animals, but they also have a longer life span. Theoretically, in physiological time (fraction of life span), the half-life of dioxin is the same in all species. Thus, the longer half-life of dioxin in humans is not a valid justification for not using daily intake as a measure of dose for interspecies extrapolation. In fact, Equation (1) above demonstrates that under steady-state conditions, the two approaches are equivalent.

The reason that body burden is a superior dose metric is that daily intake is rarely constant over time, and body burden provides an integrated measure of historical dose. Using body burden (calculated from body lipid concentration) and formula (1), one can calculate the average historical daily intake required to produce that body burden. Thus, one is free to use either dose metric in performing interspecies extrapolation and they should both produce the same result. Statements throughout the document that the use of daily intake as a dose metric is incorrect because of the long half-live of dioxin in the body are misleading.

- **Justification of Body Burden as Dose Metric.** EPA does not do a good job of justifying the use of body burden as the proper dose metric for interspecies extrapolation of toxic effect. One is not free to select whatever dose metric one wants for use in interspecies extrapolation. When selecting a measure of dose (blood concentration, tissue concentration, body burden, etc.), for use in interspecies extrapolation, one must remain with the bounds established by empirical data on extrapolation of toxic effect and the known laws governing allometric scaling.

  Data sets on interspecies extrapolation of toxicity are scarce. Travis and White (1988) analyzed the acute toxicity of 27 chemotherapeutic drugs in mice, rats, dogs, monkeys and man. Travis and Bowers (1991) analyzed anesthetic potency for 11 drugs in nine species, including humans. The strength of these data sets is that they both include human data; their weakness is that only two toxicological endpoints are addressed: lethality and anesthesia. Both data sets indicate that toxicity is approximately constant across species when administered dose is measured in mg/kg $^{0.75}$/day. This, and other theoretical arguments, is the basis for EPA's current use of body weight to the ¾ power as the proper measure of applied dose for use in interspecies extrapolation.

  It can be shown, using equations similar to in Part II, Chapter 1, section 1.4.2, page 1-47, that when dose is administered in units of mg/kg $^{0.75}$ /day, body burden (mg/kg) is the same in all species. Thus, equal quantities of body burden (mg/km) will produce equal toxic effects across species. Another way to look at this is that equal tissues concentrations produce approximately equal toxic effects in all species (realizing that adjustments must be made for differences in tissue sensitivities; for example, tissue-specific differences in the number or binding capacity of Ah receptors).

Curtis Travis

Topic 3 Mechanisms and Mode of Action
Question 4
General Reviewer

How might the discussion of mode-of-action of dioxin and related compounds be improved?

Part II, Chapter 2 provides a description of the Mechanism(s) of action of dioxin. The chapter is well written and provides a clear and well-balanced view of the complexity of understanding the mode of action for dioxin and related compounds. The primary weakness of the Chapter is lack of discussion of the mode-of-action for several TCDD-related effects referred to elsewhere in the document. Thus, the Chapter does not provide the detailed mechanistic understanding necessary to justify many of the statements made elsewhere in the document. Various parts of the documentation discuss the disruptive effects of dioxin on the hormonal system, cell growth, promotion, cell differentiation, and signal transduction (See for example, Part II, Chapter 6, page 6-15). These effects are not discussed in sufficient detail in Chapter 2.

Cell Cycle. Most of the emphasis in Part II, Chapter 2 concerns formation of the TCDD-Ah receptor-Arnt complex and its binding to DNA. Little attention is given to summarizing information concerning genes involved in the cell cycle that are activated by TCDD. For example, Chapter 2 does not contain information of the effect of TCDD on cytokines as stated in Part II, Chapter 6, page 6-1. The EPA lists (Part III, page 11 and Figure 2-2) c-fos and jun as gene products known to be mediated by TCDD. However, these genes are not discussed in Chapter 2. A background discussion of the broad molecular steps that are known to control the cell cycle and the effect of TCDD on these steps would increase understanding of the effects of TCDD on cell cycle control. . Also, the document should provide a complete list of gene products involved in cell cycle control that are affected by TCDD. For example, the Ah receptor is known to sequester the retinoblastoma protein, a key protein involved in control of the cell cycle. What is the effect of TCDD on this sequestering and what are the possible implications for the cell cycle?

Promotion. EPA has classified TCDD as "a potent promoting agent with weak or no initiating activity" (Part II, Chapter 6, page 6-28, paragraph 3) EPA provides an alternate description as "a strong cancer promoter and weak direct or indirect imitator" (Part III, page 87). TCDD does not form DNA adducts and is negative in short-term tests for genetic toxicity. Since the carcinogenicity of TCDD is a major issue and its major mode of action appears to be through promotion, Chapter 2 should discuss the known mechanisms leading to TCDD's promotional activity. What are the possible modes of action for TCDD's promotional activity? What is known about TCDD's alteration of gene products, if anything, which might explain its strong promotional activity? Chapter 2 repeatedly states that much is known about TCDD's mechanisms and mode of action, but fails to discuss one of the most important issues relative to the mode of action: how does TCDD cause the observed increase in cell proliferation?

Part II, Chapter 6, page 6-16 discusses the fact that TCDD simulates cell proliferation 10 fold in intact rats receiving 100 ng/kg TCDD, but does not stimulate cell proliferation in ovariectomized rats. As a possible explination,the document states that TCDD induced a

loss of plasma membrane epidermal growth factor receptor (EGFR) in intact rats, but not in ovariectomized rats. It is not clear how a loss of EGFR can lead to increased cell proliferation.

While EPA classifies TCDD as a strong cancer promoter, it acknowledges that this is an operational definition: TCDD acts as a promoter in initiation/promotion animal bioassays. EPA fails to highlight that most of the mechanistic data indicate that TCDD actually appears to decrease or stop cellular proliferation. The document notes that increased TCDD-induced cellular proliferation is restricted to liver foci and that it is only observed after 30 weeks of exposure. In fact, at 14 weeks cellular proliferation is reduced in TCDD exposed animals. This is consistent with the observation that TCDD actually stops the cell cycle at G1. Only after additional alterations in foci (which may be TCDD-independent) does TCDD appear to act as a promoter. A mutation of the hr (hairless) gene locus is required for TCDD's action as a promoter in mouse skin.

Current understanding of the molecular-level control of the cell cycle is fairly advanced and molecular tools are now available to identify the specific changes caused by TCDD in cell cycle control genes and gene products. EPA should recommend that these studies be done. This is a key issue in whether dioxin may be carcinogenic in humans. In particular, attention should be given to determining the effect of TCDD on expression and phosphorylation of the P53 protein, both because of its role in cell proliferation and for its known role in controlling one of the pathways to apoptosis. Studies on other non-genotoxic chemical carcinogens have show that they cause the inappropriate phosphorylation of P53.

Cell Differentiation. The document stresses that it is known that the TCDD, through its interaction with the Ah receptor, affects signal transduction pathways involved in cell differentiation. For example, the following are a few quotes:

Part II, Chapter 2, page 24, paragraph 2 states that " We can now appreciate that the Ah receptor is a member of a family of proteins that are conserved in evolution and involved in growth and differentiation processes".

Part II, Chapter 2, page 24, paragraph 2. The document states " Furthermore, we know that the genes regulated by the receptor are involved not only in xenobiotic metabolism, but also in cell growth and differentiation processes".

Part II, Chapter 6, and page 6-1, paragraph 2 states "there have been numerous reports on TCDD-induced modifications of growth factor signaling pathways and cytokines in experimental animal and cell systems…. Many of these pathways are involved in cell proliferation and differentiation."

The Chapter 2 does not discuss the possible mechanisms for TCDD's effect on cellular differentiation.

Curtis Travis

Topic 6 Cancer Effects
Question 10
Secondary Reviewer

Do you agree with the characterization in this document that dioxin and related compounds are carcinogenic hazards for humans?

General Comments

EPA believes that a weight-of-evidence evaluation suggests that mixtures of dioxin and related compounds (CCDs, CDFs, and dioxin-like PCBs) are strong cancer promoters and weak direct or indirect imitators and likely to present a cancer hazard to humans. EPA believes that TCDD is carcinogenic to humans. By this, EPA means that the weight of evidence indicates that there is a causal relationship between TCDD exposure and cancer hazard in humans. Because dioxin and related compounds always occur in the environment and in humans as complex mixtures of individual congeners, EPA believes that it is appropriate that mixtures of dioxin and related compounds also be classified as carcinogenic to humans. The weight-of-evidence approach relies on three lines of evidence: mode of action, animal data, and human epidemiological data. EPA believes that taken as a whole, these data provide strong evidence that dioxin is a known human carcinogen.

There is no doubt that TCDD is an animal carcinogen. It has been shown to be a multi-site, multi-sex, and multi-species carcinogen. . TCDD is carcinogenic in both sexes of rats and mice, carcinogenic in hamsters, and a liver carcinogen in the fish Medaka. The majority of data show that TCDD is not an initiator of the cancer process; that is, it does not appear to directly damage DNA. However, it has been shown to be a promoter in initiation/promotion studies in animals involving skin and liver. Much is known about the mechanism of action of TCDD as a carcinogen. TCDD binds to the Ah receptor as a first step in a series of events resulting in inappropriate gene activation, disruption of hormonal signaling pathways, and disruption to normal biochemical, cellular, and tissue-level biological processes, particularly those known to be involved in control of growth signals for cellular division, proper functioning of the cell cycle, cellular differentiation, and possibly apoptosis. These are precisely the molecular level events whose inappropriate control and timing result in the abnormal biological state know as cancer. There is evidence that the qualitative nature of TCDD's molecular level effects is the same in humans as in animals.

The evidence for TCDD carcinogenicity in humans is weak. Despite multiple human studies, no strong evidence for the carcinogenicity of TCDD in humans has emerged. At best, TCDD is a weak human carcinogen in lung and all cancers combined. TCDD has not been show to be a liver carcinogen in humans, nor does it appear to be a skin carcinogen. (The reason for this may that exposure cohorts tent to be male, and male animals did not get liver tumors). Thus, even though animal data and current understanding of the mechanism of action of TCDD indicate that TCDD might be carcinogenic to humans, the epidemical evidence to date for the carcinogenicity of TCDD in humans is weak. Nevertheless, EPA believes that TCCD should be classified as a known human carcinogen. While EPA acknowledges that the epidemiological evidence is inconclusive, it argues " Guidance suggests that "carcinogenic to humans" is an appropriate descriptor of humans carcinogenic potential when there is an absence of

conclusive epidemiological evidence to clearly establish a cause-and-effect relationship between human exposure and cancer, but there is compelling carcinogenicity in animals and mechanistic information in animals and humans demonstrating similar modes of carcinogenic action (Part III, page 88, paragraph 3)"

I concur with the 1995 EPA Science Advisory Broad classification of dioxin as "probably carcinogenic to humans with limited supporting information from human studies". While mechanistic data gathered in the past five years has strengthened the case that dioxin may be carcinogenic to humans, we still known little about the mechanisms downstream of the Ah receptor through which dioxin causes cancer. It is thus difficult to know if these mechanisms are working in humans. My personal belief is that the designation "Known Human Carcinogen" should be reserved for chemical compounds shown to be carcinogenic in human epidemiological studies. This is not the case for dioxin. However, it is a matter of definition, as well as a policy call, as to whether the designation "Known Human Carcinogen" should be expanded to include compounds like dioxin which are strongly carcinogenic in animals and for which some understanding of the mechanism of by which they cause cancer in known and believed to operate in humans. However, if it is decided to call such compounds "Known Human Carcinogens", it should be made clear that this is a deviation from past practices and represents a change in terminology made possible by the application of recent advances in molecular biology to strengthen our understanding on the mechanism by which some compounds cause cancer.

Specific Comments.

Mode of Action. There is strong experimental evidence that TCDD's cellular impacts are mediated through the Ah receptor. Upon binding with TCDD, this receptor migrates to the nucleus, combines with the Arnt protein, and binds to DNA-response elements in upstream regulatory regions of genes. It is believed that these genes are involved in cell growth and differentiation.

Studies in different mouse strains have shown that many of the toxic effects of dioxin (e. g., enzyme induction, thymic involution, cleft palate formation) correlate with the binding of TCDD to the Ah receptor. The Ah receptor null-allele ("knockout") mouse does not display enzyme induction or other sighs of toxicity even at high doses of dioxin. Thus, I agree that there is strong evidence that the Ah receptor is the initiator of a chain of effects that leads to some of the cellular effects of dioxin.

Human cells contain an intercellular protein whose properties resemble those of the Ah receptor in other species. However, the human Ah receptor appears to have several fold less affinity for dioxin. The document does not explain why, if humans are less sensitive to dioxin, the EPA cancer potency estimates for dioxin based on animal and human data turn out approximately the same. It would seem that estimates based on human data would be several fold lower. What I am really saying here is that the document does not investigate sufficiently strongly the possible of a mechanistic basis for less sensitivity in humans.

Cell Cycle and Promotion. It is known that TCDD and its related compounds affect cell cycle states. However, the mechanism is unknown. The Ah receptor is known to sequester the retinoblastoma protein, a key protein involved in control of the cell cycle. Progression

of the cell cycle through G1 phase is regulated in part by retinoblastoma. TCDD has been shown to arrest cells in G1. Another protein, P27Kip1, which is important in regulating cell cycle transition from G1 to S, in induced by TSDD.

TCDD is an operational promoter, this is, it promotes the appearance of tumors in initiation –promotion studies in assay systems for skin and/or liver in mice and rats. The molecular mechanisms for the promotional effects of TCDD are not known. It is presumed, but not known that the Ah receptor is involved in the promotional activity of TCDD. The document does not give sufficient attention to the question of promotion. Dioxin is a potent carcinogen that works primarily, if not totally, through promotion. The document does not discuss the mechanistic basis for dioxin's promotional effects.

Cancer. The mechanism of toxicity or carcinogenicity of TCCD is not known. It is known that association of TCCD with the Ah receptor is an initiating step in the pathway to toxicity. It is not known if TCDD association with the Ah receptor is an initiating step in the pathway to carcinogenicity. The precise components of signaling pathways downstream from the Ah receptor are not known. It is known that inappropriate activation of the Ah receptor can have disruptive effects on the regulation of a variety of signal transduction pathways.

I agree with the statement by EPA in Part II, Chapter 6, page 6-1, paragraph 3: "Although there is considerable knowledge of the details regarding activation of expression of TCDD-inducible cytochrome P450 by the Ah receptor, we still know very little about many components of Ah receptor-mediated responses and their relationship to the development of adverse responses such as cancer."

There is evidence that TCDD acts in conjunction with hormones to produce some adverse effects. For example, female rats are more prone to TCDD-induced liver neoplasm that male rats. In addition, hydrocortisone and TCDD synergize in producing cleft palate in mice. Retinoic acid and TCDD produce a similar synergistic teratogenic effect.

EPA states that a weight-of-evidence evaluation suggests that mixtures of dioxin and related compounds are strong cancer promoters and weak direct or indirect initiators (Part III, Chapter 6, page 87,paragraph 4). I do not believe that the evidence indicates that dioxin is a weak direct or indirect initiator and that this part of this statement should be modified. In Part II, Chapter 6, page 6-1, EPA states " There is considerable evidence that TCDD does not damage DNA directly through the formation of DNA adducts." EPA further states "Mechanisms have been proposed that support the possibility that TCDD might be indirectly genotoxic, either through the induction of oxidative stress or by altering the DNA damaging potential of some endogenous compounds, including estrogens." However, proposing mechanisms by which TCDD might be indirectly genotoxic does not establish it as fact. The statement at the beginning of this paragraph needs to read "a weight-of-evidence evaluation suggests that mixtures of dioxin and related compounds are strong cancer promoters and may be weak direct or indirect initiators"

Animal Carcinogenicity. TCDD has been clearly shown to increase malignant tumor incidence in laboratory animals. TCDD is carcinogenic in both sexes of rats and mice. TCDD has been shown to be a carcinogen in hamsters and to be a liver carcinogen in the fish Medaka. Initiation-promotion (I-P) studies on mouse skin and rat liver have demonstrated that TCDD is a potent tumor promoter. The TCDD skin tumor promotion is AhR dependent, with the tumor promoting potencies of several dioxin related compounds reflecting their relative AhR binding.

Enzyme Induction. TCDD is known to induce the P450 enzymes CYP1A1, CYP1A2, and CYP1B1. The relationship between TCDD induction of these enzymes and cancer is complex, increasing the rate of formation of DNA damaging metabolites in some cases and decreasing it in others. However, no relationship between TCDD carcinogenicity and induction of these enzymes has been established. Benzo(a)pyrene is not carcinogenic in Ah deficient mice. However, P450 induction by TCDD is protective against the carcinogenic action of many carcinogens such as diethylnitrosamine and aflatoxin. CYP1A2 catalyzes the conversion of 17-beta-estradiol to catechol estrogen, while CYP1B1 converts it to 4-hydroxyestradiol, a potent carcinogen in Cyrian Golden hamsters. In summary, a mechanistic link between TCCD P450 induction and cancer has not been established (Part II, Chapter 6, page 6-21).

Epidermal Growth Factor Receptor. Several studies have shown that TCDD decreases the binding capacity of the plasma membrane EGF receptor for its ligan without decreasing the number of EGF receptors. The document concludes, "TCDD produces an EGF-receptor like response consistent with the idea that TCDD enhances the generation of cellular mitotic signals" (Part II, Chapter, page 24). The justification for this statement is not clear. If TCDD decreases the binding capacity of the EGF receptor, it would seem to decrease the mitotic signals originating with this receptor. In addition, TCDD decreases the naturally occurring autophosphorylation of the EGF receptor by EGF, indicating a decrease in natural mitotic signals.

Part II, Chapter 6, page 6-24 states, "…TCDD-mediated decreases in plasma membrane EGF receptor are ovarian hormone dependent." The document had not previously established that TCDD produces a decrease in the number of EGF receptors, only that it produces a decrease in their binding capacity and autophosphorylation.

Part II, Chapter 6, page 6-24, paragraph 4 states, " incubation of human keratinocytes with TCDD decreases plasma membrane EGF receptor, and this effect is associated with an increased synthesis of TGF-alpha." The TCDD-induced increase of TGF-alpha had not been mentioned before. Since TGF-alpha binds strongly with the EGF receptor, this may explain the TCDD induced decrease in EGF receptor and the concurrent decrease in EGF-stimulated autophosphorylation (presumably because of a decrease in the number of EGF receptors).

Thyroid Hormone. TCDD induces the UGT-1 gene, which leads to a decrease in the circulating levels of thyroxin and an increase in thyroid-stimulating hormone (TSH). Prolonged increase in TSH levels can produce thyroid tumors, possibly explaining the observed increased incidence of thyroid tumors in TCDD bioassays.

Estrogen Receptor. Chronic TCDD exposure decreases the number and binding capacity of rat hepatic ER. Synthesis of rat hepatic ER, unlike other tissues, is under pituitary

control (TCDD is known to effect the pituitary through decreased levels of thyroxin). TCDD decreases in ER are Ah dependent.

Part II, Chapter 6, page 27 states, " The observation that TCDD decreases hepatic ER is in apparent contradiction to the finding that TCDD increases hepatocyte proliferation, because the ER is thought to produce mitotic signals." However, on page 6-17, the document notes that increased TCDD-induced cellular proliferation is restricted to liver foci and that it only observed after 30 weeks of exposure. In fact, at 14 weeks cellular proliferation is reduced in TCDD exposed animals. This observation is consistent with the observed TCCD-induced decrease in ER and EGFR. That is, in subchronic exposure, TCDD is not a promoter of hepatocyte poliferation. However, in subchronic exposure TCDD may block apoptosis of foci hepatocytes, thus leading to an expansion of liver foci without increased proliferation. It is only after 30 weeks that there is an observed increase in hepatocyte proliferation, and this may result from TCDD-independent genetic changes late in the cancer process.

As mentioned earlier, TCCD induction of CYP1A2 enhances conversion of 17-beta-estradiol to catechol estrogen, a possible genotoxic agent. This may explain why TCCD produces liver cancer in female intact rats but not in ovariectomized rats, and the fact that male rats do not appear to get TCCD-induced liver cancer.

Part II, Chapter 6, page 6-28 states, " This comparison has lead investigators to conclude that the antiestrogenic action of dioxin are primarily caused by effects on ER levels in reproductive tract tissues." The document has not established that TCCD reduces ER levels in any tissues except the liver. If TCDD reduces ER levels in other tissues, this needs to be stated.

Epidemiological Data.

Soft Tissue Sarcoma. The evidence for a causal relationship between TCDD exposure and Soft Tissue Sarcoma (STS) comes from multiple studies by different researchers using different research methods. However, the statistical significance of the associations is consistently weak and confounded by co-exposure the other compounds, some known to cause STS in their own right. Several important studies, such as the Fingerhut study of 5,000 production workers and the 10 and 15-year follow up of the Seveso accident workers are supportive of an association between TCDD exposure and STS, but not conclusive. While EPA acknowledges that the association between TCDD exposure and STS is weak, it argues that "no persuasive case has been made that the entirety of the association in these cases is not real (Part II, Chapter 7a, page 7A-64)". It is telling that the chapter on dose-response modeling did not use the STS data in determining the potency of dioxin. The dose-response modeling (and EPA' new estimate of TCDD potency) is based on all cancers combined and lung cancer.

Lung cancer. The evidence of a causal association between TCDD exposure and lung cancer comes from three cohort follow-up studies, all of which provide good TCDD exposure surrogates and some blood data. All three studies showed increased risk high exposure groups. Increased incidence of lung cancer in the male victims of the Japanese rice oil poisoning accident is supportive of this finding.

Overall Cancer. The evidence for a causal relationship between TCDD exposure and overall cancer rates comes from the four major production worker cohort studies. The increased relative risk in these studies for overall cancer is small, but consistent. The proposed mechanism of action is that of "general carcinogenicity" and is consistent with TCDD's known tumor promoter effects in animal studies.

Topic 9 Relative Risks of Breast Feeding

Question 17
Primary Reviewer

Has EPA adequately characterized how nursing affects short-term and long-term body burdens of dioxins and related compounds?

I believe that the EPA has adequately characterized how nursing affects short-term and long-term body burdens of dioxins and related compounds in infants. Data in both animals and humans suggests that breast-feeding results in a relatively high intake of dioxin by infants for a short, but developmentally important, span of their lives. Animal studies show that dioxin is known to affect a number of metabolic, endocrine, and immunological systems that may have an impact on developing infants. Thus, infant exposures during nursing resulting from background levels of dioxin in mother's breast milk are of concern and need to be reevaluated periodically. While little is known about the effect of pre- and post-natal exposures on cancer later in life, it is generally believed that the risk is low. Theoretical studies show that by age 10, both children who nursed and children who did not nurse have approximately the same body burdens.

Given the importance of breast-feeding in the overall health of infants, I agree with EPA that current levels of dioxin and related compounds in breast milk should not discourage the use of breast-feeding. There is no evidence that background levels of dioxin in breast-milk are harmful.

Topic 10 Risk Characterization Summary Statement

Question 18
General Reviewer-All

Does the summary and analysis support the conclusion that enzyme induction, changes in hormone levels, and indicators of altered cellular function seen in humans and laboratory animals, represent effects of unknown clinical significance, but that may be early indicators of toxic responses?

Chapter 6 provides the best evidence that enzyme induction, changes in hormone levels, and altered cellular function may be early indicators of toxic response. In, general, the presentation in the summary document could be improved. I found the quality of the writing in the summary to be somewhat less than in the analysis chapters.

Question 19
General Reviewer-All

Has the short summary statement in the risk and hazard characterization on page 107 adequately captured the important conclusions, and the areas where further evaluation is needed? What additional points should be made in this short statement?

I agree that the short summary statement in Part III, page 107 provides a reasonable statement of the fact that TCDD and related compounds may being causing adverse impacts on human health at current general population background levels. However, I believe that a few additional points should be made. First, the question above mentions the identification of areas where further evaluation is needed. The summary of page 107 provides no areas where addition evaluation is needed. Second, this section is titled Risk Characterization Statement and characterizes TCDD and related compounds as potent toxicants in animals, but gives no indication of the type of health effects that one might expect to result from dioxin exposure. I believe that some statement like "Low level TCDD exposure is likely to produce subtle changes in the normal functioning of hormonal, metabolic and cellular control systems, the clinical significance of which is currently unknown." The Risk Characterization Summary should also state that currently there is inconclusive epidemiological evidence to clearly establish a cause-and-effect relationship between human exposure and cancer, but, based on mechanistic data, the possibility cannot be ruled out.

Sources
Question 20
Secondary Reviewer

Are these sources adequately described and are the relationships to exposure adequately explained?

The EPA has done an adequate job of characterizing the U. S. sources of dioxin. Because existing information is incomplete, it is not possible to provide a totally realistic estimate of the magnitude of release for dioxin-like compounds into the environment from all sources. As EPA points out, the inventory presented by EPA is likely to underestimate total releases. In the future, attention should be given to better characterizing reservoir sources of dioxin. The summary document does a good job of summarizing environmental fate and potential pathways of exposure.

Curtis Travis

Question 21
General Reviewer- All

Please provide any other comments or suggestions to the two review documents, as interest and time allows.

Estimates of Slope Factors.

The Integrated Summary does not do a good job of explaining why the cancer slope of dioxin has increased by a factor of 10. The document should be explicit in explaining the cause of the increase. How much did the reevaluation of the animal bioassay change the slope factor? How much did changing the mathematical way that low dose extrapolation is done change the slope factor? How much did changing the dose metric to body burden change the slope factor? This information should be clearly presented in one place.

Background Cancer Risk.

Based on the human cancer (all cancers) data and the average human body burdens of 5 ng TEQ/kgBW, EPA estimates the risk of cancer from background dioxin levels to range from 1.4 E-2 to 1.3 E-3. The range of upper bound risk for the general population has increased an order of magnitude for the risk described in EPA's 1994 reassessment.

The EPA bases part of its classification of dioxin as a known human carcinogen on the association between exposure to dioxin and certain types of cancer in occupational cohorts with average body burdens of TCDD approximately 10 – 1,000 times higher than TCDD body burdens in the general population. These occupational cohort body burdens are 10-100 times the TEQ background body burdens of the general population. If background risks are in the range 10^{-3} to 10^{-2}, then the cancer risk in the occupational studies must be in the range 10^{-2} to 1. That is, based on EPA's cancer potency estimates, these occupational exposures should have resulted in nearly a 100% probability of developing cancer. If this is the case, why was not the evidence of the carcinogenicity of dioxin stronger in these studies? It would seem that the weak epidemiological data with regard to the carcinogenicity of dioxin argues against the validity of the current, higher EPA estimate of the cancer potency of dioxin. EPA should address this point in the summary document.

Specific Comments on Part II, Chapter 8.

Part II, Chapter 8, page 8-10, paragraph 2.The document states, " These critical events, the first of which is binding to the Ah receptor, are generally response-independent." It is not clear what is meant by "response-independent".

Part II, Chapter 8, page 8-10, paragraph 2. The document states, " If binding to the AhR is essential but not sufficient for effects to occur, then the dose-response curve for this event should be a better predictor of biological action than external dose as long as the shapes of the dose-response curves for these subsequent actions as similar to those of receptor binding curves." It is not clear what this sentence means.

Curtis Travis

Part II, Chapter 8, page 8-11, paragraph 1. The document states, " This outcome is inevitable for the application of the technology of mechanism-based modeling to a new area." This is a poorly written sentence.

Part II, Chapter 8, page 8-11, paragraph 4. This paragraph is a jumble of ideas. It should be rewritten to present the ideas in a more ordered fashion.

Part II, Chapter 8, page 8-12, paragraph 2. The document states, "Large differences between species and the half-life of TCDD, … " It is not clear what this means.

Part II, Chapter 8, page 8-13, paragraph 1. The document states, " At least for this comparison, the most appropriate metric for comparison is the steady-state body burden." It is not clear what is meant by "At least of this comparison". I assume you mean the comparison of some measure of dose in humans with the minimum carcinogenic dose in rats. How do you know that body burden provides the most appropriate comparison? Also, your calculated body burden is not a steady-state body burden. You mention in the next sentence that it is declining over time. Thus, it cannot be steady state.

Part II, Chapter 8, page 8-13, paragraph 1. The document states, " the current daily intake for humans is likely lower than historical levels and is biased downward because of unknown sources…" What does "biased downward because of unknown sources" mean?

Part II, Chapter 8, page 8-14, paragraph 2. The document states, " Ideally, the best dose metric is that which is directly and clearly related to the toxicity of concern by a well-defined mechanism." The meaning of this sentence is unclear. How can a dose metric be related to toxicity by a well-defined mechanism?

Part II, Chapter 8, page 8-13, paragraph 2. The document states, " For mechanism-based cancer modeling, instantaneous values of a dose metric are because they can be used as surrogates for mutation rates and growth rates…" I do not see how dose can be used as a surrogate for a mutation rate or growth rate. One might assume that mutation rates or growth rates are proportional to dose, but that is not what this sentence says.

Part II, Chapter 8, page 8-15, paragraph 4. The document states, "Given the various types of exposure scenarios and different types of exposures, it is difficult to determine a single dose metric for TCDD that can be used to compare all endpoints and species." Most of this chapter, up to this point has been used to argue this point. However, EPA gives no evidence that this statement is true. It is generally believed in toxicology that tissue concentration, adjusted for tissue sensitivity, is the appropriate dose metric in most situations. In fact, after all the discussion of why it is not possible to use a single dose metric, EPA suggests body burden (mg/kg) as the dose metric of choice. The body burden dose metric as suggested by EPA is misnamed (true body burden would be total mg in the body). What EPA is suggesting as the appropriate dose metric for TCDD is actually the average TCDD tissue concentration in a particular species; exactly the dose metric suggest by most toxicologist! It would seem better to start out by stating that average tissue concentration is the most appropriate dose metric, and then point out that there may be exceptions in certain cases.

Curtis Travis

Matti Viluksela

TOPIC #3, Question 4
Secondary reviewer

Binding affinity of dioxins to AhR and binding of the activated receptor to DRE have been discussed as possible factors explaining sensitivity differences between strains and species. Discussion on the role of the C-terminal end (the transactivation domain) of AhR, however, is completely missing. In recent studies a point mutation at the 3'-end of the *AhR* gene, resulting in altered mRNA splicing and loss of amino acids from the transactivation domain of the AhR protein, was shown to be associated with the exceptional resistance of Han/Wistar rats to TCDD (LD50 >9 600 µg/kg) (Pohjanvirta *et al.*, Mol. Pharmacol. 54:86-93, 1998). The resistance to TCDD segregated with the mutated AhR (Tuomisto *et al.*, Toxicol. Appl. Pharmacol. 155:71-81, 1999). The altered AhR was shown to normally mediate some effects of TCDD (CYP 1A1 induction, thymus atrophy), but to increase resistance to other effects, such as short term mortality and hepatotoxicity. Interestingly, in hamster, the best known dioxin resistant species, the AhR transactivation domain was also shown to be restructured (Korkalainen *et al.*, Biochem. Biophys. Res. Commun. 273:272-281, 2000). These finding suggest that the C-terminal end of AhR may be pivotal in determining sensitivity to dioxin toxicity, and therefore discussion of these findings would improve the mode-of-action section.

TOPIC #3, Question 5
Secondary reviewer

This issue is adequately discussed.

TOPIC #4, Question 7
Secondary reviewer

Basic principles of using, calculating and interpreting TEFs are clearly established. It might be helpful to give a few examples (in a Table or Appendix) how some of the current TEFs have been estimated/calculated based on different types of data.

TOPIC #8, Question 16
Primary reviewer

Possibility for increased sensitivity to dioxin-induced health effects during childhood is reasonably discussed in the light of available data. This is clearly one of the main areas where further research is needed. Most important data gaps and types of studies needed (e.g. follow-ups of exposed cohorts, mechanisms and consequences of altered thyroid homeostasis) should be specified more clearly.

TOPIC #1, Question 1
General reviewer

p. 10, lines 1-3
"As discussed in the following section, the dose associated with this plethora of effects is best compared across species using a common measurement unit of body burden of TCDD and other dioxin-like compounds, as opposed to the level or rate of exposure/intake."

More accurately effects are probably related to concentrations and not the amount of TCDD in the body. This becomes important if the amount of fat is very different in different species / individuals. Why not prefer concentration in lipid which is in balance with the mobile pool?

p. 67, lines 5-8
"Because body burden incorporates differences between species in TCDD half-life (these differences are large between rodent species and humans [Table 8.2], this dose metric appears to be the most practical for this class of compounds (DeVito et al., 1995: Aylward et al., 1996)."

Effects of a chemical are usually correlated to its concentration in critical tissues or sites. This again is usually in equilibrium with the concentrations in other tissues, not to the body burden as such (cf. lead).

p. 67, lines 31-33
"TEQ levels are calculated for PCDD, PCDF, and PCBs, based on TEQ_{DFP}-WHO_{98} values, and assume a constant 25% body fat ratio when converting from serum lipid ppt to ng/kg body burden."

Fig. 5-1 and other data provide a reasonable database of lipid concentrations of dioxins. Why try to calculate this to body burdens by using arbitrary factors, if the concentration is actually a better metric than amount.

p. 68, lines 28-31
"This will be important if, as demonstrated for some chronic effects in animals and as assumed when relying on average body burden as a dose metric, cancer and other noncancer effects are a consequence of average tissue level over a lifetime."

What is the evidence for this?

TOPIC #6, Question 10

General reviewer

The evidence for carcinogenicity of dioxins and related compounds in humans is largely based on data from industrially exposed cohorts. The exposure of these cohorts, however, has been substantially higher compared to the background exposure of the general population. Individual exposure data based on direct measurements are seldom available. Moreover, occupational exposures are never pure dioxin exposures, and the exposure of these cohorts to the main chemical is usually 10 000 - 1 000 000 times higher than the exposure to dioxins. These facts have not been adequately addressed in the document when extrapolating the data to the general population.

p. 19, lines 15-17
"Although exposure to dioxins may influence cancer response directly or indirectly, positively or negatively, it is unlikely that such data will be available to argue that dioxin exposure a net benefit to human health."

The wording illustrates that the authors are not unbiased in their treatise, but they seek to prove TCDD carcinogenic. This is not good science. A good scientist is sceptical to both directions. Carcinogenic effects of TCDD have only been observed at relatively high dose levels. It could be theoretically conceivable that enzyme induction at lower exposure levels would result in increased elimination of potentially carcinogenic xenobiotics and decreased risk for cancer. Cf. p. 84, lines26-28: *"Induction of activating/metabolizing enzymes at or near background levels, for instance, may be adaptive, and in some cases, beneficial..."*

p. 19, lines 33-34
"... mechanistic information in animals and humans demonstrating similar modes of carcinogenic action."

Please explain what is this mechanistic information? To my understanding the mechanism of carcinogenicity of TCDD is far from being clear. Differences in tumor types between animals and humans do not really speak for similar modes of action in animals and humans.

p. 14, lines 28-32
"Nonetheless, the incidence of soft tissue sarcoma is elevated in several of the most recent studies..." One of the references is Lampi *et al.*, 1992

In the study of Lampi *et al.* (1992) increased incidence of soft tissue sarcoma has nothing to do with PCDD/PCDFs. The observed soft tissue sarcomas and non-Hodgkin's lymphomas were associated with chlorophenol exposure via drinking water, and it was shown in a separate study that the PCDD/PCDF levels were not elevated in fat samples of chlorophenol exposed subjects (Vartiainen *et al.* Chemosphere 30(8):1429-1438). Contrary to chlorophenols, PCDD/PCDFs are not water soluble and therefore are never present in

drinking water. These studies emphasize the significance of other (non-dioxin) chemicals in mixed exposures as well as the importance of exposure assessment.

p.16, lines 25-27
"Since this issue was last reviewed by the Agency in 1988, TCDD has been shown to be a carcinogen in hamsters (Rao et al., 1988), which are relatively resistant to the lethal effects of TCDD."

Carcinogenicity of TCDD in hamsters is only seen at very high dose levels.

p. 18, lines 5-6
"Liver tumors are ovary dependent, but ovaries appear to protect against TCDD-mediated tumor promotion in lungs."

HeptaCDD was recently shown to induce lung tumors in female Sprague-Dawley rats (Rozman *et al.*, The Toxicologist 54 (1): 1297, 2000).

p. 65, lines 27-29
"At this time, the knowledge of the mechanism of action of dioxin, receptor theory, and the available dose-response data do not firmly establish a scientific basis for replacing a linear procedure for estimating cancer potency."

Nor do they establish a scientific basis for using it!

p.87, lines 34-36
"With regard to carcinogenicity, a weight of evidence evaluation suggests that mixtures of dioxins and related compounds (CDDs, CDFs, and dioxin-like PCBs) are strong cancer promoters and weak direct or indirect initiators and likely to present a cancer hazard to humans."

All evidence suggests that this may be the case **at high exposures**.

TOPIC #6, Question 12
General reviewer

The document is too preoccupied with "upper bound" effects. The first step should always be the determination of the most likely risk (which usually also means average risk). Upper bound effects belong to safety margin discussion, which is only partly science and mostly administrations and politics (risk management).

p. 89, lines 33-36
"...many of these biochemical effects can be hypothetisized to be the key events in a generalized dioxin mode-of-action model. These analyses do not argue for significant departures from linearity below a calculated ED_{01} for endpoints potentially related to cancer response, for at least one or two orders of magnitude lower response."

Linearity of a biochemical effect does not tell anything about the linearity of the toxic effect. In such a simple phenomenon as carbon monoxide poisoning the biochemical effect (concentration of COHb) is linear to CO-exposure, but lethality is not.

p. 90, lines 8-12
"... Consequently, the Agency, although fully recognizing this range and the public health conservative nature of the slope factors that make up the range, suggests the use of 5×10^{-3} per pg TEQ/kgBW/day as an estimator of upper bound cancer risk for both background intakes and incremental intakes above background."

Science should be kept science and risk management risk management. Using explicit conservative assumptions is bad science. Science is after truth, this means the best judgement of the state of affairs, not the conservative assumption. Conservative allowances belong to risk management. If they are included along the way in the scientific assessment, nobody knows at the end what is the truth. In the text the most likely result is not even given to the reader, let alone that it would be the basis of assessment.

p. 90, lines 17-19
"Based on these slope factor estimates, upper bound cancer risk from average current background body burdens (5 ng/kgBW) resulting from average intakes of approximately 3 pg TEQ/kgBW/day are in the range of 10^{-3} to 10^{-2}."

This is the worst case assumption. What is the most likely assumption?

TOPIC #7, Question 15
General reviewer

Tissue levels are highly age-dependent, and this should be stated more clearly.
25 ppt (ng/kg) for the average tissue level is very uninformative.

In spite of high dioxin exposure of infants their body burdens are relatively low, partly due to faster elimination of dioxins. According to a German study the elimination may be substantially faster in infants compared with adults. Based on a physiological toxicokinetic model Kreuzer *et al.* (Arch. Toxicol. 71:383-400, 1997) estimated that the half-life of elimination of TCDD in a newborn is 4 months (compared with about 5 years in adults).

p. 61, lines 19-20
"Although this scenario seems reasonable, no supporting data could be found for such a highly exposed subpopulation in the United States."

Such subpopulation of fishermen eating frequently (at least twice weekly) fish from the Baltic sea was recently found in Finland (Kiviranta *et al.* Lancet 355:1883-1885). In this population PCDD/PCDF concentrations up to 420 I-TEQ pg/g fat were measured. Highest concentrations are comparable to those found in inhabitants of Seveso A zone.

TOPIC #10, Question 18
General reviewer

p. 84, lines 24-26
"Enzyme induction, changes in hormone levels, and indicators of altered cellular function seen in humans and laboratory animals represent effects of unknown clinical significance but that may be early indicators of toxic response."

That is not at all clear; e.g. enzyme induction may have nothing to do with toxicity, because toxicity may depend on expression of completely separate genes. For comparison, corticosteroids also induce multiple effects: they can induce congenital malformations, but most effects of corticosteroids are not at all related with malformations. So, they may or may not!

p. 84, lines 20-22
"...to support the inference that humans are likely to respond with a broad spectrum of effects from exposure to dioxin and related compounds."

One should also appreciate at the same time that even huge exposures (Seveso, two ladies in Vienna) resulted in surprisingly few clinical findings (chloracne, porphyria).

Question 21
General reviewer

"second" p. 9, lines 32-34
"Even though not all observed effects may be characterized as "adverse" effects (i.e., some may be adaptive and of neutral consequence), they represent a continuum of response expected from the fundamental changes in biology caused by exposure to dioxin-like compounds."

There is no evidence on this! If CYP 1A1 gene has nothing to do with dioxin toxicity, it does not represent a continuum of response.

p. 11-12, lines 35, 1-2
"In fact, for every biochemical response that has been well studied, the data are consistent with the particular response being dependent of the AhR."

This is oversimplification, and suggests that all contradictory data have been ignored.

p. 31, lines 20-21
"Perinatal exposure of experimental animals to TCDD results in suppression of primarily T cell immeune functions, with evidence of suppression persisting into adulthood."

Please indicate the dose range for this effect.

p. 36, lines 7-10
"As adult-onset diabetes is also associated with overweight, and body composition has been shown to modify the apparent half-life of dioxin, could the rate of elimination of dioxins be lowered in people with diabetes, causing them to have higher body burdens?"

This is a misunderstanding of kinetics and accumulation of body burden. More body fat at similar daily intake would compete for dioxins in central space, and therefore prolong half-life and therefore delay the time to reach steady state. So, at higher body burden, concentration would never be higher than in lean subjects.

p. 38, lines 15-17; p. 95, lines 17-19
"Early in the disruption process, the body can overcompensate for the loss of T4, which may result in a small excess of circulating T4 ***in response*** *to the increased TSH."*

It may, but will it? This is not in line with the principles of endocrinological feedback.

p. 86, lines 20-22
"The scientific community has identified and described a series of common biological steps that are necessary for most, if not all, of the observed effects of dioxins and related compounds in vertebrated including humans."

It remains to be seen, if there is any chemical so specific that it would have only one mechanism of action. This is against all toxicological wisdom.

p. 87, lines 26-27
"The position taken in this reassessment is that these 1998 TEFs should be adopted for use by the Agency."

One should note that the TEF concept and TEQs are inherently administrative measures and not scientific. Therefore they should not be used alone in scientific context, but the original absolute results should always be available.

TECHNICAL COMMENTS, TYPOS ETC.

CHAPTER 9

List of abbreviations would be useful.
Please, use abbreviations consistently. Now there is variation, e.g. **AhR** and **Ah receptor** are used in turns.

p.9-15, line 13: Rudd**i**ck

p. 9-19, line 28-29: There is no ref. for Viluksela et al., 1997. It should be Viluksela et al., 1998ab

p. 9-19, line 30: Viluks**e**la

p. 9-25, line 32: transthy**r**etin

p. 9-25, line 34: delete one "decrease in"

p. 9-28, line 15: Putzrath, 199**7**

p. 9-30, line 5: 1,**2**,3,7,8-PCDD

p. 9-30, line 29: mat**t**er

p. 9-37, line 2: po**l**ychlorinated

PART 3

List of abbreviations would be useful.

p. 59, line 16: Wrong cross reference; should be Chapter 5 or 4-5.

p. 89, line 34: to be **the** key events

p. 103, line 6: POTW not explained

p. 130, Fig. 4-2. EDC/VC not explained.