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EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments, Volume 1

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In Support of Summary Information on the Integrated Risk Information System (IRIS)

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ABSTRACT

This document comprises the first of two EPA reports (*f.d. V aR's Rvanalysis of Kv^ Issues Related to Dioxin Toxicity and Response to NAS Comments Volumes 1 and 2 [Reanalysis Volumes 1 and 2]*) that, together, will respond to the recommendations and comments on 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) dose-response assessment included in the 2006 NAS report, *Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment.* This document, Reanalysis Volume 1, includes (1) a systematic evaluation of the peer-reviewed epidemiologic studies and rodent bioassays relevant to TCDD dose-response analysis; (2) dose-response analyses using a TCDD physiologically based pharmacokinetic model that simulates TCDD blood concentrations following oral intake; and (3) an oral reference dose (RfD) for TCDD. An RfD of 7×10^{-10} mg/kg-day is derived based on two epidemiologic studies: (a) a study that associated TCDD exposures with decreased sperm concentration and sperm motility in men who were exposed during childhood and (b) a study that associated increased thyroid-stimulating hormone levels in newborn infants born to mothers who were exposed to TCDD. A qualitative discussion of uncertainties in the RfD and a focused quantitative uncertainty analysis of the choices made in the development of points of departure for RfD derivation are also provided.

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LIST OF ABBREVIATIONS AND ACRONYMS

Ah	aryl hydrocarbon
AhR	aryl hydrocarbon receptor
AIC	Akaike Information Criterion
ANL	Argonne National Laboratory
AUC	area under the curve
BMD	benchmark does
BMDLs	benchmark dose lower confidence bounds
BMDS	Benchmark dose software
BMI	body mass index
BMR	benchmark response
BW	body weight
CADM	concentration- and age-dependent elimination model
CYP	cytochrome P450
DLC	dioxin-like compounds
ED_x	effective dose eliciting x percent response
EPA	Environmental Protection Agency
FSH	follicle stimulating hormone
GD	gestation day
GI	gastrointestinal
HED	human equivalent dose
IDD	iodine deficiency disease
ILSI	International Life Sciences Institute
IQ	intelligence quotient
IRIS	Integrated Risk Information System
KO	knockout
LASC	lipid-adjusted serum concentration
LOAEL	lowest-observed-adverse-effect-level
$LOAEL_{HED}$	HED estimate based on LOAELs
MOA	mode of action
NAS	National Academy of Sciences
NCEA	National Center for Environment Assessment
NIOSH	National Insitute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect-level
NTP	National Toxicology Program
OSF	oral slope factor
PA	permeability × area
PBPK	physiologically based pharmacokinetic
PCB	polychlorinated biphenyl
PCBs	polychlorinated biphenyls
PCDFs	polychlorinated dibenzofuran
PK	pharmacokinetics
POD	point of departure

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

RfD	reference dose
SAB	Science Advisory Board
SD	standard deviation
TC	total cholesterol
TCDD	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
TEF	toxicity equivalence factors
TEQ	toxicity equivalence
TK	toxicokinetic
TSH	thyroid stimulating hormone
TWA	time-weighted average
UF	uncertainty factor
UF	uncertainty factor
UF_A	interspecies extrapolation factor
UF_D	database factor
UF_H	human interindividual variability
UF_L	LOAEL-to-NOAEL UF
UF_S	subchronic-to-chronic UF
Vd	Volume of distribution
WHO	World Health Organization

PREFACE

This report was developed by the U.S. Environmental Protection Agency's (EPA) Office of Research and Development (ORD), National Center for Environmental Assessment (NCEA).

In 2003, EPA, along with other federal agencies, asked the National Academy of Sciences (NAS) to review aspects of the science in EPA's draft dioxin reassessment titled, *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds* ("2003 Reassessment"). In 2004, EPA sent the 2003 draft Reassessment to the NAS for their review. In 2006, the NAS released the report of their review titled, *Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment*. The NAS identified three areas in EPA's 2003 draft Reassessment that required improvement: (1) justification of approaches to dose-response modeling for cancer and noncancer endpoints; (2) transparency and clarity in selection of key data sets for analysis; and (3) transparency, thoroughness, and clarity in quantitative uncertainty analysis. The NAS provided EPA with recommendations to address their key concerns.

In 2008, EPA, in collaboration with the Department of Energy's Argonne National Laboratory (ANL), developed and published a literature database of peer-reviewed studies on TCDD toxicity, including in vivo mammalian dose-response studies and epidemiologic studies. EPA subsequently requested public comment on this database. EPA and ANL then convened a scientific workshop in 2009. The workshop goals were to identify and address issues related to the dose-response assessment of TCDD and to ensure that EPA's response to the NAS focused on the key issues and reflected the most meaningful science.

In May 2010, EPA released a draft report titled *VaR's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments* ("Reanalysis") that provided a technical response to the 2006 NAS report. The draft Reanalysis (1) developed a study selection process to evaluate studies reporting cancer and noncancer effects; (2) utilized a TCDD physiologically based pharmacokinetic (PBPK) model in its development of dose-response analyses of TCDD toxicological and epidemiologic literature; (3) presented new analyses of both the potential cancer and noncancer human health effects that may result from exposures to TCDD; (4) developed an oral reference dose (RfD) for TCDD; and (5) developed a new cancer oral slope factor for TCDD. Federal agencies and White House offices were provided an opportunity for review and comment on the draft Reanalysis prior to its public release; their comments are available at www.epa.gov/iris. The draft Reanalysis received public comments and was provided to EPA's Science Advisory Board (SAB) for independent external peer review. The SAB convened an expert panel composed of scientists knowledgeable about technical issues related to dioxins and risk assessment. For their review, the SAB held public meetings in June, July, and October 2010, and in March and June 2011.

The SAB released their final review report on August 26, 2011. In their final report, the SAB panel: (1) commended the comprehensive and rigorous process that was used to identify and evaluate the TCDD literature; (2) agreed that EPA's choice of kinetic model provided the best available basis for the dose metric calculations; (3) supported EPA's selection of two coprincipal epidemiologic studies for the derivation of the RfD for TCDD; and (4) generally agreed with EPA's characterization of TCDD as *carcinogenic to humans* in accordance with EPA's 2005 *Guidelines for Carcinogen Risk Assessment* and with EPA's selection of the critical study for the quantitative cancer assessment. However, the SAB found that the draft Reanalysis did not respond adequately to the NAS recommendation to adopt both linear and nonlinear

methods of extrapolation to account for the uncertainty in the cancer dose-response curve for TCDD. Also, the SAB report conveyed disagreement with EPA's position in the draft Reanalysis that a comprehensive uncertainty analysis was infeasible and suggested a number of methods that could be used for this purpose.

Based on the SAB review, EPA decided to separate the dioxin Reanalysis into two volumes. This document, Volume 1, systematically evaluates the epidemiologic studies and rodent bioassays relevant to TCDD dose response, including studies evaluating cancer and noncancer responses. It uses a TCDD PBPK model to simulate TCDD blood concentrations, the dose metric used in all dose-response analyses for TCDD in this volume. Volume 1 also develops an oral reference dose (RfD) based on two epidemiologic studies that associated TCDD exposures with adverse health effects. The first study reports decreased sperm concentration and sperm motility in men who were exposed to TCDD during childhood during the Seveso accident (Mocarelli et al., 2008), and the second reports increased thyroid-stimulating hormone levels in newborns born to mothers who were exposed to TCDD during the Seveso accident (Baccarelli et al., 2008). Volume 1 also provides a focused quantitative uncertainty analysis of the decisions made in the development of points of departure for TCDD RfD derivation.

In Volume 2, EPA will complete the evaluation of cancer mode-of-action, cancer dose-response modeling, including justification of the approaches used for dose-response modeling of the cancer endpoints, and an associated quantitative uncertainty analysis. The information provided in Volume 1 will be used in three ways: (1) as the first of two reports that contain EPA's response to the NAS (2006b) report, (2) as the Support Document for the TCDD noncancer IRIS Summary and TCDD oral RfD, and (3) as technical support for the dioxin Reanalysis Volume 2. The summaries of the cancer studies included in Volume 1 are presented for use related to noncancer effects. These summaries are not intended to inform regulatory or other decision-making purposes related to carcinogenesis; further, no quantitative dose-response assessments are developed for cancer studies in Volume 1.

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EXECUTIVE SUMMARY

OVERVIEW

Dioxins and dioxin-like compounds (DLCs), including polychlorinated dibenzo-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls, are structurally and toxicologically related halogenated dicyclic aromatic hydrocarbons.[1] Dioxins and DLCs are released into the environment from several industrial sources such as chemical manufacturing, combustion, and metal processing; from individual activities including the burning of household waste; and from natural processes such as forest fires. Dioxins and DLCs are widely distributed throughout the environment and typically occur as chemical mixtures. They do not readily degrade; therefore, levels persist in the environment, build up in the food chain, and accumulate in the tissues of animals. Human exposure to these compounds occurs primarily through the ingestion of contaminated foods (Lorber et al., 2009), although exposures to other environmental media and by other routes and pathways do occur.

The health effects from exposures to dioxins and DLCs have been documented extensively in epidemiologic and toxicological studies. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is one of the most toxic members of this class of compounds and has a robust toxicological database. Characterization of TCDD toxicity is critical to the risk assessment of mixtures of dioxins and DLCs because it has been selected repeatedly as the ―index chemical" for the dioxin toxicity equivalence factors (TEF) approach. In this approach, the toxicity of individual components of dioxin and DLC mixtures is scaled to that of TCDD. Then, the dose-response information for TCDD is used by the U.S. Environmental Protection Agency (EPA) and other organizations to evaluate risks from exposure to mixtures of DLCs (U.S. EPA, 2010b; Van den Berg et al., 2006; Van den Berg et al., 1998).

To provide guidance on the use of the TEF approach in environmental health risk assessments, EPA published a report titled, *Recommended Toxicity Equivalence Factors (TEFs) for Human Health Risk Assessments of 2,3,7,8-Tetrachlorodibenzo-p-dioxin and Dioxin-Like Compounds* (TEF report) (U.S. EPA, 2010b). The TEF report describes EPA's updated approach for evaluating the human health risks from exposures to environmental media containing DLCs. In the TEF report, EPA recommends use of the consensus TEF values for

[1] For further information on the chemical structures of these compounds, see U.S. EPA (U.S. EPA, 2010b, 2008b, 2003).

TCDD and DLCs published in 2005 by the World Health Organization (Van den Berg et al., 2006) for all cancer and noncancer effects mediated through aryl hydrocarbon receptor binding. Further, EPA recommends that the TEF methodology, a component mixture method, be used to evaluate human health risks posed by these mixtures, using TCDD as the index chemical; therefore, it is imperative to correctly assess the dose response of TCDD and understand the uncertainties and limitations therein.

In 2003, EPA completed a comprehensive human health assessment external review draft titled, *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds* (~~"~~2003 Reassessment"). As part of EPA's commitment to the development of health assessment information of the highest scientific integrity, scientific peer review is an integral component of the process EPA uses to generate high quality toxicity and exposure assessments of environmental contaminants. To this end, EPA asked the National Academy of Sciences (NAS) to review the 2003 draft Reassessment. In 2006, NAS released their report titled, *Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment* (NAS, 2006a). In this review, the NAS identified three key recommendations requiring improvement to support a scientifically robust characterization of human responses to exposures to TCDD. These three key areas are (1) improved transparency and clarity in the selection of key data sets for dose-response analysis, (2) further justification of approaches to dose-response modeling for cancer and noncancer endpoints, and (3) improved transparency, thoroughness, and clarity in quantitative uncertainty analysis. NAS also encouraged EPA to calculate an oral noncancer reference dose (RfD), and provided specific comments on various aspects of EPA's 2003 draft Reassessment.

In May 2009, EPA Administrator Lisa P. Jackson announced the *Science Plan for Activities Related to Dioxins in the Environment* (~~"~~Science Plan") that addressed the need to finish EPA's dioxin reassessment and provide a completed health assessment on this high profile chemical to the American public.[2] The Science Plan stated that EPA would release a draft report responding to the recommendations and comments included in the NAS review of EPA's 2003 draft Reassessment.

As outlined in the Science Plan, in 2009, EPA developed a draft report titled *VaR's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments*

[2] Available online at http://www.epa.gov/dioxin/scienceplan.

(–Reanalysis") that responded to the key comments and recommendations in the NAS report (U.S. EPA, 2010a). The draft Reanalysis focused on TCDD dose-response issues and included analyses of relevant new studies and the derivation of an oral noncancer RfD and an oral slope factor (OSF) for cancer. The draft Reanalysis was reviewed internally by EPA scientists and was provided for review to other federal agencies and White House offices. On May 21, 2010, the draft Reanalysis was released for public review and comment and independent external peer review by EPA's Science Advisory Board (SAB).

For their review, the SAB held public meetings in June, July, and October 2010, and in March and June 2011. They released their final report reviewing the draft Reanalysis on August 26, 2011 (SAB, 2011).[3] In their report, the SAB communicated the following overarching observations:

- They found that the draft Reanalysis was clear, logical, and responsive to many—but not all—of the NAS recommendations; they were impressed with the comprehensive and rigorous study selection process that was used to identify, review and evaluate the scientific literature on TCDD dose response;
- They agreed with the choice of the Emond physiologically based pharmacokinetic (PBPK) model for dose metric calculations and with the selection of whole blood as the dose metric;
- They agreed with the choice of two epidemiologic studies as coprincipal studies whose developmental toxicity data were used to derive the RfD for TCDD;
- They agreed with EPA's cancer weight of evidence classification of TCDD as *carcinogenic to humans* (with the exception of one panelist with a dissenting view);

The SAB also identified two deficiencies in EPA's draft Reanalysis with respect to the completeness of the consideration of two critical elements:

- Nonlinear dose response for TCDD carcinogenicity; and
- Uncertainty analysis

[3] Available online at http://yosemite.epa.gov/sab/sabproduct.nsf/2A45B492EBAA8553852578F9003ECBC5/$File/SAB-11-014-unsigned.pdf.

The SAB recommended that EPA fully evaluate both linear and nonlinear dose-response approaches to TCDD cancer dose-response assessment—including a discussion of carcinogenic mode of action. The SAB also recommended a number of approaches to quantitative uncertainty analysis that could be implemented by EPA, including the use of sensitivity analyses and probability trees.

In August 2011, EPA announced a plan for moving forward to complete the draft Reanalysis.[4] Per this plan, the current document is the first of two EPA reports (*f.d. V aR's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments Volumes 1 and 2 [Reanalysis Volumes 1 and 2]*) that, together, will respond to the recommendations and comments on TCDD dose-response assessment included in the NAS review of EPA's 2003 draft Reassessment. Both Volumes focus on TCDD only. This report, Reanalysis Volume 1, completes and publishes EPA's study selection criteria and study selection results for both noncancer and cancer TCDD dose-response assessment; choice of kinetic model; noncancer RfD for TCDD; and a qualitative discussion of uncertainties in the RfD with a focused quantitative uncertainty analysis. Reanalysis Volume 1 responds to key comments and recommendations pertaining to noncancer TCDD dose-response assessment published by the NAS in their review (NAS, 2006b).

The information and analyses in this Volume have undergone revisions in response to SAB comments and recommendations as well as comments provided by the public (see Appendix A). Reanalysis Volume 2 will address the two deficiencies identified by the SAB, i.e., nonlinear dose response for TCDD carcinogenicity and quantitative uncertainty analysis for TCDD carcinogenicity. In Volume 2, EPA will complete the evaluation of cancer mode of action, cancer dose-response modeling, including an updated literature search, justification of the approaches used for dose-response modeling of the cancer endpoints, and an associated quantitative uncertainty analysis. The information provided in Volume 1 will be used in three ways: (1) as the first of two reports that contain EPA's response to the NAS (2006b) report, (2) as the Support Document for the TCDD noncancer IRIS Summary and TCDD oral RfD, and (3) as technical support for Reanalysis Volume 2. The summaries of the cancer studies included in Volume 1 are presented for use related to non-cancer effects. They also provide information on the complete literature review and study selection process that EPA conducted in preparing

[4] Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=209690.

the draft Reanalysis, which included information on both cancer and noncancer effects. These summaries are not intended to inform regulatory or other decision-making purposes related to carcinogenesis; further, no quantitative dose-response assessments are developed for cancer studies in Volume 1. The final cancer analysis will be included in EPA's Reanalysis, Volume 2.

The three key NAS recommendations specifically pertain to dose-response assessment and uncertainty analysis. Therefore, EPA's response to the NAS in this document is focused on these issues.

EPA thoroughly considered the recommendations of the NAS and, in Reanalysis Volume 1, responds with an evaluation of TCDD hazard identification and dose-response data via the following:

- An updated literature search that identified new TCDD dose-response studies (see Section 2);
- A workshop that included the participation of external experts in TCDD health effects, toxicokinetics, dose-response assessment and quantitative uncertainty analysis; these experts discussed potential approaches to TCDD dose-response assessment and considerations for EPA's response to the NAS (U.S. EPA, 2009a) (see Appendices B and I);
- Development of a detailed study selection process including criteria and considerations for the selection of key epidemiologic and animal bioassay studies (see Section 2.3) for quantitative TCDD dose-response assessment (see Section 2.4.1/Appendix C and Section 2.4.2/Appendix D, respectively);
- Kinetic modeling that quantifies appropriate dose metrics for use in TCDD dose-response assessment (see Section 3 and Appendices E and F);
- A sensitivity analysis performed on each of the Emond animal and human PBPK models that identify the most sensitive variables in each model (see Section 3.3.4);
- Dose-response modeling for all appropriate noncancer data sets (see Section 4.2/Appendix G);
- A thorough and transparent evaluation of the selected TCDD data for use in the derivation of an RfD, including justification of approaches used for dose-response modeling of noncancer endpoints (see Section 4.2 and Appendix H);
- The development of an RfD (see Section 4.3);

- A qualitative discussion of the uncertainty in the RfD and a focused quantitative uncertainty analyses of the RfD (see Sections 4.4 and 4.5, respectively); and
- Responses to the comments and recommendations made by the SAB in their final report (SAB, 2011) (see Appendix A).

Those activities and analyses are briefly described in this Executive Summary, and they are described in detail in the related sections of this document.

In addition to this document, several additional EPA activities address other TCDD issues, specifically related to the application of dioxin TEFs and to TCDD and DLC background exposure levels. Information on the application of the dioxin TEFs is published elsewhere by EPA for both ecological (U.S. EPA, 2008b) and human health assessment (U.S. EPA, 2010b). As a consequence, EPA does not directly address TEFs herein but makes use of the concept of toxicity equivalence as applicable to the analysis of exposure dose uncertainty in epidemiologic studies and an animal bioassay. Furthermore, this document does not address the NAS recommendations pertaining to the assessment of human exposures to TCDD and other dioxins. Information on updated background levels of dioxin in the U.S. population has been recently reported (Lorber et al., 2009). In 2006, EPA also released a report titled *An Inventory of Sources and Environmental Releases of Dioxin-Like Compounds in the United States for the Years 1987, 1995, and 2000*, which presents an evaluation of sources and emissions of dioxins, dibenzofurans, and coplanar polychlorinated biphenyls (PCBs) to the air, land and water of the United States (U.S. EPA, 2006a).

PRELIMINARY ACTIVITIES UNDERTAKEN BY EPA TO ENSURE THAT THE REANLAYSIS VOLUMES 1 AND 2 REFLECT THE CURRENT STATE-OF-THE-SCIENCE

As part of the development of this document, EPA undertook two activities that involved the public: an updated literature search and a scientific expert workshop. The adverse health effects associated with TCDD exposures are documented extensively in epidemiologic and toxicologic studies. As such, the database of relevant information pertaining to the dose-response assessment of TCDD is vast and constantly expanding. Responding directly to the NAS recommendation to use the most current and up-to-date scientific information related to TCDD, EPA, in collaboration with the Department of Energy's Argonne National Laboratory

(ANL), developed an updated literature database of peer-reviewed studies on TCDD toxicity, including in vivo mammalian dose-response studies and epidemiologic studies. An initial literature search for studies published since the development of the 2003 draft Reassessment was conducted to identify studies published between January 1, 2000, and October 31, 2008. EPA published the initial literature search results in the Federal Register in November 2008 and invited the public to review the list and submit additional, relevant, peer-reviewed studies. Additional studies identified by the public and through continued work on this response were incorporated into the final set of studies for TCDD dose-response assessment (updated through October 2009). Since release of the draft Reanalysis for public comment and external peer review in 2010, EPA has collected a limited number of additional studies that inform EPA's derivation of an RfD for TCDD. These studies were identified by EPA scientists, the SAB, and the public, and they have been used to further evaluate the biological significance of the endpoints used to derive the RfD and to develop information on uncertainty in the RfD. These additional studies are cited in the appropriate sections of this document. None of the data sets collected since October 2009 was used quantitatively in the noncancer dose-response assessment of TCDD.

To assist in responding to the NAS, EPA, in collaboration with ANL, convened a scientific expert workshop ("Dioxin Workshop") in February 2009 that was open to the public. The primary goals of the Dioxin Workshop were to identify and address issues related to the dose-response assessment of TCDD and to ensure that EPA's response to the NAS focused on the key issues, while reflecting the most meaningful science. EPA and ANL assembled expert scientists and asked them to identify and discuss the technical challenges involved in addressing the NAS comments, discuss approaches for addressing these key recommendations, and to assist in the identification of important published and peer-reviewed literature on TCDD. The workshop was structured into seven scientific topic sessions as follows: (1) quantitative dose-response modeling issues, (2) immunotoxicity, (3) neurotoxicity and nonreproductive endocrine effects, (4) cardiovascular toxicity and hepatotoxicity, (5) cancer, (6) reproductive and developmental toxicity, and (7) quantitative uncertainty analysis of dose response. External cochairs (i.e., scientists who were not members of EPA or ANL) were asked to facilitate the sessions and then prepare summaries of discussions occurring in each session. The session

summaries formed the basis of a final workshop report (U.S. EPA, 2009a) (see Appendix B). Some of the key outcomes from the workshop include the following recommendations:

- Further develop study selection criteria for evaluating the suitability of developing dose-response models based on animal bioassays and human epidemiologic studies;
- Use kinetic modeling to identify relevant dose metrics and dose conversions between test animal species and humans, and between human internal dose measures and human intakes;
- Consider newer human or animal bioassay (NTP, 2006a) publications when evaluating quantitative dose-response models for cancer;
- Consider both linear and nonlinear modeling in the cancer dose-response analysis.

The discussions held during the Dioxin Workshop helped inform, guide, and focus EPA's response to the NAS.

EPA'S APPROACH TO CONSIDERING TRANSPARENCY AND CLARITY IN THE SELECTION OF KEY STUDIES AND DATA SETS FOR DOSE-RESPONSE MODELING

One of the key NAS recommendations to EPA was to utilize a clear and transparent process for the selection of key studies and data sets for dose-response assessment. EPA agrees with the NAS and believes that clear delineation of the study selection process and decisions regarding key studies and data sets will facilitate communication of critical decisions made in the TCDD dose-response assessment. EPA developed detailed processes and TCDD-specific criteria and considerations for the selection of key dose-response studies. These criteria and considerations are based on current guidance for point of departure (POD) identification and RfD and OSF derivation (U.S. EPA, 2005a, b, 2000, 1998, 1996, 1991, 1986a, b); they also consider issues specifically related to TCDD. These criteria reflect EPA's goal of developing noncancer and cancer toxicity values for TCDD through a transparent study selection process. Following the selection of key studies, EPA employed additional processes to further select and identify cancer and noncancer data sets from these key studies for use in dose-response analysis of TCDD.

Figure ES-1 presents EPA's study selection process for the evaluation of the epidemiologic studies considered for this TCDD dose-response assessment, including specific

study inclusion criteria (see Section 2.3.1). EPA applied its TCDD-specific epidemiologic study inclusion criteria to all studies published on TCDD. For all peer reviewed studies, EPA

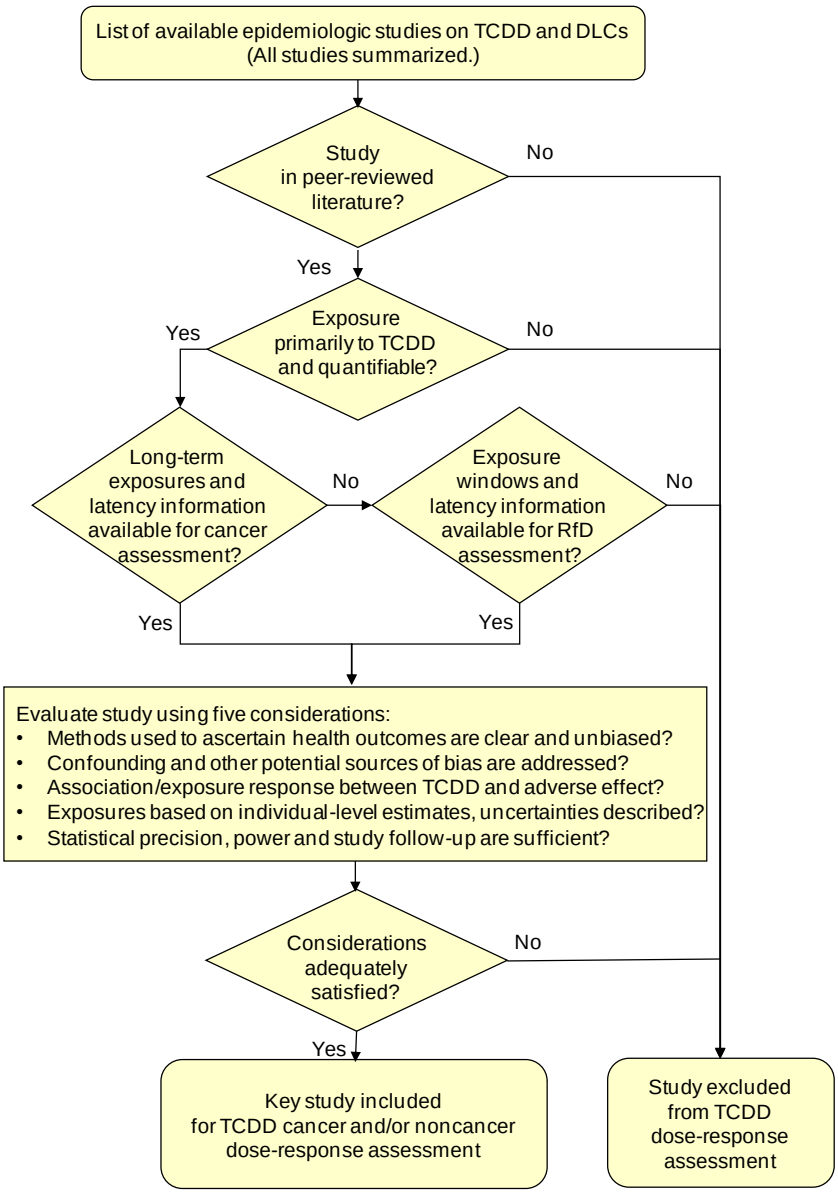

Figure ES-1. EPA's selection process to evaluate available epidemiologic studies using study inclusion criteria and other epidemiologic considerations for use in the dose-response analysis of TCDD.
EPA applied its TCDD-specific epidemiologic study inclusion criteria to all studies published on TCDD and DLCs. For all peer reviewed studies, EPA examined whether the exposures were primarily to TCDD and if the TCDD exposures could be quantified so that dose-response analyses could be conducted. Then, EPA required that the effective dose and oral exposure be estimable: (1) for cancer, information is required on long-term exposures, (2) for noncancer, information is required regarding the appropriate time window of exposure that is relevant for a specific, nonfatal health endpoint, and (3) for all endpoints, the latency period between TCDD exposure and the onset of the health endpoint is needed. Finally, studies were evaluated using five considerations

regarded as providing the most relevant kind of information needed for quantitative human health risk analyses. Only studies meeting these criteria and adequately satisfying the considerations were selected for EPA's TCDD dose-response analysis.

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Figure ES-2 presents EPA's study selection process for the evaluation of mammalian bioassays considered for TCDD dose-response assessment—including the specific study inclusion criteria (see Section 2.3.2). EPA evaluated all available in vivo mammalian bioassay studies on TCDD. Studies had to be published in the peer-reviewed literature. Studies on genetically altered species were excluded as their direct relevance to human health is not known. Next, EPA applied dose requirements to each study's lowest tested average daily dose, with specific requirements for cancer (≤1 μg/kg-day) and noncancer (≤30 ng/kg-day) studies. EPA also required that the animals were exposed via the oral route to only TCDD. Finally, the studies were evaluated for quality and summarized to ensure the most relevant information for quantitative analyses was provided. Only studies meeting all of the criteria were included in EPA's TCDD dose-response analysis.

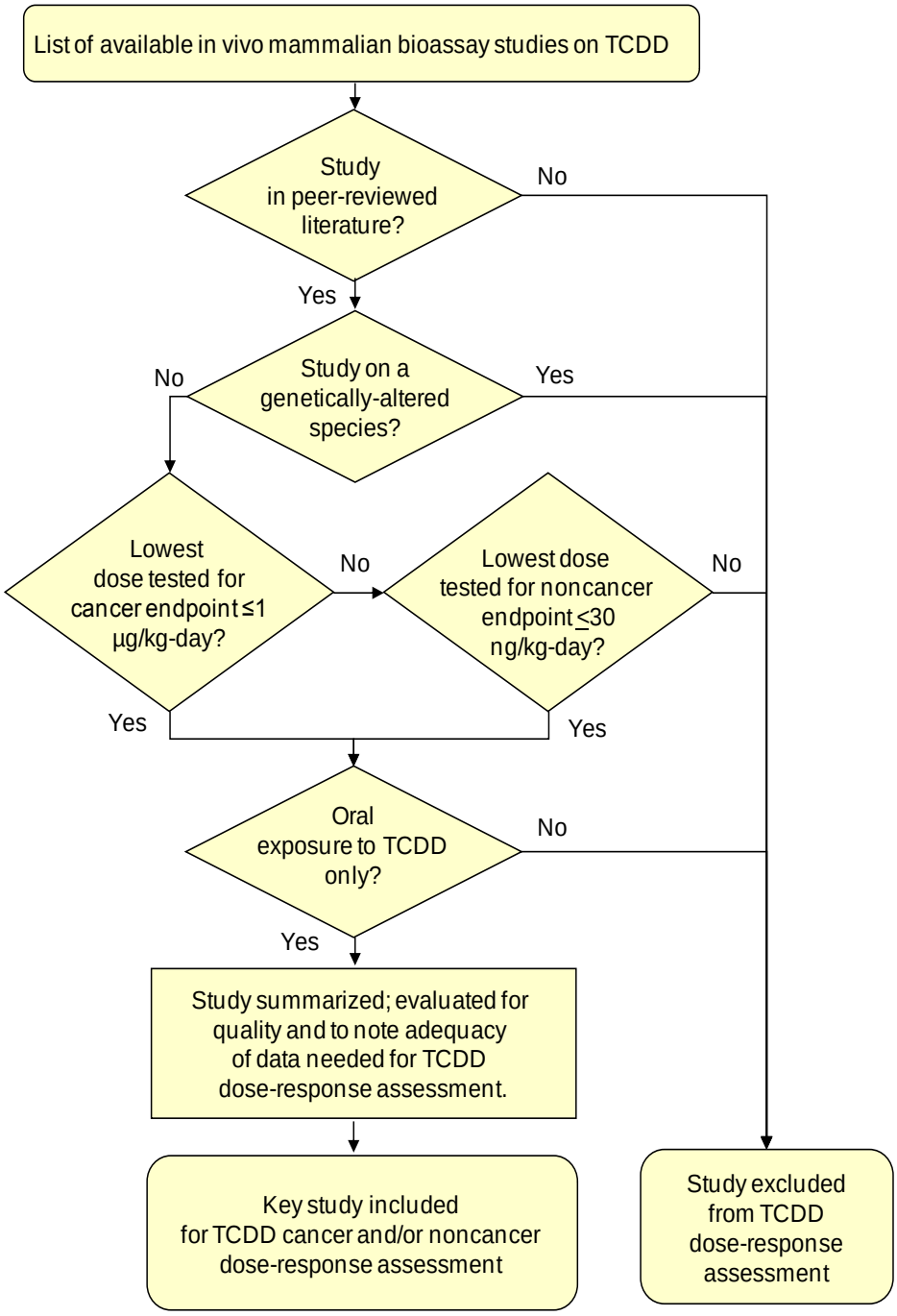
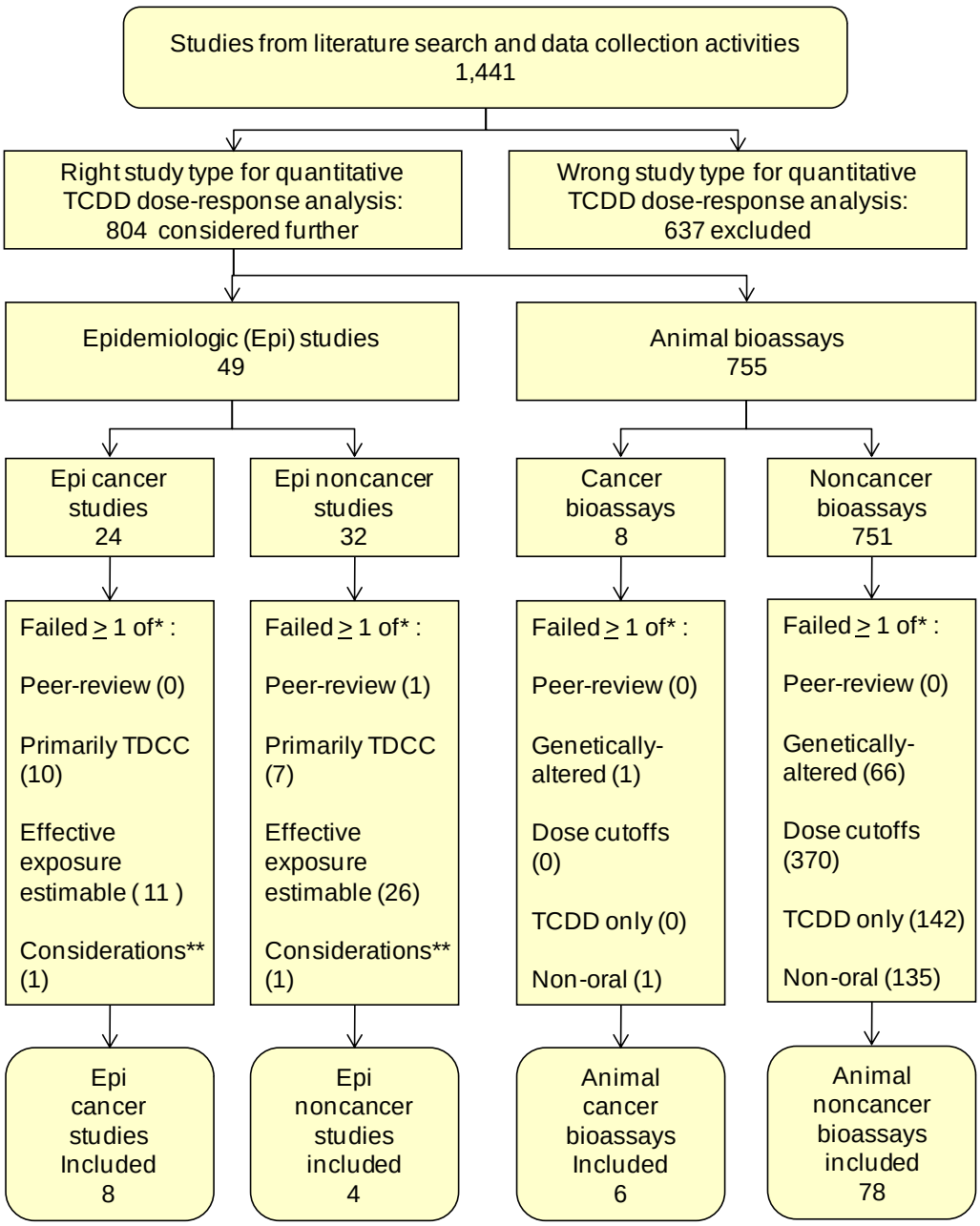


Figure ES-2. EPA's process to evaluate available animal bioassay studies using study inclusion criteria for use in the dose-response analysis of TCDD.
EPA evaluated all available in vivo mammalian bioassay studies on TCDD. Studies had to be published in the peer-reviewed literature. Studies on genetically-altered species were excluded as their relevance to human health is not known. Next, EPA applied dose requirements to each study's lowest tested average daily dose, with requirements for cancer (≤1 µg/kg−day) and noncancer (≤30 ng/kg−day) studies. EPA also required that the animals were exposed via the oral route to only TCDD. Finally, the studies were evaluated for quality and summarized to ensure providing the most relevant information for quantitative human health risk analyses. Only studies meeting all of the criteria were selected for EPA's TCDD dose-response analysis.

Figure ES-3 shows the results of EPA's process to select and identify in vivo mammalian bioassays and epidemiologic studies for quantitative TCDD dose-response assessment. A total of 1,441 studies were examined. Of these, 637 studies were eliminated from consideration as they were not suitable study types; these included, in vitro bioassays, review articles, PBPK modeling studies, and studies that evaluated dioxin-like compounds (DLCs) other than TCDD. Of the remaining studies, 49 were epidemiologic studies (7 studies contained both cancer and noncancer endpoints), and 755 were animal bioassays (4 studies contained both cancer and noncancer endpoints). These epidemiologic studies and animal bioassays were then evaluated using EPA's study inclusion criteria. Appendices C and D detail EPA's study summaries and evaluations for the epidemiologic studies and animal bioassays, respectively. Results of the study selection process for the epidemiologic studies are shown in Tables 2-1 and 2-2 (preliminary cancer studies and final noncancer studies, respectively) and for the animal bioassays are shown in Tables 2-3 and 2-4 (preliminary cancer bioassays and final noncancer bioassays, respectively). Through this study selection process, EPA was able to identify a group of studies for TCDD dose-response evaluation that spanned the types of adverse health effects associated with TCDD exposures and encompass the range of doses in the lower end of the dose-response region most relevant to the development of an RfD. The summaries of the cancer studies are presented for use related to non-cancer effects in this document. Quantitative dose-response assessments will be developed for the cancer studies in the Reanalysis, Volume 2.



*Failed criteria are not mutually exclusive; more than one can fail for a given study.
**Indicates those studies that passed all three criteria but were not selected based on study considerations.

Figure ES-3. Results of EPA's process to select and identify in vivo mammalian and epidemiologic studies for use in the dose-response analysis of TCDD.

Four animal studies and seven epidemiologic studies contained both cancer and noncancer endpoints. Two epidemiologic cancer studies, Steenland et al. (1999) and Flesch-Janys et al. (1998), passed all criteria, but were still not selected because they were superseded by other studies on the same cohort for which an improved analysis was done. One noncancer epidemiologic study, Baccarelli et al. (2005), passed all criteria, but was excluded because the health endpoint, chloracne, is considered to be an outcome associated with high TCDD exposures.

For the selected studies, EPA conducted additional evaluations to determine which study/endpoint data sets were the most appropriate for development of the RfD for TCDD. During the study selection process, EPA identified four epidemiologic studies and 78 animal bioassays that met the study inclusion criteria and adequately satisfied the considerations for TCDD dose−response analyses. From the epidemiologic studies, one was eliminated because EPA could not assess the biological significance of the finding and could not establish a LOAEL; EPA derived three candidate RfDs from the other studies. Figure ES-4 overviews the disposition of the 78 noncancer animal bioassays selected for TCDD dose-response. Of these, EPA eliminated those studies that contained no toxicologically relevant endpoints for RfD derivation (see Appendix H and Section 4.2.1). EPA then identified PODs from the remaining bioassays and eliminated from further analysis those studies with PODs above specified dose limits. (See additional details on POD development in the section below on Derivation of an RfD for TCDD.) These dose limits were imposed to limit the size of the analysis yet ensure representation of all important health effects associated with TCDD exposure. EPA derived 37 candidate RfDs from the remaining 48 animal studies, with 11 studies presented as supporting information.

In summary, EPA conducted a transparent study selection process to select epidemiologic studies and animal bioassays for TCDD quantitative dose-response analyses. From these selected studies, EPA identified 40 candidate RfDs, three from the epidemiologic studies and 37 from the animal bioassays.

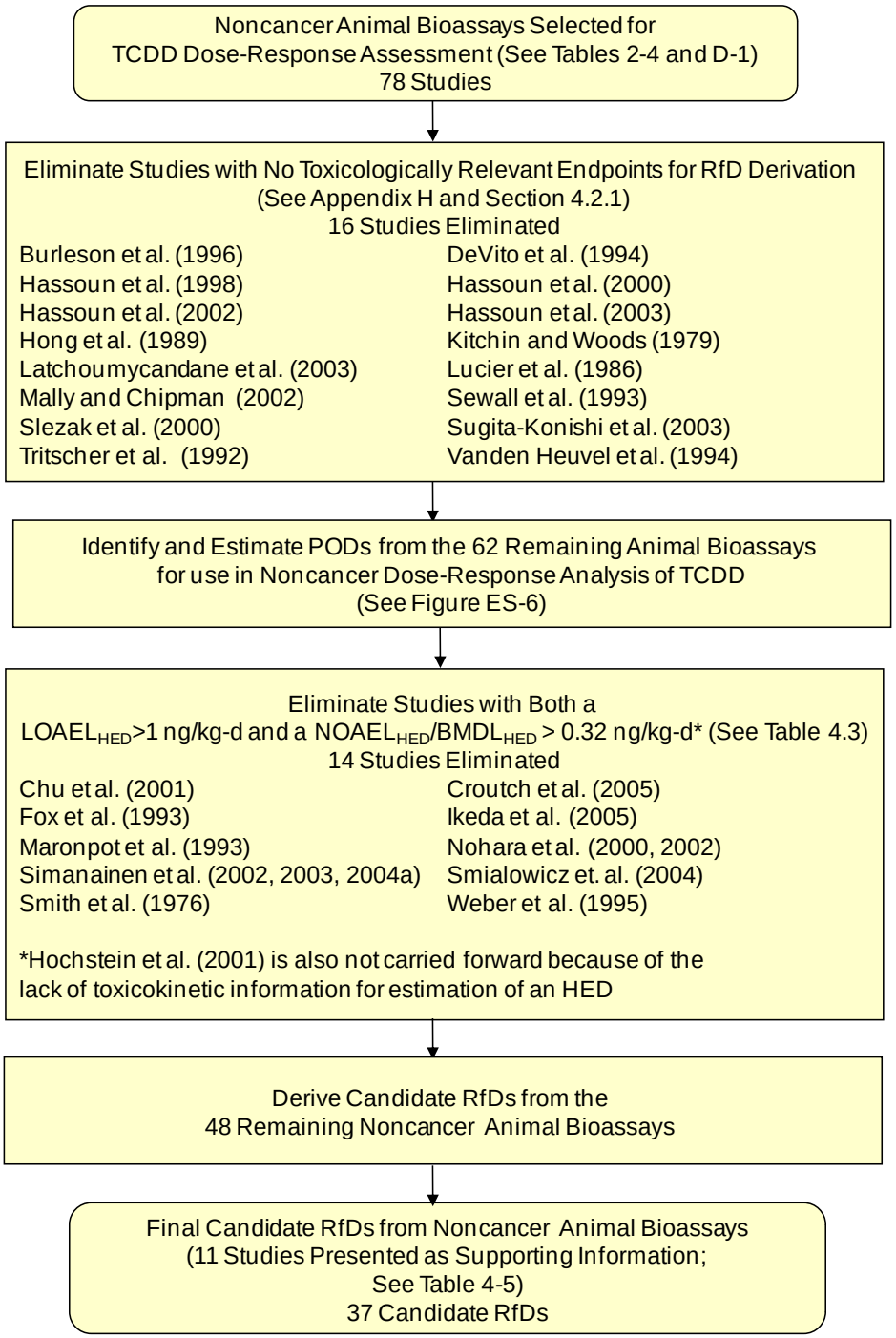


Figure ES-4. Disposition of animal noncancer bioassays selected for TCDD dose-response analysis.

EPA evaluated each noncancer endpoint found in the 78 studies that passed the study inclusion criteria. From this evaluation, EPA eliminated 16 studies that contained no toxicologically relevant endpoints for RfD derivation. Then, as detailed in Figure 4-3, EPA selected and identified PODs for use in deriving candidate RfDs. EPA then eliminated 13 studies based on dose limits for the PODs' HEDs; one study was also not carried forward because of the lack of toxicokinetic information for estimation of an HED. Of the remaining 48 studies, EPA derived 37 RfD candidates, with 11 studies presented as supporting information.

USE OF KINETIC MODELING TO ESTIMATE TCDD HUMAN EXPOSURES AND DOSES IN ANIMAL BIOASSAYS

The NAS recommended that EPA utilize state-of-the-science approaches to finalize the 2003 draft Reassessment. Although the NAS concurred with EPA's use of first-order body burden models in the 2003 draft Reassessment, analyses of recent TCDD literature and comments by experts at the Dioxin Workshop suggested that the understanding of TCDD kinetics had increased significantly since the release of EPA's 2003 draft Reassessment. These advances led to the development of several pharmacokinetic models for TCDD (Emond et al., 2006; Aylward et al., 2005a; Emond et al., 2005; Emond et al., 2004) and resulted in EPA's incorporation of TCDD pharmacokinetics in the dose-response assessment of TCDD.

The evaluation of internal dose in exposed humans and other species is facilitated by an understanding of pharmacokinetics (i.e., absorption, distribution, metabolism, and excretion). TCDD pharmacokinetics are influenced by three distinctive features: (1) TCDD is highly lipophilic, (2) TCDD is slowly metabolized, and (3) TCDD induces binding proteins in the liver. The overall impact of these factors results in preferential storage of TCDD in adipose tissue, a long half-life of TCDD in blood due to slow metabolism, and sequestration in liver tissue when binding induction becomes significant. As these kinetic features control target tissue levels of dioxin, they become important in relating toxicity in animals to possible effects in humans.

Consideration of pharmacokinetic mechanisms is critical to the selection of the dose metrics of relevance to dose-response modeling of TCDD. Earlier assessments for TCDD—including the 2003 Reassessment—used estimates of body burden as the dose metric for extrapolation between animals and humans. These body burden calculations used a simple one-compartment kinetic model based on the assumption of a first-order decrease in the levels of administered dose as a function of time. However, the assumption of a constant half-life value for the clearance of TCDD from long-term or chronic exposure is not well-supported biologically given the dose-dependent elimination observed in rodents and humans. The dynamic disposition and redistribution of TCDD between blood, fat, and liver as a function of time and dose is better described using biologically-based models. Additionally, these models provide estimates for other dose metrics (e.g., serum, whole blood, or tissue levels) that are more biologically relevant to response than body burden estimated based on an assumption of first-order elimination over time.

For extrapolation from rodents to humans, EPA considered the following possible dose metrics for TCDD: administered dose, first-order body burden, lipid-adjusted serum concentration (LASC), whole blood concentration, tissue concentration, and functional-related metrics of relevance to the mode of action (MOA) (e.g., receptor occupancy) (see Section 3.3.4.1). After evaluation of these dose metrics, EPA chose to use TCDD concentration in whole blood, modeled as a function of administered dose, as the dose metric for assessing TCDD dose response in this document. LASC is commonly used in the epidemiologic literature as the metric of choice because TCDD is highly lipid-soluble and LASC accounts for individual differences in the size of the serum lipid compartment. However, whole blood concentration was chosen because of the structure of the Emond PBPK model, in which the liver and other tissue compartments are connected to the whole blood compartment rather than to the serum compartment; LASC is estimated only as a result of model simulations by multiplying whole-blood concentrations by a conversion constant. EPA used the time-weighted average whole-blood concentration over the relevant exposure periods for all animal bioassay dosing protocols, dividing the area under the time-course concentration curve (AUC) by the exposure duration. Because all of the epidemiologic studies evaluated by EPA reported TCDD exposures as LASC rather than whole-blood concentrations, oral intakes were modeled using LASC as the dose metric. In most cases, the reported TCDD LASC was extrapolated both forward and backward in time to simulate the actual exposure scenario.[5]

Several biologically-based kinetic models for TCDD exist in the literature. The more recent pharmacokinetic models explicitly characterize the concentration-dependent elimination of TCDD (Emond et al., 2006; Aylward et al., 2005a; Emond et al., 2005; Emond et al., 2004; Carrier et al., 1995a, b). The biologically based pharmacokinetic models describing the concentration-dependent elimination (i.e., the pharmacokinetic models of Aylward et al. (2005a) and Emond et al. (2006; 2005)) are relevant for application to simulate the TCDD dose metrics in humans and animals exposed via the oral route. The rationale for considering the application of the Aylward et al. (2005a) and Emond et al. (2006; 2005; 2004) models was largely based on the fact that both models reflect research results from recent peer-reviewed publications, and both models are formulated with dose-dependent hepatic elimination consistent with the

[5] For the Seveso cohort, which had a high single TCDD exposure followed by low-level background exposures leading to a gradual decline in the internal TCDD concentrations, EPA estimated both peak and average exposures over a defined critical exposure window (see Section 4.2.2).

physiological understanding of TCDD kinetics. Dose-response modeling based on body burden of TCDD in adult animals and humans can be conducted with either of the models.[6] The predicted slope and body burden over a large dose range are quite comparable between the two models (generally within a factor of two).

Results of simulations of serum lipid concentrations or liver concentrations vary for the two models to a larger extent (up to a factor of 7), particularly for simulations of short duration. These differences reflect two characteristics of the Emond et al. (2006) model: first, quasi-steady-state is not assumed in the Emond et al. (2006) model; second, the serum lipid composition used in the model is not the same as the adipose tissue lipids. The Aylward et al. (2005a) model does not account for differential solubility of TCDD in serum lipids and adipose tissue lipids, nor does it account for the diffusion-limited uptake by adipose tissue. Based on this evaluation, EPA determined that the Emond et al. (2006) provided more applicability than the Aylward et al. (2005a) model with respect to the ability to simulate serum lipid and tissue concentrations during exposures that do not lead to the onset of steady-state condition in the exposed organism. Of the two selected models, the pharmacokinetic model developed by Emond et al. (2006) is more physiologically based, as compared to the Aylward et al. (2005a) model. The Emond et al. (2006) pharmacokinetic model simulates the blood compartment directly in the rat, mouse, and human, but the Aylward et al. (2005a) model does not. Finally, there are also gestational and life-time nongestational forms of the Emond et al. (2006) model, but not for the Aylward et al (2005a) model. As a result, in this document, EPA chose the Emond rodent PBPK model to estimate blood TCDD concentrations based on administered doses (see Section 3.3.4, Appendix E).

To enhance the biological basis of the PBPK model of Emond et al. (2006), three minor modifications were made before its use in the computation of dose metrics for TCDD: (1) recalculation of the volume of the ―rest of the body compartment" after accounting for volume of the liver and fat compartments; (2) calculation of the rate of TCDD excreted via urine by multiplying the urinary clearance parameter by blood concentration in the equation instead of by the concentration in the rest of the body compartment; and (3) recalibration for the human gastric nonabsorption constant to match oral bioavailability data in humans (Poiger and

[6] The Aylward et al. (2005a) model cannot be used to estimate TCDD body burden when the duration of the rodent bioassay is less than 1 month,

Schlatter, 1986) (see Section 3.3.4.4 for details). The modified PBPK model was evaluated against all published data used in the original model. EPA assumed that the same blood TCDD levels that led to effects in animals would also lead to effects in humans; therefore, the Emond human PBPK model was used to estimate the lifetime average daily oral doses (consistent with the chronic RfD) that would correspond to the blood TCDD concentrations estimated to have occurred during the animal bioassays. EPA used the same Emond human PBPK model to estimate the lifetime average daily doses that would correspond to the TCDD blood or tissue concentrations reported in the epidemiologic studies (see Appendix F). These estimates are the Human Equivalent Doses (HEDs) that are used to develop candidate RfDs for TCDD.

A sensitivity analysis was performed on each of the animal and human Emond PBPK models to determine the most sensitive variables (see Section 3.3.4.3.2.5). In each case, all input variables in each model were included in the analysis; the sensitivity analysis was conducted by varying each parameter one at a time. For the rat and mouse nongestational models and rat and mouse gestational models for the low and high doses when variables were increased by +5%, predicted TCDD blood concentrations were very sensitive to the Hill coefficient (see *h* in Eq. 3-20, Section 3.3.4.3.2.2). Other influential PBPK model variables are associated with the overall dioxin elimination/sequestration rate, including the CYP1A2 induction rates, the liver weight, the binding capacity and affinity, and the gastric and intestinal excretion rates. For the gestational model dosing protocols, the Hill coefficient remains the most sensitive variable but the elasticity decreases compared with the nongestational analysis. Otherwise, many of the most sensitive variables remain those associated with elimination. Additional parameters related to the adipose tissue blood flow and with the adipose diffusional permeability fraction are also relatively influential. For the human gestational and nongestational models, additional variables associated with the adipose compartment partition coefficient, the body weight, and the fractional adipose tissue volume are also relatively influential variables at the RfD and POD dose compared with the animal models. For all models, the elasticities are relatively similar across the different doses evaluated.

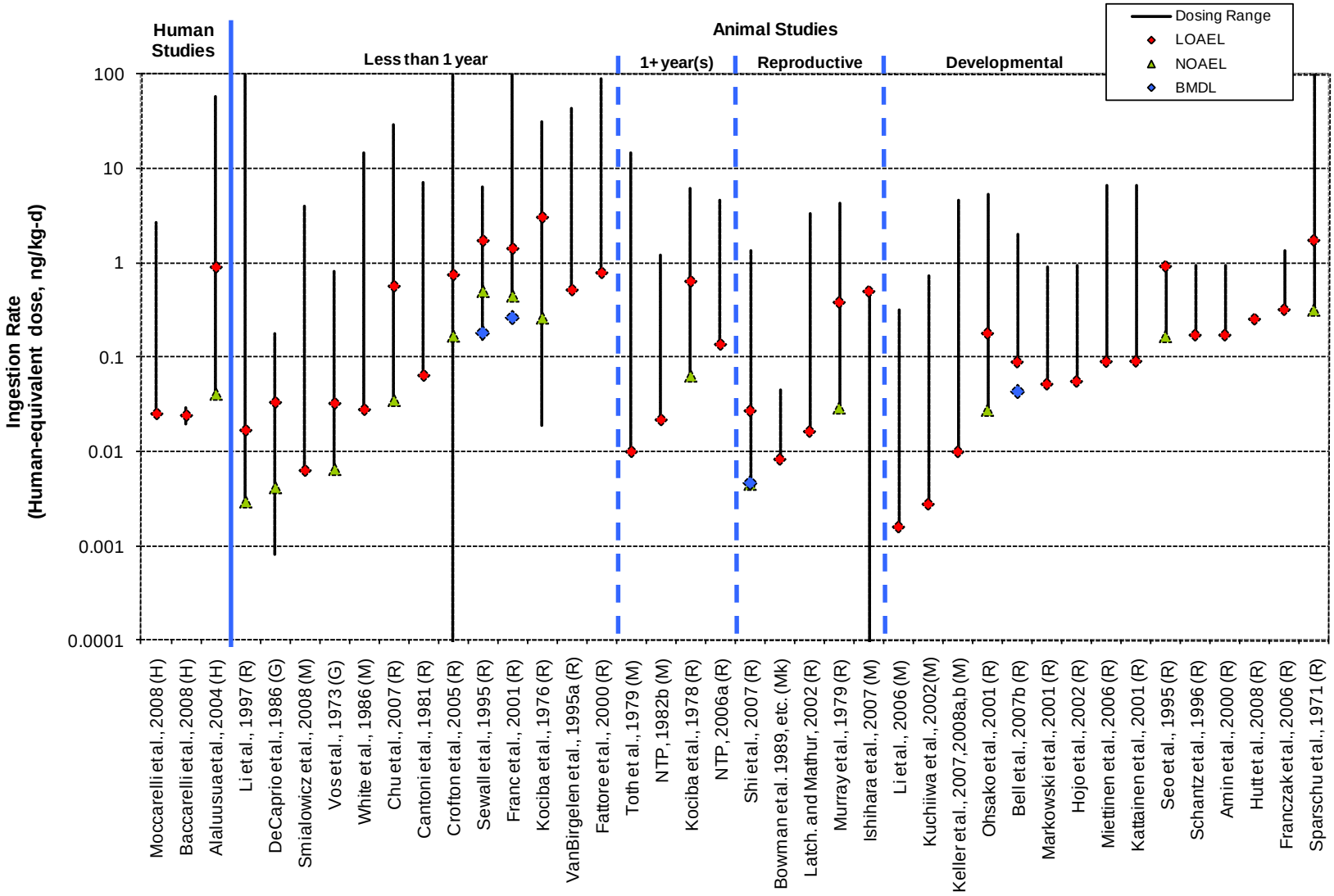
For variables which are optimized, a sensitivity analysis which varies each parameter one at a time may overestimate the model uncertainty associated with the variable. In this analysis, the most sensitive variable in all the models is the Hill parameter. The elasticity is high in part because the Hill parameter is an exponent; thus, small changes in the value can lead to larger

changes in the whole blood concentration. The Hill coefficient (as it is used in the PBPK models) can only be estimated with high confidence when optimized against in vivo hepatic CYP1A2 induction data in response to TCDD exposure. This type of data is found in animal experiments only. When this coefficient is optimized against human blood levels of TCDD, it is influenced by other parameters describing the dose-dependent elimination mechanism of the chemical; these data cannot be evaluated in vivo in humans.

This analysis highlights several important research needs. While the disposition of TCDD following high exposures is reasonably understood and simulated in current models, the current scientific understanding of disposition following TCDD exposures near current background dietary intakes (likely the primary source of TCDD exposure for most of the U.S. population) are not understood as well at present. This uncertainty affects the estimation of TCDD intake rates corresponding to the lower blood TCDD levels associated with LOAELs and NOAELs. The disposition of DLCs following exposures at background levels is similarly not well understood.

DERIVATION OF AN RFD FOR TCDD

The NAS specifically recommended that EPA derive an RfD for TCDD. Through a transparent study selection process, EPA identified key studies from both epidemiologic studies and animal bioassays. EPA then identified PODs for RfD derivation from those key human epidemiologic studies and animal bioassays. Figure ES-5 (exposure-response array) shows the PODs for TCDD graphically in terms of human-equivalent intake (ng/kg-day). The human study endpoints are shown at the far left of the figure and, to the right, the rodent endpoints are arranged by the following study categories: less than 1 year, greater than 1 year, reproductive, and developmental.



G = guinea pig; H = human; M = mouse; Mk = monkey; R = rat

Figure ES-5. Exposure-response array for ingestion exposures to TCDD.

For each noncancer epidemiologic study that EPA selected, EPA evaluated the dose-response information developed by the study authors to determine whether the study provided noncancer effects and TCDD-relevant exposure data for a toxicologically-relevant endpoint. If such data were available, EPA identified a NOAEL or LOAEL as a POD. Then, EPA used the Emond human PBPK model to estimate the continuous oral daily intake (ng/kg-day) that would lead to the relevant blood TCDD concentrations associated with the POD. If all of this information was available, then the result was included as a POD.

Through this process, EPA identified adverse health effects from the following four epidemiologic studies to be considered as the basis for the RfD: Eskenazi et al. (2002b) (menstrual cycle effects) Alaluusua et al. (2004) (developmental—tooth development), Mocarelli et al. (2008) (reproductive—decreased sperm concentrations and motility [semen quality]), and Baccarelli et al. (2008) (developmental—increased thyroid-stimulating hormone levels in neonates [neonatal TSH]). All four studies are from the Seveso cohort, whose members were exposed environmentally to high peak concentrations of TCDD as a consequence of an industrial accident. For each of the menstrual cycle, tooth development, and semen quality endpoints, EPA calculated a POD for derivation of a candidate RfD by estimating dose as the mean of the peak exposure (following the accident) and the average exposure over a defined critical exposure window for that endpoint. For neonatal TSH, EPA calculated the POD from estimates of maternal exposure during pregnancy reported by the study authors (Baccarelli et al., (2008) (see Section 4.2.3). The PODs estimated for both menstrual cycle and tooth development were well above those estimated for semen quality and neonatal TSH.

Figures ES-4 and ES-6 together present the strategy EPA used to evaluate the study/endpoint combinations found in the animal bioassays that met EPA's study inclusion criteria, estimate PODs, and develop a final set of candidate RfDs for TCDD. Figure ES-4 overviews the disposition of the 78 animal noncancer studies selected for TCDD dose-response analyses. Of these studies, 16 were eliminated because EPA determined that they contained no toxicologically relevant endpoints that could be used to derive a candidate RfD (see Appendix H and Section 4.2.1). EPA then identified PODs from the remaining bioassays; at this point, Figure ES-4 refers to Figure ES-6, which is a flow chart of the iterative process used to estimate PODs and compare them within and across studies to arrive at a final set of PODs from these bioassays (see additional details below). From this final set of PODs, Figure ES-4 shows that

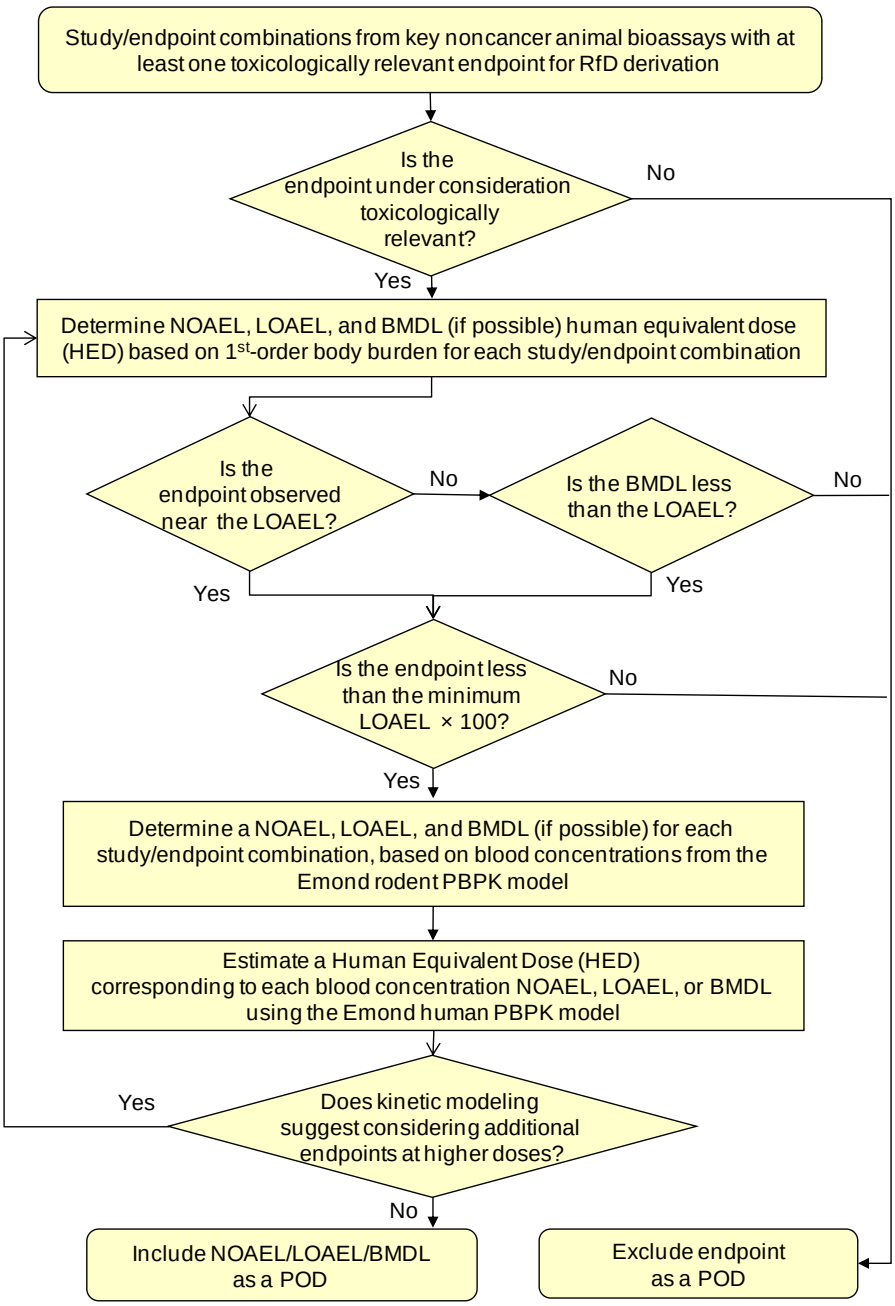


Figure ES-6. EPA's process to identify and estimate PODs from key animal bioassays for use in noncancer dose-response analysis of TCDD.
For the studies with at least one toxicologically relevant endpoint, EPA first determined if each endpoint was toxicologically relevant. If so, EPA determined the NOAEL, LOAEL, and BMDL Human Equivalent Dose (HED) based on 1st-order body burdens for each endpoint. Within each study, these potential PODs were included when the endpoint was observed near the LOAEL and if the BMDL was less than the LOAEL. Then, if the endpoint was less than the minimum LOAEL ×100 across all studies, EPA calculated PODs based on blood concentrations from the Emond rodent PBPK model and, for all of the PODs, HEDs were estimated using the Emond human PBPK model. If the kinetic modeling results suggested considering additional endpoints at higher doses, the process was repeated. Finally, the lowest group of the toxicologically relevant PODs was selected for final use in derivation of candidate RfDs.

EPA then eliminated 13 studies from further analysis because both of the following conditions were met: human equivalent dose (HED) $LOAEL_{HED}$ >1 ng/kg-day and $NOAEL_{HED}/BMDL_{HED}$ >0.32 ng/kg-day (see Table 4-3). One additional study was also not carried forward because of the lack of toxicokinetic information for estimation of an HED.

Figure ES-6 summarizes the strategy employed for identifying and estimating PODs from the 62 animal bioassays with at least one toxicologically relevant endpoint for RfD derivation. For the noncancer endpoints within these studies, EPA first evaluated the toxicological relevance of each endpoint, rejecting those judged not to be relevant for RfD derivation. Next, initial PODs based on the first-order body burden metric (see Section 3.3.4.2) and expressed as HEDs (i.e., $NOAEL_{HED}$, $LOAEL_{HED}$, $BMDL_{HED}$) were determined for all relevant endpoints (summarized in Table 4-3). Because there were very few NOAELs and BMDL modeling was largely unsuccessful due to data limitations (see Section 4.2), the next stage of evaluation was carried out using LOAELs only. Within each study, effects not observed at the LOAEL (i.e., reported at higher doses) with $BMDL_{HED}$s greater than the $LOAEL_{HED}$ were eliminated from further analysis, as they would not be considered as candidates for the final POD on either a BMDL or NOAEL/LOAEL basis (i.e., the POD would be higher than the PODs of other relevant endpoints). In addition, all endpoints with $LOAEL_{HED}$ estimates beyond a 100-fold range of the lowest identified $LOAEL_{HED}$ across all studies were (temporarily) eliminated from further consideration, as they would not be POD candidates either (i.e., the POD would be higher than the PODs of other relevant endpoints). For the remaining endpoints, EPA then determined final potential PODs based on TCDD whole-blood concentrations obtained from the Emond rodent PBPK models. HEDs were then estimated for each of these PODs using the Emond human PBPK model. At this point, if the PBPK modeling results suggested considering additional endpoints at higher doses, the process was repeated. From the final set of HEDs, a POD was selected[7] for each study, to which appropriate uncertainty factors (UFs) were applied following EPA guidance (see Section 4.3.3). The resulting candidate RfDs were then considered in the final selection process for the RfD. Other endpoints occurring at slightly higher doses representing additional effects associated with TCDD exposure (beyond the 100-fold $LOAEL_{HED}$

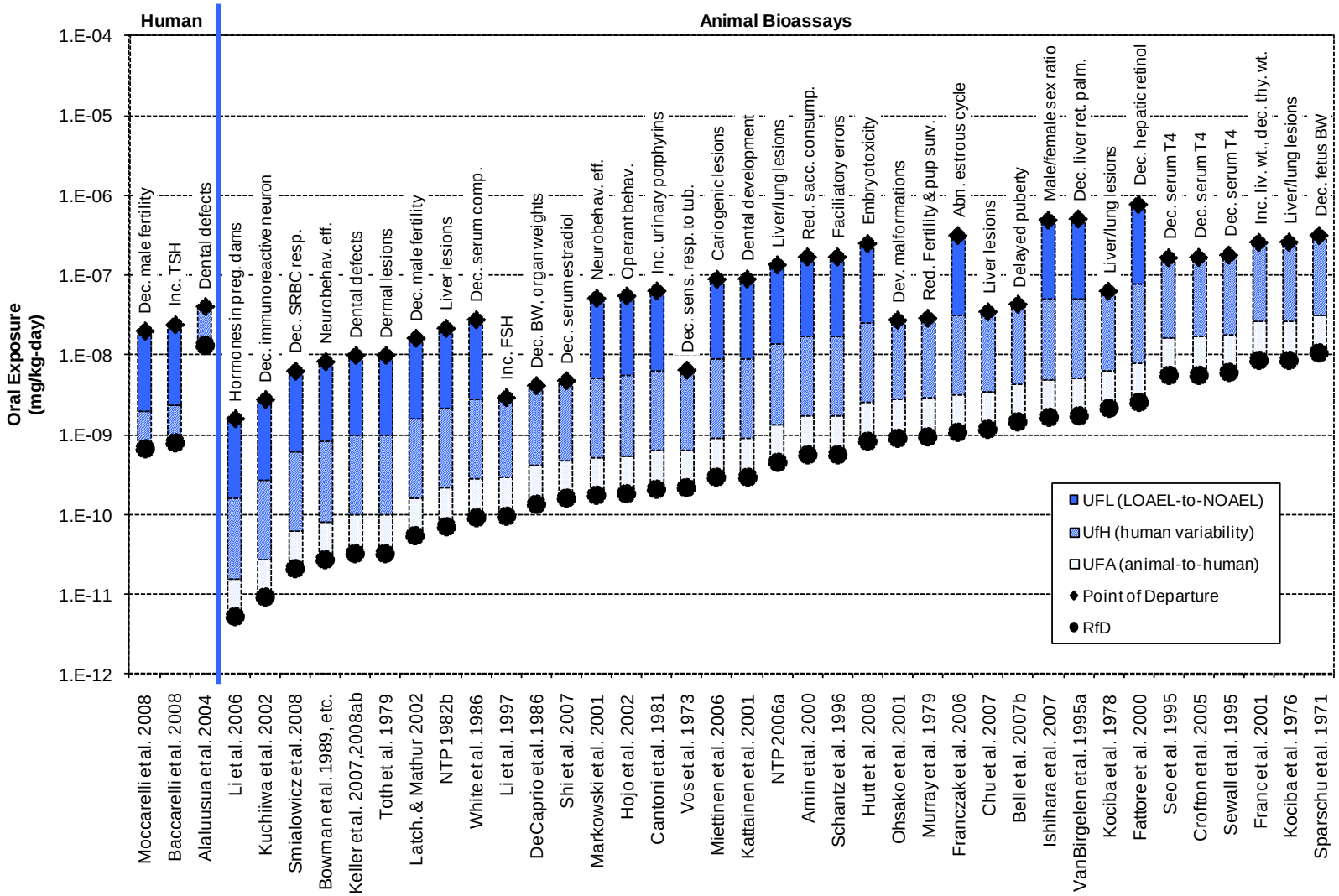
[7] In the standard order of consideration: BMDL, NOAEL, and LOAEL.

range) were evaluated, modeled, and included in the final candidate RfD array[8] to examine endpoints not evaluated by studies with lower PODs. In addition, Benchmark Dose (BMD) modeling based on administered dose was performed on all endpoints for comparison purposes.

For BMD modeling, EPA used a 10% BMR for dichotomous data for all endpoints; no developmental studies were identified with designs that incorporate litter effects, for which a 5% BMR would be used (U.S. EPA, 2000). For continuous endpoints in this document, EPA used a BMR of 1 standard deviation from the control mean whenever a specific toxicologically-relevant BMR could not be defined. Importantly, the 2003 Reassessment defined the ED_{01} as 1% of the maximal response for a given endpoint, not as a 1% change from control. Because RfD derivation is one goal of this document, the noncancer modeling effort undertaken here differs substantially from the modeling in the 2003 Reassessment. Evaluation of BMD modeling performance, goodness-of-fit, dose-response data, and resulting BMD and BMDL estimates included statistical criteria as well as expert judgment of their statistical and toxicological properties. EPA has reported and evaluated the BMD results using the standard suite of goodness-of-fit measures from the benchmark dose modeling software (BMDS 2.1). (see Appendix H and Section 4.2 for more information on the BMD modeling criteria and results.)

For selection of the POD to serve as the basis of the RfD, EPA gave the epidemiologic studies the highest consideration because human data are preferred in the derivation of an RfD. This preference for epidemiologic study data also is consistent with recommendations of panelists at the Dioxin Workshop (U.S. EPA, 2009a) (see Appendix B). Figure ES-7 arrays the candidate RfDs from both the human and animal bioassays in units of human-equivalent intake (mg/kg-day). The human studies included in Figure ES-7 (Baccarelli et al., 2008; Mocarelli et al., 2008; Alaluusua et al., 2004) each evaluate a segment of the Seveso civilian population (i.e., not an occupational cohort) exposed directly to TCDD released from an industrial accident. EPA designated the (Baccarelli et al., 2008; Mocarelli et al., 2008; Alaluusua et al., 2004) studies as coprincipal in deriving the RfD (see Section 4.3). In the Seveso cohort, exposures were primarily to TCDD, the chemical of concern, with apparently minimal DLC exposures beyond those associated with background intake, qualifying these studies for use in the RfD derivation

[8] However, studies with a lowest dose tested greater than 30 ng/kg-day were not included in the expanded evaluation.



Dec = decreasing effect; Inc = increasing effect

Figure ES-7. Candidate RfD Array

for TCDD. In addition, by using PODs derived from human data, the uncertainty of interspecies extrapolation is eliminated. The study subjects included newborns (exposed in utero) and adults who were exposed when they were less than 10 years of age, identifying effects in potentially vulnerable lifestages, accounting for at least some part of the uncertainty in extrapolation of effect levels to sensitive human populations and lifestages.

For Baccarelli et al. (2008), EPA defined the LOAEL (in LASC terms) as the maternal TCDD LASC of 235 ppt corresponding to a neonatal TSH level of 5 μU/mL, determined by the regression modeling performed by the study authors. The World Health Organization (1994) established the 5 μU/mL standard as a benchmark indicator for medical follow-up for investigation of potential congenital hypo-thyroidism. This benchmark was intended to address potential iodine deficiencies, but it is equally applicable to TCDD exposure for evaluating the equivalent effect. Baccarelli et al. (2008) discounted iodine status in the population as a confounder. For TCDD, the toxicological concern is not likely to be iodine uptake inhibition, but rather increased metabolism and clearance of the thyroid hormone, thyroxine (T4). An increased TSH level is an indicator of a potential decrease in circulating T4 levels, which could eventually lead to neurological deficiencies. TCDD has been associated with reductions in T4 in a number of animal studies[9] as discussed in Section 4.3.6.1. Adequate levels of thyroid hormone are essential in the newborn and young infant as this is a period of active brain development (Zoeller and Rovet, 2004; Glinoer and Delange, 2000). Thyroid hormone disruption during pregnancy and in the neonatal period can lead to irreversible neurological deficiencies.

Baccarelli et al. (2008) did not provide oral intakes associated with TCDD serum concentrations. EPA estimated the maternal TCDD intake corresponding to the LASC LOAEL of 235 ppt (at delivery) by use of the Emond human PBPK model the continuous daily intake from birth to age 30, the average age of the maternal cohort at delivery, that resulted in a 235 ppt maternal LASC at delivery. The resulting modeled maternal daily intake rate of 0.020 ng/kg-day established the LOAEL POD for the RfD. EPA did not define a NOAEL because it is not clear what maternal intake should be assigned to the group below 5 μU/mL.

For Mocarelli et al. (2008), EPA defined the LOAEL as the lowest exposed group (1st-quartile) median TCDD LASC of 68 ppt, corresponding to decreased sperm concentrations

[9]Sewall et al. (1995), Seo et al. (1995), Van Birgelen et al. (1995a; 1995b), Crofton et al. (2005), and NTP (2006a).

(25%) and decreased motile sperm counts (12%) in men who were 1−9 years old at the time of the Seveso accident (initial TCDD exposure event). There is no clear adverse effect level indicating male fertility problems for either of these sperm effects. As sperm concentration decreases, the probability of pregnancy from a single ejaculation also decreases; infertile conditions arise when the number of normal sperm per ejaculate is consistently and sufficiently low. Previously, the incidence of male infertility was considered increased at sperm concentrations less than 20 million sperm/mL (WHO, 1980). More recently, Cooper et al. (2010) suggested that the 5th percentile for sperm concentration (15 million/mL) could be used as a limit by clinicians to indicate needed follow-up for potential infertility. Skakkebaek (2010) suggests the following two limits for human sperm concentrations: 15 million sperm/mL, based on Cooper et al. (2010) and 40 million sperm/mL. Skakkebaek justifies the upper level of 40 million sperm/mL citing a study by Bonde et al. (1998) of couples planning to become pregnant for the first time; in the Bonde study, pregnancy rates declined when sperm concentrations were below 40 million sperm/mL. Skakkebaek suggests that 15 million sperm/mL may be too low of a cut off for normal fertility and that sperm concentrations between 15 million sperm/mL and 40 million sperm/mL may indicate a range of reduced fertility. For fertile men, between 50% and 60% of sperm are motile (Swan et al., 2003; Slama et al., 2002; Wijchman et al., 2001). Any impacts on these reported levels could become functionally significant, leading to reduced fertility. Low sperm counts are typically accompanied by poor sperm quality with respect to morphology and motility (Slama et al., 2002).

EPA judged that the impact on sperm concentration and quality reported by Mocarelli et al. (2008) is biologically significant given the potential for functional impairment. Although a decrease in sperm concentration of 25% likely would not have clinical significance for a typical individual, EPA's concern with the reported decreases in sperm concentration and total number of motile sperm (relative to the comparison group) is that such decreases associated with TCDD exposures could lead to shifts in the distributions of these measures in the general population. Because male fertility is susceptible to reductions in both the number and quality of sperm produced, such shifts in the population could result in decreased fertility in men at the low ends of these population distributions. Further, in the group exposed due to the Seveso accident, individuals 1 standard deviation below the mean had sperm concentrations of 21.8 million/mL;

this concentration falls at the low end of the range of reduced fertility (15 million and 40 million sperm/mL) suggested by Skakkebaek (2010).

For Mocarelli et al. (2008), TCDD LASC levels were measured within approximately 1 year of the initial exposure event. Because effects were only observed in men who were under 10 years of age at the time of exposure, EPA assumed a maximum 10-year critical exposure window for elicitation of these effects. Using the Emond human PBPK model, EPA has estimated a continuous daily oral intake of 0.020 ng/kg-day associated with the (LASC) LOAEL of 68 ppt (see Section 4.2.3.2). The reference group is not designated as a NOAEL because there is no clear zero-exposure measurement for any of these endpoints, particularly considering the contribution of background exposure to DLCs, which further complicates the interpretation of the reference group response as a true ―control" response (see discussion in Section 4.4). However, males less than 10 years old can be designated as a being in a sensitive lifestage as compared to older males who were not affected.

The two PODs based on the Baccarelli et al. (2008) and Mocarelli et al. (2008) studies, are adjusted LOAELs with the same value of 0.020 ng/kg-day, providing mutual quantitative support. Because these two studies define the most sensitive endpoints evaluated in the epidemiologic literature, they are designated as coprincipal studies for the RfD. Increased TSH in neonates (Baccarelli et al., 2008) and male reproductive effects (decreased sperm count and motility) (Mocarelli et al., 2008) are designated as cocritical effects. The adjusted LOAEL of 0.020 ng/kg-day is designated as the POD for the RfD. EPA used a composite UF of 30 for the RfD. A factor of 10 for UF_L was applied to account for lack of a NOAEL. A factor of 3 ($10^{0.5}$) for UF_H was applied to account for human interindividual variability because the effects were elicited in sensitive lifestages. A UF of 1 was not applied because the sample sizes in these two epidemiologic studies were relatively small, which, combined with uncertainty in exposure estimation, may not fully capture the range of interindividual variability. In addition, potential chronic effects are not well defined for humans and could possibly be more sensitive. The resulting RfD for TCDD in standard units is 7×10^{-10} mg/kg-day.

Although the human data are preferred, Figure ES-7 presents a number of candidate RfDs derived from animal bioassays that are lower than the human RfDs. Two of the rat bioassays among this group of studies—Bell et al. (2007b) and NTP (2006a)—are of particular note. Both studies were recently conducted and very well designed and conducted, using 30 or more

animals per dose group; both also are consistent with and, in part, have helped to define the current state of practice in the field of toxicology. Bell et al. (2007b) evaluated several reproductive and developmental endpoints, initiating TCDD exposures well before mating and continuing through gestation. NTP (2006a) is the most comprehensive evaluation of TCDD chronic toxicity in rodents to date, evaluating dozens of endpoints at several time points in all major tissues. Thus, proximity of the candidate RfDs derived from these two high quality, recent studies, provide additional support for the RfD derived from the two coprincipal epidemiologic studies.

EPA also developed cross-species comparison tables and figures of selected toxicological endpoints for all the animal and human studies that met the EPA selection criteria (see Appendix D.3). The endpoints include male and female reproductive effects, thyroid hormone levels and developmental dental effects, all of which have been reported for humans. In addition, immunological and neurological effects are shown because they are sensitive effects in experimental animal studies, although not evident in humans. The analysis presented in Appendix D.3 supports the conclusion that there is a substantial amount of qualitative concordance of effects between rodents and humans, but a much lower quantitative concordance.

There are several animal bioassay candidate RfDs at the lower end of the RfD range in Figure ES-7 that are more than 10-fold below the human-based RfDs. Two of these studies report effects that are analogous to the endpoints reported in the three human studies and support the RfDs based on human data. Specifically, decreased sperm production in Latchoumydandane and Mathur (2002) is consistent with the decreased sperm counts and other sperm effects in Mocarelli et al. (2008), and missing molars in Keller et al. (2008a; 2008b; 2007) are similar to the dental defects seen in Alaluusua et al. (2004). Thus, because these endpoints have been associated with TCDD exposures in humans, these animal studies would not be selected for RfD derivation in preference to human data showing similar effects.

Another characteristic of the remaining studies in the lower end of the candidate RfD distribution is that they are dominated by mouse studies (comprising 7 of the 9 lowest rodent-based RfDs). EPA has less confidence in the candidate RfD estimates based on mouse data than either the rat or human candidate RfD estimates. EPA has less confidence in the Emond mouse PBPK model than the other Emond PBPK models used to estimate the PODs because of the lack of key mouse-specific data, particularly for the gestational component (see

Section 3.3.4.3.2.5). The $LOAEL_{HED}$s identified in mouse bioassays are low primarily because of the large toxicokinetic interspecies extrapolation factors used for mice, for which there is more potential for error. In addition, each one of the mouse studies has other qualitative limitations and uncertainties that make them less desirable candidates as the basis for the RfD than the human studies.

EPA conducted additional sensitivity analyses of two groups of studies. Using variable sensitivity trees, EPA further analyzed the impacts of some sources of uncertainty encountered in the development of candidate RfDs based on Baccarelli et al. (2008), Mocarelli et al. (2008) and NTP (2006a), specifically examining the sensitivity of the POD value to choices made for estimating possible contributions associated with exposures to DLCs, exposure uncertainties and PBPK model variables and inputs (see Section 4.5.1). In Section 4.5.2, EPA also evaluated a number of endpoints presented in seven other Seveso cohort studies to estimate the range of potential PODs based on uncertainties in exposure duration, exposure averaging protocols and DLC background exposures. Included among those seven study/endpoint combinations are two studies that satisfied all the study selection criteria and considerations—developmental dental effects (Alaluusua et al., 2004) and duration of menstrual period (Eskenazi et al., 2002b)—a new developmental study on semen quality (Mocarelli et al., 2011) that was published after the study selection process was completed but is useful in this uncertainty analysis of the POD ranges, and four studies that did not satisfy all the study inclusion criteria and considerations.[10]

Overall, the results of these sensitivity analyses increase the confidence in the TCDD RfD—both qualitatively and quantitatively. EPA's sensitivity analyses show some POD estimates that are higher than the POD used to derive the RfD, while other analyses show POD estimates lower than the POD used to derive the RfD. These sensitivity analyses also highlight several important research needs. They highlight that the current scientific understanding of disposition following TCDD exposures that are closer to current background dietary intakes are not understood as well as the disposition of high TCDD exposures at present. There is also toxicological uncertainty regarding several of the endpoints; additional studies corroborating

[10] Mocarelli (2000), Eskenazi et al. (2005), and Warner et al. (2007; 2004). See Appendix C for study descriptions.

these outcomes and their toxicological significance would further increase their utility in refining the TCDD RfD.

1. INTRODUCTION

Dioxins and dioxin-like compounds (DLCs), including polychlorinated dibenzo-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls are structurally and toxicologically related halogenated dicyclic aromatic hydrocarbons.[11] Dioxins and DLCs are released into the environment from several industrial sources such as chemical manufacturing, combustion, and metal processing; from individual activities including the burning of household waste; and from natural processes such as forest fires. Dioxins and DLCs are widely distributed throughout the environment and typically occur as chemical mixtures. They do not readily degrade; therefore, levels persist in the environment, build up in the food chain, and accumulate in the tissues of animals. Human exposure to these compounds occurs primarily through the ingestion of contaminated foods (Lorber et al., 2009), although exposures to other environmental media and by other routes and pathways do occur.

The health effects from exposures to dioxins and DLCs have been documented extensively in epidemiologic and toxicological studies. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is one of the most toxic members of this class of compounds and has a robust toxicological database. Characterization of TCDD toxicity is critical to the risk assessment of mixtures of dioxins and DLCs because it has been selected repeatedly as the ―index chemical" for the dioxin toxicity equivalence factors (TEF) approach. In this approach, the toxicity of individual components of dioxin and DLC mixtures is scaled to that of TCDD. Then, the dose-response information for TCDD is used by the U.S. Environmental Protection Agency (EPA) and other organizations to evaluate risks from exposure to mixtures of DLCs (U.S. EPA, 2010b; Van den Berg et al., 2006; 1998) (also see the World Health Organization's Web site for the dioxin TEFs).[12]

To provide guidance on the use of the TEF approach in environmental health risk assessments, EPA published a report titled, *Recommended Toxicity Equivalence Factors (TEFs) for Human Health Risk Assessments of 2,3,7,8-Tetrachlorodibenzo-p-dioxin and Dioxin-Like Compounds* (TEF report) (U.S. EPA, 2010b). The TEF report describes EPA's updated approach for evaluating the human health risks from exposures to environmental media containing DLCs. In the TEF report, EPA recommends use of the consensus TEF values for

[11] For further information on the chemical structures of these compounds, see U.S. EPA (2010b, 2008b, 2003).
[12] Available online at http://www.who.int/ipcs/assessment/tef_update/en/.

TCDD and DLCs published in 2005 by the World Health Organization (Van den Berg et al., 2006) for all cancer and noncancer effects mediated through aryl hydrocarbon receptor binding. Further, EPA recommends that the TEF methodology, a component mixture method, be used to evaluate human health risks posed by these mixtures, using TCDD as the index chemical. The TEFs are factors that scale individual DLC exposures to toxicity equivalence (TEQ)[13] units of TCDD. To assess health risks for a given exposure to a mixture of DLCs, the TEQ's of those DLCs are summed, and the sum (i.e., total TEQ) is compared to dose-response information for TCDD. Therefore, it is imperative to correctly assess the dose response of TCDD and understand the uncertainties and limitations therein.

In 2003, EPA produced an external review draft of the multiyear comprehensive reassessment of dioxin exposure and human health effects titled, *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds* (U.S. EPA, 2003). This draft report, herein called the "2003 Reassessment," consisted of (1) a scientific review of information relating to sources of and exposures to TCDD, other dioxins, and DLCs in the environment; (2) detailed reviews of scientific information on the health effects of TCDD, other dioxins, and DLCs; and (3) an integrated risk characterization for TCDD and related compounds.

In 2004, EPA asked the National Research Council of the National Academy of Sciences (NAS) to review the 2003 Reassessment. The NAS Statement of Task was as follows:

[13] TEQ is the product of the concentration of an individual DLC in an environmental mixture and the corresponding TCDD TEF for that compound. These products are summed to yield the TEQ of the mixture.

> The National Academies' National Research Council will convene an expert committee that will review EPA's 2003 draft reassessment of the risks of dioxins and dioxin-like compounds to assess whether EPA's risk estimates are scientifically robust and whether there is a clear delineation of all substantial uncertainties and variability. To the extent possible, the review will focus on EPA's modeling assumptions, including those associated with the dose-response curve and points of departure; dose ranges and associated likelihood estimates for identified human health outcomes; EPA's quantitative uncertainty analysis; EPA's selection of studies as a basis for its assessments; and gaps in scientific knowledge. The study will also address the following aspects of EPA's 2003 Reassessment: (1) the scientific evidence for classifying dioxin as a human carcinogen; and (2) the validity of the nonthreshold linear dose-response model and the cancer slope factor calculated by EPA through the use of this model. The committee will also provide scientific judgment regarding the usefulness of toxicity equivalence factors (TEFs) in the risk assessment of complex mixtures of dioxins and the uncertainties associated with the use of TEFs. The committee will also review the uncertainty associated with the 2003 Reassessment's approach regarding the analysis of food sampling and human dietary intake data, and, therefore, human exposures, taking into consideration the Institute of Medicine's report Dioxin and Dioxin-Like Compounds in the Food Supply: Strategies to Decrease Exposure. The committee will focus particularly on the risk characterization section of EPA's 2003 Reassessment report and will endeavor to make the uncertainties in such risk assessments more fully understood by decision makers. The committee will review the breadth of the uncertainty and variability associated with risk assessment decisions and numerical choices, including, for example, modeling assumptions, including those associated with the dose-response curve and points of departure. The committee will also review quantitative uncertainty analyses, as feasible and appropriate. The committee will identify gaps in scientific knowledge that are critical to understanding dioxin reassessment (NAS, 2006, p. 43, Box 1-1).

In 2006, the NAS published its review of EPA's 2003 Reassessment titled *Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment* (NAS, 2006b).

1.1. SUMMARY OF KEY NAS (2006B) COMMENTS ON DOSE-RESPONSE MODELING IN THE 2003 REASSESSMENT

While recognizing the effort that EPA expended to prepare the 2003 Reassessment, the NAS committee identified three key areas that they believed required improvement to support a scientifically robust health assessment. These three key areas are

- Transparency and clarity in selection of key data sets for analysis;
- Justification of approaches to dose-response modeling for cancer and noncancer endpoints; and
- Transparency, thoroughness, and clarity in quantitative uncertainty analysis.

In their Public Summary, the NAS made the following overall recommendations to aid EPA in addressing their key concerns:

- EPA should identify the most important data sets to be used for quantitative risk assessment for each of the four key end points (cancer, immunotoxicity, reproductive effects, and developmental effects). EPA should specify inclusion criteria for the studies (animal and human) used for derivation of the benchmark dose (BMD) for different noncancer effects and potentially for the development of RfD (reference dose) values and discuss the strengths and limitations of those key studies; describe and define (quantitatively to the extent possible) the variability and uncertainty for key assumptions used for each key end-point-specific risk assessment (choices of data set, POD [point of departure],[14] model, and dose metric); incorporate probabilistic models to the extent possible to represent the range of plausible values; and assess goodness-of-fit of dose-response models for data sets and provide both upper and lower bounds on central estimates for all statistical estimates. When quantitation is not possible, EPA should clearly state it and explain what would be required to achieve quantitation (NAS, 2006b, p. 9).

- EPA should continue to use body burden as the preferred dose metric but should also consider physiologically based pharmacokinetic modeling as a means to adjust for differences in body fat composition and for other differences between rodents and humans (NAS, 2006b, p. 9).

- When selecting a BMD as a POD, EPA should provide justification for selecting a response level (e.g., at the 10%, 5%, or 1% level). In either case, the effects of this choice on the final risk assessment values should be illustrated by comparing point estimates and lower bounds derived from selected PODs (NAS, 2006b, p. 9).

- EPA should compare cancer risks by using nonlinear models consistent with a receptor mediated mechanism of action and by using epidemiologic data and the new National Toxicology Program (NTP) animal bioassay data (NTP, 2006a). The comparison should include upper and lower bounds, as well as central estimates of risk. EPA should clearly communicate this information as part of its risk characterization (NAS, 2006b, p. 9).

- Although EPA addressed many sources of variability and uncertainty qualitatively, the committee noted that the 2003 Reassessment would be substantially improved if its risk characterization included more quantitative approaches. Failure to characterize variability and uncertainty thoroughly can convey a false sense of precision in the conclusions of the risk assessment (NAS, 2006b, p. 5).

[14] Point of departure: The dose-response point that marks the beginning of a low-dose extrapolation. This point can be the lower bound on dose for an estimated incidence or a change in response level from a dose-response model (BMD), or a NOAEL (no-observed-adverse-effect-level) or LOAEL (lowest-observed-adverse-effect-level) for an observed incidence, or change in level of response (available online at http://www.epa.gov/iris/help_gloss.htm#p).

Importantly, the NAS encouraged EPA to calculate an RfD as the 2003 Reassessment does not contain an RfD derivation. The committee suggested that:

> …estimating an RfD would provide useful guidance to risk managers to help them (1) assess potential health risks in that portion of the population with intakes above the RfD, (2) assess risks to population subgroups, such as those with occupational exposures, and (3) estimate the contributions to risk from the major food sources and other environmental sources of TCDD, other dioxins, and DLCs for those individuals with high intakes (NAS, 2006b, p. 6).

The NAS made many other thoughtful and specific recommendations throughout their review; additional NAS recommendations and comments pertaining to the dose-response assessment of TCDD will be presented and addressed in various sections throughout this document.

1.2. EPA'S SCIENCE PLAN

In May 2009, EPA Administrator Lisa P. Jackson announced the "*Science Plan for Activities Related to Dioxins in the Environment*" ("Science Plan") that addressed the need to finish EPA's dioxin reassessment and provide a completed health assessment on this high profile chemical to the American public.[15]

The Science Plan outlined EPA's interim milestones for addressing several issues related to dioxins and DLCs. With regard to EPA's response to the NAS comments on the 2003 Dioxin Reassessment, the Science Plan stated the following:

1. EPA will release a draft report that responds to the recommendations and comments included in the NAS 2006 review of EPA's 2003 Dioxin Reassessment.
    a. EPA's National Center for Environment Assessment (NCEA) in the Office of Research and Development, will prepare a limited response to key comments and recommendations in the NAS report.
    b. The draft response will focus on dose-response issues raised by the NAS and will include an analysis of relevant new key studies.

[15] Available at http://www.epa.gov/dioxin/scienceplan.

2. EPA will provide the draft response to comments report for internal and external review.

    a. The draft response to comments report will also undergo both internal EPA review and interagency review.

    b. The draft response will be provided for public review and comment and independent external peer review.

3. The EPA Science Advisory Board (SAB) will review the science content of the response to comments report.

As outlined in the Science Plan, in 2009, EPA developed a draft report titled *VaR's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments* (draft Reanalysis) that responded to the key comments and recommendations in the NAS report (U.S. EPA, 2010a). The draft Reanalysis focused on TCDD dose-response issues and included analyses of relevant new studies and the derivation of an oral RfD. The draft Reanalysis was reviewed internally by EPA scientists and externally by other federal agencies and White House Offices. On May 21, 2010, the draft Reanalysis was released for public review and comment and independent external peer review by EPA's SAB.

1.3. SAB (SCIENCE ADVISORY BOARD) REVIEW OF EPA'S DRAFT REANALYSIS

For their review, the SAB convened an expert panel composed of scientists knowledgeable about technical issues related to dioxins and risk assessment. The SAB held public meetings in June, July, and October 2010 and March and June 2011. They released their final report reviewing the draft Reanalysis on August 26, 2011 (SAB, 2011).[16] In their report, the SAB made the following overarching observations:

- They found that the draft Reanalysis was clear, logical and responsive to many, but not all, of the NAS recommendations; they were impressed with the comprehensive and rigorous study selection process that was used to identify, review and evaluate the scientific literature on TCDD dose response;
    - …the SAB finds that the *Report* is generally clear, logical, and responsive to many but not all of the recommendations of the NAS. The SAB has, however,

[16] Available online at http://yosemite.epa.gov/sab/sabproduct.nsf/2A45B492EBAA8553852578F9003ECBC5/$File/SAB-11-014-unsigned.pdf.

provided many recommendations to further improve the clarity, organization, and responsiveness of various parts of the *Report*. The SAB was impressed with the process that EPA used to identify, review, and evaluate the relevant literature. The SAB finds that EPA's process was comprehensive and rigorous and included public participation. (SAB, 2011, p. 1)

- They agreed with the choice of the Emond physiologically based pharmacokinetic (PBPK) model for dose metric calculations and with whole blood as the appropriate dose metric;
    - The SAB agrees with EPA's use of blood TCDD concentration as a surrogate for tissue exposure to TCDD. Blood TCDD concentration is a better choice than using body burden (as in the 2003 Reassessment) because it is more closely related to the biologically relevant dose metric: the free concentration of dioxin in the target tissues. It is important to recognize, however, that TCDD distribution within tissues such as the liver can be nonuniform. The SAB further agrees that the PBPK model developed by Emond et al. (2006; 2005; 2004) provides the best available basis for the dose metric calculations in the assessment. (SAB, 2011, p. 2)
- They agreed with the choice of two epidemiologic studies as co-critical studies whose developmental toxicity data were used to derive the RfD for TCDD;
    - The SAB supports EPA's selection of the Mocarelli et al. (2008) and Baccarelli et al. (2008) studies for identifying "eocritical" effects for the derivation of the RfD. These two human epidemiologic studies are well designed and provide sufficient exposure information, including biological concentrations that could be used to establish acceptable lifetime daily exposure levels. (SAB, 2011, p. 3)
- They agreed with EPA's evaluation of TCDD carcinogenicity (with the exception of one panelist with a dissenting view);
    - The SAB agrees with EPA's conclusion that TCDD is "*Carcinogenic to Humans*." (SAB, 2011, p. 5).

The SAB also noted two deficiencies in EPA's draft Reanalysis with respect to the completeness of the consideration of two critical elements:

- Nonlinear dose response for TCDD carcinogenicity, and
- Uncertainty analysis

The SAB recommended that EPA fully evaluate both linear and nonlinear dose-response approaches to TCDD cancer dose-response assessment, including a discussion of carcinogenic mode of action. The SAB also recommended a number of approaches to quantitative uncertainty analysis that could be implemented by EPA, including the use of sensitivity analyses and probability trees.

- The SAB finds that the Report did not respond adequately to the NAS recommendation to adopt —both linear and nonlinear methods of risk characterization to account for the uncertainty of dose-response relationship shape below the ED_{01} (effective does eliciting *x* percent response)." EPA should present both linear and nonlinear risk assessment approaches. In the absence of a definitive nonlinear mode of action, the linear option results can serve as the baseline for comparison with other estimates. (SAB, 2011, p. 6)

- …the SAB does not agree with EPA's argument that conducting a unified quantitative uncertainty analysis for TCDD toxicity is unfeasible…..EPA argues that a complete quantitative uncertainty analysis would require data and resources not available. The SAB disagrees with this logic. While EPA may lack an adequate empirical basis for full Monte-Carlo propagation of input distributions, there are other options available. More limited evaluations can, and should, be implemented to inform critical issues in the dioxin reassessment. (SAB, 2011, p. 7)

The SAB made many additional thoughtful comments and specific recommendations throughout their review pertaining to the dose-response assessment of TCDD (SAB, 2011).

1.4. SCOPE OF EPA'S REANALYSIS VOLUMES 1 AND 2

In August 2011, EPA announced a plan for moving forward to complete the draft Reanalysis.[17] Per this plan, the current document comprises the first of two EPA reports (*U.S. VaR's Rvanalysis of \ v^ Zssues Relatvd to Dioxin Toxicity and Rvsponse to NAS Comments Volumes 1 and 2 [Reanalysis Volumes 1 and 2]*) that together will respond to the recommendations and comments on TCDD dose-response assessment included in the NAS review of EPA's 2003 draft Reassessment. Both Volumes focus on TCDD only. This report, Reanalysis Volume 1, completes and publishes EPA's study selection criteria and results for both noncancer and cancer TCDD dose-response assessment; choice of kinetic model; noncancer RfD

[17] Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=209690.

for TCDD; and a qualitative discussion of uncertainties in the RfD with a focused quantitative uncertainty analysis.

These information and analyses have undergone revisions in response to SAB comments and recommendations (see Appendix A). Reanalysis Volume 2 will address the two deficiencies identified by the SAB, i.e., nonlinear dose response for TCDD carcinogenicity and quantitative uncertainty analysis. In Volume 2, EPA will complete the evaluation of cancer mode-of-action, cancer dose-response modeling, including justification of the approaches used for dose-response modeling of the cancer endpoints, and an associated quantitative uncertainty analysis. The information provided in Volume 1 will be used in three ways: (1) as the first of two reports that contain EPA's response to the NAS (2006b) report, (2) as the Support Document for the TCDD noncancer Integrated Risk Information Systems (IRIS) Summary and TCDD oral RfD, and (3) as technical support for Reanalysis Volume 2. The summaries of the cancer studies included in Volume 1 are presented for use related to noncancer effects. These summaries are not intended to inform regulatory or other decision-making purposes related to carcinogenesis; further, no quantitative dose-response assessments are developed for cancer studies in Volume 1.

1.5. OVERVIEW OF EPA'S RESPONSE TO NAS (2006B)

In their key recommendations, the NAS commented that EPA should thoroughly justify and communicate approaches to dose-response modeling, increase transparency in the selection of key data sets, and improve the communication of uncertainty (particularly quantitative uncertainty). They also encouraged EPA to calculate an RfD. These main areas of improvement refer to issues specifically related to TCDD dose-response assessment (and uncertainty analysis); therefore, as noted in the Science Plan, EPA's response to the NAS is particularly focused on these issues.

EPA thoroughly considered the recommendations of the NAS and, in Reanalysis Volume 1, responds with scientific and technical evaluation of TCDD dose–response data via the following:

- An updated literature search that identified new TCDD dose-response studies (see Section 2/Appendix I);

- A workshop that included the participation of external experts in TCDD health effects, toxicokinetics, dose-response assessment and quantitative uncertainty analysis; these experts discussed potential approaches to TCDD dose-response assessment and considerations for EPA's response to NAS (U.S. EPA, 2009a) (see Appendix B);

- Detailed study inclusion criteria and processes for the selection of key studies (see Section 2.3) and epidemiologic and animal bioassay data for quantitative TCDD dose-response assessment (see Section 2.4.1/Appendix C and Section 2.4.2/Appendix D respectively);

- Kinetic modeling that quantifies appropriate dose metrics for use in TCDD dose-response assessment (see Section 3 and Appendices E and F);

- Sensitivity analyses that were performed on each of the animal and human Emond PBPK models that identify the most sensitive variables in each model (see Section 3.3.4);

- Dose-response modeling for all appropriate noncancer data sets (see Section 4.2/Appendix G);

- Thorough and transparent evaluation of the selected TCDD data for use in the derivation of an RfD, including justification of approaches used for dose-response modeling of noncancer endpoints (see Section 4.2 and Appendix H);

- The development of an RfD (see Section 4.3);

- A qualitative discussion of the uncertainty in the RfD and a focused quantitative uncertainty analyses of the RfD (see Sections 4.4 and 4.5, respectively); and

- Responses to the comments and recommendations made by the SAB in their final report (SAB, 2011) (see Appendix A).

Each of those activities is described in detail in subsequent sections of this document. The majority of the risk assessment terms used in this document are typically used in IRIS documents. Definitions can be located by referring to the IRIS online glossary, available at http://epa.gov/iris/help_gloss.htm. In addition to this document, it should be noted that several additional EPA activities address other TCDD issues, specifically related to the application of dioxin TEFs and to TCDD and DLC background exposure levels. Information on the application of the dioxin TEFs is published elsewhere by EPA for both ecological (U.S. EPA, 2008b) and human health risk assessment (U.S. EPA, 2010b). As a consequence, EPA does not directly address TEFs herein, but makes use of the concept of toxicity equivalence as applicable to the analysis of exposure dose in epidemiologic studies. Furthermore, this document does not

address the NAS recommendations pertaining to the assessment of human exposures to TCDD and other dioxins. Information on updated background levels of dioxin in the U.S. population has been recently reported (Lorber et al., 2009). In 2006, EPA also released a report titled *An Inventory of Sources and Environmental Releases of Dioxin-Like Compounds in the United States for the Years 1987, 1995 and 2000*, which presents an evaluation of sources and emissions of dioxins, dibenzofurans, and coplanar polychlorinated biphenyls (PCBs) to the air, land and water of the United States (U.S. EPA, 2006b).

1.5.1. TCDD Literature Update

EPA has developed a literature database of peer-reviewed studies on TCDD toxicity, including in vivo mammalian dose-response studies and epidemiologic studies for use in quantitative TCDD dose-response assessment and supporting qualitative discussions. An initial literature search for studies published since the 2003 Reassessment was conducted by the U.S. Department of Energy's Argonne National Laboratory (ANL) through an Interagency Agreement with EPA. ANL used the online National Library of Medicine database (PubMed) and identified studies published between the year 2000 and October 31, 2008 (see Appendix I). Supporting references published since the release of the 2003 Reassessment were also identified. Supporting studies were classified as studies pertaining to TCDD kinetics, TCDD mode-of-action, in vitro TCDD studies, and TCDD risk assessment approaches. The literature search strategy explicitly excluded studies addressing: (1) analytical/detection data and cellular screening assays; (2) environmental fate, transport and concentration data; (3) dioxin-like compounds and toxic equivalents; (4) nonmammalian dose-response data; (5) human exposure analyses only, including body burden data; and (6) combustor or incinerator or other facility-related assessments absent primary dose-response data.

EPA published the initial literature search results in the Federal Register on November 24, 2008 (73 FR 70999; November 24, 2008) and invited the public to review the list and submit additional peer-reviewed in vivo mammalian dose-response studies for TCDD, including epidemiologic studies that were absent from the list (U.S. EPA, 2008a). Submissions were accepted by the EPA through an electronic docket, email, and hand delivery, and they were evaluated for use in TCDD dose-response assessment. The literature search results and subsequent submissions were used during a 2009 scientific workshop, which was open to the

public and featured a panel of experts on TCDD toxicity and dose-response modeling (discussed below). Additional studies identified during the workshop, and those collected by EPA scientists during the development of this report through October 2009, have been incorporated into the final set of studies for TCDD quantitative dose-response assessment.

Since release of the draft Reanalysis for public comment and external peer review in 2010, EPA has collected a limited number of additional studies published since October 2009 that also inform EPA's derivation of an RfD for TCDD. These studies were identified by EPA scientists, the SAB, and the public, and they have been used to further evaluate the biological significance of the endpoints used to derive the RfD and to develop information on uncertainty in the RfD. These additional studies are cited in the appropriate sections of this document. None of the data sets collected since October 2009 was used quantitatively in the noncancer dose-response assessment of TCDD.

1.5.2. EPA's 2009 Workshop on TCDD Dose Response

To assist EPA in responding to the NAS, EPA and ANL convened a scientific workshop (the "Dioxin Workshop") on February 18–20, 2009, in Cincinnati, OH. The goals of the Dioxin Workshop were to identify and address issues related to the dose-response assessment of TCDD and to ensure that EPA's response to the NAS focused on the key issues and reflected the most meaningful science. The Dioxin Workshop included seven scientific sessions: quantitative dose-response modeling issues, immunotoxicity, neurotoxicity and nonreproductive endocrine effects, cardiovascular toxicity and hepatotoxicity, cancer, reproductive and developmental toxicity, and quantitative uncertainty analysis of dose response. During each session, EPA asked a panel of expert scientists to perform the following tasks:

- Identify and discuss the technical challenges involved in addressing the NAS comments related to the dose-response issues within each specific session topic and the TCDD quantitative dose-response assessment.
- Discuss approaches for addressing the key NAS recommendations.
- Identify important published, independently peer-reviewed literature—particularly studies describing epidemiologic studies and in vivo mammalian bioassays expected to be most useful for informing EPA's response.

The sessions were followed by open comment periods during which members of the audience were invited to address the expert panels. The session's Panel Cochairs were asked to summarize and present the results of the panel discussions—including the open comment periods. The summaries were intended to reflect the core of the panel discussions and incorporated points of agreement as well as minority opinions. Final session summaries were prepared by the session Panel Co-chairs with input from the panelists, and they formed the basis of a final workshop report (U.S. EPA, 2009a) (Appendix B of this report). Because the sessions were not designed to achieve consensus among the panelists, the summaries do not necessarily represent the opinions of all the scientists that attended the meeting. Some of the key discussion points from the workshop that influenced EPA's development of this document are listed below (see Appendix B for detail):

- In the development of study selection criteria, more relevant exposure-level decision points using tissue concentrations could be defined.
- A linear approach to body-burden estimation, which was utilized in the 2003 Reassessment (U.S. EPA, 2003), does not fully consider key toxicokinetic issues related to TCDD—e.g., sequestration in the liver and fat, age-dependent elimination, and changing elimination rates over time. Thus, kinetic/mechanistic modeling could be used to quantify tissue-based metrics. In considering human data, lipid-adjusted serum levels may be preferable over body burden, although the assumptions used in the back calculation of the body burden in epidemiologic cohorts are of concern. In considering rat bioassay data, lipid-adjusted body-burden estimates may be preferable.
- New epidemiologic studies on noncancer endpoints have been published since the 2003 Reassessment that may need to be considered (e.g., thyroid dysfunction literature from Wang et al. (2005) and Baccarelli et al. (2008).
- The 1% of maximal response (ED_{01}) that was utilized in the 2003 Reassessment has not typically been used in dose-response assessment. Some alternative ideas were as follows: (1) the POD should depend on the specific endpoint; (2) for continuous measures, the benchmark response (BMR) could be based on the difference from control and consider the adversity level; and (3) for incidence data, the BMR should be set to a fixed-risk level.
- The quantitative dose-response modeling for cancer could be based on human or animal data. There are new publications in the literature for four epidemiological cohort studies (Dutch cohort, NIOSH (National Institue for Occupational Saftey and Health) cohort, BASF accident cohort, and Hamburg cohort). The increase in total cancers could be considered for modeling human cancer data. However, non-Hodgkin lymphoma and

lung tumors are the main TCDD-related cancer types seen from human exposure. In reviewing the rat data, the NTP (2006a) data sets are new and can be modeled. Although the liver and lungs are the main target organs, modeling all cancers, as well as using tumor incidence in lieu of individual rats as a measure, should be considered.

- Both linear and nonlinear model functions should be considered in the cancer dose-response analysis because there are data and rationales to support use of either below the POD.

- For quantitative uncertainty analysis, consider the impacts of choices among plausible alternative data sets, dose metrics, models, and other more qualitative choices. Issues to consider include how much difference these choices make and, also, how much relative credence should be put toward each alternative as a means to gauge and describe the landscape of imperfect knowledge with respect to possibilities for the true dose response. This may be difficult to do quantitatively because the factors are not readily expressed as statistical distributions. However, the rationale for accepting or questioning each alternative in terms of the available supporting evidence, contrary evidence, and needed assumptions, can be delineated.

1.5.3. Organization of EPA's Response to NAS Recommendations (Reanalysis Volume 1)

The remainder of this document, Reanalysis Volume 1, is divided into three sections that address the three primary areas of concern resulting from the NAS (2006b) review. Section 2 describes EPA's approach to the recommendation for transparency and clarity during selection of key data sets suitable for TCDD dose-response assessment—including criteria for the selection of key dose-response studies and results of the evaluations of the important epidemiologic studies and animal bioassays (Appendices C and D contain study summaries and additional details on study evaluations for the epidemiologic and animal bioassays, respectively). Sections 3 and 4 present EPA's response to the NAS recommendation to better justify the approaches used in dose-response modeling of TCDD for noncancer endpoints. Section 3 discusses the toxicokinetic modeling EPA conducted to support the dose-response analyses. Section 4 presents EPA's noncancer data set selection, the noncancer dose-response modeling results, the RfD derivation for TCDD, a qualitative discussion of the uncertainties associated with the RfD, and a focused quantitative uncertainty analysis of the PODs considered for RfD derivation.

2. TRANSPARENCY AND CLARITY IN THE SELECTION OF KEY DATA SETS FOR DOSE-RESPONSE ANALYSIS

This section addresses transparency and clarity in the study selection process and identifies key data sets for TCDD dose-response analysis. Section 2.1 summarizes the NAS committee's comments specifically regarding this issue. Section 2.2 presents EPA's response to those comments and describes EPA's approach to ensuring transparency and clarity in the selection of studies for subsequent dose-response analyses. Section 2.3 describes the TCDD-specific study inclusion criteria and study quality evaluation process EPA used in this document for determining the eligibility of both epidemiologic and experimental animal studies for TCDD dose-response analysis. Section 2.4 summarizes the results of applying the study inclusion criteria to the epidemiologic studies (see Section 2.4.1, Tables 2-1 and 2-2) and the in vivo mammalian bioassays (see Section 2.4.2, Tables 2-3 and 2-4). These results present the key TCDD epidemiologic and animal bioassays that were identified using the study inclusion criteria. Additional details on this process can be found in Appendices C and D. Appendix C summarizes all of the available epidemiologic studies, evaluates the suitability of these studies for TCDD dose-response analyses, and presents the study selection process results. Appendix D summarizes only the animal bioassay data that have met the study inclusion criteria for TCDD dose-response assessment and, in Tables D-1 and D-2, shows the results of the study selection process for all of the animal bioassays identified by EPA. Study/endpoint combination data sets for developing TCDD toxicity values for noncancer effects are further evaluated in Section 4 of this document. Based on the cancer studies identified in this document, study/endpoint combination data sets for developing toxicity values for cancer effects will be explored in a separate document, Volume 2 of this effort. The summaries and study evaluations for the cancer studies presented in this section and in Appendices C and D for epidemiologic studies and animal bioassays, respectively, are presented for use related to noncancer effects. These summaries are not intended to inform regulatory or other decision-making purposes related to carcinogenesis; further, no quantitative dose-response assessments are developed for cancer studies in Volume 1.

2.1. SUMMARY OF NAS COMMENTS ON TRANSPARENCY AND CLARITY IN THE SELECTION OF KEY DATA SETS FOR DOSE-RESPONSE ANALYSIS

The NAS committee proposed that EPA develop a clear and readily understandable methodology for evaluating and including epidemiologic and animal bioassay data sets in dose-response evaluations. The NAS committee recommended the development and application of transparent initial criteria to judge whether or not specific epidemiologic or animal bioassay studies be included in TCDD dose-response analysis.

Specific NAS comments on the topic of study evaluation and inclusion criteria include the following:

> EPA should specify inclusion criteria for the studies (animal and human) used for derivation of the benchmark dose (BMD) for different noncancer effects and potentially for the development of RfD values and discuss the strengths and limitations of those key studies (NAS, 2006b, p. 27).
>
> …in its [EPA's] evaluation of the epidemiological literature of carcinogenicity, it did not outline eligibility requirements or otherwise provide the criteria used to assess the methodological quality of other included studies (NAS, 2006b, p. 56).
>
> With regard to EPA's review of the animal bioassay data, the committee recommends that EPA establish clear criteria for the inclusion of different data sets (NAS, 2006b, p. 191).
>
> …the committee expects that EPA could substantially improve its assessment process if it more rigorously evaluated the quality of each study in the database (NAS, 2006b, p. 56).
>
> EPA could also substantially improve the clarity and presentation of the risk assessment process for TCDD…by using a summary table or a simple summary graphical representation of the key data sets and assumptions…(NAS, 2006b, p. 56).

2.2. EPA'S RESPONSE TO NAS COMMENTS ON TRANSPARENCY AND CLARITY IN THE SELECTION OF KEY DATA SETS FOR DOSE-RESPONSE ANALYSIS

EPA agrees with the NAS committee regarding the need for a transparent and clear process with criteria identified for selecting studies and key data sets for TCDD dose-response analyses. The delineation of the study selection process and decisions regarding key data sets will facilitate communication regarding critical decisions made in the TCDD dose-response assessment. In keeping with the NAS committee's recommendation to use a transparent process and improve clarity and presentation of the health assessment process for TCDD, Figure 2-1

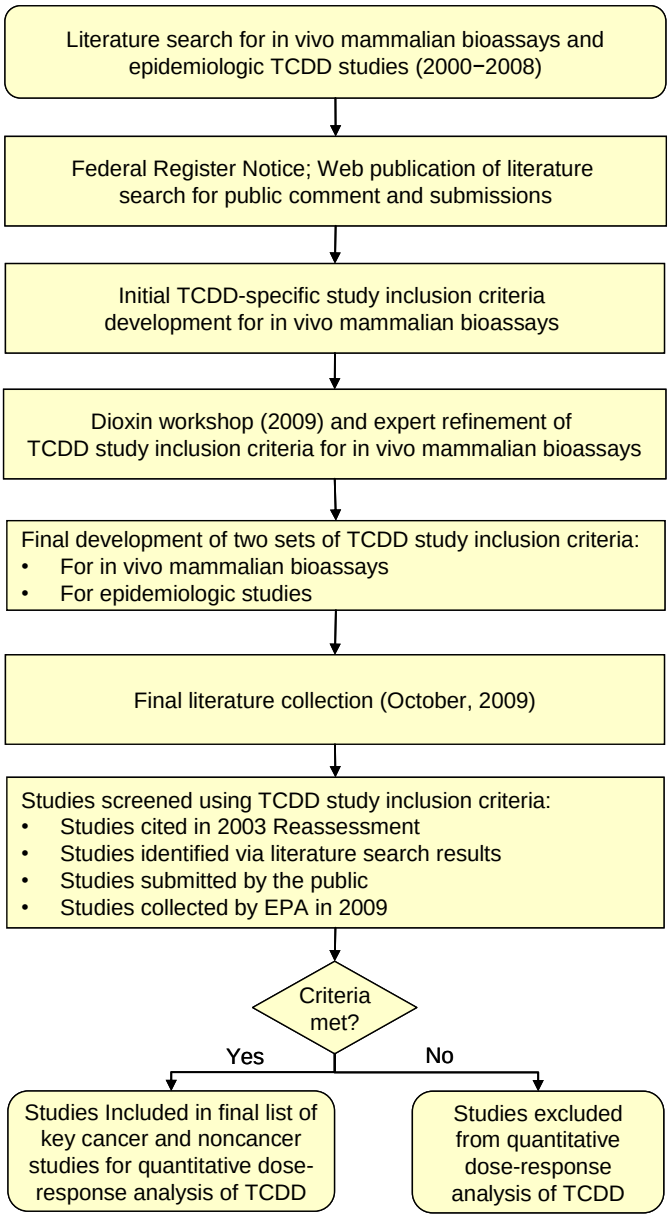


Figure 2-1. EPA's process to select and identify in vivo mammalian and epidemiologic studies for use in the dose-response analysis of TCDD.
EPA first conducted a literature search to identify studies published since the 2003 Reassessment. Results were published, and additional study submissions were accepted from the public. Next, EPA developed TCDD-specific study inclusion criteria for in vivo mammalian studies and held a Dioxin Workshop where these criteria were discussed and refined. Third, EPA developed two final sets of study inclusion criteria, one for in vivo mammalian studies and another for epidemiologic studies. Finally, EPA applied these two sets of criteria to all studies from the literature search, public submissions, 2003 Reassessment, and additional studies identified by EPA after the Dioxin Workshop through October 2009. The studies that met these criteria formed a list of key studies for EPA's consideration in TCDD dose-response assessment.

provides an overview of the approach that EPA has used in this document to develop a final list of key cancer and noncancer studies for quantitative dose-response analysis of TCDD. The steps in Figure 2-1 are further explained below.

Literature search for in vivo mammalian and epidemiologic TCDD studies (2000−2008): EPA conducted a literature search to identify peer-reviewed, dose-response studies for TCDD that have been published since the 2003 Reassessment. This search included in vivo mammalian and epidemiologic studies of TCDD from 2000 to 2008. Additional details describing the conduct of this literature search are presented in Section 1.5.1 of this document.

Federal Register Notice—Web publication of literature search for public comment: In November 2008, EPA published a list of citations from results of this literature search (U.S. EPA, 2008a) and invited the public to review this preliminary list of dose-response citations for use in TCDD dose-response assessment. EPA requested that interested parties identify and submit peer-reviewed studies for TCDD that were absent from this list. Two parties identified additional references that were not included in the 2008 Federal Register notice and submitted additional references for EPA to consider. These references were included in the final TCDD literature database considered by EPA for TCDD dose-response analysis.

Initial study inclusion criteria development for TCDD in vivo mammalian bioassays: EPA developed an initial set of draft criteria for evaluating the extensive TCDD database of in vivo mammalian bioassays. These initial study inclusion criteria had three purposes. First, they provided a method to transparently and rigorously evaluate the scientific quality of each study in EPA's database, a deficiency in the 2003 Reassessment identified by the NAS committee. Second, their application provided an efficient way to initially screen the vast number of TCDD mammalian bioassays for consideration in TCDD dose-response analyses. Third, they served as a starting point for discussions of study inclusion criteria by expert panelists who were convened by EPA for its scientific workshop on TCDD dose-response analysis (the Dioxin Workshop), described next [also see the workshop report in Appendix B, U.S. EPA (2009a)].

Dioxin Workshop and expert refinement of TCDD in vivo mammalian study inclusion criteria: In February 2009, EPA convened "A Scientific Workshop to Inform EPA's Response to NAS Comments on the Health Effects of Dioxin in EPA's 2003 Dioxin Reassessment" [see workshop details in Section 1.5.2 and Appendix B (U.S. EPA, 2009a)]. At the workshop, EPA presented the draft set of study inclusion criteria; the workshop panelists evaluated the study inclusion criteria in relation to the various toxic endpoints that were discussed and made recommendations for their revision.

Final development of study inclusion criteria for TCDD in vivo mammalian studies: Based on discussions and recommendations made at the Dioxin Workshop, the initial

draft study inclusion criteria for evaluating the TCDD mammalian bioassay literature were revised and are presented in Section 2.3.2.

Development of study inclusion criteria for epidemiologic studies: Following the Dioxin Workshop, EPA determined that an evaluation process was also needed for selection of epidemiologic studies for TCDD dose-response assessment. These criteria were developed and are detailed in Section 2.3.1.

Final literature collection (October 2009): Additional literature was collected as it was identified by EPA following the Dioxin Workshop through October 2009 to ensure the consideration of all recently published data for this report.

Studies screened using study inclusion criteria: The two sets of TCDD-specific study inclusion criteria for epidemiologic studies and in vivo animal bioassays presented in Sections 2.3.1 and 2.3.2, respectively, were used to evaluate all studies included in the 2003 Reassessment, studies identified in the 2000−2008 literature search, studies identified through public comment and submission, and studies collected in 2009 as identified by EPA during the development of this document. Section 2.4 and Appendices C and D present results of EPA's evaluation of epidemiologic and mammalian bioassay literature for both cancer and noncancer endpoints.

Final list of key noncancer studies and preliminary list of cancer studies for quantitative dose-response analysis of TCDD: Application of the study inclusion criteria concludes in Section 2.4 with development of a final list of key noncancer studies and a preliminary list of cancer studies to be considered for quantitative dose-response analyses of TCDD. In Section 4, PODs are developed and evaluated for all biologically relevant noncancer study/endpoint combinations from the final key noncancer study lists, and key data sets and PODs for the development of TCDD noncancer toxicity values are identified. Similar analyses will be undertaken in Volume 2 of this effort for TCDD cancer dose-response assessment.

2.3. STUDY SELECTION PROCESS FOR TCDD DOSE-RESPONSE ANALYSIS

In this section, EPA describes the study selection process that includes both TCDD-specific study selection criteria and methodological considerations that have been developed to evaluate epidemiologic studies and animal bioassays for quantitative TCDD dose-response assessment. These criteria and considerations reflect EPA's goal of developing noncancer and cancer toxicity values for TCDD through a transparent study selection process; they are intended to be used by EPA for TCDD dose-response assessment only. The TCDD in vivo mammalian literature base differs from most other chemicals in magnitude and comprehensiveness. It comprises ~1,500 studies that evaluate multiple cancer and noncancer endpoints, many species including humans, and covers an expansive dose range, including doses

at and below 1 nanogram per kilogram body weight per day (ng/kg-day). Thus, the study inclusion criteria and considerations developed in this document are specific to evaluating the TCDD literature and cannot necessarily be generically applied to other chemicals. Further, TCDD has a long half-life in humans (~7 years) and bioaccumulates in fat tissue, resulting in the specification of study inclusion criteria for estimating exposures during the critical windows for adverse health effects. In this effort, EPA sought to identify a group of studies for TCDD dose-response evaluation that would span the types of adverse health effects associated with TCDD exposures and encompass the range of doses in the lower end of the dose-response region most relevant to human health protection. Detailed study inclusion criteria have been developed that consider TCDD-specific issues and reflect EPA methods for POD identification, RfD derivation, and oral slope factor (OSF) derivation. (The effort in this document contrasts with EPA's 2003 Reassessment where the focus was on individual endpoints and the goal was to compare dose response across studies.)

The study inclusion criteria and considerations were applied to each of the studies listed in the "Preliminary Literature Search Results and Request for Additional Studies on 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) Dose-Response Studies" (U.S. EPA, 2008a); studies identified and submitted by the public and by participants in the Dioxin Workshop (U.S. EPA, 2009b); studies included in the 2003 Reassessment; and other relevant published studies collected by EPA scientists through October 2009. In this effort, the goal was to identify the most relevant studies for TCDD quantitative human health risk analyses. Those that did not qualify were not used quantitatively, but some of these were still considered relevant to the qualitative evaluations of TCDD noncancer and cancer assessments. Similarly, some types of studies were not screened, i.e., studies on DLCs, mixtures toxicity, mode of action, in vitro toxicity, nonmammalian toxicology, and risk assessment; however, they were considered to be important supplemental information to be used as needed, for example, in discussions of biological significance.

For the study selection process, EPA has focused on TCDD studies and has not included studies on DLCs or DLC mixtures because inclusion of the DLC literature would likely increase the uncertainty in TCDD dose response unnecessarily, given that the TCDD database is quite robust. In addition, EPA believes that using studies evaluating information primarily or exclusively on TCDD dose response provides the most appropriate data for the risk assessment

of dioxins and DLCs using the TEF approach. EPA is concerned that: (1) using the TEQ data to estimate TCDD toxicity values would not accurately reflect TCDD dose response; and (2) uncertainty in the underlying data used to derive the TEF values would complicate the extrapolation of TEQ dose-response data to inform TCDD dose response.

Because TCDD is used as the index chemical in the TEF approach, the most relevant and accurate information that specifically addresses quantitative dose response of individual TCDD exposures is needed. The WHO (World Health Organization) expert panel assigned TEF values from a conservative perspective that was intended to be health protective (Van den Berg et al., 2006). In the development of the TEFs, the WHO expert panel considered data from Haws et al. (2006a, b), who present summary statistics of relative potency values assembled from selected in vivo and in vitro studies. For each individual DLC, the WHO expert panel typically assigned TEF values using an in vivo study whose relative potency value was above the 50th percentile of the ranges presented by Haws et al. (2006a, b). Thus, when these TEFs are used in a dose-response study, they produce total TEQ estimates that may be biased high for certain combinations of DLCs. If a RfD for TCDD were derived based on TEQ dose-response data, that RfD would likely also be biased high and, in that case, would underestimate health risk from environmental exposures. Thus, using the TEQ data to estimate TCDD toxicity values would not accurately reflect TCDD dose response.

Finally, there is uncertainty in how the underlying data were used to derive the TEF values that complicates the extrapolation of TEQ dose-response data to inform TCDD dose response. The kinds of information available for calculating relative potencies within a study are highly variable across DLCs, including many types of and numbers of in vivo (including different test species) and in vitro studies. In addition, a number of different methods are employed to calculate the range of relative potencies presented by Haws et al. (2006a, b), ranging from comparing dose-response curves, to developing ratios of effective doses that cause an effect in 50% of the test units (ED_{50s}), to estimating values from graphs of dose-response data. The uncertainty in the TEFs can be a substantial issue for dose-response modeling when effect levels in a study occur at doses close to background TEQ levels and TCDD is not a dominant component of the mixture. In this case, the contribution of TCDD dose to the observed toxic effect may not be feasible to estimate as it is confounded by other TEQ concentrations and impacted by other TEF uncertainties.

EPA has undertaken different approaches for epidemiologic versus in vivo animal bioassay study evaluation and key data set selection. The significant differences between animal and human health effects data and their use in EPA health assessment support development of separate study inclusion criteria and different approaches to study evaluation. For example, animal bioassays on TCDD are closely controlled experiments where dose and effect are precisely measured and causality can be more easily inferred; thus, the animal criteria contain precise dose limits and specific limitations on elements of the experimental design. Because epidemiologic studies on TCDD are carried out within a population setting, these observational studies employ statistical and other analytical techniques to estimate exposures/doses, and to assess dose-response relationships after controlling or accounting for confounding factors and other potential sources of bias. Thus, the epidemiologic criteria contain requirements for being able to reasonably quantify the exposure-response relationship for the biologically-relevant exposure window.[18]

Section 2.4 and Appendices C and D present the results of the study selection process. In Appendix C, all of the available epidemiologic studies on TCDD are summarized and evaluated for suitability for dose-response modeling using the TCDD-specific study inclusion criteria described in Section 2.3.1 below; only studies meeting the study inclusion criteria and study quality considerations are presented as key studies in Section 2.4.1 (see Tables 2-1 and 2-2 for the cancer and noncancer endpoints, respectively). In Appendix D, because summarizing all of the available animal bioassays on TCDD was prohibitive, only studies first meeting the in vivo animal bioassays study inclusion criteria described in Section 2.3.2 below are summarized; Tables D-1 and D-2 present the results of the study selection process evaluations for the studies that met and did not meet the study inclusion criteria, respectively. The selected animal studies are presented as key studies in Section 2.4.2 (see Tables 2-3 and 2-4 for cancer and noncancer endpoints, respectively).

[18] Critical exposure windows can be identified either through conceptual understanding of the timing of the affected biological process, such as a susceptible life-stage during which the effect is manifested, or empirically, when such critical windows are evident from the results of an epidemiological study. Note that the conceptual understanding can be obtained independently of the epidemiologic study in question.

2.3.1. Study Inclusion Criteria for TCDD Epidemiologic Studies

This section describes the process EPA used to select epidemiologic studies for identifying PODs for TCDD quantitative dose-response assessment.[19] This selection process includes specific criteria based on EPA's approaches for deriving OSFs and RfDs (see Text Box 2-1). Additional considerations used in selecting epidemiologic data for quantitative dose-response modeling are also necessary, particularly given EPA's preference to use human studies over animal studies whenever possible (U.S. EPA, 2005a). As described by Hertz-Picciotto (1995), key components needed for the use of an epidemiologic study as a basis for quantitative risk assessment include issues regarding exposure assessment and overall study quality. Exposure assessments need to be well-quantified with exposures linked to individuals. Different types of biases (e.g., confounding) also need to be eliminated in these studies. For example, biases related to inclusion criteria for membership in the study population and follow-up procedures need to be ruled out or considered to have a neglible impact on study findings. In addition, confounding should be controlled for or at least likely to be limited. The strength of the association, either within the full study or within a high exposure subgroup, can also be considered in the evaluation of suitability for dose-response modeling (Hertz-Picciotto, 1995). Stayner et al. (1999), however, note that even weak associations could be useful in terms of providing an estimate of a potential upper bound for a quantitative risk estimate.

Text Box 2-1. EPA Risk Assessment Guidelines and Guidance Documents for Toxicity Assessment
Guidelines for Mutagenicity Risk Assessment (U.S. EPA, 1986a)
Guidelines for the Health Risk Assessment of Chemical Mixtures (U.S. EPA, 1986b)
Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991)
Guidelines for Reproductive Toxicity Risk Assessment (U.S. EPA, 1996)
Guidelines for Neurotoxicity Risk Assessment (U.S. EPA, 1998)
Benchmark Dose Technical Guidance Document [external review draft] (U.S. EPA, 2000)
Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a)
Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens (U.S. EPA, 2005b)

EPA's study selection process included applying TCDD-specific study inclusion criteria to epidemiologic data which met the five following considerations (also see Figure 2-2 for more details):

[19] In general, for these epidemiologic studies, EPA is evaluating tissue concentrations of TCDD that have been used in conjunction with kinetic modeling to estimate previous TCDD exposures.

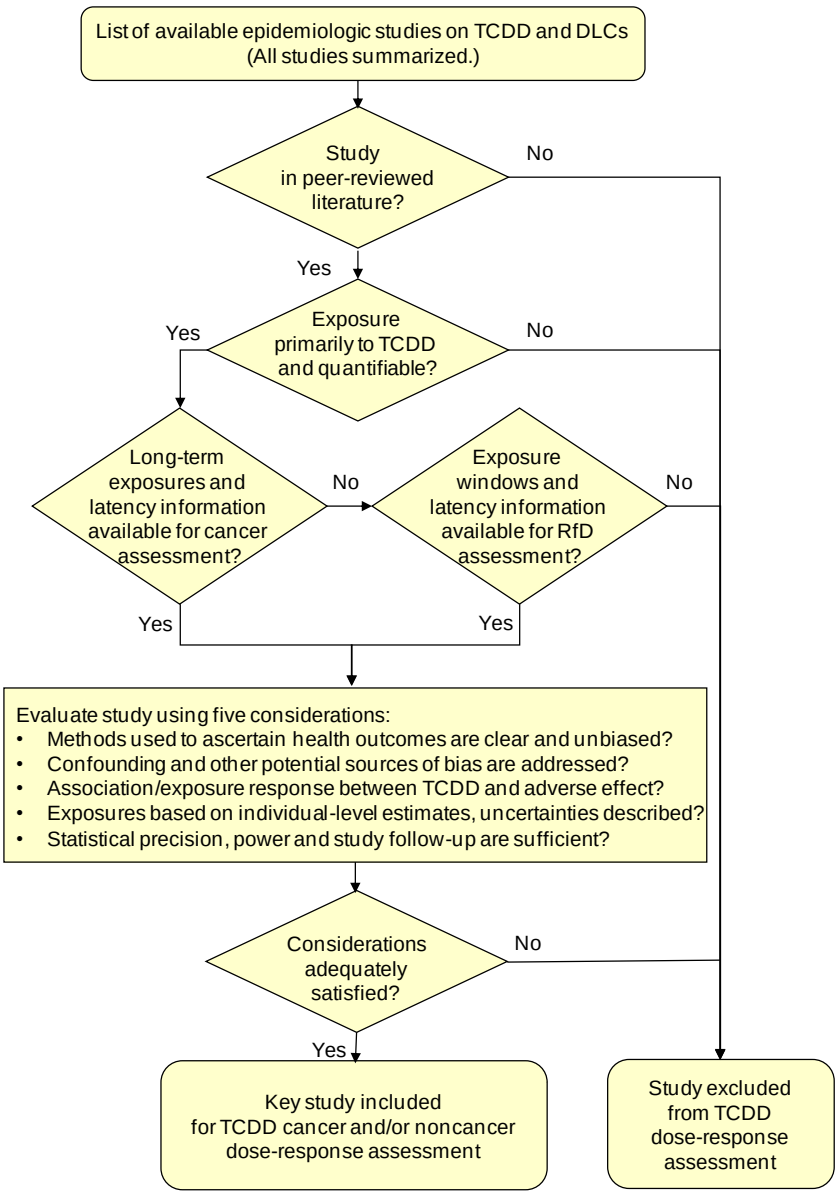


Figure 2-2. EPA's selection process to evaluate available epidemiologic studies using study inclusion criteria and other epidemiologic considerations for use in the dose-response analysis of TCDD.
EPA applied its TCDD-specific epidemiologic study inclusion criteria to all studies published on TCDD and DLCs. For all peer-reviewed studies, EPA examined whether the exposures were primarily to TCDD and if the TCDD exposures could be quantified so that dose-response analyses could be conducted. Then, EPA required that the effective dose and oral exposure be estimable: (1) for cancer, information is required on long-term exposures, (2) for noncancer, information is required regarding the appropriate window of exposure that is relevant for a specific, nonfatal health endpoint, and (3) for all endpoints, the latency period between TCDD exposure and the onset of the health endpoint is needed. Finally, studies were evaluated using five considerations regarded as providing the most relevant kind of information needed for quantitative human health risk analyses. Only studies meeting these criteria and adequately satisfying the considerations were selected for EPA's TCDD dose-response analysis.

1. The methods used to ascertain health outcomes are clearly identified and unbiased (e.g., outcome classification was made ~~"b~~linded" to exposure levels of the study participants).

2. The risk estimates generated from the study are not susceptible to important biases arising from an inability to control or account for confounding factors or other sources of bias (e.g., selection or information bias) arising from limitations of the study design, data collection, or statistical analysis.

3. The study demonstrated an association between TCDD and an adverse health endpoint (assuming minimal misclassification of exposure and absence of important biases) with some suggestion of an exposure-response relationship.

   This consideration addresses the use of null studies (i.e., studies reporting no association between TCDD and the health endpoint of interest) for the quantitative dose-response assessment used to derive an RfD; such studies are still used in qualitative assessments. Theoretically, a no-observed-adverse-effect level (NOAEL) can be identified from a null study and used to derive an RfD; that is, the highest available exposure dose from such a study could provide a NOAEL, which could serve as a basis for an RfD after appropriate uncertainty factors were applied. However, a NOAEL from a study in which no adverse effects have been observed is not usually chosen for RfD derivation when other available studies demonstrate lowest-observed-adverse-effect levels (LOAELs). The large and comprehensive database available to assess quantitative TCDD dose response provides many positive studies that are considered stronger candidates for derivation of an RfD than the studies for which only a NOAEL can be identified. [However, null studies are used by EPA to discuss the biological significance of the critical endpoint(s) used as the basis for deriving an RfD.]

4. The exposure assessment methodology is clearly described and can be expected to provide adequate characterization of exposure, with assignment of individual-level exposures within a study (e.g., based on biomarker data, or based on a job-exposure-matrix approach[20]). Limitations and uncertainties in the exposure assessment are considered.

5. The size and follow-up period of a cohort study are large enough and long enough, respectively, to yield sufficiently precise estimates for use in development of quantitative risk estimates and to ensure adequate statistical power to limit the possibility of not detecting an association that might be present. Similar considerations regarding sample size and statistical precision and power apply to other study designs such as case-control studies.

[20] A job-exposure matrix approach consists of a number of related methods for the quanitfication of occupational exposures that can be used to help assess potential risk.

In addition to these five study considerations, three specific study inclusion criteria were used to select studies for further evaluation and potential TCDD quantitative dose-response assessment:

1. The study is published in the peer-reviewed scientific literature and provides an appropriate discussion of data collection and analysis methods, as well as sufficient detail to allow consideration of its strengths and limitations.

2. The exposure is primarily to TCDD, rather than DLCs, and can be quantified so that dose-response relationships can be assessed for non-fatal adverse endpoints.[21] Because all epidemiologic cohorts have background exposures to DLCs, in which TCDD is a minor component, only those studies for which TCDD exposure is well above background will qualify for dose-response modeling. To the extent to which background DLC exposure becomes more significant with respect to TCDD exposure, limited quantitative assessment of DLC background exposures may be necessary.

3. The effective dose and oral exposure must be quantifiable. The timing of the measurement of health endpoints (i.e., the response) also must be consistent with current biological understanding of the endpoint and its progression.

   For cancer endpoints, EPA assumes that cumulative TCDD dose estimates are toxicologically relevant measures. Thus, cancer studies must provide information about long-term TCDD exposure levels. Further, for measures of cancer occurrence or death, sufficient follow-up is needed to allow for examination of latency between the end of effective exposure and cancer detection or death.

Text Box 2-2.
Critical Exposure Window

In this document, a biologically-relevant critical exposure window of susceptibility (~~c~~ritical exposure window" or ~~c~~ritical window") is defined as an exposure period during some specific life stage over which an individual is susceptible to the agent (e.g., TCDD) for a particular health endpoint. In utero and early lifetime exposures are often identified as critical exposure windows for many defects in anatomical and physiological processes under development during those periods. Critical exposure windows can be identified either through conceptual understanding of the timing of the affected biological process, such as a susceptible life-stage during which the effect is manifested, or empirically, when such critical windows are evident from the results of an epidemiologic study. An example of the latter is the semen quality effects associated with early exposure to TCDD for boys under 10 years of age compared to boys 10−17 years of age at the time of TCDD exposure (Morarelli et al. [2008]; see Appendix C for study details).Identifying such critical windows is important for TCDD in the practical sense of defining a reasonable duration over which to average internal exposures that vary greatly from an initial high peak exposure to a much lower terminal exposure, as is the case for almost of the epidemiologic studies under consideration for TCDD. EPA considers the internal exposures following the actual TCDD exposure incident to be relevant for averaging because of the relatively slow elimination of TCDD and the possibility that these concentrations could still be affecting the processes leading to the adverse health outcome.

[21] The IRIS Program does not generally base RfDs on highly severe effects, such as mortality.

> For noncancer endpoints, exposure estimates and analysis must allow for examination of issues of latency and other issues regarding the appropriate time window of exposure relevant for specific endpoints. That is, there must be sufficient information, either in the study or elsewhere, to allow for the identification of a biologically-relevant ~~"c~~ritical exposure window" of susceptibility (see Text Box 2-2).

Those studies that satisfied these three study inclusion criteria and, in addition, adequately satisfied the study quality provisions specified in the five considerations were considered to be suitable for quantitative TCDD dose-response analyses (see results in Section 2.4.1 and Appendix C).

2.3.2. Study Inclusion Criteria for TCDD In Vivo Mammalian Bioassays

This section identifies the criteria EPA applied to select nonhuman in vivo mammalian studies for defining PODs for use in TCDD dose-response modeling. These criteria are specifically developed to evaluate the TCDD literature and are not necessarily generic, however, they are based on EPA's approaches for deriving OSFs and RfDs from bioassay data (see Text Box 2-1). EPA agrees with the NAS committee regarding the utility of an oral RfD and the need for reevaluation of the OSF for TCDD, specifically in light of data that have been published since the 2003 Reassessment was released. RfDs and OSFs are generally derived using data sets that demonstrate the occurrence of adverse effects, or their precursors, in the low-dose range for that chemical. RfDs and OSFs are derived from a health-protective perspective for chronic exposures. Thus, when a group of studies is available on a chemical for which a number of effects are observed at various doses across those studies, the studies using the lowest doses that show effects will typically be selected as the basis of the RfD and OSF derivations, all other considerations being equal. Studies conducted at higher doses relative to other available studies are used as supporting evidence for the final RfD or OSF because they were conducted at doses too high to impact the numeric derivations of toxicity values.

EPA expresses RfDs and OSFs in terms of average daily doses, usually as mg/kg-day and per mg/kg-day, respectively. Thus, the study inclusion criteria for the animal bioassay data presented in this section include requirements that average daily exposures in the studies are within a low-dose range where, relative to other studies, they could be considered for development of a toxicity value. These low-dose requirements do not imply that TCDD studies conducted at higher doses are of poor quality, simply that they are not quantitatively useful in the

development of toxicity values because other studies with lower exposures will be selected as the basis of the RfD and OSF derivations under current EPA guidance (see Text Box 2-1). Because EPA has identified hundreds of in vivo mammalian studies that may be considered for quantitative TCDD dose-response assessment, the development and application of these study inclusion criteria have been critical to moving the health assessment process forward.

EPA's method for applying TCDD-specific study inclusion criteria for mammalian bioassays is detailed below and in Figure 2-3. Four specific study inclusion criteria were used to select studies for further evaluation and potential TCDD quantitative dose-response analyses and identification of PODs:

1. The study is published in the peer-reviewed scientific literature.
2. The study was not conducted on a genetically-altered species.
3. The lowest dose level tested is ≤1 μg/kg-day for cancer studies and ≤30 ng/kg-day for noncancer studies.
4. The study design consists of orally administered TCDD-only doses.

Those studies that satisfied these four criteria (see results in Section 2.4.2 and Appendix D) were considered suitable for quantitative TCDD dose-response analysis.

In evaluating the selected in vivo animal studies, EPA considered study quality issues to ensure that the study provided important information needed to assess the relevance of the study's endpoints and to quantify the dose-response relationship. Each study needed to test a mammalian species and identify the strain, gender, and age of the tested animals. The study had to clearly document its testing protocol, including dosing frequency, duration, and timing of dose administration relative to age of the animals. For example, the control group or groups had to be well characterized and appropriate, given the testing protocol. Also, clinical and pathological examinations conducted during the study needed to be endpoint-appropriate, particularly for negative findings. EPA used the results of these study evaluations in drafting study summaries for all of the animal bioassays that met the study inclusion criteria (see Appendix D).

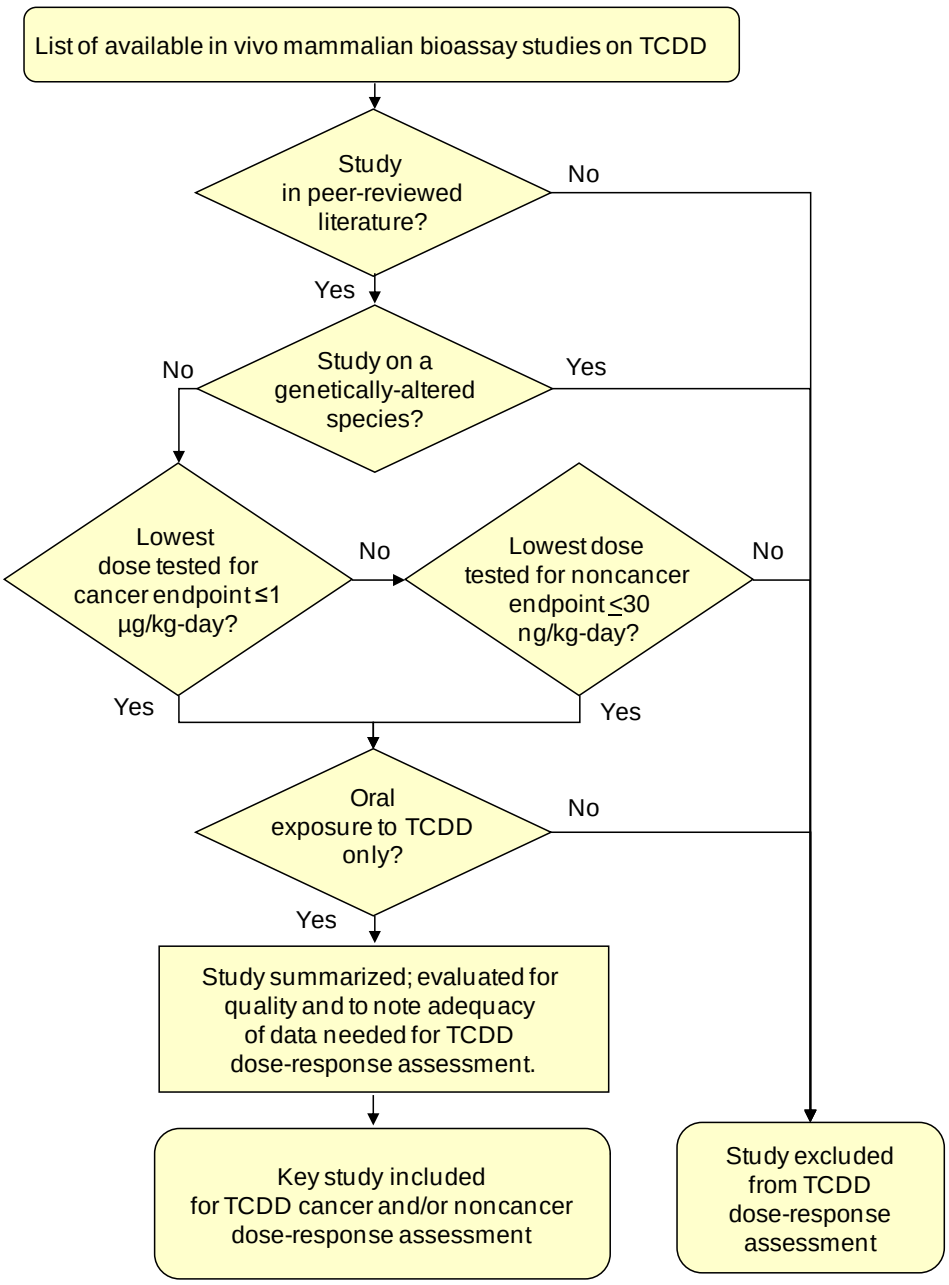


Figure 2-3. EPA's process to evaluate available animal bioassay studies using study inclusion criteria for use in the dose-response analysis of TCDD.
EPA evaluated all available in vivo mammalian bioassay studies on TCDD. Studies had to be published in the peer-reviewed literature. Studies on genetically-altered species were excluded as their relevance to human health is not known. Next, EPA applied dose requirements to each study's lowest tested average daily dose, with requirements for cancer (≤1 µg/kg−day) and noncancer (≤30 ng/kg−day) studies. EPA also required that the animals were exposed via the oral route to only TCDD. Finally, the studies were evaluated for quality and summarized to ensure providing the most relevant information for quantitative human health risk analyses. Only studies meeting all of the criteria were selected for EPA's TCDD dose-response analysis.

The criteria for dose requirements are intended to be reasonable limits that restrict the number of studies that would need to be considered while ensuring that all study/data set combinations that could be candidates for deriving a cancer or noncancer toxicity value were analyzed. Thus, the dose range under consideration allows for liberal ranges of NOAELs, LOAELs, and benchmark dose lower confidence bounds (BMDLs) for assessment of both cancer and noncancer effects. The dose requirements for cancer and noncancer studies were set after EPA conducted a brief review of typical dose levels in studies analyzed in the 2003 Reassessment and in some of the more recent studies found through EPA's literature search.

For cancer studies, the low-dose limit was selected liberally so as not to exclude a study that might possibly report a sensitive tumor endpoint. Given that the limit of 1 μg/kg-day is 3 orders of magnitude higher than the lowest-tested dose in one of the most sensitive animal bioassays (Kociba et al., 1978) evaluated in U.S. EPA (2003), it is virtually impossible that a study with a low dose of 1 μg/kg-day or greater would ever be considered for deriving a cancer toxicity value. Following identification of new animal cancer bioassays, no studies were eliminated based on this limit.

For noncancer studies, the identification of a low-dose limit is more complicated because of the variety of exposure protocols and endpoints and the consequent varied degree of toxicokinetic extrapolation to human equivalent exposures. However, EPA is confident that the low-dose limit of 30 ng/kg-day will not exclude any study from which a POD could be derived that would be low enough to be considered for the RfD. A preliminary screening of the literature indicated that, for all study types (e.g., acute, developmental, chronic), there are many studies with apparent effect levels well below 30 ng/kg-day. Effects observed above 30 ng/kg-day, therefore, would have no chance of being considered as the basis for an RfD.

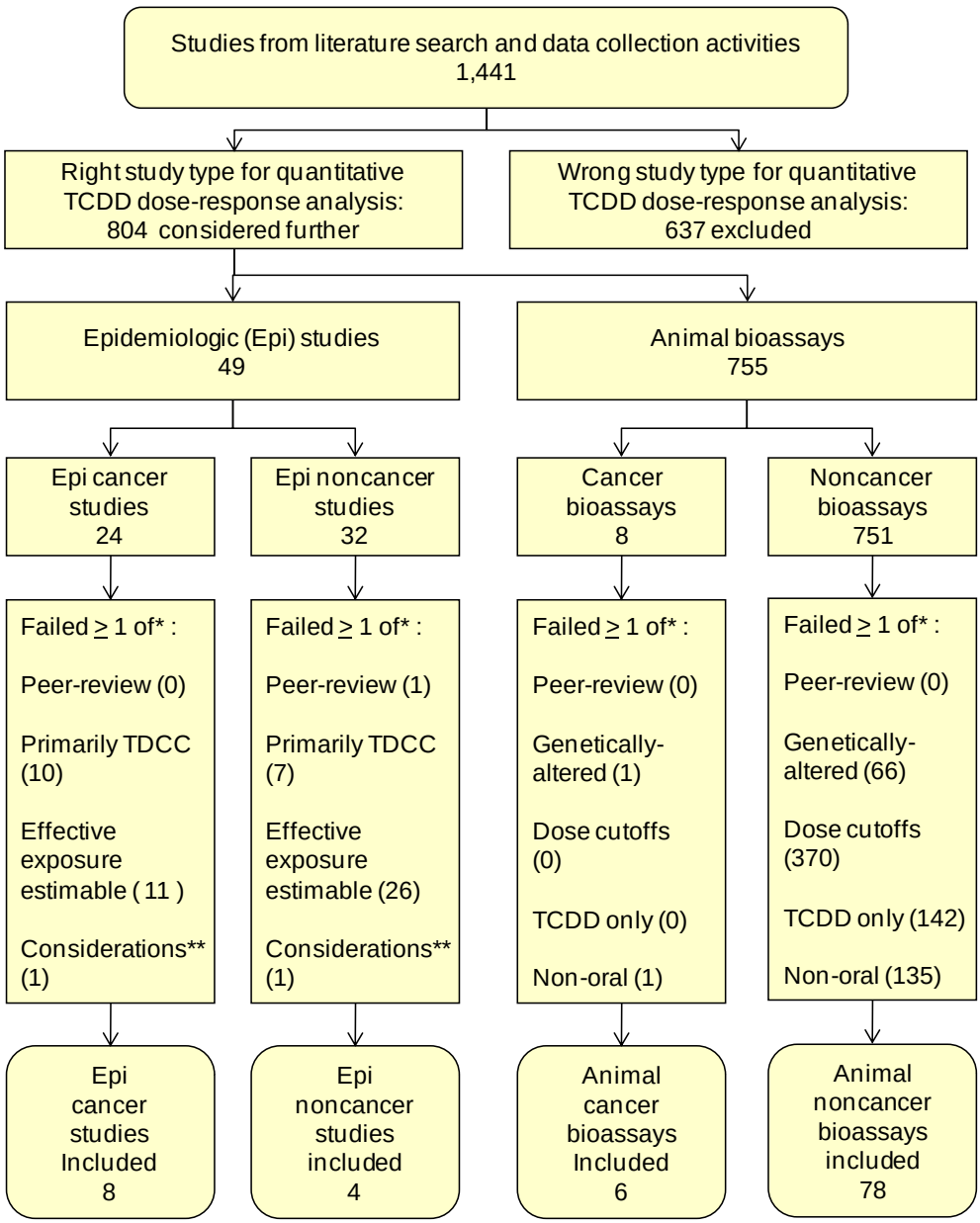
2.4. SUMMARY OF KEY DATA SET SELECTION FOR TCDD DOSE-RESPONSE MODELING

To meet the NAS' concerns regarding transparency and clarity in the identification of TCDD studies for dose-response assessment, EPA has developed and applied two sets of criteria for epidemiologic studies and animal bioassays. EPA collected these studies through October, 2009, including studies from the 2003 Reassessment and newer studies found via literature searches and through public submissions (see Section 2.2 and Figure 2-1). Based on these activities, a total of 1,441 studies were examined for their potential to be used in TCDD

quantitative dose-response analysis. Of these, Figure 2-4 shows that 637 studies were eliminated from consideration as they were not suitable study types; these included, in vitro bioassays, review articles, PBPK modeling studies, and studies that evaluated PCBs or other dioxin-like compounds other than TCDD. Of the remaining studies, 49 were epidemiologic studies (7 studies contained both cancer and noncancer endpoints), and 755 were animal studies (4 studies contained both cancer and noncancer endpoints). These epidemiologic and animal studies were then evaluated using EPA's study inclusion criteria.

Detailed results of EPA's evaluations and study summaries are shown in Appendices C and D for the epidemiologic studies and animal bioassays, respectively. Final results in tabular form are shown in this section. Tables 2-1 and 2-2 contain the preliminary list of cancer studies and the final list of key noncancer studies, respectively, that have met EPA's study inclusion criteria for epidemiologic data. Tables 2-3 and 2-4 provide the preliminary list of cancer bioassays and the final list of key noncancer bioassays, respectively, that have met EPA's study inclusion criteria for animal bioassay data. Collectively, Tables 2-2 and 2-4 contain the final set of key studies that EPA has selected for development of the noncancer dose-response assessment for TCDD presented in Section 4 of this document, Reanalysis, Volume 1. Tables 2-1 and 2-3 provide preliminary lists of cancer studies that will be useful in developing the cancer dose-response assessment to be presented in Reanalysis, Volume 2.

Through this study selection process, EPA has identified a relevant group of studies that spans the possible risk analytic choices for human health protection. Each study provides important TCDD dose-response information but also is associated with limitations and uncertainties that must be considered and characterized during TCDD dose-response evaluations. EPA has benefited from this effort by greatly reducing the scope of dose-response modeling and analyses to a manageable size, and by focusing on the most important studies from the perspective of developing cancer and noncancer toxicity values. Results of applying the study inclusion criteria showed that exposure information was a primary factor in study selection (see Figure 2-4). In the epidemiologic studies, exposure needed to be primarily to TCDD and quantifiable on an individual level. In addition, the identification of critical exposure windows (see Text Box 2-2) and the availability of latency information in the epidemiologic studies were vital data for developing human exposure estimates. In the animal studies, dose limits were the most important criteria.



*Failed criteria are not mutually exclusive; more than one can fail for a given study.
**Indicates those studies that passed all three criteria but were not selected based on study considerations.

Figure 2-4. Results of EPA's process to select and identify in vivo mammalian and epidemiologic studies for use in the dose-response analysis of TCDD.

Four animal studies and seven epidemiologic studies contained both cancer and noncancer endpoints. Two epidemiologic cancer studies, Steenland et al. (1999) and Flesch-Janys et al. (1998), passed all criteria, but were still not selected because they were superseded by other studies on the same cohort for which an improved analysis was done. One noncancer epidemiologic study, Baccarelli et al. (2005), passed all criteria, but was excluded because the health endpoint, chloracne, is considered to be an outcome associated with high TCDD exposures.

Table 2-1. Epidemiologic studies selected for TCDD cancer dose-response modeling

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases or deaths	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Akhtar et al. (2004)	Mortality and incidence for all cancers and for site-specific cancers including prostate and melanoma	Vietnam 1962−1971	Ranch Hand (RH) cohort including 1,196 U.S. military males exposed by spraying Agent Orange during Vietnam war in Southeast Asia (SEA); comparison (C) cohort matched by age, race, and military occupation.	Cumulative serum lipid concentrations (CSLC) of TCDD based on serum levels collected from veterans in 1987, 1992, 1997, and a first-order kinetic model with a 7.6-year half-life. CSLC estimates for 1,009 RH cohort and 1,429 C cohort veterans.	CSLC (ppt-years) RH and C ≤2 yrs in SEA: All site Comparison ≤10 Low >10-118.5 High >118.5 Continuous (Log TCDD) Melanoma Comparison ≤10 Low >10-118.5 High >118.5 Continuous (Log TCDD) Prostate Comparison ≤10 Low >10-118.5 High >118.5 Continuous (Log TCDD)	 No.,% 34, 5.9 28, 9.8 22, 14.6 15, 8.6 No., % 3, 0.5 4, 1.4 4, 2.7 3, 1.7 No., % 7, 1.2 10, 3.5 6, 4.0 5, 2.9	 RR (95% CI) 1.0 1.44 (0.82−2.53) 2.23 (1.24−4.00) 2.02 (1.03−3.95) 1.24 (1.01−1.53) *p* = 0.04 1.0 2.99 (0.53-16.8) 7.42 (1.34-41.04) 7.51 (1.12-50.21) 2.24 (1.29-3.89) *p* = 0.004 1.0 1.5 (0.51-4.40) 2.17 (0.68-6.87) 6.04 (1.48-24.61) 1.48 (0.93-2.35)* *p* = 0.10	Adjusted for age at tour, military occupation, smoking, skin reaction to sun exposure, eye color, number of years in SEA. Also stratified analyses by year of tour of duty. Restricted to ≤2 years in SEA, white Air Force veterans, 0% and 100% time in Vietnam for RH and C Cohorts, respectively.	Used multiplicative Poisson regression models to compare cancer incidence and cancer mortality with national rates and proportional hazards models to contrast cohorts with regard to cancer incidence.

Table 2-1. Epidemiologic studies selected for TCDD cancer dose-response modeling (continued)

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases or deaths	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Becher et al. (1998)	Mortality from all cancers combined	Hamburg, Germany, production period was 1950−1984, and mortality follow-up extended through 1992	Boehringer cohort including approximately 1,189 workers employed in the production of herbicides.	CSLC of TCDD based on area under curve (in µg/kg years); back-extrapolation to date of last employment took into account age and percentage body fat; half-life value was 7.2 years.	Categorical exposures (Cox model) 0− <1 1− <4 4− <8 8− <16 16− <64 64+	124	RR (95% CI) 1.0 1.12 (0.70−1.80) 1.42 (0.70−2.85) 1.77 (0.81−3.86) 1.63 (0.73−3.64) 2.19 (0.76−6.29) p = 0.03	Available: year of entry, age of entry, duration of employment, birth cohort, β-HCH; TEQ other than TCDD.	Included in U.S. EPA (2003). A large number of models were fitted. These included models for 5 different latency intervals (0, 5, 10, 15, and 20 years), as well as multiplicative, additive, and power models, and different offset variables (person years and expected deaths).
					Continuous exposure TCDD (µg/kg years)	124	β = 0.0089, p = 0.0047	Available: year of entry, age of entry, duration of employment, birth cohort, β-HCH; TEQ other than TCDD.	
Cheng et al. (2006)	Mortality from all cancers	USA, 1942–1993	NIOSH cohort including 3,538 occupationally exposed male workers at 8 plants in the United States; 256 cancer deaths.	CSLC of TCDD based on work histories, job-exposure matrix, and concentration and age-dependent two-compartment model of elimination kinetics.	No exposure categories provided	256 cancer deaths	The slope (β) was 3.3 × 10^{-6} for lag of 15 years excluding upper 5% of TCDD exposures. The slopes ranged two orders of magnitude depending on modeling assumption.	Available: age, year of birth, and race. Risks adjusted for: year of birth, age, and race. Indirectly examined other potential confounders such as smoking and other occupational exposures.	Confounding by smoking was considered indirectly by analysis of smoking-related and smoking-unrelated cancers. Other occupational exposures were considered indirectly by repeated analyses removing one plant at a time. Based on indirect evaluation, there was no clear evidence of confounding.

Table 2-1. Epidemiologic studies selected for TCDD cancer dose-response modeling (continued)

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases or deaths	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Collins et al. (2009)	Mortality from all cancers and specific cancer types	Midland, MI, USA. Follow-up period: 1942–2003. Serum collection period: 2004–2005	Subset of NIOSH cohort including 1,615 occupationally exposed male workers at 1 plant in the United States; 177 cancer deaths.	CSLC of TCDD based on work histories, job-exposure matrix, and concentration and age-dependent two-compartment model of elimination kinetics. Serum samples were obtained from 280 former workers collected during 2004−2005.	Part per billion-year estimates of cumulative TCDD exposure	177 cancer deaths	The slope of a proportional hazards regression model for fatal soft tissue sarcoma was 0.05872 (95% CI not provided but for Chi-square $p = 0.0060$) for every 1-part per billion-year increase in cumulative exposure of TCDD. Slope estimates for all fatal cancers (0.00161, $p = 0.78$), fatal lung (-0.00173, $p = 0.89$), fatal prostate (0.01294, $p = 0.30$), fatal leukemias (-0.12822, $p = 0.34$), and fatal non-Hodgkin lymphomas (0.01081, $p = 0.68$) were not statistically significant.	Hazard ratios adjusted for age, year of birth, and hire year. Stratified analyses used to examine potential impact of pentachlorophenol exposure on mortality.	Confounding by smoking was not considered directly due to a lack of data. Relatively long follow-up period (average = 36 years). Potential outcome misclassification for soft tissue sarcoma due to potential inaccuracies on death certificates. Data analyzed from one plant reduces heterogeneity associated with multiplant analyses. More serum samples ($n = 280$) analyzed than used to derive TCDD estimates for other NIOSH cohort analyses.

Table 2-1. Epidemiologic studies selected for TCDD cancer dose-response modeling (continued)

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases or deaths	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Michalek and Pavuk (2008)	Cancer incidence, all sites combined	Vietnam 1962−1971	RH cohort including 1,196 U.S. military males exposed by spraying Agent Orange during Vietnam war in Southeast Asia (SEA); C cohort matched by age, race, and military occupation.	CSLC of TCDD based on serum levels collected from veterans in 1987, 1992, 1997, 2002, and a first-order kinetic model with a 7.6-year half-life. CSLC estimates for 986 RH cohort and 1,597 C cohort veterans.	CSLC (ppt-years) Results stratified by ≤1968, ≥30 days pre-1967, ≤2 yrs in SEA: Comparison ≤10 Low >10-91 High >91	Continuous exposure: Log (TCDD) No.,% 67, 12.6 Categorical TCDD No., % 30, 11.2 10, 8.3 12, 24.5 15, 16.1	1.4 (1.1-1.7) $p = 0.005$ RR (95% CI) 1.0 0.5 (0.2−1.1) 1.7 (0.8−3.5) 2.2 (1.1−4.4).	Cox regression proportional hazards models adjusted for year of birth, eye color, race, smoking, body mass index at the qualifying tour, military occupation, and skin reaction to sun exposure. Also stratified analyses by years of service in SEA, days of herbicide spraying, calendar period of service.	Without stratification, there was no significant increase in the risk of cancer with log (TCDD) in the combined cohort.

Table 2-1. Epidemiologic studies selected for TCDD cancer dose-response modeling (continued)

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases or deaths	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Ott and Zober (1996)	Mortality and incidence for all cancers combined, as well as for specific cancer sites	Ludwig-shafen, Germany, 1954−1992	BASF cohort, 243 men exposed from accidental release that occurred in 1953 during production of trichlorophenol, or who were involved in clean-up activities.	CSLC of TCDD expressed in µg/kg based on TCDD half-life of 5.1−8.9 years, Cox regression model.	Internal comparisons based on continuous measure of TCDD. External comparisons exposure categories (for malignant neoplasms): <0.1, 0.1−0.99 1.0−1.99 >2 µg/kg	*Internal cohort analysis* 31 All cancer deaths 47 All incident cancers *External cohort analyses* Deaths 8 8 8 7	RR (95% CI) 1.22 (95% CI: 1.00−1.50) 1.11 (95% CI: 0.91−1.35) SMR (95% CI) 0.8 (0.4−1.6) 1.2 (0.5−2.3) 1.4 (0.6−2.7) 2.0 (0.8−4.0)	Available: age, BMI, smoking status, and history of occupational exposure to aromatic amines and asbestos.	Included in U.S. EPA (2003) Positive associations noted for digestive cancer, but not for respiratory cancer. Association between TCDD and increased SMRs found only among current smokers. Last published account of this cohort.
Steenland et al. (2001)	Mortality from all cancers	USA, 1942–1993	NIOSH cohort including 3,538 male workers, 256 cancer deaths.	CSLC of TCDD based on work histories, job-exposure matrix, and a simple one-compartment, first-order pharmacokinetic elimination model with 8.7-year half-life.	CSLC (ppt-years) <335 335−520 520−1,212 1,212−2,896 2,896−7,568 7,568−20,455 ≥20,455	64 29 22 30 31 32 48	RR (95% CI) 1.00 1.26 (0.79−2.00) 1.02 (0.62−1.65) 1.43 (0.91−2.25) 1.46 (0.93−2.30) 1.82 (1.18−2,82) 1.62 (1.03−2,56)	Available: date of birth and age. Adjusted for date of birth, and age was used as time scale in Cox model.	Included in U.S. EPA (2003)

Table 2-1. Epidemiologic studies selected for TCDD cancer dose-response modeling (continued)

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases or deaths	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Warner et al. (2002)	Breast cancer incidence	Italy 1976–1998	981 women from Zones A and B with available archive serum samples, 15 breast cancer cases.	CSLC of TCDD (ppt) collected between 1976 and 1981. For most samples collected after 1977, serum TCDD levels were back-extrapolated using a first-order kinetic model with a 9-year half-life.	Categorical <20 ppt 20.1−44 ppt 44.1−100 ppt >100 ppt Continuous (Log_{10}TCDD)	Cases 1 2 7 5 15	RR (95% CI) 1.0 1.0 (0.1−10.8) 4.5 (0.6−36.8) 3.3 (0.4−28.0) p = 0.07 2.1 (1.0−4.6)	Available: gravidity, parity, age at first pregnancy, age at last pregnancy, lactation, family history of breast cancer, age at menarche, current body mass index, oral contraceptive use, menarcheal status at explosion, menopause status at diagnosis, height, smoking, alcohol consumption. Adjusted for age, which was used as time scale in Cox model; other covariates were evaluated but were not identified as confounders.	Included in U.S. EPA (2003)

CI = confidence interval; CSLC = cumulative serum lipid concentration; HCH = hexachlorocyclohexane.

Table 2-2. Epidemiologic studies selected for TCDD noncancer dose-response modeling

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Alaluusua et al. (2004)	Dental defects	Seveso, Italy, Dental exams administered in 2001 among those exposed to TCDD in 1976	65 subjects <9.5 years old at time of Seveso explosion and residing in Zones ABR (i.e., the most heavily contaminated area in decreasing order); 130 subjects recruited from the non-ABR region (i.e. the unexposed).	Serum TCDD (ng/kg) from 1976 samples for those who resided in Zones ABR; no serum levels for non-ABR residents (unexposed). TCDD exposure represent levels as of 1976 (after accident).	Non-ABR Zone 31−226 ng/kg 238−592 ng/kg 700−26,000 ng/kg <5 years of age at time of accident Non-ABR Zone or 31−226 ng/kg serum TCDD 238−26,000 ng/kg serum TCDD	10 1 5 9 25	Dental defect % 26% 10% 45% 60% *p*-value = 0.016 33% *p*-value = 0.0009 Odds Ratios (95% CI) (among those <5 years of age at time of accident) 1.0 2.4 (1.3−4.5) *p*-value = 0.007	Available: medical history, age, sex, education, smoking.	Dose-response pattern observed with dental defects in the ABR zone; however, the control population had a much higher prevalence of dental defects (26%) than those in the lowest exposure group (10%). Also assessed hypodontia and other dental and oral aberrations, but these were too rare to allow modeling by ABR zone.

Table 2-2. Epidemiologic studies selected for TCDD noncancer dose-response modeling (continued)

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Baccarelli et al. (2008)	b-TSH measured 72 hours after birth from a heel pick (routine screening for all newborns in the region)	Italy, 1976; children, 1994–2005	*Population-based study:* 1,041 singletons (56 from Zone A, 425 from Zone B, and 533 from reference) born between Jan. 1, 1994-June 30, 2005. *Plasma dioxin study:* 51 children born to 38 women of fertile age who were part of the Seveso Chloracne Study.	Based on zone of residence, estimated mean values from a previous study. Maternal plasma TCDD levels estimated at the date of delivery using a first-order pharmacokinetic model and elimination rate estimated in Seveso women (half-life = 9.8 years).	*Population-based study:* Reference Zone A Zone B *Plasma dioxin study:* Continuous maternal plasma TCDD	533 births 56 births 425 births	*Population-based study* Geometric Mean b-TSH (log-transformed) Reference: 0.98 (95% CI: 0.90−1.08) Zone B: 1.66 (95% CI: 1.19−2.31) Zone A: 1.35 (95% CI: 1.22−1.49) Association between neonatal b-TSH with plasma TCDD: adjusted β = 0.75 ($p < 0.001$)	Available: gender, birth weight, birth order, maternal age at delivery, hospital, type of delivery. There was limited evidence of confounding, so mean TSH results presented here are unadjusted.	An association with serum TCDD levels of mothers was found with b-TSH among the 51 births in the plasma dioxin study.
Eskenazi et al. (2002b)	Menstrual cycle characteristics: menstrual cycle length.	Seveso, Italy, follow-up interview conducted in 1996-1997 of women exposed to TCDD in the 1976 accident	Women who were <40 years from Zones A or B in 1976.	Serum TCDD (ng/kg) from 1976 samples. TCDD exposure level was back-extrapolated to 1976 using the Filser or the first-order kinetic models.	Interquartile range was 64−322 ppt TCDD examined as continuous measure (per 10-fold increase in serum levels).		Lengthening of the menstrual cycle by 0.93 days (95% CI: -0.01, 1.86)	Interview data: medical history, personal habits, work history, reproductive history, age, smoking, body mass index, alcohol and coffee consumption, exercise, illness, abdominal surgeries.	A positive association between menstrual cycle length and serum TCDD was found among women who were premenarcheal at the time of accident ($n = 134$).

Table 2-2. Epidemiologic studies selected for TCDD noncancer dose-response modeling (continued)

Reference	Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases	Effect measure/ trend tests (*p*-value)	Risk factors	Comments
Mocarelli et al. (2008)	Sperm conc. (million/mL) Progressive motility (%) Serum E_2 (pmol/L)	Italy, 1976, 1998	Among the 257 exposed (from Zone A), men 1−26 in 1976 with serum levels <2000 ppt in 1976, 135 (53%) were included. Among the 372 nonexposed invitees, 184 (49%) men aged 1−26 in 1976 were included.	Serum TCDD (in ppt) from 1976−1977 samples (for exposed men); background values were assumed for unexposed men based on serum analysis of residents in uncontaminated areas.	Median serum TCDD levels (in ppt) by quartile for men aged 1−9 in 1976 (68; 142; 345; 733 ppt)		Men exposed between the ages 1−9 had reduced semen quality 22 years later. Reduced sperm quality included decreases in sperm count ($p = 0.025$), progressive sperm motility ($p = 0.001$), and total number of motile sperm ($p = 0.01$) relative to the comparison group.	Available: age, abstinence time, smoking status, education, alcohol use, maternal smoking during pregnancy, employment status, BMI, chronic exposure to solvents and other toxic substances. Adjusted for smoking status, organic solvents, age at time of tests, BMI, alcohol use, education, employment status, and abstinence (days) for sperm data. Hormone data not adjusted for education level, employment status, and abstinence time.	Results stratified by timing of exposure (1−9 yrs old vs. 10−17 yrs old in 1976).

b-TSH = blood thyroid-stimulating hormone; CI = confidence interval.

Table 2-3. Animal bioassays selected for cancer dose-response modeling

Reference	Species/strain	Sex exposure route/duration	*n*	Average daily dose levels (ng/kg-day)	Cancer types	Statistical significant tumors (pairwise with controls or trend tests)
Della Porta et al. (1987)	Mouse/ $B6C3F_1$	Male/female Oral gavage once per week; 52 weeks	~40 to 50 in each dose group including controls	0, 351, and 714	Females and males: hepatocellular adenomas and carcinomas	Liver: adenomas and carcinomas in females and carcinomas in males (using incidental tumor statistical test)
Kociba et al. (1978); Goodman and Sauer (1992)	Rat/Sprague-Dawley	Male/female Oral-lifetime feeding; 2 years	50 each (86 each in vehicle control group)	0, 1, 10, or 100	Females: liver, lung, oral cavity Males: adrenal, oral cavity, tongue	Adrenal cortex: adenoma Liver: hepatocellular adenoma(s) or carcinoma(s); hyperplastic nodules Lung: keratinizing squamous cell carcinoma Oral cavity: stratified squamous cell carcinoma of hard palate or nasal turbinates Tongue: stratified squamous cell carcinoma
NTP (1982c)	Mouse/ $B6C3F_1$	Male/female Oral-gavage twice per week; 104 weeks	50 each (75 each in vehicle control group)	0, 1.4, 7.1, or 71 for males; 0, 5.7, 28.6, or 286 for females	Females: hematopoietic system, liver, subcutaneous tissue, thyroid Males: liver, lung	Hematopoietic system: lymphoma or leukemia Liver: hepatocellular adenoma or carcinoma Lung: alveolar/bronchiolar adenoma or carcinoma Subcutaneous tissue: fibrosarcoma Thyroid: follicular-cell adenoma
NTP (1982c)	Rat/Osborne-Mendel	Male/female Oral-gavage twice per week; 104 weeks	50 each (75 each in vehicle control group)	0, 1.4, 7.1, or 71	Females: adrenal, liver, subcutaneous tissue, thyroid Males: adrenal, liver, thyroid	Adrenal: cortical adenoma, or carcinoma or adenoma, NOS Liver: neoplastic nodule or hepatocellular carcinoma Subcutaneous tissue: fibrosarcoma Liver: neoplastic nodule or hepatocellular carcinoma Thyroid: follicular-cell adenoma or carcinoma

Table 2-3. Animal bioassays selected for cancer dose-response modeling (continued)

Reference	Species/strain	Sex exposure route/duration	*n*	Average daily dose levels (ng/kg-day)	Cancer types	Statistical significant tumors (pairwise with controls or trend tests)
NTP (2006a)	Rat/Harlan Sprague-Dawley	Female Oral-gavage 5 days per week; 2 years	53 or 54	0, 2.14, 7.14, 15.7, 32.9, or 71.4	Liver Lung Oral mucosa Pancreas	Liver: hepatocellular adenoma Liver: cholangiocarcinoma Lung: cystic keratinizing epithelioma Oral mucosa: squamous cell carcinoma Pancreas: adenoma or carcinoma
Toth et al. (1979)	Mouse/ Outbred Swiss/H/Riop	Male Gastric intubation once per week; 1 year	43 or 44 (vehicle control group = 38)	0, 1, 100, or 1,000	Liver	Liver: tumors

Table 2-4. Animal bioassay studies selected for noncancer dose-response modeling

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Reproductive toxicity studies									
Bowman et al.(1989a; 1989b); Schantz and Bowman (1989); Schantz et al. (1992; 1986)	Monkey/ Rhesus	Daily dietary exposure in female monkeys (3.5−4 years)	F (F0, F1, F2, F3)	3 to 7 (F1)	0, 0.12, or 0.67	None	0.12	Reproductive and developmental effects	Neurobehavioral effects (e.g., discrimination-reversal learning affected)
Franc et al. (2001)	Rat/Sprague-Dawley, Long-Evans, Han/Wistar	Biweekly oral gavage (22 weeks)	Female	8	0, 10, 30 or 100	10	30	Body weight, relative liver weight, relative thymus weight	Increased relative liver weight in Sprague-Dawley and Long-Evans Rats; Increased relative thymus weight in Sprague-Dawley, Han/Wistar, and Long-Evans Rats
Hochstein et al (2001)	Mink	Daily dietary exposure (132 days)	F	12	0.03 (control), 0.8, 2.65, 9, or 70	None	2.65	Reproductive effects	Reduced kit survival
Hutt et al. (2008)	Rat/Sprague-Dawley	Oral gavage (GDs 14 and 21, postpartum days 7 and 14), (Pups: once per week for 3 months)	Female (F0 and F1)	3 (F0 and F1)	0 or 7.14	None	7.14	Developmental effects	Lower proportion of morphologically normal pre-implantation embryos during compaction stage

Table 2-4. Animal bioassay studies selected for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Reproductive toxicity studies (continued)									
Ikeda et al. (2005)	Rat/ Holtzman	Corn oil gavage (initial loading dose followed by weekly dose during mating, pregnancy, and lactation–about 10 weeks)	F (F0) F and M (F1 and F2)	12 (F0) Not specified (F1 and F2)	0 or 16.5	None	16.5 (maternal exposure)	Reproductive and developmental effects	Decreased development of the ventral prostrate (F1), decreased sex ratio (percentage of males) (F2)
Ishihara et al. (2007)	Mouse/ICR	Sesame oil gavage (initial loading dose followed by weekly doses for 5 weeks)	M (F0)	42 or 43	0, 0.095, or 950	0.1	100	Reproductive effects	Decreased male/female sex ratio (percentage of males) (F1)
Latchoumy-candane and Mathur (2002) and related Latchoumy-candane et al. (2003, 2002a; 2002b)	Rat/Wistar albino	Olive oil gavage (daily for 45 days)	M	6	0, 1, 10, or 100	None	1	Reproductive effects	Reduced sperm production, decreased reproductive organ weights
Reproductive toxicity studies (continued)									
Murray et al. (1979)	Rat/Sprague-Dawley	Daily dietary exposure (3 generations)	F and M, (F0) F and M, (F1 and F2)	10–32 (F0) 22 (F1) 28 (F2)	0, 1, 10, or 100	1	10	Reproductive and developmental effects	Decrease in fertility, decrease in the number of live pups, decrease in gestational survival; decrease in postnatal survival, decreased postnatal body weight in one or more generations

Table 2-4. Animal bioassay studies selected for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Shi et al. (2007)	Rat/Sprague-Dawley	Maternal corn oil gavage (weekly on GDs 14 and 21; PNDs 7 and 14) Offspring corn oil gavage (weekly for 11 months)	F (F0) F (F1)	3 (F0) 10 (F1)	0, 0.14, 0.71, 7.14, or 28.6	0.14	0.71	Reproductive effects	Decrease serum estradiol levels (F1)
Yang et al. (2000)	Rhesus monkey/ Cynomolgus	Fed gelatin capsules (5 days/week for 12 months)	F	6 (treatment) 5 (controls)	0, 0.71, 3.57, or 17.86	17.86	None	Endometriosis effects	Increased endometrial implant survival, increased maximum and minimum implant diameters, growth regulatory cytokine dysregulation
Developmental toxicity studies									
Amin et al. (2000)	Rat/Harlan Sprague-Dawley	Corn oil gavage (GDs 10−16)	F (F0)	80−88 (F1)	0, 25, or 100	None	25	Developmental effects	Decreased preference in the consumption of 0.25% saccharin solution (F1)

Table 2-4. Animal bioassay studies selected for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Bell et al. (2007b)	Rat/CRL:WI (Han)	Maternal daily dietary exposure for an estimated 20 weeks (12 weeks prior to mating through parturition)	F (F0) M (F1)	65 (F0 treatments) 75 (F0 controls) at study initiation; following interim sacrifice ~30 animals were allowed to litter; F1 on PND 21 was ~7	0, 2.4, 8, or 46	None	2.4 (maternal exposure)	Reproductive and developmental effects	Delayed BPS (F1)
Franczak et al. (2006)	Rat/Sprague-Dawley	Maternal corn oil gavage (GDs 14 and 21; PNDs 7 and 14) Offspring corn oil gavage (weekly for 8 months)	F (F0 and F1)	2 or 3 (F0) 7 (F1)	0, 7.14, or 28.6	None	7.14	Developmental effects	Decreased serum estradiol levels (F1)
Developmental toxicity studies (continued)									
Hojo et al. (2002) and related Zareba et al. (2002)	Rat/Sprague-Dawley	Maternal single corn oil gavage (GD 8) Offspring exposed during gestation and lactation (35 days)	F (F0) F and M (F1)	12 (F0) 50 or 60 (F1)	0, 20, 60, or 180	None	20 (maternal exposure)	Developmental effects	Abrogation of sexually dimorphic neuro-behavioral responses (F1)

Table 2-4. Animal bioassay studies selected for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Kattainen et al. (2001)	Rat/ Han/Wistar and Long-Evans	Maternal single corn oil gavage (GD 15)	F (F0) F and M (F1)	4 to 8 (F0) 3F/3M per treatment group (F1)	0, 30, 100, 300, or 1,000	None	30 (maternal exposure)	Developmental effects	Reduced mesiodistal length of the lower third molar (F1)
Keller et al. (2008a; 2008b; 2007)	Mouse/ C57BL/6J, BALB/cByJ, A/J, CBA/J, C3H/HeJ, and C57BL/10J	Maternal single corn oil gavage (GD 13)	F (F0) F and M (F1a, b, c)	Dams not specified (F0); 23−36 (F1a); 4−5 (F1b); 107−110 (F1c)	0, 10, 100, or 1,000	None	10 (maternal exposure)	Developmental effects	Variation in M1 morphology in C57BL/10J males and females (F1a); decreased mandible shape and size in C3H/HeJ males (F1b); variation in molar shape in C3H/HeJ males (F1c) (2008a; 2008b; 2007)
Developmental toxicity studies (continued)									
Kuchiiwa et al. (2002)	Mouse/ddY	Maternal olive oil gavage (weekly for 8 weeks prior to mating)	F (F0) M (F1)	7 (F0) 3 (F1 immuno-cytochemical analysis) 6 (F1 cell number count)	0, 0.7, or 70	None	0.7 (LOEL) (maternal exposure)	Neurotoxicity	Decreased serotonin-immunoreactive neurons in raphe nuclei of male offspring (F1)
Li et al. (2006)	Mouse/NIH (pregnant and pseudo-pregnant)	Maternal sesame oil gavage daily for 8 days (GDs 1−8)	F	10	0, 2, 50, or 100	None	2	Developmental effects	Decreased progesterone and increased serum estradiol levels
Markowski et al. (2001)	Rat/Holtzman	Maternal single olive oil gavage (GD 18)	F (F0 and F1)	4−7 (F0 and F1)	0, 20, 60, or 180	None	20 (maternal exposure)	Behavioral effects	Decreased training responses (F1)

Table 2-4. Animal bioassay studies selected for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Miettinen et al. (2006)	Rat/Line C	Maternal single corn oil gavage (GD 15)	F (F0) F and M (F1)	24−32 (treatment) 12−48 (controls)	0, 30, 100, 300, or 1,000	None	30 (maternal exposure)	Developmental effects	Increase in dental caries (F1)
Nohara et al. (2000)	Rat/ Holtzman	Maternal single corn oil gavage (GD 15)	F (F0) M (F1)	Not specified (F0) 5 males and 3 females (F1)	0, 12.5, 50, 200, or 800	800 (maternal exposure)	None	Immunotoxicity	Decreased spleen cellularity (F1)
Ohsako et al. (2001)	Rat/ Holtzman	Maternal single corn oil gavage (GD 15)	F (F0) M (F1)	6 (F0) 5 males and 3 females (F1)	0, 12.5, 50, 200, or 800	12.5 (maternal exposure)	50 (maternal exposure)	Developmental effects	Decreased anogenital distance (F1)
Developmental toxicity studies (continued)									
Schantz et al. (1996)	Rat/Harlan Sprague-Dawley	Maternal corn oil gavage (GDs 10−16	F(F0)	~4 (F0); 80−88 (F1)	0, 25, or 100	None	None	Developmental effects	Facilitatory effect on radial arm maze learning (F1)
Seo et al. (1995)	Rat/Sprague-Dawley	Maternal corn oil gavage (GDs 10−16)	F and M (F1)	~15 (F0); 5−9 (F1)	0, 25, or 100	25	100	Developmental effects	Decreased thymus weight
Simanainen et al. (2004)	Rat/TCDD-resistant Han/Wistar bred with TCDD-sensitive Long-Evans	Maternal corn oil gavage (GDs 15)	F (F0) M (F1)	5−8 (F0)	0, 30, 100, 300, or 1,000	100	300	Reproductive effects	Reduction in daily sperm production and cauda epididymal sperm reserves
Sparschu et al. (1971)	Rat/Sprague-Dawley	Maternal corn oil gavage (GDs 6-15)	F (F0)	31 (controls) 10-14 (F0)	0, 30, 125, 500, 2,000, or 8,000	50	125	Maternal toxicity; Developmental effects	Decreased body weight in dams and male fetuses; fetal intestinal hemorrhage and subcutaneous edema

Table 2-4. Animal bioassay studies selected for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Smith et al. (1976)	Mouse/CF-1	Maternal corn oil gavage (GDs 6-15)	F (F0)	14-41 (F0)	0, 1.0, 10, 100, 1,000, or 3,000	1,000 (maternal) 100 (fetal)	3,000 (maternal) 1,000 (fetal)	Teratogenic and developmental effects	Increased relative liver weight (F0 dams); increased incidence of cleft palate (fetuses)
Developmental toxicity studies (continued)									
Sugita-Konishi et al. (2003)	Mouse/C57/6N Cji	Maternal drinking water exposure (daily for 17-day lactational period)	F (F0) F and M (F1)	8 (F0) Not specified (F1)	0, 1.14, or 11.3	1.14 (NOEL) (maternal exposure)	11.3 (LOEL) (maternal exposure)	Immunotoxicity	Increased susceptibility to *Listeria* (F1 males and females); increase in thymic CD4+ cells (F1 males); decreased spleen weight (F1 males)
Acute toxicity studies									
Burleson et al. (1996)	Mouse/B6C3F$_1$	Corn oil gavage (single exposure)	F	20	0, 1, 5, 10, 50, 100, or 6,000	5	10	Immunotoxicity	Increased mortality from influenza infection 7 days after a single TCDD exposure
Crofton et al. (2005)	Rat/Long-Evans	Corn oil gavage (4 consecutive days)	F	14, 6, 12, 6, 6, 6, 6, 6, and 4, respectively, in control and treated groups	0, 0.1, 3, 10, 30, 100, 300, 1,000, 3,000, or 10,000	30	100	Thyroid effects	Reduction in serum T4 levels
Kitchin and Woods (1979)	Rat/Sprague-Dawley	Corn oil gavage (single dose)	F	4 (treated); 9 (control)	0, 0.6, 2, 4, 20, 60, 200, 600, 2,000, 5,000, or 20,000	0.6 (NOEL)	2 (LOEL)	Enzyme induction	Increased benzo(a)pyrene hydroxylase (BPH)
Acute toxicity studies (continued)									
Li et al. (1997)	Rat/Sprague-Dawley	Corn oil dose via oral gastric intubation (single dose)	F	10	0, 3, 10, 30, 100, 300, 1,000, 3,000, 10,000, or 30,000	3	10	Hormonal effects	Increased serum FSH (1997)

Table 2-4. Animal bioassay studies selected for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Lucier et al. (1986)	Rat/Sprague-Dawley	Corn oil gavage or TCDD-contaminated soil (single dose)	F	6	0, 15, 40, 100, 200, 500, 1,000, 2,000, or 5,000 in corn oil 0, 15, 44, 100, 220, 500, 1,100, 2,000, or 5,500 in contaminated soil	None	15 (LOEL)	Enzyme induction	Induction of aryl hydrocarbon hydroxylase (at low dose in both treatment protocols)
Nohara et al. (2002)	Mouse/ $B6C3F_1$, BALB/c, C57BL/6N and DBA2	Corn oil gavage (single dose)	M, F	10−40	0, 5, 20, 100, or 500	500	None	Mortality and body-weight changes	No increased mortality of virus-infected mice or treatment-related changes in body weight
Simanainen et al. (2002)	Rat/TCDD-resistant Han/Wistar bred; TCDD-sensitive Long-Evans	Corn oil gavage (single dose)	M, F	9−11	30–100,000	100	300	General toxicological endpoints, organ weights, dental defects	Reduction in serum T4 levels
Acute toxicity studies (continued)									
Simanainen et al. (2003)	Rat/TCDD-resistant Han/Wistar bred with TCDD-sensitive Long-Evans	Corn oil gavage (single dose)	M, F	5−6	Line A: 30−3,000,000 Line B: 30−1,000,000 Line C: 30−100,000	100	300	General toxicological endpoints, organ weights, dental defects	Decreased thymus weight
Smialowicz et al. (2004)	Mouse/ C57BL/6N CYP1A2 (+/+) wild-type	Corn oil gavage (single dose)	F	Not specified	0, 30, 100, 300, 1,000, 3,000, or 10,000	300	1,000	Immunotoxicity	Decreased antibody response to SRBCs

Table 2-4. Animal bioassay studies selected for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Vanden Heuvel et al. (1994b)	Rat/Sprague-Dawley	Corn oil gavage (single dose)	F	5−15	0, 0.05, 0.1, 1, 10, 100, 1,000, or 10,000	0.1 (NOEL)	1 (LOEL)	Liver effects	Increase in hepatic EROD activity and CYP1A1 mRNA levels
Acute toxicity studies (continued)									
Weber et al. (1995)	Inbred Mouse/ C57BL/6	Corn oil gavage (single dose on Day 0) Sacrificed on Day 8	M	4-7	0, 30, 100, 300, 1,000, 3,000, 9,400, 37,500, 75,000, 100,000, 133,00, or 235,000	1,000	3,000	Hepatic and renal enzyme and hormone alterations; liver and kidney weight	Increased relative liver weight
	Inbred Mouse/ DBA/2	Corn oil gavage (two doses on Days -1 and 0) Sacrificed on Day 8	M	4-7	0, 1,000, 10,000, 97,500, 375,000, 1,500,000, 1,950,000, or 3,295,000	10,000	97,500		
Subchronic toxicity studies									
Chu et al. (2001)	Rat/Sprague-Dawley	Corn oil gavage (daily for 28 days)	F	5	0, 2.5, 25, 250, or 1,000	250	1,000	Body- and organ-weight changes	Decreased body weight, increased relative liver weight and related biochemical changes, decreased relative thymus weight
Chu et al. (2007)	Rat/Sprague-Dawley	Corn oil gavage (daily for 28 days)	F	5	0, 2.5, 25, 250, or 1,000	2.5	25	Liver effects	Alterations in thyroid, thymus, and liver histopathology

Table 2-4. Animal bioassay studies selected for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Subchronic toxicity studies (continued)									
DeCaprio et al. (1986)	Guinea pig/ Hartley	Daily dietary exposure (90 days)	M, F	10/sex	0, 0.12, 0.61, 4.9, or 26 (males); 0, 0.12, 0.68, 4.86, or 31 (females)	0.61	4.9	Body- and organ-weight changes	Decreased body weight (male and females); increased relative liver weights (males); decreased relative thymus weight (males)
DeVito et al. (1994)	Mice/B6C3F$_1$	Corn oil gavage (5 days/week for 13 weeks)	F	5	0, 1.07, 3.21, 10.7, 32.1, or 107	None	1.07 (LOEL)	Body- and organ-weight changes; enzyme induction	Increased EROD, ACOH and phosphotyrosyl proteins at all doses
Fattore et al. (2000)	Rat/Iva:SIV 50-Sprague-Dawley	Daily dietary exposure (13 weeks)	M, F	6	0, 20, 200, or 2,000	None	20	Liver effects	Reduced hepatic vitamin A levels
		Daily dietary exposure (13 weeks)	M, F	6	0 or 200				
		Daily dietary exposure (13 weeks)	M, F	6	0, 200, or 1,000				
		Daily dietary exposure (13 weeks, 26, and 39 weeks)	F	6	0 or 100				
Subchronic toxicity studies (continued)									
Fox et al. (1993)	Rat/Sprague-Dawley	Gavage loading/ maintenance doses (every 4 days for 14 days)	M, F	6	0, 0.55, 307, or 1,607	0.57	327	Body- and liver-weight changes; hepatic cell proliferation	Increased absolute and relative liver weight

Table 2-4. Animal bioassay studies selected for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Hassoun et al. (1998)	Mouse/ $B6C3F_1$	Corn oil gavage (5 days/week for 13 weeks)	F	Not specified	0, 0.32, 1.07, 10.7, or 107	None	0.32 (LOEL)	Brain effects	Induction of biomarkers of oxidative stress at all doses
Hassoun et al. (2000)	Rat/Harlan Sprague-Dawley	Corn oil gavage (5 days/week for 13 weeks)	F	6	0, 2.14, 7.14, 15.7, 32.9, or 71.4	None	2.14 (LOEL)	Liver and brain effects	Induction of biomarkers of oxidative stress at all doses in liver and brain
Hassoun et al. (2003)	Rat/Harlan Sprague-Dawley	Corn oil gavage (5 days/week for 13 weeks)	F	12	0, 7.14, 15.7, or 32.9	None	7.14 (LOEL)	Brain effects	Induction of biomarkers of oxidative stress at all doses
Subchronic toxicity studies (continued)									
Kociba et al. (1976)	Rat/Sprague-Dawley	Corn oil gavage (5 days/week for 13 weeks)	M, F	12	0, 0.71, 7.14, 71.4, or 714	7.14	71.4	Liver effects, body-weight changes, and hematologic and clinical effects	Reduced body weight and food consumption, slight liver degeneration, lymphoid depletion, increased urinary porphyrins and delta aminolevulinic acid, increased serum alkaline phosphatase and bilirubin
Mally and Chipman (2002)	Rat/F344	Corn oil gavage (2 days/week for 28 days)	F	3	0, 0.71, 7.14, or 71.4	None	0.71 (LOEL)	Clinical signs and histopathology	Decreased Cx32 plaque number and area in the liver
Slezak et al. (2000)	Mouse/ $B6C3F_1$	Corn oil gavage (5 days/week for 13 weeks)	F	Not specified	0, 0.11, 0.32, 1.07, 10.7, or 107.14	1.07 (NOEL)	10.7 (LOEL)	Liver, lung, kidney, and spleen effects	Increased hepatic superoxide anion

Table 2-4. Animal bioassay studies selected for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Smialowicz et al. (2008)	Mouse/ $B6C3F_1$	Corn oil gavage (5 days/week for 13 weeks)	F	8−15	0, 1.07, 10.7, 107, or 321	None	1.07	Immunotoxicity and organ weight	Reduced antibody response to SRBC, increased relative liver weight
Van Birgelen et al. (1995a; 1995b)	Rat/Sprague-Dawley	TCDD in diet (13 weeks)	F	8	0, 14, 26, 47, 320, or 1,024	None	14	Multiple end-points	Decreased absolute and relative thymus weights, decreased liver retinoid levels
Subchronic toxicity studies (continued)									
Vos et al. (1973)	Guinea pig/ Hartley	Corn oil gavage (weekly for 8 weeks)	F	10	0, 1.14, 5.71, 28.6, or 143	1.14	5.71	Immunotoxicity	Decreased total leukocytes and lymphocyte count, decreased absolute thymus and weight, increase in primary serum tetanus antitoxin
White et al. (1986)	Mouse/ $B6C3F_1$	Corn oil gavage (daily for 14 days)	F	6−8	0, 10, 50, 100, 500, 1,000, or 2,000	None	10	Immunotoxicity	Reduction of serum complement activity
Chronic toxicity studies									
Cantoni et al. (1981)	Rat/CD-COBS	Corn oil gavage (weekly for 45 weeks)	F	4	0, 1.43, 14.3, or 143	None	1.43	Hepatic porphyria	Increased urinary porphyrin excretion
Croutch et al. (2005)	Rat/Sprague-Dawley	Loading/ maintenance dose (every 3 days for different durations up to 128 days)	F	5	0, 0.85, 3.4, 13.6, 54.3, or 217 (28-day duration)	54.3 (28-day duration)	217 (28-day duration)	Body-weight changes and changes in PEPCK activity and IGF-I levels	Decreased body weight, decreased PEPCK activity, and reduced IGF-I levels

Table 2-4. Animal bioassay studies selected for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Hassoun et al. (2002)	Rat/Sprague-Dawley	Corn oil gavage (5 days/week for 30 weeks)	F	6	0, 2.14, 7.14, 15.7, 32.9, or 71.4	None	2.14 (LOEL)	Brain effects	Induction of biomarkers of oxidative stress at all doses
Chronic toxicity studies (continued)									
Hong et al. (1989)	Rhesus monkeys.	Daily dietary (4 years)	F	7-8	0, 0.12, or 0.67	None	None	Immunotoxic effects	None
Kociba et al. (1978)	Rat/Sprague-Dawley	Daily dietary exposure (2 years)	M, F	50	0, 1, 10, or 100	1	10	Multiple endpoints measured	Increased urinary porphyrins, hepatocellular nodules, and focal alveolar hyperplasia
Maronpot et al. (1993)	Rat/Sprague-Dawley	Biweekly gavage (30 weeks)	F	9	0, 3.5, 10.7, 35, or 125	10.7	35	Body- and organ-weight changes, clinical chemistry, hepatocellular proliferation	Increased relative liver weight
NTP (1982c)	Mouse/ $B6C3F_1$; Rat/Osborne Mendel	Corn oil gavage (2 days/week for 104 weeks)	M, F	50	0, 1.4, 7.1, or 71 for rats and male mice; 0, 5.7, 28.6, or 286 for female mice	None	1.4	Liver and body-weight changes	Increased incidences of liver lesions in mice (males and females)
NTP (2006a)	Rat/Sprague-Dawley	Corn oil gavage (5 days/week for 105 weeks)	F	53	0, 2.14, 7.14, 15.7, 32.9, or 71.4	None	2.14	Liver and lung effects	Increased absolute and relative liver weights, increased incidence of hepatocellular hypertrophy, increased incidence of alveolar to bronchiolar epithelial metaplasia

Table 2-4. Animal bioassay studies selected for noncancer dose-response modeling (continued)

Reference	Species/ strain	Exposure protocol	Sex (exposure group)	*n*	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)
Chronic toxicity studies (continued)									
Sewall et al. (1993)	Rat/Sprague-Dawley	Biweekly gavage (30 weeks)	F	9	0, 3.5, 10.7, 35, or 125	None	3.5 (LOEL)	EGFR kinetics and auto-phosphorylation, hepatocellular proliferation	Decrease in EGFR maximum binding capacity
Sewall et al. (1995)	Rat/Sprague-Dawley	Biweekly gavage (30 weeks)	F	9	0, 0.1, 0.35, 1, 3.5, 10.7, 35, or 125	10.7	35	Thyroid function	Decreased serum T^4 levels
Toth et al. (1979)	Mouse/Swiss/ H/Riop	Sunflower oil gavage (weekly for 1 year)	M	38−44	0, 1, 100, or 1,000	None	1	Skin effects	Dermal amyloidosis and skin lesions
Tritscher et al. (1992)	Rat/Sprague-Dawley	Initiated with i.p. injection of diethylnitrosamine (175 mg/kg) or saline, followed 2 weeks later by biweekly TCDD in corn oil gavage (30 weeks)	F	At least 9 per group	3.5, 10.7, 35.7, or 125	None	None	CYP induction	None

ND = not determined; ACOH = acetanilide-4-hydroxylase; BPS = balanopreputial separation; EGFR = epidermal growth factor receptor; EROD = 7-ethoxyresorufin-O-deethylase; FSH = follicle stimulating hormone; IGF = insulin-like growth factor; i.p. = intraperitoneal; PEPCK = phosphoenolpyruvate carboxykinase; PND = postnatal day.

2.4.1. Key Epidemiologic Data Sets

The studies listed in Tables 2-1 and 2-2, for cancer and noncancer, respectively, are those studies that have met the epidemiologic TCDD study inclusion criteria (see Section 2.3.1). Summaries for all of the epidemiologic studies evaluated are also provided in Appendix C and are organized by epidemiologic cohort. Following a brief summary of each cohort, its associated studies are then summarized chronologically, assessed for methodological considerations relative to epidemiologic cohorts and studies, and evaluated for suitability for TCDD dose-response assessment. Further, Appendix C presents explicit details regarding whether the considerations and criteria were met (see summary Tables C-2 and C-3, followed by Tables C-4 though C-57, which provide details for each study).

The cancer epidemiologic studies on TCDD that were subjected to the study selection process include 24 peer-reviewed publications from 8 cohorts. An evaluation of these against EPA's study inclusion criteria resulted in selecting 8 studies from the NIOSH, Boehringer, BASF, Ranch Hand, and Seveso cohorts for further consideration in TCDD quantitative cancer dose-response assessment (see Table 2-1). All of these studies had serum TCDD measurements on individual study participants, used kinetic models to refine exposure estimates, and accounted for latency or appropriate exposure windows in their analyses. As shown in Figure 2-4, most of the other studies were excluded because exposures were not primarily to TCDD and not quantifiable on an individual level; many studies also failed to provide information on an appropriate latency period or window of exposure for cancer (see Table C-2). In addition, two studies (Steenland et al., 1999; Flesch-Janys et al., 1998) passed all criteria but were not selected because they were superseded by other studies on the same cohort for which an updated analysis was done [i.e., Steenland et al. (2001) and Becher et al. (1998), respectively]. The Baccarelli et al. (2006) study also passed all of the criteria but was not selected because of an issue identified during evaluation of the study considerations (i.e., lack of an obvious adverse health endpoint). The noncancer epidemiologic studies (see Table C-3) on TCDD that were subjected to the study selection process include 32 peer-reviewed publications from 10 cohorts. An evaluation of these against EPA's study inclusion criteria resulted in selecting four studies from the Seveso cohort for further consideration in TCDD quantitative noncancer dose-response assessment (see Table 2-2). The 4 Seveso cohort studies passed all criteria primarily because TCDD serum levels were available for individuals in the studies, and the critical windows of

exposure were identifiable for the endpoints that served as PODs [e.g., the 9 months of pregnancy for exposed mothers clearly defined the window of exposure for the fetus in Baccarelli et al. (2008)]. As shown in Figure 2-4, many of the excluded studies failed to provide enough information on expected latency for the nonfatal endpoints or failed to provide data on the critical period of exposure to quantitatively estimate an oral human dose. A number of studies also had exposures that were not primarily to TCDD. One study, Baccarelli et al. (2005), passed all criteria but was excluded because the health endpoint, chloracne, is considered to be an outcome associated with high TCDD exposures; thus this study was not considered further in RfD derivation. The Warner et al. (2004) study also passed all criteria but was not selected because EPA could not assess the biological significance of this finding and could not establish a LOAEL for this effect (i.e., it did not satisfy one of the study considerations).

2.4.2. Key Animal Bioassay Data Sets

The studies listed in Tables 2-3 and 2-4, for cancer and noncancer, respectively, are those studies that have met the in vivo animal bioassay TCDD study inclusion criteria (see Section 2.3.2 and Figure 2-3). Appendix D provides study summaries, is organized by reproductive studies, developmental studies, and general toxicity studies (subdivided by duration), and summarizes the experimental protocol, the results, and the NOAELs and LOAELs EPA has identified for each study. The doses shown in Tables 2-3 and 2-4 are expressed as average daily administered intakes in units of nanograms per kilogram body weight per day (ng/kg-day), adjusted for continuous exposure when necessary.[22] Tables D-1 and D-2 present the results of the study selection evaluations for the studies that met and did not meet the study inclusion criteria, respectively.

A total of eight animal cancer bioassays were available for evaluation using EPA's study inclusion criteria (see Section 2.3.2 and Figure 2-3). Table 2-3 presents the 6 studies that met these criteria and are considered suitable for quantitative TCDD dose-response modeling. As shown in Figure 2-4, only 2 of the available cancer bioassays did not meet EPA's study inclusion criteria (and are not summarized in Appendix D). These include Eastin et al. (1998) (genetically

[22] Standard EPA guidance was applied for adjustment of intermittent gavage protocols and dietary exposures as indicated in each specific study description in Appendix D.

altered mouse strain) and Rao et al. (1988) (intraperitoneal injection instead of oral route of exposure).

A total of 751 animal bioassays on a noncancer endpoint were available for evaluation using EPA's study inclusion criteria (see Section 2.3.2 and Figure 2-3). As shown in Figure 2-4, 673 of the available noncancer studies were excluded based on one or more of the following reasons: (1) 66 studies used genetically-altered animals; (2) 370 studies had a lowest tested dose that was too high (i.e., greater than 30 ng/kg-day); (3) 142 studies tested chemicals that were not TCDD only or used an unspecified TCDD dose; and (4) 135 studies did not use an oral dosing method. Table D-2 of Appendix D shows these studies and identifies the study inclusion criteria that were not met. For many studies, more than one reason for exclusion was found and identified. Conversely, in some cases, at least one identified criterion was not met, and, given the study was then excluded based on that one criterion, not all of the other criteria for exclusion were further evaluated and articulated. Tables 2-4 and D-1 of Appendix D present the 78 studies that were selected as key data sets for TCDD noncancer dose-response analyses.

In Section 4, additional evaluations are made to determine which study/endpoint data sets are the most appropriate for development of the RfD for TCDD. For further consideration in the RfD derivation process, only the toxicologically-relevant endpoints from the studies in Table 2-4 are carried forward to Section 4 (see Section 4.2.1 and Appendix H for details on study/endpoint combinations not used in RfD derivation for this reason). For some entries in Table 2-4, there are several publications from the peer-reviewed literature shown in the same row of the table. In these cases, the publications are grouped together because they are based on the same noncancer animal bioassay. Additionally, in Table 2-4, the noncancer adverse effects in the animal studies listed under the heading, "endpoints examined," are presented as general categories of effects, such as "developmental effects," "liver effects," or "thyroid function." In Section 4, more detailed descriptors of the specific endpoints associated with such adverse health effects are articulated and evaluated to develop PODs for the derivation of an oral RfD for TCDD. Final candidate study/endpoint data sets are selected in Section 4 based on factors such as toxicological relevance of the endpoints (see Section 4.2.1 and Appendix H), dose-response modeling results, and POD comparisons across studies, as illustrated in Figures 4-1 and 4-3 for epidemiologic and toxicological data, respectively.

3. THE USE OF TOXICOKINETICS IN THE DOSE-RESPONSE MODELING FOR CANCER AND NONCANCER ENDPOINTS

A key recommendation from the NAS for improving the 2003 Reassessment was that EPA should justify its approaches to dose-response modeling for cancer and noncancer endpoints. Further, the NAS suggested that EPA incorporate the most up-to-date and relevant state of the science for the TCDD dose-response assessment.

While EPA believes that at the time of its release, the 2003 Reassessment offered a substantial improvement over the general state-of-the-science regarding dose-response modeling, EPA agrees with the NAS that the justification of the approaches to dose-response modeling can be improved and the methodologies updated to reflect the most current EPA guidance (see Text Box 2-1) and science. In Section 3, EPA describes the use of toxicokinetic (TK)[23] information in the dose-response modeling of TCDD. Section 3.1 summarizes the NAS comments regarding the use of TK in the dose-response approaches for TCDD. Section 3.2 overviews EPA's responses to the NAS comments. Section 3.3 discusses TCDD kinetics, including TK models developed to simulate disposition of this compound in rodents and humans (see Section 3.3.4), alternative measures of dose that could be used in a TCDD dose-response analysis (see Section 3.3.4), and uncertainties in the TCDD dose estimates (see Section 3.3.5). Section 4 of this document incorporates the TK information into noncancer dose-response modeling.

3.1. SUMMARY OF NAS COMMENTS ON THE USE OF TOXICOKINETICS IN DOSE-RESPONSE MODELING APPROACHES FOR TCDD

The NAS commented on the appropriate use of TK models in dose-response modeling for TCDD. Specifically, the committee requested that EPA consider using such models to provide refined estimates of dose, for example, as the underlying science and predictive capabilities of these models improved.

> [Discussing Kinetic models]…the committee encourages further development and use of these models as data become available to validate and further develop them (NAS, 2006b, p. 59).

[23] Toxicokinetics (TK) is the branch of the pharmacokinetics (PK) that examines the disposition of toxins and toxicants.

Although the NAS agreed with EPA's use of body burden as a dose metric in the 2003 Reassessment (e.g., see NAS, 2006b, p. 7), the NAS was concerned about the limitations of first-order kinetic models, such as the one used in the 2003 Reassessment, to estimate TCDD body burdens.

> TCDD, other dioxins, and DLCs act as potent inducers of cytochrome P450 (CYP), a property that can affect both the hepatic sequestration of these compounds and their half-lives. Hepatic sequestration of dioxin may influence the quantitative extrapolation of the rodent liver tumor results because the body-burden distribution pattern in highly dosed rats would differ from the corresponding distribution in humans subject to background levels of exposure. EPA should consider the possible quantitative influence of dose-dependent toxicokinetics on the interpretation of animal toxicological data (NAS, 2006b, p. 129).

The NAS also asked EPA to evaluate the impact of kinetic uncertainty and variability on dose-response assessment. The NAS committee asked EPA to use TK models to examine both interspecies and human interindividual differences in the disposition of TCDD, which would better justify EPA dose-response modeling choices.

> The Reassessment does not adequately consider the use of a PBPK model to define species differences in tissue distribution in relation to total body burden for either cancer or noncancer end points (NAS, 2006b, p. 62).
>
> EPA …should consider physiologically based pharmacokinetic modeling as a means to adjust for differences in body fat composition and for other differences between rodents and humans (NAS, 2006b, p. 10).
>
> The Reassessment does not provide details about the magnitudes of the various uncertainties surrounding the decisions EPA makes in relation to dose metrics (e.g., the impact of species differences in percentage of body fat on the steady-state concentrations present in nonadipose tissues). The committee recommends that EPA use simple PBPK models to define the magnitude of any differences between humans and rodents in the relationship between total body burden at steady-state concentrations (as calculated from the intake, half-life, bioavailability) and tissue concentrations. The same model could be used to explore human variability in kinetics in relation to elimination half-life. EPA should modify the estimated human equivalent intakes when necessary (NAS, 2006b, p.73).

Finally, the NAS asked EPA to use TK considerations to better justify its choice of dose metric.

> EPA makes a number of assumptions about the appropriate dose metric and mathematical functions to use in the Reassessment's dose-response analysis but does not adequately comment on the extent to which each of these assumptions could affect the resulting risk estimates…EPA did not quantitatively describe how this particular selection affected its estimates of exposure and therefore provided no overall quantitative perspective on the relative importance of the selection (NAS, 2006b, p. 51).

3.2. OVERVIEW OF EPA'S RESPONSE TO THE NAS COMMENTS ON THE USE OF TOXICOKINETICS IN DOSE-RESPONSE MODELING APPROACHES FOR TCDD

In response to the NAS recommendations regarding TCDD kinetics and choice of dose metrics, this document presents an in-depth evaluation of TCDD TK models, exploring their differences and commonalities and their possible application for the derivation of dose metrics relevant to TCDD. Initially, EPA discusses the application of first-order kinetics to estimate body burden as a dose metric for TCDD. This first-order kinetic model is used to predict TCDD body burden for all of the studies identified as Key Studies (see Section 2.4); this model uses a constant half-life to simulate the elimination of TCDD from the body. However, given the observed data indicating early influence of cytochrome P450 1A2 (CYP1A2) induction and binding to TCDD in the liver and later redistribution of TCDD to fat tissue, the use of a constant half-life for TCDD clearance following long-term or chronic TCDD exposure is not biologically supported. Therefore, using half-life estimates based on observed terminal steady state levels of TCDD will not account for the possibility of an accelerated dose-dependent clearance of this chemical during early stages following elevated TCDD exposures. The biological processes leading to dose-dependent TCDD excretion are better described using PBPK models than by simple first-order kinetic models. Additionally, as part of its preparation for developing this document, EPA evaluated recent TCDD kinetic studies as NAS advocated. Although the NAS agreed with continued use of body burden metric as the dose metric of choice, EPA believes that the state-of-the-practice has advanced sufficiently to justify the consideration of alternative dose metrics (other than administered dose) based on an application of a physiologically based TK model.

EPA identified a number of advances in the overall scientific understanding of TCDD disposition; many of these are documented in a summary discussion introducing the section on TCDD kinetics (see Section 3.3). The increased understanding warranted an evaluation of current kinetic modeling of TCDD to determine if the use of such models would improve the dose-response assessment for TCDD. Justification of the final PBPK model choice is detailed in Section 3.3. Through the choice of a published PBPK model to estimate dose metrics for dioxin, EPA has addressed several of the NAS concerns. The PBPK model can be applied to estimate dose metrics other than body burden that may be more directly related to response, e.g., tissue levels, serum levels, blood concentrations, or dose metrics related to TCDD-protein receptor binding. The selected PBPK model included an explicit description of physiological and biochemical parameters; therefore, it can also provide an excellent tool for investigating differences in species uptake and disposition of TCDD. One of the criteria used to select a PBPK model for TCDD kinetics was the availability of both human and animal models so that differences in species uptake and disposition of TCDD can be investigated. Additionally, the PBPK model includes quantitative information that is suitable for addressing the impact of physiological (e.g., body weight [BW] or fat tissue volume), or biochemical (e.g., induction of CYP1A2) variability on overall risk of TCDD between species, in response to another area of concern in the NAS report. The sensitivity analysis and uncertainty in dose metrics derived for the health assessment of TCDD are also presented in Section 3.3. A detailed discussion on the uncertainty in choice of PBPK model-driven dose metrics is also provided in Section 3.3.

3.3. PHARMACOKINETICS (PK) AND PK MODELING

3.3.1. Pharmacokinetics (PK) Data and Models in TCDD Dose-Response Modeling: Overview and Scope

In general, the use of measures of internal dose in dose-response modeling is considered to be superior to that of administered dose (or uptake) because the former is more closely related to the response. The evaluation of internal dose, or dose metric, in exposed humans and other animals is facilitated by an understanding of pharmacokinetics (i.e., absorption, distribution, metabolism, and excretion). When measurements of internal dose (e.g., blood concentration, tissue concentration) are not available in animals and humans, pharmacokinetic models can be used to estimate them. The available data on the pharmacokinetics of TCDD in animals and

humans have been reviewed (NAS, 2006b; U.S. EPA, 2003; van Birgelen and van den Berg, 2000).

It is evident based on these reviews and other analyses that three distinctive features of TCDD play important roles in determining its pharmacokinetic behavior, as discussed below:

- **TCDD is very highly lipophilic** and thus is more soluble in fat or other relatively nonpolar organic media than in water. The *n*-octanol/water partition coefficient is a commonly used measure of lipophilicity equal to the equilibrium ratio of a substance's concentration in *n*−octanol (a surrogate for biotic lipid) to the substance's concentration in water (Leo et al., 1971). For TCDD, this coefficient is on the order of 10,000,000 or more (ATSDR, 1998). It follows that the solubility of TCDD in the body's lipid fraction, i.e., the fatty portions of various tissues, including adipose, organs, and blood, is extremely high.
- **TCDD is very slowly metabolized** compared to many other organic compounds, with an elimination half-life in humans on the order of years following an initial period of distribution in the body (Michalek and Pavuk, 2008; Carrier et al., 1995a). Most laboratory animals used for toxicological testing tend to eliminate TCDD much more quickly than humans, although even in animals, TCDD is eliminated much more slowly than most other chemicals.
- **TCDD induces binding proteins in the liver** that have the effect of sequestering some of the TCDD. The ability of TCDD to alter gene expression and the demonstration that the induction of CYP1A2 is responsible for hepatic TCDD sequestration suggest that both pharmacokinetic and pharmacodynamic events must be incorporated for a quantitative description of TCDD disposition (Santostefano et al., 1998). The induction of these proteins implies that TCDD tends to be eliminated more rapidly in the early years following short-term, high-level exposures than it is after those initial levels have declined. Leung et al. (1988) and Andersen et al. (1993), in their PBPK modeling, have taken into consideration the issue of liver protein binding. Recent efforts of pharmacokinetic modeling have supported the concentration-dependent elimination of TCDD in animals and humans (Emond et al., 2006; Aylward et al., 2005b).

Sections 3.3.2 and 3.3.3 present the salient features of TCDD pharmacokinetics in animals and humans, respectively, with particular focus on mechanisms and data of relevance to interspecies and intraspecies variability. Section 3.3.4 describes the various dose metrics for the dose-response modeling of TCDD and the characteristics of pharmacokinetic models potentially useful for estimating these metrics. Finally, Sections 3.3.5 and 3.3.6 summarize uncertainty in the dose estimate and the application of pharmacokinetic models associated with the predictions of dose metrics used in dose-response modeling, respectively. Dose metrics derived via PBPK

modeling approaches are utilized in Section 4 of this document for noncancer TCDD dose-response modeling.

3.3.2. Pharmacokinetics (PK) of TCDD in Animals and Humans

3.3.2.1. *Absorption and Bioavailability*

When administered via the oral route in the dissolved form, TCDD appears to be well absorbed. Animal studies indicate that oral exposure to TCDD in the diet or in an oil vehicle results in the absorption of >50% of the administered dose (Olson et al., 1980; Nolan et al., 1979). Human data from Poiger and Schlatter (1986) indicate that >87% of the oral dose (after ingestion of 105 ng [^{3}H]−2,3,7,8−TCDD [1.14 ng/kg BW] in 6 mL corn oil) was absorbed from the gastrointestinal tract. Lakshmanan et al. (1986), investigating the oral absorption of TCDD, suggested that it is absorbed primarily by the lymphatic route and transported predominantly by chylomicrons.

Oral absorption is generally less efficient when TCDD is more tightly bound in soil matrices. Based on experiments in miniature swine, Wittsiepe et al. (2007) reported an approximately 70% reduction in bioavailability when TCDD was administered in the form of contaminated soil, relative to TCDD after extraction from the same soil matrix with solvents. Working with soil from the prominent contamination site at Times Beach, Missouri, Shu et al. (1988) reported an oral bioavailability of approximately 43% based on experiments in rats. Percent dose absorbed by the dermal route is reported to be less than the oral route, whereas absorption of TCDD by the transpulmonary route appears to be efficient (Banks and Birnbaum, 1991) (see for example; Roy et al., 2008; U.S. EPA, 2003; Diliberto et al., 1996; Nessel et al., 1992; Banks et al., 1990).

3.3.2.2. *Distribution*

TCDD in systemic circulation equilibrates and partitions into the tissues where it is then accumulated, bound, or eliminated. Whereas the bulk of the body tissues are expected to equilibrate in a matter of hours, the adipose tissue will approach equilibrium concentrations with blood much more slowly. Consistent with these assertions, a number of experimental and modeling studies in rats and humans have shown that TCDD has a large volume of distribution (Vd), i.e., the apparent volume in which it is distributed. The Vd corresponds to the volume of

blood plus the product of internal tissue volumes and the corresponding tissue:blood partition coefficients. This parameter is a key determinant of the elimination rate of TCDD in exposed organisms. The tissue:blood partition coefficients of TCDD, in turn, are determined by the relative solubility of TCDD in tissue and blood components (including neutral lipids, phospholipids, and water).

Column 2 in Table 3-1 presents the tissue:blood partition coefficients for TCDD (Emond et al., 2005; Wang et al., 1997). Column 3 of this table lists the physical volume of each tissue, scaled to a person weighing 60 kg. The last column shows the implications of the tissue volumes and tissue:blood partition coefficients for the effective volumes of distribution for each tissue and for the body as a whole. It can be seen that, purely on the basis of solubility space, the fat should be expected to contain about 94% of the TCDD in the body, and that the body as a whole behaves as if it is about 1,200 L in terms of blood-equivalents (i.e., approximately 22−fold larger than its physical volume).

Table 3-1. Partition coefficients, tissue volumes, and volume of distribution for TCDD in humans

Tissue	Tissue:blood partition coefficient	Tissue volume (liters, for a 60-kg person)	Effective volume of distribution (Vd—liters of blood equivalent)	Percent total Vd
Blood	1	3	3	0.25
Fat	100	11.4	1.140	94.19
Liver	6	1.56	9	0.77
Rest of the body	1.5	38.64	58	4.79
	Total	**54.6**[a]	**1.210**	**100.00**

[a]The total tissue volume presented here represents only 91% of body weight because some of the weight and volume of the body is occupied by bone and other structures where TCDD uptake and accumulation do not occur to a significant extent.

Source: Wang et al. (1997), Emond et al. (2006; 2005).

Maruyama et al. (2002) have published another set of tissue:blood partition coefficients for TCDD and other dioxin congeners based in part on observations of tissue concentrations measured in autopsy specimens from eight Japanese people without known unusual exposures to TCDD. Their estimates of TCDD partition coefficients seem to be rather large and variable,

with a fat:blood value of 247 ± 78 (standard deviation [SD]), a liver:blood value of 9.8 ± 5.7, and a muscle:blood value of 18 ± 10.6. Depending on time of autopsy, tissue samples may not be an accurate source of information on observed, in vivo partition coefficients because weight loss is likely to occur pre and post mortem. In particular, a decline in the fat stores volume could lead to an increased concentration of dioxin in fat in autopsy specimens relative to what would be observed in vivo.

The calculations shown in Table 3-1 do not include the additional amount that will be bound to induced proteins in the liver. That induction and binding will tend to increase the contribution of the liver on the effective volume of distribution (Birnbaum, 1986).

It is also of interest to point out some basic implications of the data in Table 3-1 for the expected rates of perfusion-mediated transfer of TCDD between blood and each of the organ/tissues. The rate of loss from a tissue (occurring primarily via blood flow) and the corresponding half-life can be calculated using the following equations:

$$\text{Rate constant for loss (hour}^{-1}) = \frac{\text{Blood flow (liters / hour)}}{\text{Tissue volume (liters)} \times \text{Tissue / Blood Partition Coefficent}} \quad \text{(Eq. 3-1)}$$

$$t_{1/2} \text{ for tissue perfusion loss} = \frac{\ln(2)}{\text{Rate constant for loss}}$$

$$= \frac{\ln(2) \times \text{Tissue volume (liters)} \times \text{Tissue/Blood Partition Coefficent}}{\text{Blood flow (liters/hour)}} \quad \text{(Eq. 3-2)}$$

Because TCDD is highly lipophilic, its concentration in the aqueous portion of the blood is very small, and TCDD tends to partition from blood components into cellular membranes and tissues, probably in large part via diffusion. As a result, full equilibrium concentrations of TCDD are not attained by the end of the transit time through organs from the arterial to venous blood. For organs in which this occurs, diffusion coefficients or ―permeability factors" have been estimated to assess the fractional attainment of equilibrium concentration that occurs by the time the blood leaving each organ reaches the venous circulation. Table 3-2 presents the permeability factors and implications for perfusion half-lives for TCDD, per Emond et al. (2006; 2005).

Table 3-2. Blood flows, permeability factors, and resulting half lives (t½) for perfusion losses for humans as represented by the TCDD PBPK model of Emond et al. (2006; 2005)

Tissue	Permeability (fraction of compartment blood flow)	Rate constant for compartmental elimination (hour−1)	t½ (hrs)
Fat	0.12	0.0049	143
Liver	0.03	0.77	0.90
Rest of the body	0.35	3.84	0.18

Despite the high lipid bioconcentration potential of TCDD, the adipose tissue does not always have the highest concentration (Abraham et al., 1988; Geyer et al., 1986; Poiger and Schlatter, 1986). Further, the ratios of tissue:tissue concentrations of TCDD and related compounds (e.g., the liver:adipose ratio) may not remain constant during nonsteady-state conditions. TCDD concentrations have been observed to decrease more rapidly in the liver than in adipose tissue. For example, Abraham et al. (1988) found that the liver:adipose tissue concentration ratio in female Wistar rats exposed to a subcutaneous TCDD dose of 300 ng/kg decreased from 10.3 at 1 day postexposure to 0.5 at 91 days postexposure. It should be noted that even at a ratio of 0.5, the amount of TCDD in the liver is greater than that based on lipid content of the tissue alone, consistent with the presence of hepatic TCDD-binding proteins. The liver:adipose tissue concentration ratio also was dose-dependent, such that the liver TCDD burden increased from ~11% of the administered dose at low doses (i.e., 1−10 ng/kg) to ~37% of the dose at an exposure level of 300 ng/kg. The increase in TCDD levels in liver, accompanied by a decrease in concentration in the adipose tissue, is a particular behavior to be considered in high-dose to low-dose extrapolations. This behavior is essentially a result of dose-dependent hepatic processes, as described below.

3.3.2.3. *Metabolism and Protein Binding*

The metabolism of TCDD is slow, particularly in humans, and it is thought to be mediated by the CYP1A2 enzyme that is inducible by TCDD (Weber et al., 1997; Olson et al., 1994; Wendling et al., 1990; Ramsey et al., 1982). The low rate of metabolism in combination with sequestration appear to account for the retention of TCDD in liver, and these processes collectively contribute to the long half-life for elimination of TCDD from the body.

Dynamic changes in TCDD binding in liver and partitioning to adipose tissues have been studied extensively in rats and mice (Diliberto et al., 2001; Diliberto et al., 1995). Figure 3-1 shows observations by Diliberto et al. (1995) of the ratio of liver concentrations to adipose tissue concentrations for mice given doses spread over a 100−fold range and studied at four different times following exposure. It can be seen that even for the lowest dose studied, the liver:adipose concentration ratio is higher than would be expected based on the lipid contents of the tissues (i.e., 6:100, corresponding to the ratio of human liver:blood and adipose:blood partition coefficients; see Table 3-1). Moreover, the relative concentration in the liver consistently rises with dose, with the steepest rise observed during the first 2 weeks after dosing. If the distribution of TCDD were governed solely by passive partitioning into adipose, there should be no such change in relative concentrations with dose. However, data presented in Figure 3-1 illustrate that at longer time points, the ratio of TCDD in the liver to TCDD in adipose decreases, indicating that a redistribution of the chemical occurs as time goes on for each applied dose. The redistribution of TCDD tissue levels from liver to adipose with increasing time suggests that binding of the chemical in the liver (including via induction of CYP1A2) is an important kinetic consideration at early exposure points with relatively high applied doses.

Experiments with CYP1A2 ―knock-out" mice (i.e., congenic strains differing in only a single gene that is ―knocked out" in one of the strains) indicate that the inducible binding of TCDD is attributable to CYP1A2 (Diliberto et al., 1999, 1997). As noted previously, this enzyme is believed to make an important contribution to metabolism of TCDD. Given the critical role of CYP1A2 induction in the kinetics of TCDD, dose-and time-dependent induction of this protein in rats has been examined and modeled (Emond et al., 2006, 2004; Santostefano et al., 1998; Wang et al., 1997). Accordingly, the amount of CYP1A2 in the liver can be computed as the time-integrated product of inducible production and a simple first-order loss process (Wang et al., 1997):

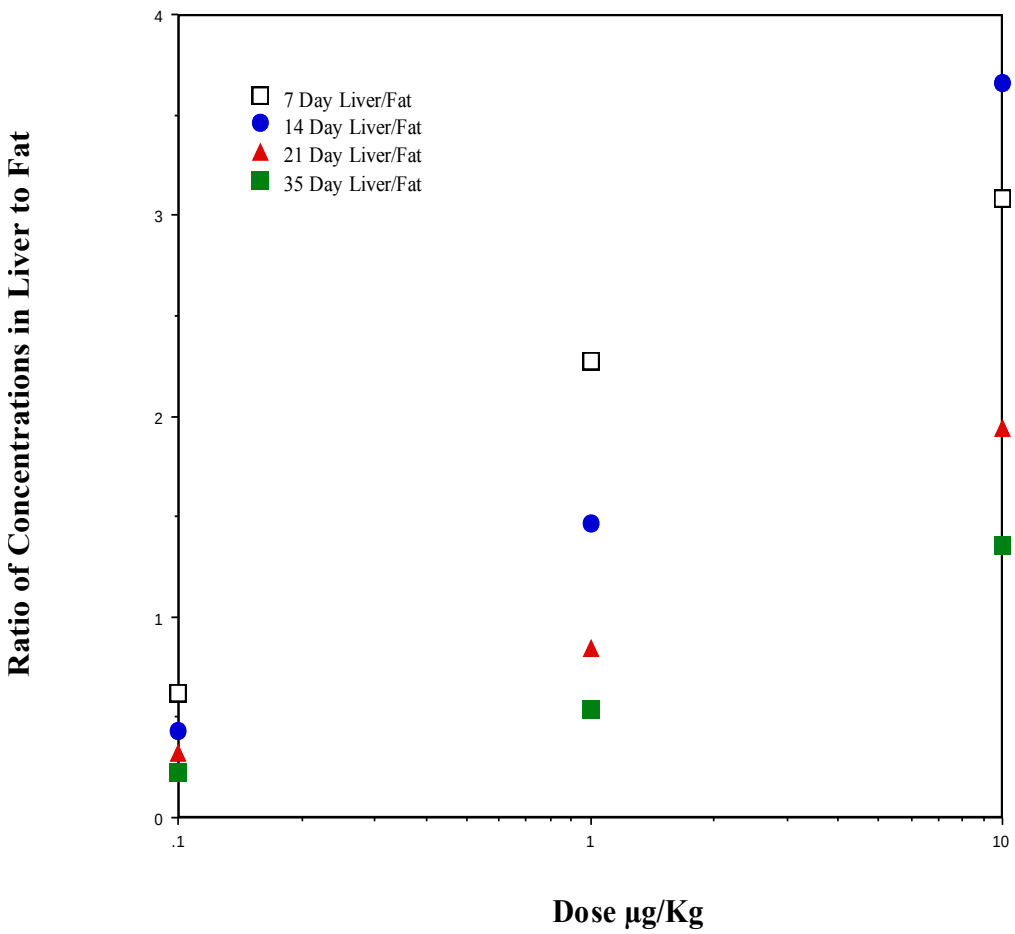


Figure 3-1. Liver/fat concentration ratios in relation to TCDD dose at various times after oral administration of TCDD to mice.

Source: Dilberto et al. (1995).

$$\frac{dCYP_{2A1}}{dt} = S(t)K_0 - K_2 C_{A2t} \qquad \text{(Eq. 3-3)}$$

where CYP_{2A1} is the concentration of the enzyme, K_2 is the rate constant for the first-order loss, C_{A2t} is the concentration of CYP1A2 in the liver, K_0 is the basal rate of production of CYP1A2 in the liver, and $S(t)$ is a multiplicative stimulation factor for CYP1A2 production in the form of a Hill-type function:

$$S(t) = 1 + \frac{In_{A2}(C_{Ah\text{-}TCDD})^h}{(IC_{A2})^h + (C_{Ah\text{-}TCDD})^h} \qquad \text{(Eq. 3-4)}$$

where IC_{A2} corresponds to the concentration of the aryl hydrocarbon (Ah)-TCDD complex at which half of the maximum fold stimulation of CYP2A production is reached, and h, the Hill exponent, determines the curvature of the stimulation in relation to concentration of the Ah-TCDD complex at relatively low doses. A value of 0.6 as the Hill exponent has been used by Wang et al. (2000; 1997) and Emond et al. (2006; 2005; 2004), indicative of a negative cooperation, i.e., the curve is convex-upward (supralinear), depicting a faster increase in the low-dose region compared to a straight line. Additional parameters in this expression include In_{A2}, the maximum fold increase in the CYP1A2 synthesis rate over the basal rate that can occur at high levels of TCDD, and ($C_{Ah\text{-}TCDD}$), the concentration of TCDD bound to the aryl hydrocarbon receptor (AhR). This concentration in turn depends on the concentration of TCDD in the liver (C_{Lif}), the concentration of the AhR (Ah_{Li}) in liver, and the dissociation constant for the Ah-TCDD receptor complex, K_{DAh}:

$$C_{Ah\text{-}TCDD} = \frac{Ah_{Li} \times C_{Lif}}{K_{DAh} + C_{Lif}} \qquad \text{(Eq. 3-5)}$$

3.3.2.4. *Elimination*

Estimated elimination half-lives (i.e., the time taken for the concentration to be reduced to one-half of its initial level) of TCDD range from 11 days in the hamster to 2,120 days in humans (U.S. EPA, 2003). Hepatic metabolism and binding processes, fecal excretion, and accumulation in adipose tissue collectively determine the dose-dependent elimination half-lives in various species. Aylward et al. (2005a) depicted the relationship between the elimination rate versus initial level of lipid-corrected TCDD in serum for 36 people (see Figure 3-2). Even though this analysis was done using the initial TCDD level, rather than the geometric mean or midpoint level in the decline for each person, it indicated a concentration-dependency of the half-life and elimination of TCDD in exposed individuals.

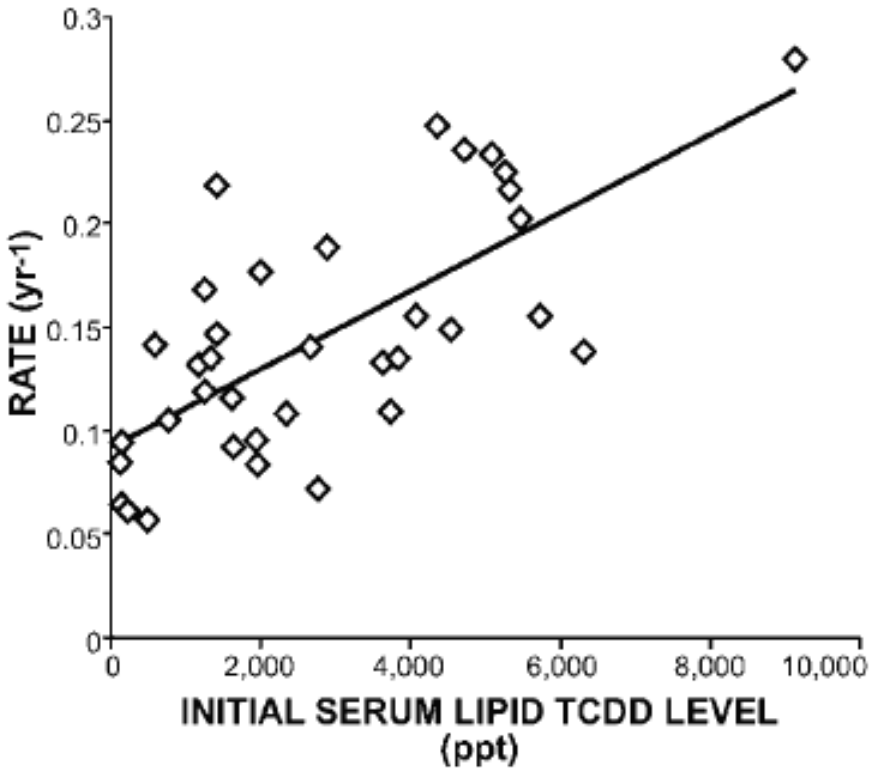


Figure 3-2. First-order elimination rate fits to 36 sets of serial TCDD sampling data from Seveso patients as function of initial serum lipid TCDD.

Source: Aylward et al. (2005b).

3.3.2.5. *Interspecies Differences and Similarities*

Among the pharmacokinetic determinants of TCDD, some are known to vary markedly among species whereas others are not characterized sufficiently in this regard. Overall, the qualitative determinants of the body burden and elimination half-lives appear to be similar across species. Based on empirical observations for TCDD as well as with other polychlorinated dibenzofurans (PCDFs), Carrier et al. (1995a, b) argued that in rats, monkeys, and humans, the dose-dependent changes in the fraction contained in liver and adipose tissue follow a similar pattern across species. The authors suggested that the half-saturation body burden is around 100 ng/kg, and the plateau of liver dose (as fraction of body burden) appears to occur around 1,000 ng/kg. Literature also indicates that aryl hydrocarbon receptor (AhR) is conserved phylogenetically (Harper et al., 2002; Fujii-Kuriyama et al., 1995; Nebert et al., 1991) and is present in mammalian species, including experimental animals and humans (Okey et al., 1994;

Lorenzen and Okey, 1991; Manchester et al., 1987; Roberts et al., 1986; Roberts et al., 1985). These qualitative similarities in pharmacokinetic determinants and outcome support the use of animal data to infer general patterns of the pharmacokinetic behavior of TCDD in humans. However, quantitative differences in determinants, including physiological, physicochemical, and biochemical, need to be taken into account. Even though species-specific physiological parameters can be obtained from the literature, key data on species-specific biochemical parameters (particularly binding constants, maximal capacity, induction rates, and other parameters) are not available for humans at this time. However, these can be inferred by using a pharmacokinetic model fit to in vivo data on the rate of TCDD elimination from specific compartments in humans (Emond et al., 2006; Aylward et al., 2005b; Emond et al., 2005; Emond et al., 2004; Carrier et al., 1995a, b).

3.3.3. Pharmacokinetics (PK) of TCDD in Humans: Interindividual Variability

TCDD pharmacokinetics and tissue doses vary across the human population as a function of the interindividual variability of the key kinetic determinants. Because the NAS comments focused on health effects associated with chronic, lifetime exposure, the key kinetic determinants for such exposures include clearance, binding, and temporal changes in volume of distribution. When considering the interindividual variability in pharmacokinetics and dose metrics of TCDD, it is important to recognize that the elevated lipid-corrected serum concentrations in highly exposed persons are associated with greater elimination rates, probably due to greater degrees of induction of CYP1A2 in the liver and possibly other related metabolic enzymes (Emond et al., 2006; Aylward et al., 2005b; Abraham et al., 2002; Grassman et al., 2000).

The interindividual variability in adipose content is a critical parameter in pharmacokinetic models given the characteristics of TCDD (see Section 3.3.2). Both metabolic elimination and elimination via the GI tract depend on the fraction of TCDD in the body that is available outside of adipose tissue. As body fat content rises, a smaller portion of the total body TCDD will be contained in the relatively available fraction outside of the adipose tissue. Because elimination of TCDD by both metabolism and fecal excretion depends on the small proportion of TCDD that exists outside of fat tissue, people with larger proportions of body fat—including many older people—will tend to require longer times to reduce TCDD levels by a

given proportion than leaner people (Emond et al., 2006; Rohde et al., 1999; Van der Molen et al., 1998; Van der Molen et al., 1996).

The sections that follow highlight key aspects of interindividual variability in TCDD pharmacokinetics, with an emphasis on the available data related to elimination half-lives and volume of distribution.

3.3.3.1. *Life Stage and Gender*

The influence of the variability of fat content in human population on the distribution and clearance of TCDD has been evaluated by several investigators. There are data showing an inverse dependency of TCDD elimination rate on percent body fat. Figure 3-3 shows this relationship in a study in which TCDD elimination via feces was measured in six people in relation to their body fat content (Rohde et al., 1999). Observations of TCDD elimination rates in a small number of men and women in the Seveso cohort (Aylward et al., 2005a) provide a modest opportunity to compare TCDD elimination rates with actual human data. Based on the partition coefficients reported by Emond et al. (2006), the elimination rates for the men in the sampled group are expected to be greater than the elimination rates in the women. Taking into consideration values similar to those shown in Table 3-2, and fat proportions inferred from body mass indices using the equations of Lean et al. (1996), the Seveso men studied are expected to have an overall average of about 3.92% of their TCDD body burden outside of fat, whereas the women are expected to have an average of only 2.36% outside of fat. On this basis, the TCDD elimination rates in the men are expected to be 3.92/2.36 = 1.66 times faster than the elimination rates in the women. By comparison, Michalek et al. (2002) reported observed elimination rates in men and women that result in a slightly lower ratio:

$$\frac{\text{men:}0.111\,\text{year}^{-1} \pm 0.010\ \text{(std.error)}}{\text{women:}0.071\,\text{year}^{-1} \pm 0.010\ \text{(std.error)}} = 1.56 \qquad \text{(Eq. 3-6)}$$

The central estimates for the elimination rates correspond to half lives of 6.5 and 9.6 years for men and women, respectively.

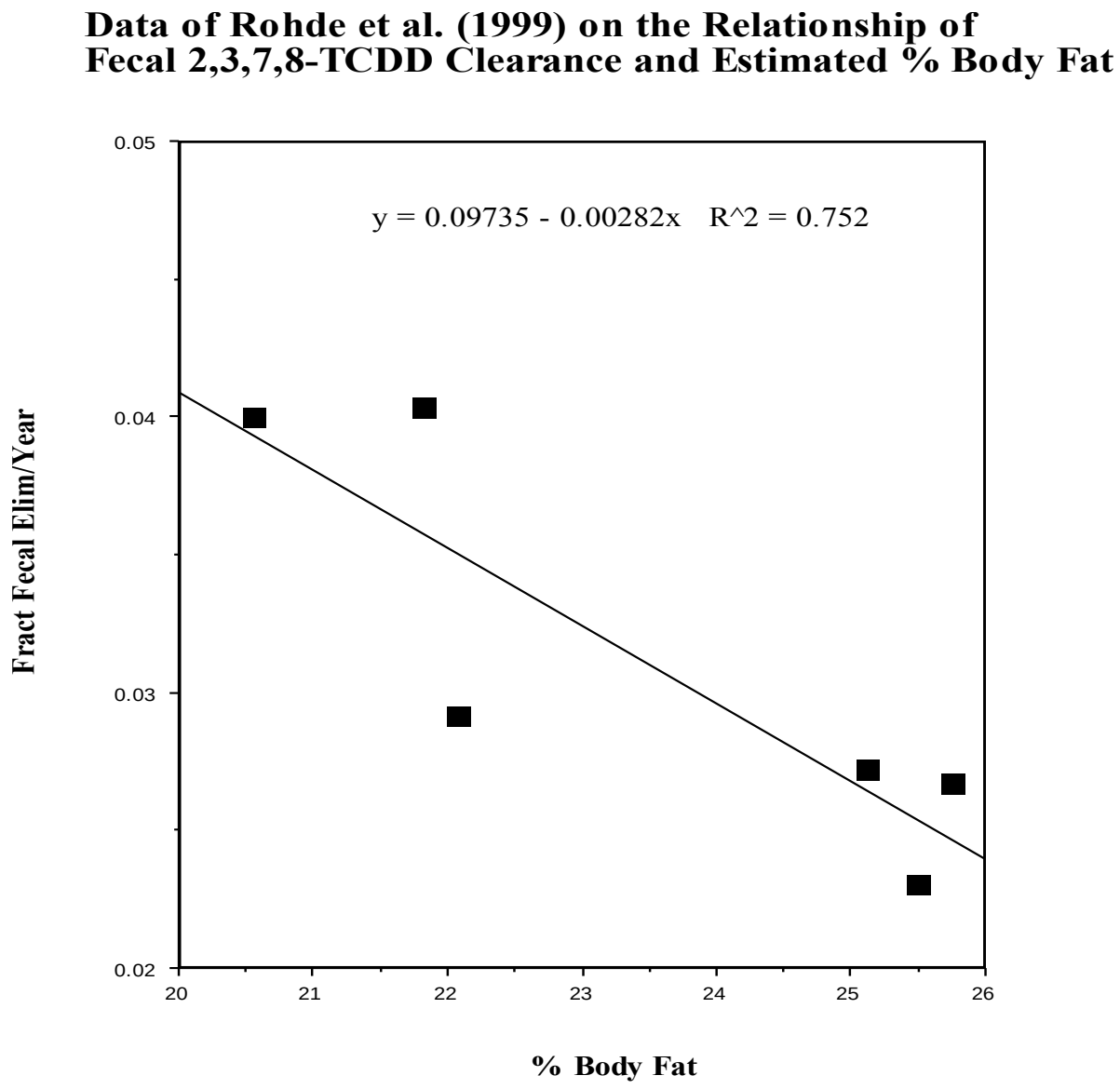


Figure 3-3. Observed relationship of fecal 2,3,7,8-TCDD clearance and estimated percent body fat.

Source: Rohde et al. (1999).

A further point of comparison can be derived using the observed body mass index (BMI)[24] and TCDD elimination rate of each of the male Ranch Hand military veterans, whose TCDD elimination rates were observed between 9 and 33 years after their time in Vietnam. The average BMI over that time was 29.44 (based on 287 measurements for the 97 veterans, tabulated in three periods by Michalek et al., 2002), and their average age was about 44.5 for the

[24] The BMI is calculated as the body weight in kilograms divided by the square of the height in meters.

measurements. Based on these data, the corresponding average estimated percent body fat is 29.7% using the Lean et al. (1996) formula for men. The observed average TCDD elimination rate constant for these men for the period was 0.092 $year^{-1}$ ± 0.004 (standard error), corresponding to a half-life of 7.5 years. This half-life is slightly longer than the central estimate of the half-life of 6.2 years (i.e., ln(2)/0.111) for the smaller group of Seveso males with their slightly smaller estimated percent body fat. Figure 3-4 shows a simple plot of these data and a fitted unweighted regression line characterizing the relationship between estimated fat content and TCDD elimination rates. Variation in metabolic enzyme activities and other routes of loss is also likely to be important, but there is little human quantitative information available on these issues.

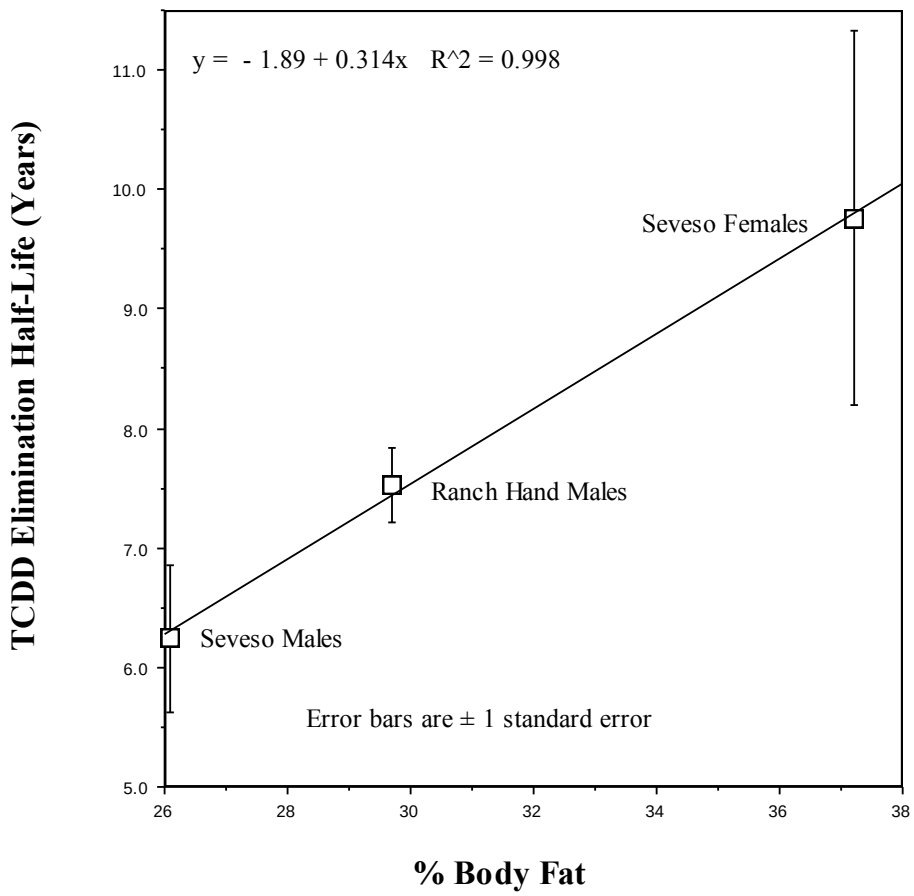


Figure 3-4. Unweighted empirical relationship between percent body fat estimated from body mass index and TCDD elimination half-life—combined Ranch Hand and Seveso observations.

More recently, Kerger et al. (2006) estimated the slope of the relationship between half-life and age to be 0.12 years (95% confidence interval, 0.10−0.14), which corresponds to the rate of increase in TCDD half-life for each year of age. The authors speculated that although age explained most of the variance in the individual half-life trends, it was also correlated with TCDD concentration, BMI, and body fat mass. The regression model developed by these authors discriminated between the high and low TCDD exposures or concentrations. Thus, after accounting for the TCDD (concentration × age) term's effect on the slope of age, the final model for TCDD concentration ≤700 ppt was

$$t_{1/2} = 0.35 + 0.12 \times Age \qquad \text{(Eq. 3-7)}$$

For TCDD concentration >700 ppt, the final model was

$$t_{1/2} = 0.35 + 0.088 \times Age \qquad \text{(Eq. 3-8)}$$

where $t_{1/2}$ is the half-life and Age is the age at time of subsequent sampling. Pharmacokinetic information relevant to specific age groups is presented in the sections that follow.

3.3.3.1.1. *Prenatal period*

Data to estimate TCDD elimination rates for fetuses are not available. Levels of TCDD in fetal tissues for rats were experimentally estimated at different gestational periods and utilized in a developmental model by Emond et al. (2004). There is information on body composition that is relevant to prediction of TCDD dose to fetus. These data, summarized as part of the radiation dosimetry model of the International Commission on Radiological Protection, are consistent with the idea that early fetuses are nearly all water and less than 1% lipid, and lipid levels rise toward parity with protein near the time of normal delivery.

Bell et al. (2007a) reported that the disposition of TCDD into the fetus shows dose dependency, with a greater proportion of the dose reaching the fetus at lower doses of TCDD. Further, both CYP1A1 and CYP1A2 are highly inducible (~103−fold) in fetal liver, whereas CYP1A2 shows much lower induction (10−fold) in maternal liver. It has been speculated that this is due to the lower basal levels of CYP1A2 in fetal liver, as compared to maternal liver (Bell

et al., 2007a). The greater relative disposition to the fetus at low doses may be the result of higher bioavailability due to less hepatic sequestration and elimination in the mother.

3.3.3.1.2. *Infancy and childhood*

Hattis et al. (2003) describe the general pattern of change of body fat content with age in children. Central tendency values for percent body fat begin at about 12% at birth and rise steeply to reach about 26% near the middle of the first year of life. Fat content then falls to reach a minimum of approximately 15% at 5−8 years of age, followed by a sex-dependent ―adiposity rebound" that takes females to about 26% body fat while the males remain near 16−17% on average by age 20. The interindividual variability distributions about these central values are complex, as some children experience the ―adiposity rebound" earlier than others, and this creates patterns that are not simply interpretable as unimodal normal distributions. Hattis et al. (2003) did find it possible to fit distributions of body fat content inferred from National Health and Nutrition Examination Survey skin fold measures to mixtures of two normal distributions for children between age 5 and 18.

At least two groups of authors have published PBPK modeling results indicating generally more rapid clearance of TCDD in children than in adults, a trend that is consistent with the generally lower fat content of children (Leung et al., 2006; Van der Molen et al., 2000; Kreuzer et al., 1997). The rapid expansion of the adipose tissue compartment can contribute, in part, to the reduced apparent half-life in children (Clewell et al., 2004). This reduction may also be due to varying rates of metabolism and/or fecal lipid excretion (Kerger et al., 2007; Abraham et al., 1996).

Furthermore, very young children have different modes and quantities of TCDD exposure compared to adults. Lakind et al. (2000) characterize distributions of milk intake for nursing infants to characterize distributions of TCDD exposure. This is also a corresponding route of loss of TCDD stores for lactating women, as described in Section 3.3.3.2 below.

3.3.3.1.3. *Adulthood and old age*

The fraction of fat in relation to body weight in adulthood and old age can be computed as a function of the BMI and age (e.g., Lean et al., 1996):

$$\%\,Body\,Fat\,(\text{males}) = 1.33 \times BMI + 0.236 \times Age - 20.2 \qquad \text{(Eq. 3-9)}$$

$$\%\,Body\,Fat\,(\text{females}) = 1.21 \times BMI + 0.262 \times Age - 6.7 \qquad \text{(Eq. 3-10)}$$

The above equations are the result of analysis of data based on underwater weighing of 63 men and 84 women (age range 16.8−65.4). The salient observation with respect to TCDD for these data is that age and BMI-dependent variability in fat content have implications for the variability in TCDD elimination rates and internal dose among adults.

3.3.3.2. *Physiological States: Pregnancy and Lactation*

Data on body fat content in pregnant women at various stages of gestation (Pipe et al., 1979) have potential implications for TCDD elimination rates during pregnancy, even though the relationship between these parameters has not been formally analyzed.

Lactation is viewed as an additional route of elimination for some chemicals such as TCDD. According to a recent study, a breast-feeding woman expels through lactation an estimated 8.76 kg fat per year [q_f (kg/day), 0.8 kg milk/day with an average 3% lipid], and the partition coefficient between blood lipid and milk fat (K_{BM}) for TCDD is 0.92 (Milbrath et al., 2009; Wittsiepe et al., 2007). The estimated rate of elimination of TCDD due to breast-feeding (k_{bfed}) can then be computed as follows (Milbrath et al., 2009):

$$k_{bfed} = \frac{q_f \times \Delta t_{bfed}}{K_{BM} \times \frac{pbf_i}{100} \times BW_i} \qquad \text{(Eq. 3-11)}$$

where

Δt_{bfed} (unitless)	= the fraction of the year during which the woman was actively breast-feeding;
pbf_i	= woman's percent body fat; and
BW	= woman's body weight in kg.

Assuming no interaction between breast-feeding and other half-life determinants Milbrath et al. (2009), the authors predicted a half-life of 4.3 years for TCDD in a 30-year-old,

nonsmoking woman with 30% body fat if she did not breast-feed that year, and a half-life of 1.8 years if she breast fed for 6 months.

3.3.3.3. *Lifestyle and Habits*

One of the factors related to lifestyle and habits that could influence TCDD kinetics is smoking. Smoking has been reported to enhance the elimination of dioxin and dioxin-like compounds (Ferriby et al., 2007; Flesch-Janys et al., 1996). Milbrath et al. (2009) accounted for interindividual variation in body composition as well as smoking habits in an empirical model. The predicted half-life (years) for an individual *i* as a function of age, smoking status, and percent body fat *i* was as follows

$$t_{1/2}(age, smoke, pbf)_i = [\beta_{(0age)} + \beta_{(age)} \times age_i] \times SF_i \times \frac{pbf_i}{pbf_{ref(age_i)}} \qquad \text{(Eq. 3-12)}$$

where

$\beta_{(0age)}$ = intercept constant derived from regressed data;

$\beta_{(age)}$ = slope constant derived from regressed data;

age_i = specific age *i* (years);

pbf_i = individual percent body fat;

$pbf_{ref(age_i)}$ = reference percent body fat; and

SF_i = the unitless, multiplicative smoking factor.

3.3.3.4. *Genetic Traits and Polymorphism*

One particular genetic locus that is potentially related to TCDD pharmacokinetics and tissue dose is the gene for the AhR. Eight candidate AhR polymorphisms have been identified to date (Connor and Aylward, 2006; Harper et al., 2002). Given the role of AhR in regulating the induction of CYP1 isozymes (Connor and Aylward, 2006; Toide et al., 2003; Baron et al., 1998), the polymorphism might lead to interindividual differences in metabolic clearance, the significance of which would depend upon the dose, fat content, and exposure scenario. In this regard, it should be noted that the inducibility of aromatic hydrocarbon hydroxylase in human

tissues has been reported to be highly variable, up to 100−fold (Connor and Aylward, 2006; Smart and Daly, 2000; Wong et al., 1986).

The scientific literature contains values of K_d (the dissociation constant of the TCDD−AhR complex) ranging from about 1 to much higher values (corresponding to lower binding affinity) (reviewed in Connor and Aylward, 2006). This provides suggestive evidence for a heterogeneous human AhR, with functionally important polymorphisms (Micka et al., 1997; Roberts et al., 1986), even though some of the range may be attributed to experimental procedural differences and to other factors (Connor and Aylward, 2006; Harper et al., 2002; Lorenzen and Okey, 1991; Manchester et al., 1987).

The various pharmacokinetic processes and determinants (see Sections 3.3.2 and 3.3.3), individually or together, might influence the dose metrics of relevance to the dose-response modeling of TCDD.

3.3.4. Dose Metrics and Pharmacokinetic Models for TCDD

3.3.4.1. *Dose Metrics for Dose-Response Modeling*

The **dose metric** related to a toxicological endpoint can range from the maximal concentration, the area under a time-course curve (area under the curve [AUC]), or the time-averaged concentration of the toxic moiety in the body, blood, or target tissue, to an appropriate measure of the resulting interactions in the target tissue (e.g., receptor occupancy or functional biomarkers related to specific effects). A single dose metric, however, is unlikely to be sufficient for all endpoints and exposure durations. Consideration of these issues is critical to the selection of the dose metrics of relevance to dose-response modeling of TCDD.

Figure 3-5 lists a range of alternative dose metrics for TCDD in terms of their relevance based on considerations of pharmacokinetic mechanisms and mode of action (MOA). The **administered dose** or daily intake (ng/kg-day) is the least relevant dose metric for dose-response modeling of TCDD. This dose adjusts only for body-weight differences between species. The administered dose, when used with an uncertainty factor for kinetics (or kinetic adjustment factor, such as $BW^{3/4}$) and an uncertainty factor for dynamics, can also account for allometrically predicted pharmacokinetic (clearance) and pharmacodynamic differences between species in deriving the human equivalent dose (HED). In effect, the use of kinetic and dynamic adjustment or uncertainty factors facilitates the computation of HED. Such a calculation of HED is

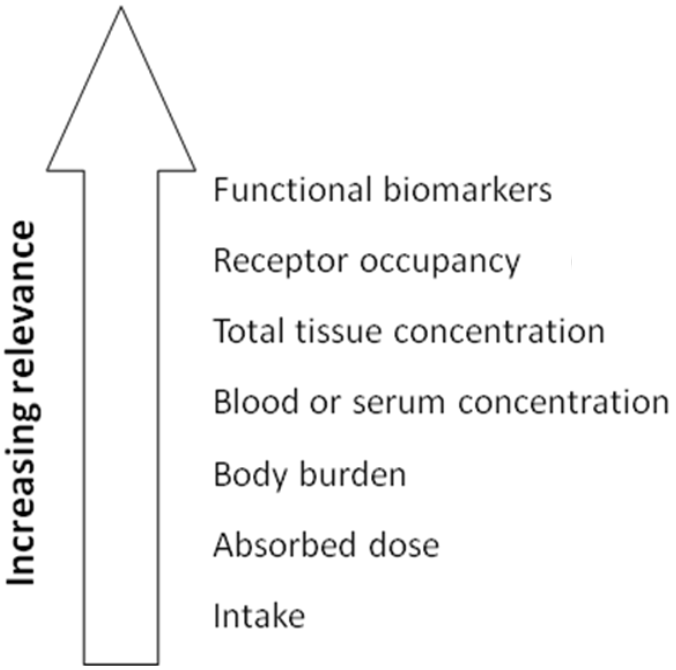


Figure 3-5. Relevance of candidate dose metrics for dose-response modeling, based on mode of action and target organ toxicity of TCDD.

associated with the steady-state blood concentration of parent chemical in rats by accounting for species differences in metabolic clearance. This is generally done by relating to body surface area or metabolic rates, with no corresponding temporal changes in the volume of distribution (see, for example, Krishnan and Andersen, 1991). Such calculations of HED for TCDD may not be appropriate given that (1) steady-state was not attained in all critical toxicological studies chosen for the assessment, (2) the clearance is mainly due to enzyme(s) and processes whose levels/rates do not necessarily vary across species or life stages as a function of body surface differences, and (3) there is a likelihood of change in volume of distribution over time. Furthermore, the use of administered dose does not explicitly account for the dose-dependent elimination of TCDD from tissues as demonstrated in multiple studies (reviewed in Sections 3.3.2 and 3.3.4). The use of administered dose in TCDD dose-response modeling is unlikely to facilitate the characterization of the true relationship between the response and the relevant measures of internal dose that are influenced by dose-dependent elimination and binding processes. Additionally, the use of administered dose to extrapolate across species or life stages

would not effectively take into account the differences in fat content or the demonstrated dose-dependent and species-dependent differences in elimination half-life of TCDD.

Dose metrics for TCDD may include absorbed dose, body burden, serum or whole blood concentration, tissue concentration, and possibly functional-related metrics of relevance to the MOA (e.g., receptor occupancy, change in protein levels). These measures can be calculated as a current (terminal), average (over a defined period), or integral quantity. The applicability of the integral measures, such as the AUC (i.e., the area under the curve of a plot of blood or plasma concentration vs. time), traditionally used for analyzing chronic toxicity data, is questionable in the case of TCDD. This is because of differences in lifespan and uncertainties regarding the appropriateness of the duration to be specified for averaging the AUC in experimental animals and humans for certain critical effects (NAS, 2006b).

Among the alternative dose metrics, the **absorbed dose** accounts for differences in body weight as well as species-specific differences in bioavailability. Thus, the **absorbed dose** is equivalent to **body burden**. **Body burden**, or more appropriately, the body concentration, represents the amount of TCDD per kg body weight. TCDD body burdens, like other dose measures, can be determined as the peak, the average over the period of the bioassays, or the level at the end of the experiments. Thus, the terminal or average body burdens can be obtained either using data or pharmacokinetic models and used in dose-response modeling. The body burden is a measure of TCDD dose that reflects the net impact of bioavailability, uptake, distribution, and elimination processes in the organism. It is essentially a function of the volume of distribution and clearance processes, and as such, it does take into account the temporal changes in volume of distribution as well as the concentration-dependent clearance. These are phenomena that are critical to the understanding of TCDD dose to the target. However, the body burden may not accurately reflect the tissue dose (NAS, 2006b), and as such, does not allow for analysis of species-specific differences in target organ sensitivity to TCDD. In essence, the body burden represents only an ―overall average" of TCDD concentration in the body, without regard to the differential partitioning and accumulation in specific tissues, including the target tissue(s).

Serum (or blood) concentration of TCDD is a dose metric that reflects both the body burden and the dose-to-target tissues. Serum or blood concentration, at steady-state, would be reflective of the impact of clearance processes and expected to be directly proportional to the tissue concentrations of TCDD (NAS, 2006b). This dose metric for lipophilic chemicals such as

TCDD is often expressed as a lipid-normalized value, to adjust for varying serum lipid content (Niskar et al., 2009; Patterson et al., 2009; DeKoning and Karmaus, 2000), particularly in human biomonitoring studies, thus of relevance to dose-response modeling; however, the serum lipid-normalized concentrations of TCDD are not routinely collected and reported in animal toxicological studies. Serum lipid-adjusted TCDD concentration is calculated as the ratio of serum TCDD content over serum lipid content per unit volume. Alternatively, TCDD serum lipid-normalized calculation can be estimated by using the formula TL = (2.27 × TC) + TG + 62.3 mg/dL where the total lipid (TL) content of each sample is estimated from its total cholesterol (TC) and triglyceride (TG) (Patterson et al., 2009). The lipid-adjusted serum concentration, however, would be reflective of the lipid-adjusted concentration of TCDD in other organs (reviewed in Aylward et al., 2008) depending upon the extent of steady-state attained and the similarity of lipid composition across tissues in each species. In essence, the serum lipid-normalized measure is representative of the amount of TCDD per specified volume of total lipids, whereas the whole blood measure will be reflective of the ensemble of free, lipid-bound and protein-bound TCDD in plasma and erythrocytes, which may be species-specific. Even though these dose metrics are thought to be more closely and directly related to the tissue concentrations associated with an effect, a less direct association might occur at increasing doses when nonlinear processes dominate the kinetics and distribution of TCDD into organs such as the liver.

Tissue concentration of TCDD, as free, bound, or total TCDD, is a more relevant pharmacokinetic measure of dose, given that it provides a measure of exposure of the target cells to the chemical. In this regard, the CYP1A2-bound fraction may be considered as a relevant dose metric for certain toxic effects; however, the available data contain mixed results regarding the mechanistic linkage of this dose metric to toxicity and carcinogenicity (reviewed in Budinsky et al., 2006). In such cases, the use of alternative dose metrics (e.g., bound concentration as well as the serum concentration) in dose-response modeling could be considered. Other function-related biomarkers and dose metrics could facilitate the additional consideration of pharmacodynamic aspects reflecting tissue- and species-specific sensitivity. These metrics may represent the most relevant measures of tissue exposure and sensitivity to TCDD. For example, receptor occupancy and functional biomarkers as dose metrics for TCDD require a clear

understanding of mode of action of TCDD and availability of relevant data. In the absence of such information, these possible dose metrics cannot be utilized at the present time.

Empirical time-course data on the alternative dose metrics of TCDD associated with epidemiologic and experimental (animal) studies are not available, requiring the use of pharmacokinetic models to obtain estimates of these dose metrics. These models may be simple, based on first-order kinetics or more complex based on physiochemical, biochemical, and physiological parameters for simulating uptake, distribution (including sequestration to proteins), and clearance of TCDD (see Section 3.3.4.3).

3.3.4.2. *First-Order Kinetic Modeling*

Figure 3-6 illustrates the process of estimating a human-equivalent TCDD oral exposure from an experimental animal-administered dose, based on the assumption that body burden is the effective dose metric for TK equivalence across species. The primary assumption is that the time-weighted average (TWA) TCDD body burden over some critical time period is the proximate toxicokinetically effective dose eliciting a toxicological effect.[25] The process consists of estimating the effective average body burden in the experimental animal over some time t_A (generally the experimental duration) using a TK model, then ~~"b~~ack-calculating" a daily human exposure level that would result in that average body burden over some time t_H (the human equivalent to t_A).

The following closed-form equation is the general formula used to calculate a TCDD terminal body burden in an experimental animal or human at time (t).

$$BB(t) = BB(0) + \frac{d(1 - e^{-kt})fa}{k} \qquad \text{(Eq. 3-13)}$$

where

$BB(t)$ = the body burden at time t (ng/kg);

$BB(0)$ = the initial body burden (ng/kg);

d = the daily dose (ng/kg-day);

k = the whole-body elimination rate (days^{-1});

[25] The conversion depicted in Figure 3-6 does not account for toxicodynamic differences between species.

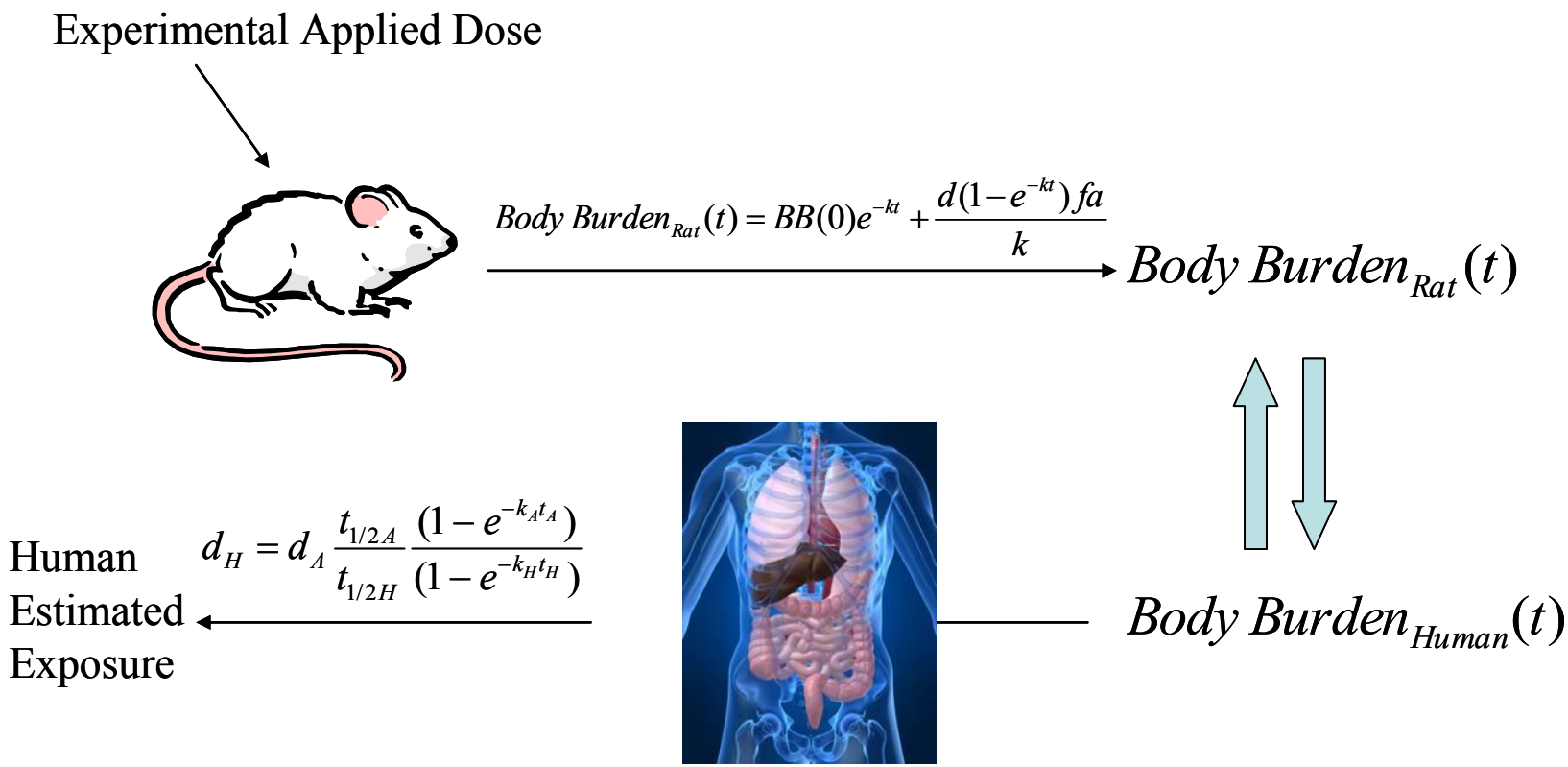


Figure 3-6. Process of estimating a human-equivalent TCDD lifetime average daily oral exposure (d_H) from an experimental animal average daily oral exposure (d_A) based on the body-burden dose metric.
The arrows represent mathematical conversions based on toxicokinetic modeling. BB_A (TWA animal body burden) and BB_H (TWA human body burden) are assumed to be toxicokinetically equivalent. See text for further explanation.

t = the time at which the body burden is determined (days); and

fa = the fraction of oral dose absorbed (unitless).

For the experimental animal, *BB(t)* is $BB_A(t) = BB_A(0)e^{-k_A t_A} + \frac{d_A(1-e^{-k_A t_A})fa_A}{k_A}$,

and for humans, this parameter is $BB_H(t) = BB_H(0)e^{-k_H t_H} + \frac{d_H(1-e^{-k_H t_H})fa_H}{k_H}$.

Setting $BB_H(t) = BB_A(t)$ obtains the following expression:

$$BB_H(0)e^{-k_H t_H} + \frac{d_H(1-e^{-k_H t_H})fa_H}{k_H} = BB_A(0)e^{-k_A t_A} + \frac{d_A(1-e^{-k_A t_A})fa_A}{k_A} \quad \text{(Eq. 3-14)}$$

Rearranging and solving for d_H yields:

$$d_H = d_A \frac{k_H}{k_A}\frac{fa_A}{fa_H}\frac{(1-e^{-k_A t_A})}{(1-e^{-k_H t_H})} + BB_A(0)e^{-k_A t_A} - BB_H(0)e^{-k_H t_H} \quad \text{(Eq. 3-15)}$$

Assuming that initial body burdens are very small compared to *BB(t)* and that the fraction of TCDD absorbed is the same for humans and experimental animals, and using the relationship $k = \frac{\ln(2)}{t_{1/2}}$, where $t_{½}$ is the whole-body half-life, a simplified solution for d_H is obtained.

$$d_H = d_A \frac{t_{1/2A}}{t_{1/2H}}\frac{(1-e^{-k_A t_A})}{(1-e^{-k_H t_H})} \quad \text{(Eq. 3-16)}$$

The term $1-e^{-|t}$ is the daily fraction eliminated. Therefore, d_H can be seen to be the average daily administered dose to the experimental animal times the ratio of the animal:human half-life times the ratio of the animal:human daily fraction eliminated over the respective times, t_A and t_H. For both species at (theoretical) steady state ($t \rightarrow \infty$; daily fraction eliminated $\rightarrow 1$),

the latter ratio approaches unity, reducing the animal:human conversion factor to the ratio of the half-lives.

However, for less-than-lifetime exposures eliciting noncancer effects, specific values for t_A and t_H must be considered. Furthermore, Eq. 3-16 computes d_H on the basis of *terminal* body burdens at times t_A and t_H. The more representative metric for toxicokinetic equivalence based on average body burden over the respective time periods is given in Eq. 3-17.

$$BB(t) = BB(0)\frac{1}{t}\int_0^t e^{-k\tau}d\tau + d\frac{fa}{k}\frac{1}{t}\int_0^t (1-e^{-k\tau})d\tau = BB(0)\frac{\left(1-e^{-kt}\right)}{kt} + d\frac{fa}{k}\left[1-\frac{\left(1-e^{-kt}\right)}{kt}\right] \quad \text{(Eq. 3-17)}$$

Solving for d in Eq. 3-17 by assuming minimal initial body burden (BB(0) ~ 0) and setting $d = d$ yields:

$$d_H = d_A \frac{t_{1/2A}}{t_{1/2H}} \frac{\left[1-\frac{\left(1-e^{-k_A t_A}\right)}{k_A t_A}\right]}{\left[1-\frac{t_{H0}}{t_H}-\frac{\left(e^{-k_H t_{H0}}-e^{-k_H t_H}\right)}{k_H t_H}\right]} \quad \text{(Eq. 3-18)}$$

where t_{H0} is the initial human exposure time.

The value of t_A is the duration of the experimental exposure period. For some gestational exposures, if a critical exposure window is defined, t_A will be the duration of the critical exposure window. The value of t_H is the human-equivalent duration corresponding to t_A. However, for t_A less than lifetime (less than 2 years in rodents) and no defined susceptible life stage, t_H cannot begin at 0 (because typically animal experiments do not begin at age 0), but must end at 25,550 days (70 years) to include the terminal (pseudo) steady-state level, at which the $BB_H(t)$: d_H ratio is highest. Otherwise, starting t_H at 0 would not be protective for less-than-lifetime effects that could be manifest at any age in humans; the average is determined from the terminal end of the human exposure period because the daily exposure achieving the target blood concentration is smaller than for the same exposure period beginning at birth (i.e., d_H would be higher for earlier exposure periods) and is health protective for effects occurring

after shorter-term exposure.[26] Figure 3-7 depicts the relationship of daily dose to TWA body burden graphically for several exposure duration scenarios. For shorter durations occurring later in life, the average body burden over the exposure period does not differ substantially from the steady-state value. Even for half-lifetime exposures, the deviation of the average from steady state is minimal. Only for lifetime exposures does the difference become more marked, but only by about 15%. Note that in the 2003 Reassessment, a constant value of 3,000 was used for $BB_H(t)$: d_H, based on the relationship of continuous exposure to theoretical steady-state body burden (t = lifetime, $t_{½}$ = 2,593 days); this approach, while conservative, does not account for exposure scenarios of different durations and does not strictly reflect the average body burden dose metric.

The simulation in Figure 3-7 is based on a unit daily exposure to humans, such that the target body burden represents $BB_H(t_H)$:d_H as a general scalar for calculating d_H from any given d_A. Table 3-3 shows the resulting TK conversion factors for the rodent species and strains comprising the bulk of the experimental animals in TCDD studies. Monkey and mink values are not shown in this table because, for the former, only chronic exposures were evaluated and, for the latter, no TCDD half-life information is available. Monkey (Rhesus) half-life estimates range from about 200−500 days. A representative value of 365 days is used for this TCDD assessment. The d_A to d_H conversion factor for the chronic monkey exposures (3.5−4 years) in TCDD studies is 9.2−9.7 (BB_A:d_A = 279−263).

Application of first-order kinetics for the health assessment of TCDD can only be used to estimate total body burdens or back-calculate administered dose from experimental data. Body burden calculations using first-order kinetics is based on the assumption of a first-order decrease in the levels of administered dose as function of time. In that sense, any loss of TCDD from the body is described by using a rate constant that is not specific to any biological process. This constant is usually estimated from estimates of half-life of TCDD. Assuming a constant half-life value for the clearance for long-term or chronic TCDD exposure is not biologically supported given the observed data indicating early influence of CYP1A2 induction and binding to TCDD and later redistribution of TCDD to fat tissue. Abraham et al. (1988) found that the liver:adipose tissue concentration ratio in female Wistar rats exposed to a subcutaneous TCDD dose of300 ng/kg decreased from 10.3 at 1 day postexposure to 0.5 at 91 days postexposure.

[26] See the following (Section 3.3.4.3) for a more detailed discussion of this concept.

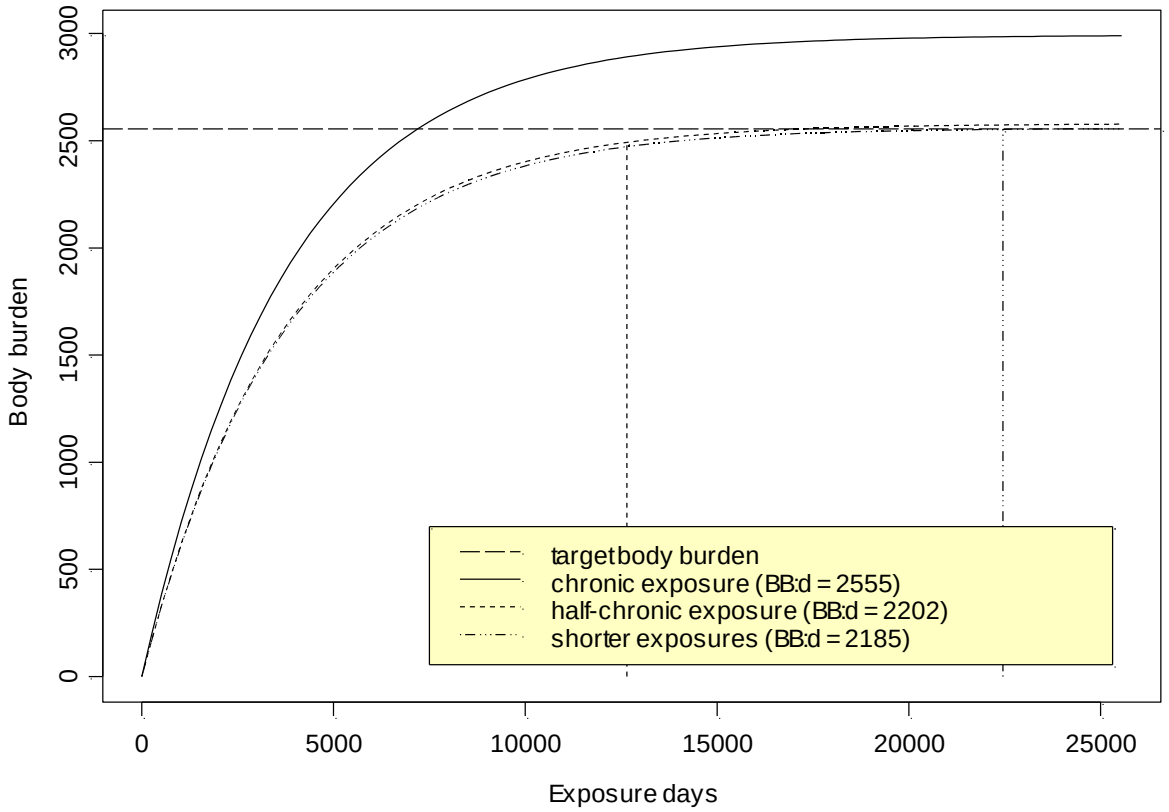


Figure 3-7. Human body burden time profiles for achieving a target body burden for different exposure duration scenarios.

BB:d is $BB_H(t_H):d_H$ in Figure 3-6. The curve depicted using the solid line illustrates the increase in the human body burden over time for a hypothetical human administered a daily TCDD dose where the time-weighted average human body burden estimate over the lifetime is equal to the target body burden attained in a rodent bioassay. When compared to shorter durations (dashed lines), a higher average daily TCDD dose is required to yield a time-weighted average human body burden over a lifetime that is equal to the target body burden attained in a rodent bioassay. The half-chronic exposure scenario (depicted using a dashed line) is equivalent to a 1-year exposure in rodents. When compared to a chronic BB_H, a lower value of d_H is needed to attain the target body burden in a rodent bioassay when the time-weighted average is over the last 35 years of life; the dose–to-plateau ratio is also smaller (i.e., $d_{H,C} < d_{H,SC}$ to attain the target body burden in a rodent bioassay). The shorter exposure scenario is equivalent to most other shorter rodent exposure durations, from 1 day to subchronic, which are indistinguishable with respect to the BB:d ratio (subchronic shown).

Table 3-3. Toxicokinetic conversion factors for calculating human equivalent doses from rodent bioassays based on first-order kinetics

	Mouse	Rat (Wistar)	Rat (other)	Guinea pig
Half-life (days)[a]	**10**	**20**	**25**	**40**
Exposure duration (days)	**Conversion factor (CF)[b] $BB_A(t_A):d_A$ given in parentheses**			
1	3,882 (0.77)	3,815 (0.79)	3,802 (0.79)	3,783 (0.79)
7	1,107 (2.71)	1,020 (2.94)	1,004 (2.99)	979 (3.07)
14	681 (4.41)	587 (5.11)	569 (5.27)	543 (5.53)
28	453 (6.62)	350 (8.56)	331 (9.06)	303 (9.90)
90	307 (9.76)	186 (16.1)	163 (18.4)	130 (23.0)
180	282 (10.6)	154 (19.5)	129 (23.2)	93 (32.1)
365	270 (11.1)	141 (21.3)	115 (26.0)	77 (38.9)
730	226 (11.3)	115 (22.2)	93 (27.4)	60 (42.5)

[a]Half-life for humans = 2,593 days (7.1 years).
[b]$d_H = d_A$/CF; $BB_H(t_H):d_H$ = 2,185 (1−180 days), 2,202 (365 days), 2,555 (730 days).

Consequently, using half-life estimates based on observed steady-state levels of TCDD will not account for the possibility of accelerated dose-dependent clearance of the chemical at the early stages and, thus, would result in estimation of lower administered levels of the chemical. The dynamic change in half-life due to dose-dependent elimination at the early stages of TCDD exposure and its later redistribution to fat tissues for steady-state levels is better described using biologically based models, such as the PBPK models and concentration- and age-dependent elimination (CADM) models (Emond et al., 2006; Aylward et al., 2005b; Emond et al., 2005; Emond et al., 2004; Carrier et al., 1995a, b). Additionally, these models provide estimates for other dose metrics (e.g., serum or tissue levels) that are more biologically relevant to response than administered dose or total body burden (see Section 3.3.4.3).

3.3.4.3. *Biologically Based Kinetic Models*

The development and evolution of biologically based kinetic models for TCDD have been reviewed by EPA (2003) and Reddy et al. (2005). The initial PBPK model of Leung et al. (1988) was developed with the consideration of TCDD binding to CYP1A2 in the liver. The next level of PBPK models by Andersen et al. (1993) and Wang et al. (1997) used diffusion-limited uptake and described protein induction by interaction of DNA-binding sites. The models of Kohn et al. (1993) and Andersen et al. (1997) further incorporated extensive

hepatic biochemistry and described zonal induction of CYP by TCDD. TCDD PBPK models have evolved to include detailed descriptions of gastrointestinal uptake, lipoprotein transport, and mobilization of fat, as well as biochemical interactions of relevance to organ-level effects (Kohn et al., 1996; Roth et al., 1994). Subsequently, developed PBPK models either used constant hepatic clearance rate (Maruyama et al., 2002; Wang et al., 2000; Wang et al., 1997) or implemented varying elimination rates as an empirical function of body composition or dose (Van der Molen et al., 2000; Van der Molen et al., 1998; Andersen et al., 1997; Kohn et al., 1996; Andersen et al., 1993). The more recent pharmacokinetic models explicitly characterize the concentration-dependent elimination of TCDD (Emond et al., 2006; Aylward et al., 2005b; Emond et al., 2005; Emond et al., 2004; Carrier et al., 1995a, b). The biologically based pharmacokinetic models describing the concentration-dependent elimination (i.e., the pharmacokinetic models of Emond et al., 2006; Aylward et al., 2005b; Emond et al., 2005) are relevant for application to simulate the TCDD dose metrics in humans and animals exposed via the oral route. The rationale for considering the Aylward et al. (2005b) and Emond et al. (2006; 2005; 2004) models for estimating dose metrics for possible application to TCDD health assessment is based on the following considerations.

- Both models were developed and calibrated using research results from the more recent peer-reviewed publications.
- Both models are relatively simple and less parameterized than earlier kinetic models for TCDD. The Aylward et al. (2005b) model is based on two-time scale TCDD kinetics described by Carrier et al. (1995a), and the Emond et al. (2006; 2005; 2004) PBPK models are reduced versions of earlier complex PBPK models. Although simple, both the Aylward et al. (2005b) and Emond et al. (2006; 2005; 2004) models are inclusive of important kinetic determinants of TCDD disposition.
- Both models are uniquely formulated with dose-dependent hepatic elimination consistent with current understanding of TCDD toxicokinetics.
- Both models and extrapolated human versions were tested against human data collected in a variety of human exposure scenarios (Aylward et al., 2005b; Emond et al., 2005).
- Both models are capable of deriving one or more of the candidate dose metrics that may be of interest to EPA's dose-response assessment of TCDD.

3.3.4.3.1. *Concentration- and age-dependent model (CADM)*

3.3.4.3.1.1. ***Model structure***

The pharmacokinetic model of Aylward et al. (2005b), referred to as CADM in this report, is based on an earlier model developed by Carrier et al. (1995a, b) that describes the dose-dependent elimination and half-lives of polychlorinated dibenzo-*p*-dioxins and furans. This model describes the TCDD levels in blood (body), liver, and adipose tissue. Blood itself is not characterized physically as a separate compartment within the model, and the distribution of TCDD to tissues other than adipose tissue and liver (usually less than 4%) is not accounted for by the model. The original structure of the Carrier et al. (1995a, b) model was modified by Aylward et al. (2005b) to include TCDD elimination through partitioning from circulating lipids across the lumen of the large intestine into the fecal content (see Figure 3-8). The most recent version of the Carrier model (2008; Aylward et al., 2005b) includes fecal excretion of TCDD from two routes: (1) elimination from circulating blood lipid through partitioning into the intestinal lumen; and (2) elimination of unabsorbed TCDD from dietary intake.

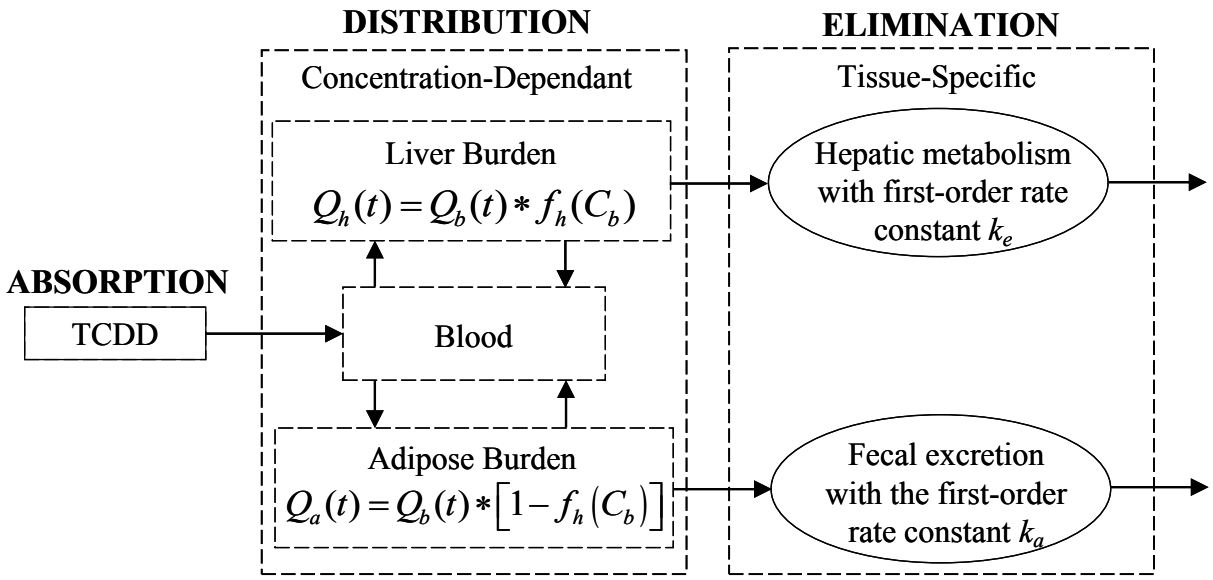


Figure 3-8. Schematic of the CADM structure.

Source: Aylward et al. (2005b).

A basic assumption of this model is that metabolic elimination of TCDD is a function of its current concentration in the liver. The current concentration of TCDD in the liver increases with increasing body burden in a nonlinear fashion as a result of the induction of (and binding of TCDD to) specific proteins (i.e., CYP1A2). Consequently, the fraction of TCDD body burden contained in the liver increases nonlinearly (with a corresponding decrease in the fraction contained in adipose tissues) with increasing body burden of TCDD (Aylward et al., 2005a; Carrier et al., 1995a).

Of particular note is that the adipose tissue compartment of the model is considered to represent the lipid contained throughout the body. It then assumes that the concentrations of TCDD in lipids of plasma and various organs are essentially equivalent to that of adipose tissue, and as such, these concentrations are included in the adipose compartment of the model. Even though this approximation is fairly reasonable given the available data, there is some concern that the adipose compartment of this model also includes the lipid content of the liver to some unknown extent. Because the equilibrium balance between free and bound TCDD in the liver is dependent on the adipose content of the tissue, removal of lipid volume from the liver would mathematically alter total hepatic concentration and, therefore, would affect the estimated levels of the chemical available for binding to proteins.

Distribution in the body is modeled to occur between hepatic and adipose/lipid compartments, with the fraction of body burden in liver increasing according to a function that parallels the induction of the binding protein CYP1A2. Elimination is modeled to occur through hepatic metabolism (represented as a first-order process with rate constant *K* that decreases with age) and through lipid-based partitioning of unmetabolized TCDD across the intestinal lumen into the gut, which is also modeled as a first-order process. As the body burden increases, the amount of TCDD in the liver increases nonlinearly, resulting in an increased overall elimination rate.

3.3.4.3.1.2. ***Mathematical representation***

The CADM model describes the distribution to tissues (including liver and adipose tissue) based on exchange from blood at time intervals of 1 month. The model is based on quasi-steady-state-approximation, and, thus, it is also based on the consideration that the intertissue processes reach their equilibrium values –quasi-instantaneously." In this regard,

absorption and internal distribution reflective of kinetics at the cellular level (e.g., diffusion, receptor binding, and enzyme induction) likely occur on a relatively fast time scale (a few hours to a few days). However, the overall body concentration (i.e., body burden) varies slowly with time such that it remains virtually unchanged during short time intervals.

The CADM model does not differentiate between binding to AhR and CYP1A2, and it lacks explicit descriptions of CYP1A2 induction, a key determinant of TCDD kinetics. However, the empirical equation in the CADM model is based on five parameters (i.e., f_{min}, f_{max}, K, W_a, and W_l; see Tables 3-4 and 3-5) that allow the successful description of the behavior of TCDD in liver and adipose tissue (i.e., TCDD half-lives in each compartment increase with decreasing body burden). This observation implies that the model adequately accounts for the ensemble of the processes. Essentially, the CADM model describes the rate of change in tissue concentrations of TCDD as a function of total body burden such that the global elimination rate decreases with decreasing body burden or administered dose.

3.3.4.3.1.3. ***Parameter estimation***

The CADM model is characterized by its simplicity and fewer parameters compared to physiologically based models. Reflecting this simplicity, hepatic extraction is computed with a unified empirical equation that accounts for all relevant processes (i.e., protein induction and binding).

The key parameters (f_{min}, f_{max}, K, and k_e) were all obtained by fitting to species-specific pharmacokinetic data. The physiological parameters (such as tissue weights) used in the model are within ranges documented in the literature. The fat content is described to vary as a function of age, sex, and BMI. However, the BMI of the model is not allowed to change during an individual simulation (which can range from 20 years to 70+ years), when in reality, the percentage of fat in humans changes over time. None of the TCDD-specific parameters were estimated *a priori* or independent of the data set simulated by the model.

Table 3-4. Equations used in the concentration and age-dependent model (CADM; Aylward et al., 2005b)[a]

Parameter	Equation
Hepatic Concentration (ng/kg)	$C_{hepatic} = \frac{Q_{body}}{W_l} * (f_{\min} + \frac{(f_{\max} - f_{\min}) * C_{body}}{K + C_{body}}$
Fat Concentration (ng/kg)	$C_{adipose} = \frac{Q_{body}}{Wa} * (1 - (f_{\min} + \frac{(f_{\max} - f_{\min}) * C_{body}}{K + C_{body}}))$
Hepatic Elimination	$Exr_hepatic = k_e * Q_{body} * (1 - (f_{\min} + \frac{(f_{\max} - f_{\min}) * C_{body}}{K + C_{body}}))$
Excretion via gut of Unchanged TCDD (Exsorption)	$Exr_gut = k_a * Q_a$
Change of TCDD due to bodyweight change	$ChangeTCDD_BW = Q_{body} * \frac{(BW(t+dt) - BW(t))}{BW(t)}$
Amount in body as a function of time	$Q_{body}(t+dt) - Q_{body}(t) = Exr_hepatic + Exr_gut + ChangeTCDD_BW$
Adipose tissue growth	$W_a = \frac{1.2 * BMI + (0.23 * Age) - 10.8 * sex}{100}$
Change of hepatic elimination constant with age	$k_e = k_{e0} - k_{eslope} * Age$

[a]For abbreviations and parameter descriptions, see Table 3-5.

Table 3-5. Parameters of the concentration and age-dependent model (CADM; Aylward et al., 2005b)

Parameter	Value	Units	Comments/sources
f_{hmin}[a]	0.01	unitless	Minimum body burden fraction in liver
f_{hmax}[a]	0.7	unitless	Maximum body burden fraction in liver
K[a]	100	ng/kg	Body burden at half-maximum of fraction liver
k_e	Calculated	per year	$k_e = k_{e0} - k_{e_slope}$ * (age) with enforced minimum of k_{e_min}
k_{e0}	0.85	per year	CADM-mean hepatic elimination base rate at age 0
k_{e_slope}	0.011	per year	Change in k_e per year of age
k_{e_min}	0.2	per year	Minimum hepatic elimination rate
w_a (adipose weight fraction)	Calculated	unitless	w_a = [(1.2*BMI)+0.23*Age-10.8*sex]/100
w_h (liver body weight fraction)	0.03	unitless	Assumed constant
k_a (adipose clearance factor)	0.0025	per month	Passive elimination rate from intestinal tract
Monthly dose	0.15507069	ng	per month
Estimated absorption fraction	0.97	unitless	From Moser and McLaghlan (2001)
Body weight	70	kg	Standard male weight
Sex	1	unitless	1 = male; 0 = female
Time of administration	840	months	
Initial Cbody	0.2	ng/kg	Estimated background young adults UMDES sampling
Absorbed monthly dose 1	0.150418569	ng	per month

[a]The values of f_{hmin}, f_{hmax}, and K were obtained by best fit of the model simulations to the experimental data with the method of least squares (Aylward et al., 2005a; Carrier et al., 1995a).

3.3.4.3.1.4. ***Model performance and degree of evaluation***

The CADM model was not evaluated for its capabilities in predicting data sets not used in its parameterization. In other words, one or more of the key input parameters (f_{hmin}, f_{hmax}, k_e, K) was obtained essentially by fitting to the species-specific pharmacokinetic data, such that there was no ―external" evaluation data set to which the model was applied. Despite the lack of emphasis on the ―external" evaluation aspect, the authors (Aylward et al., 2005a; Carrier et al., 1995a, b) have demonstrated the ability of the model to describe multiple data sets covering a range of doses and species.

The visual comparison of the simulated data to experimental values suggests that the model could, to an approximate degree, correctly reproduce the whole set of data (e.g., pharmacokinetic [PK] profile over a range of dose and time) and not just part of the PK curve, essentially with the use of a single set of equations and parameters.

The pharmacokinetic data sets for TCDD that were used to calibrate the CADM model by Aylward et al. (2005a; Carrier et al., 1995a, b) included the following:

- Adipose tissue and liver concentrations of TCDD following a single oral dose of 1 µg/kg in monkeys (McNulty et al., 1982);
- Percent dose retained in liver for a total dose of 14 ng in hamsters (Van den Berg et al., 1986);
- Elimination kinetics of TCDD in female Wistar rats following a single subcutaneous dose of 300 ng/kg (data from Abraham et al., 1988);
- Liver and adipose tissue concentrations (terminal measurements) in Sprague−Dawley rats given 1, 10, or 100 ng TCDD/kg bw per day for 2 years (Kociba et al., 1978); and
- Serum lipid concentrations of TCDD over a period of several years in 54 adults (29 men and 25 women) from Seveso and in three Austrian patients (Aylward et al., 2005a).

For illustration purposes, Figure 3-9 shows model simulations of rat data from Carrier et al. (1995a). Figure 3-2 (see Section 3.3.2.4) depicts the human data that were used by the authors to support the concentration-dependent elimination concept; the model was parameterized to provide adequate fit to these data (Aylward et al., 2005a).

The authors did not report any specialized analyses that quantitatively evaluated the uncertainty, sensitivity, and/or variability of CADM model parameters and structure.

3.3.4.3.1.5. ***Confidence inconentration- and age-dependent elimination (CADM) model predictions of dose metrics***

Using professional judgment, EPA ranked its confidence in the CADM model as low, medium, or high (or not applicable) based on model simulations of administered dose, absorbed dose, body burden, serum lipid concentration, total tissue (liver) concentration, and receptor occupancy. A qualitative level of confidence associated with the predictability and reliability of absorbed dose and body burden for oral exposures in humans (as well as several animal species) by this model can be ranked as high (see Table 3-6). This model, however, does not account for

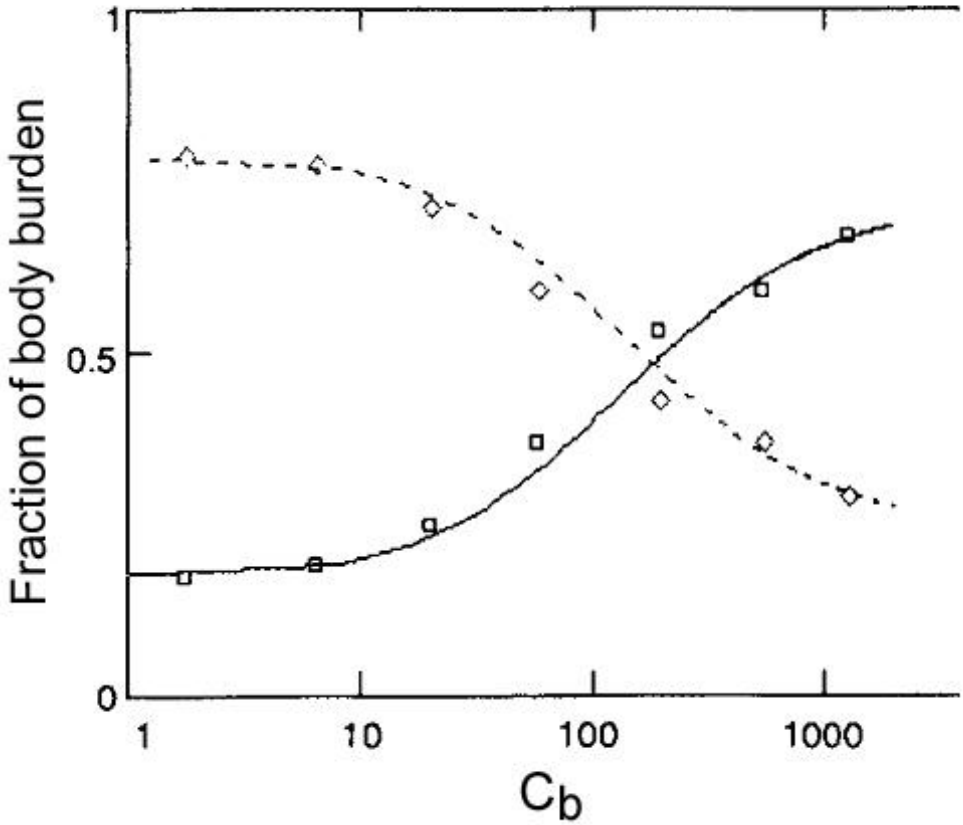


Figure 3-9. Comparison of observed and simulated fractions of the body burden contained in the liver and adipose tissues in rats.
f_h, fraction contained in liver (observation) (□); $f_{h\text{-}sim}$, fraction contained in liver (simulation) (—); f_{at}, fraction contained in the adipose tissue (observation) (◇); $f_{at\text{-}sim}$, fraction contained in the adipose tissue (simulation) (---); and C_b, body concentration in ng TCDD/kg body wt.

Source: Carrier et al. (1995a); data from Abraham et al. (1988) measured 7 days after dosing.

Table 3-6. Confidence in the CADM[a] model simulations of TCDD dose metrics[b]

Dose metric	Level of confidence
Administered dose	NA
Absorbed dose	H
Body burden	H
Serum lipid concentration	M
Total tissue (liver) concentration	L
Receptor occupancy (bound concentration)	NA

H = high, M = medium, L = low, NA = not applicable.
[a]Concentration and age-dependent model (Aylward et al., 2005b).
[b]Using professional judgment, EPA ranked its confidence in the CADM model as low, medium, or high (or not applicable) based on model simulations of administered dose, absorbed dose, body burden, serum lipid concentration, total tissue (liver) concentration, and receptor occupancy.

the differential solubility of TCDD in serum lipids and adipose tissue lipids, nor does it account for the diffusion-limited uptake by adipose tissue. Due to these limitations, the confidence associated with the predictions of the serum lipid concentration of TCDD is considered medium, particularly when it is not documented that steady-state is reached during the critical toxicological studies and human exposures. Furthermore, the CADM model does not facilitate the computation of TCDD concentrations in specific internal organs (other than liver and adipose tissue). The reliability of this model for simulating the liver concentration (free, bound, or total) of TCDD at low doses is considered to be low. This low confidence level is a result of the uncertainty associated with the key parameter, f_{hmin}. This parameter needs to be recalibrated for each study/species/population to effectively represent the free fraction of TCDD in liver and the amount of TCDD contained in the hepatic lipids and bound to the liver proteins (whose levels might be reflective of background exposures of various sources; see Carrier et al., 1995a). The uncertainty related to the numerical value of this parameter in animals and humans—particularly at very low exposures—raises concern regarding the use of this model to predict TCDD concentration (free, bound, or total) in liver as the dose metric for dose-response modeling. Although the use of the parameter f_{hmax} permits the prediction of the dose to liver at high doses, it does not specifically facilitate the simulation of the amount bound to the protein or level of induction in liver. Because the CADM model is not capable of simulating enzyme induction based on biologically relevant parameters, its reliability for predicting the concentration of

TCDD bound specifically to the AhR is not known. Finally, due to the lack of parameterization or verification with kinetic data in pregnant, lactating, or developing animals or humans, the CADM model is unlikely to be reliable in the current form for use in *predicting* potential dose metrics for these lifestages or study groups that might form the basis of PODs for the assessment.

3.3.4.3.2. *Physiologically based pharmacokinetic (PBPK) model*

3.3.4.3.2.1. ***Model structure***

Emond et al. (2006, 2004) simplified the eight-compartment rat model of Wang et al. (1997) to a four-compartmental developmental model (liver, fat, rest of body, and placenta with fetal transfer) (Emond et al., 2004), and later to a three-compartment adult model (liver, fat, rest of the body) (Emond et al., 2006) (see Figures 3-10 and 3-11). Their rationale for simplification of the model was based on evaluating, critiquing, and improving all earlier PBPK models by Wang et al. (1997). In general, the main reason for the simplification was that extrapolation of a PBPK model to humans with these many (i.e., eight compartments) compartments would be problematic due to the limited availability of relevant human data for validation (Emond et al., 2004). One major difference from earlier models, repeatedly emphasized by Emond et al. (2006; 2005), was their description (included in their simplified PBPK models) of the dose-dependent, inducible elimination of TCDD. The rationale for including TCDD binding and induction of CYP1A2 into the model was earlier described by Santostefano et al. (1998).

The most recent version of the rat and human PBPK models developed by Emond et al. (2006) describes the organism as a set of three compartments corresponding to physiological tissues—liver, fat, and rest of the body—interconnected by systemic circulation (see Figure 3-10). The liver compartment includes descriptions of CYP1A2 induction, which is critical for simulating TCDD sequestration in liver and dose-dependent elimination of TCDD. In this model, the oral absorption of TCDD from the gastrointestinal (GI) tract accounts for both the lymphatic (70%) and portal (30%) systems.

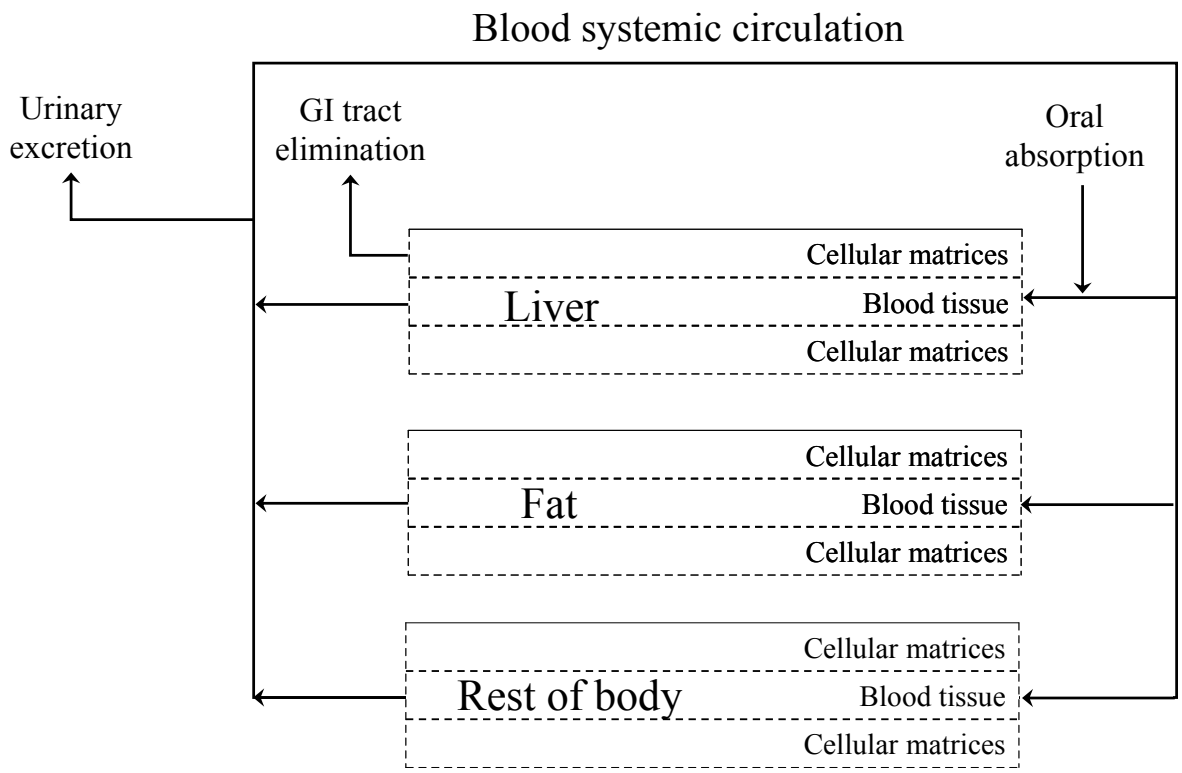


Figure 3-10. Conceptual representation of PBPK model for rat exposed to TCDD.

Source: Emond et al. (2006).

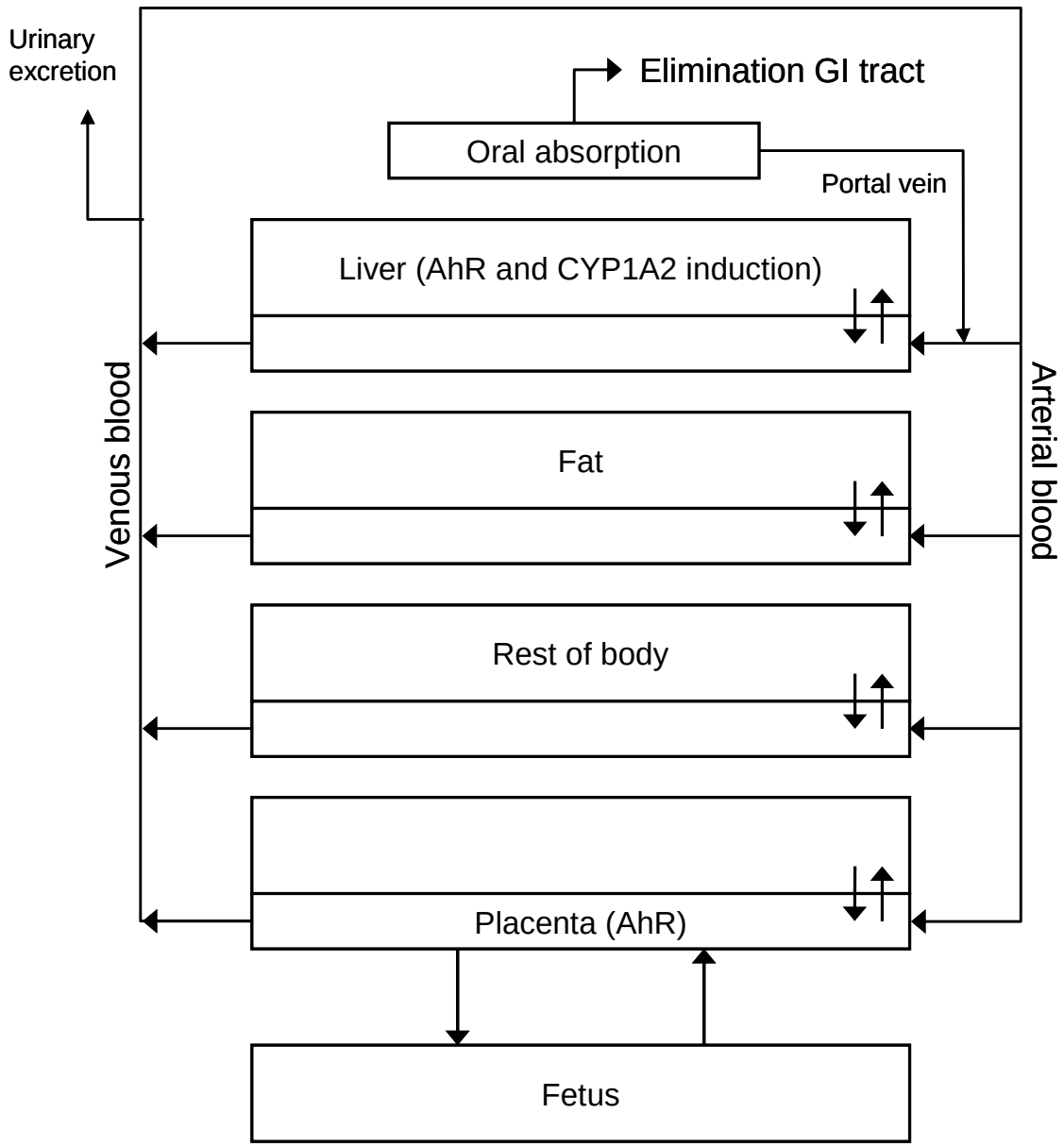


Figure 3-11. Conceptual representation of PBPK model for rat developmental exposure to TCDD.

Source: Emond et al. (2004).

The biological relationship between TCDD ―sequestration" by liver protein and its ―elimination" by the liver is not entirely clear. TCDD is metabolized slowly by unidentified enzymes. CYP1A2 is known to metabolize TCDD based on studies in CYP1A2 knockout mice (Diliberto et al., 1999, 1997), in which the metabolic profile is different compared to wild-type mice. However, because several metabolites appear in the feces of CYP1A2 knock out mice, it

is assumed that there are other enzymes involved in TCDD metabolism. TCDD binds to AhR and induces not only CYP1A2, but also CYP1A1, CYP1B1, and several UDP-glucuronosyltransferase and transporters (Gasiewicz et al., 2008). Both hydroxylated and glucuronidated hydroxyl metabolites are found in the feces of animals treated with TCDD (Hakk et al., 2009). Because the exact enzymes involved with TCDD are unknown and yet the metabolism is induced by TCDD, an assumption of increased elimination rate of TCDD in proportion to the induction of CYP1A2 is made. In the PBPK model, CYP1A2 is also needed because TCDD binds to rat, mouse, and human CYP1A2 (Staskal et al., 2005; Diliberto et al., 1999). Thus, CYP1A2 induction is necessary to describe TCDD pharmacokinetics due to TCDD binding. Hence, CYP1A2 can be used as a marker of Ah-receptor induction of ―TCDD metabolizing enzymes." Other models use AhR occupancy as a marker of induction of ―TCDD metabolizing enzymes" (Kohn et al., 2001; Andersen et al., 1997).

Figure 3-11 depicts the structure of the rat developmental-exposure PBPK model (Emond et al., 2004). This model was developed to describe the relationship between maternal TCDD exposure and fetal TCDD concentration during critical windows of susceptibility in the rat. In formulating this PBPK model, Emond et al. (2004) reduced the original 8-compartment model for TCDD in adult rats by Wang et al. (1997) to a 4-compartment (i.e., liver, fat, placenta, and rest of the body) model for maternal rat. Activation of the placental compartment and a separate fetal compartment occurs during gestation (Emond et al., 2004).

3.3.4.3.2.2. ***Mathematical representation***

The key equations of the PBPK model of Emond et al. (2004) are reproduced in Text Boxes 3-1 and 3-2, whereas those from Emond et al. (2006; 2005) are listed in Table 3-7. The rate of change of TCDD in the various tissue compartments is modeled on the basis of diffusion limitation considerations. Accordingly, mass balance equations are used to compute the rate of change in the tissue (i.e., intracellular compartment) and tissue blood (i.e., extracellular compartment). The membrane transfer of TCDD is computed using a permeation coefficient-surface area cross product (PA) for each tissue. Metabolism and binding of TCDD to the AhR and inducible hepatic protein (CYP1A2) are described in the liver. The total mass in the liver is then apportioned between free dioxin (C_{lip}) and bound forms of TCDD (see Figure 3-12). The dose- and time-dependent induction of hepatic CYP1A2 in the liver is

Table 3-7. Equations used in the TCDD PBPK model of Emond et al. (2006)

Aspect	Equation
Body-weight growth with age	$BW_{time}(g) = BW_T0 \times \left(\frac{0.41 \times time}{1402.5 + time} \right)$
Cardiac output	$Qc(mL/hr) = QCCAR \times 60 \left(\frac{BW}{1000} \right)^{0.75}$ A factor of 60 corresponds to the conversion of minutes to hours, and 1,000 is used for the conversion of BW from grams to kilograms.
Blood compartment	$Cb(nmol/mL) = \frac{\left[(Qf \times Cfb) + (Qre \times Creb) + (Qli \times Clib) + lymph \right]}{Qc} - \frac{(Cb \times CLURI)}{Qc}$
Tissue compartment (fat, rest of the body)	
Tissue blood subcompartment	$\frac{dAtb}{dt}(nmol/mL) = Qt(Ca - Ctb) - PAt\left(Ctb - \frac{Ct}{Pt} \right)$ $Ctb(nmol/mL) = \frac{Atb}{Wtb}$
Tissue cellular matrices	$\frac{dAt}{dt}(nmol/mL) = PAt\left(Ctb - \frac{Ct}{Pt} \right)$ $Ct(nmol/mL) = \frac{At}{Wt}$
Liver tissue compartment	
Tissue blood subcompartment	$\frac{dAlib}{dt}(nmol/mL) = Qli(Ca - Clib) - PALI(Clib - Clifree) + input_{oral}$ $Clib(nmol/mL) = \frac{Alib}{WLIB}$
Tissue cellular matrices	$\frac{dAli}{dt}(nmol/mL) = PALI(Clib - Clifree) - (KBILE_LI \times Clifree \times WLI)$ $Cli(nmol/mL) = \frac{Ali}{Wli}$
Free TCDD concentration in liver	$Clifree(nmol/mL) = Cli - \left[Clifree \times PLI + \left(\frac{LIBMAX \times Clifree}{KDLI + Clifree} \right) + \left(\frac{CYP1A2 \times Clifree}{KDLI1A2 + Clifree} \right) \right]$
Concentration bound to AhR in hepatic tissue	$Ct_{AhRbound}(nmol/mL) = \frac{LIBMAX \times Clifree}{KDLI + Clifree}$ All other induction processes and equations have been described and presented by Wang et al. (1997).

Table 3-7. Equations used in the TCDD PBPK model of Emond et al. (2006) (continued)

Aspect	Equation
Gastrointestinal absorption and distribution of TCDD to the portal lymphatic circulation	
Amount of TCDD remaining in lumen cavity	$\frac{dLumen}{dt}(nmol/hr) = \left[\left(KST + KABS\right) \times lumen\right] + intake$ Lumen in the amount of TCDD remaining in the GI tract (nmol); intake is the rate of intake of TCDD during a subchronic exposure (nmol/hr).
Amount of TCDD eliminated in the feces	$\frac{dFeces}{dt}(nmol/hr) = KST \times lumen$
Absorption rate of TCDD to the blood via the lymphatic circulation	$\frac{dLymph}{dt}(nmol/hr) = KABS \times lumen \times 0.7$
Absorption rate of TCDD by the liver via portal circulation	$\frac{dPortal}{dt}(nmol/hr) = KABS \times lumen \times 0.3$

Note: Key parameters and abbreviations are defined in Table 3-8.

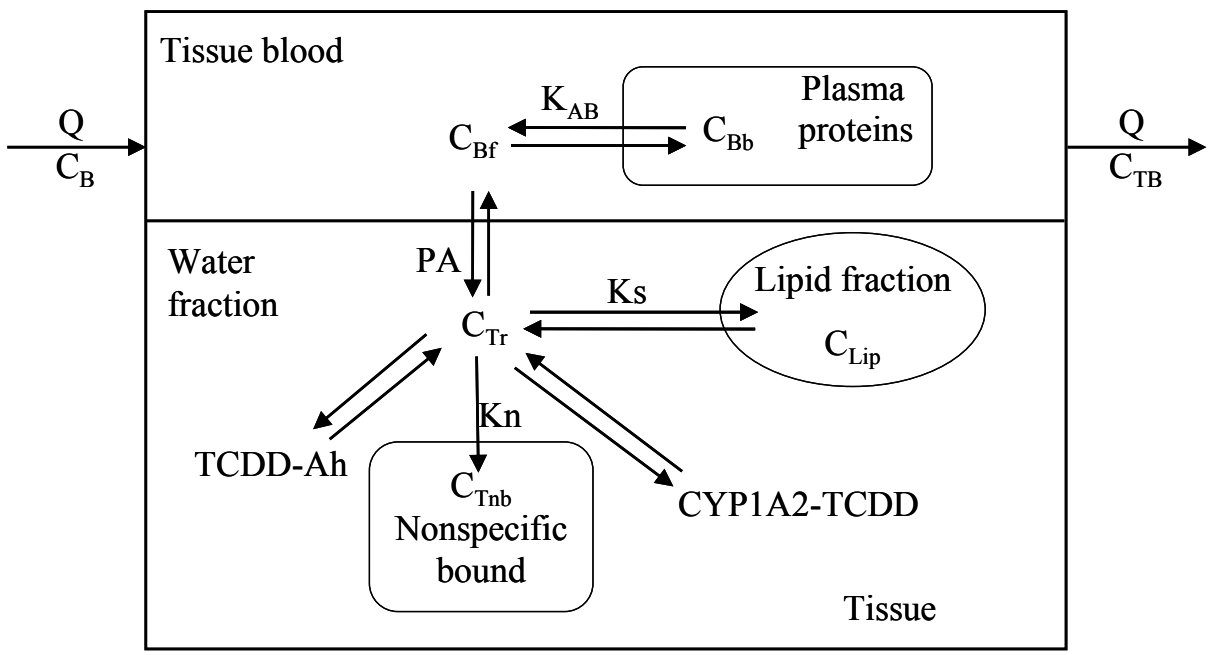


Figure 3-12. TCDD distribution in the liver tissue.

Source: Wang et al. (1997).

described per Wang et al. (1997) and Santostefano et al. (1998). Accordingly, the amount of CYP1A2 in the liver was computed as the time-integrated product of inducible production and a simple first-order loss process (Wang et al., 1997):

$$\frac{dCYP_{1A2}}{dt} = S(t)K_0 \; - \; K_2 C_{A2t} \qquad \text{(Eq. 3-19)}$$

In this expression, CYP_{1A2} is the concentration of the enzyme (nmol/g), K_2 is the rate constant for the first-order loss (hour^{-1}), C_{A2t} is the concentration of CYP1A2 in the liver (nmol/g), K_0 is the basal rate of production of CYP1A2 in the liver (nmol/g/hr), and $S(t)$ (unitless) is a multiplicative stimulation factor for CYP1A2 production in the form of a Hill-type function (see Section 3.3.2.3):

$$S(t) = 1 + \frac{In_{A2}(C_{Ah\text{-}TCDD})^h}{(IC_{A2})^h + (C_{Ah\text{-}TCDD})^h} \qquad \text{(Eq. 3-20)}$$

where, $S(t)$ is the stimulation function, In_{A2} is the maximum fold of CYP1A2 synthesis rate over the basal rate, $C_{Ah\text{-}TCDD}$ is the concentration of AhR occupied by TCDD, and IC_{A2} is the Michaelis-Menten constant of CYP1A2 induction (nM). The dose-dependent or variable elimination of TCDD was described using the relationship:

$$KBILE\ LI = \left[\frac{CYP1A2_{induced} - CYP1A2_{basal}}{CYP1A2_{basal}}\right] \times Kelv \qquad \text{(Eq. 3-21)}$$

where $CYP1A2_{induced}$ is the concentration of induced CYP1A2 (nmol/mL), $CYP1A2_{basal}$ is the basal concentration of CYP1A2 (nmol/mL), and *kelv* is the interspecies constant adjustment for the elimination rate (hour^{-1}).

There are various ways of formulating the dose-dependent elimination as a function of the level of CYP1A2, and the above equation (used by the authors) can be viewed as one means of describing this behavior quantitatively. The numerator in the equation above will always be

greater than zero when there is TCDD in the system (including TCDD derived from either background exposures or defined external sources). Consequently, the rate of elimination will correspond to a nonzero value for situations involving TCDD exposures.

It should be noted that $CYP1A2_{induced}$ should always be greater than $CYP1A2_{basal}$ for any CYP1A2-mediated elimination to take place in Eq. 3-21. This will always be the case whenever TCDD is present in the liver because the induced levels of CYP1A2 are an estimate of "total" enzyme content at any time point including basal levels. Furthermore, Eq. 3-21 is a mathematical representation of the induced elimination rate of TCDD by the liver that is numerically influenced by the scalable parameter *kelv*. Hence, the mathematical description for the elimination of TCDD by the liver is dominated by the level of CYP1A2 induction (as mathematically influenced by the Hill coefficient in Eq. 3-20) and the numerical estimation of the *kelv* constant. The interrelationship between the induction Hill coefficient (*h* in Eq. 3-20) and *kelv* becomes a critical consideration when data are used to fit both parameters as will be illustrated in the sensitivity analysis of the PBPK model.

The gestational model included mathematical descriptions for the changes in physiological parameters such as body weight, cardiac output, and tissue volumes consistent with experimental observations in pregnant rats. Additionally, this model included a fetal compartment and considered the transfer of TCDD between the placental and fetal compartments as a diffusion-limited process (rather than a perfusion-limited) process (see Text Boxes 3-1 and 3-2).[27]

[27] Diffusion limited, sometimes also known as "membrane limited," means a chemical's movement from one side of the membrane to the other is limited by the membrane. Thus, the membrane, in this case, is a limiting factor for uptake. Perfusion limited, also known as "flow limited" indicates that a chemical is so rapidly taken up (e.g., by the tissue from the blood) that the flow rate is the only limiting factor.

Text Box 3-1.

Variation of Body Weight with Age: $BW_{Time}(g) = BWinitial \times \left(\frac{0.41 \times Time}{1402.5 + Time} \right)$

Cardiac Output: $Qc(mL/h) = Qcc \times 60 \left(\frac{BWmother}{1,000} \right)^{0.75}$

A factor of 60 corresponds to the conversion of minutes to hours, and 1,000 is the conversion of body weight from g to kg.

Blood Compartment:

$$Cb(nmol/mL) = \frac{((Qf \times Cfb) + (Qre \times Creb) + (Qli \times Clib) + (Qpla \times Cplab) + Lymph)) - (Cb \times Clru)}{Qc}$$

Text Box 3-2.

Placenta Tissue Compartment

(a) Tissue-blood subcompartment

$$\frac{dAplab}{dt}\left(nmol\ /\ h\right) = Qpla(Ca - Cplab\right) + PApla(Cplab - Cplafree)$$

$$Cplab = \frac{Aplab}{Wplab}$$

(b) Tissue cellular matrices

$$\frac{dApla}{dt}\left(nmol\ /\ h\right) = PApla(Cplab - Cplafree\right) - \frac{dApla_fet}{dt} + \frac{dAfet_pla}{dt}$$

$$Cpla(nmol\ /\ mL) = \frac{Apla}{Wpla}$$

Free TCDD Concentration in Placenta

$$Cplafree(nmol\ /\ mL) = Clpla - \left[(Cplafree \times Ppla + \left(\frac{Plab\max \times Cplafree}{Kdpla + Cplafree}\right)\right]$$

Dioxin Transfer from Placenta to Fetuses

$$\frac{dAPla_fet}{dt}(nmol\ /\ h) = Cl_{Pla_fet} \times Cpla$$

Dioxin Transfer from Fetuses to Placenta

$$\frac{dAfet_Pla}{dt}(nmol\ /\ h) = Cl_{Pla_fet} \times CfetV$$

Fetal Dioxin Concentration (Fetuses 5 = Per Litter)

$$\frac{dAfet}{dt}(nmol\ /\ h) = \frac{dAPla_fet}{dt} - \frac{dAfet_Pla}{dt}$$

$$Cfet(nmol\ /\ h) = \frac{Afet}{Wfet}$$

$$CfetV(nmol\ /\ mL) = \frac{Cfet}{Pfet}$$

3.3.4.3.2.3. ***Parameter estimation***

Table 3-8 lists the numerical values of the adult rat and human PBPK models of Emond et al. (2006; 2005; 2004). Additionally, Table 3-8 lists the numerical values that can be used in a mouse PBPK model. The values for key input parameters of the rat gestational model are summarized in Table 3-8 as well as Figure 3-13.

The parameters for the rat model were obtained primarily from Wang et al. (1997) except that the value of the affinity constant for CYP1A2 was slightly changed from 0.03 to 0.04 nmol/mL to get a better fit to experimental data (Emond et al., 2004), and the variable elimination parameter (*kelv*) was obtained by optimization of model fit to kinetic data from Santostefano et al. (1998) and others (Emond et al., 2006; Emond et al., 2005; Wang et al., 1997). Wang et al. (1997) used measured tissue weights whereas the tissue blood flows and tissue blood weights were obtained from International Life Sciences Institute (ILSI, 1994). The partition coefficients (which were similar to those of Leung et al.,1990; Leung et al., 1988), the permeability × area (PA) value for tissues, the dissociation constant for binding to CYP1A2 (IC_{A2}), and the Hill coefficient (*h*) were estimated using a two-stage process of fitting to dose-response and time-course data on TCDD tissue distribution (Wang et al., 1997). In the initial stage, the experimental data of arterial blood concentrations were used as input to the individual compartment to estimate the parameters; then, with the values obtained during stage one as initial estimates, those unknown parameters were re-estimated by solving the entire model at once using an optimization route (Wang et al., 1997). The receptor concentrations and dissociation constant of TCDD bound to AhR were obtained by fitting the model to TCDD tissue concentration combined with enzyme data reported by Santostefano et al. (1998) whereas the basal CYP1A2 in liver was based on literature data (Wang et al., 1997).

The parameters for the human PBPK model were primarily based on the rat model (Emond et al., 2006; Emond et al., 2005; Wang et al., 1997). Specifically, the blood fraction in the tissues, the tissue:blood partition coefficients, tissue permeability coefficient, the binding affinity of TCDD to AhR and CYP, and the maximum binding capacity in the liver for AhR were all set equal to the values used in the rat model. The species-specific elimination constant, *kelv*, was estimated by fitting to human data (Emond et al., 2005).

Table 3-8. Parameters of the PBPK model for TCDD

Parameter description	Symbol	Parameter values					
		Human nongestational[a]	Human gestational[a]	Mouse nongestational	Mouse gestational	Rat nongestational	Rat gestational
Body weight (g)	BW	Calculated	Calculated	23-28	23-28	125-250[b]	85-190[b]
Cardiac output (mL/hour/kg)	QCCAR	15.36[c,d]	Calculated	275[c]	275[c]	311.4[e]	311.4[e]
Tissue (intracellular) volumes (fraction of BW)							
Liver	WLI0	Calculated	Calculated	0.0549[f]	0.0549[f]	0.036[e]	0.036[e]
Fat	WF0	Calculated	Calculated	0.069[e]	Calculated	0.069[e]	Calculated
Tissue blood volumes							
Liver (fraction of WLI0)	WLIB0	0.266[e]	0.266[e]	0.266[e]	0.266[e]	0.266[e]	0.266[e]
Fat (fraction of WF0)	WFB0	0.05[e]	0.05[e]	0.05[e]	0.05[e]	0.05[e]	0.05[e]
Rest of body (fraction of WRE0)	WREB0	0.03[e]	0.03[e]	0.03[e]	0.03 [e]	0.03[e]	0.03[e]
Placenta tissue fraction of tissue blood weight (unitless)	WPLAB0	N/A	0.5[g]	N/A	0.5[e]	N/A	0.5[e]
Tissue blood flow (fraction of cardiac output)							
Liver	QLIF	0.26[c]	0.26[c]	0.161[f]	0.161[f]	0.183[e]	0.183[e]
Fat	QFF	0.05[c]	0.05[c]	0.07[h]	0.07[h]	0.069[e]	0.069[e]
Placenta	QPLAF	N/A	Calculated	N/A	Calculated	N/A	Calculated
Tissue permeability (fraction of tissue blood flow)							
Liver	PALIF	0.35[e]	0.35[e]	0.35[e]	0.35[e]	0.35[e]	0.35[e]
Fat	PAFF	0.12[i]	0.12[i]	0.12[i]	0.12[i]	0.091[e]	0.091[e]
Placenta diffusional permeability fraction (unitless)	PAPLAF	N/A	0.3[g]	N/A	0.03[g]	N/A	0.3[g]
Rest of body	PAREF	0.03[e]	0.03[e]	0.03[e]	0.03[e]	0.0298[e]	0.0298[e]

Table 3-8. Parameters of the PBPK model for TCDD (continued)

Parameter description	Symbol	Parameter values					
		Human nongestational[a]	Human gestational[a]	Mouse nongestational	Mouse gestational	Rat nongestational	Rat gestational
Partition coefficient							
Liver	PLI	6[e]	6[e]	6[e]	6[e]	6[e]	6[e]
Fetus/blood partition coefficient (unitless)	PFETUS	N/A	4[j]	N/A	4[j]	N/A	4[j]
Placenta/blood partition coefficient (unitless)	PPLA	N/A	1.5[j]	N/A	3[g]	N/A	1.5[j]
Fat	PF	100[e]	100[e]	400[i]	400[i]	100[e]	100[e]
Rest of body	PRE	1.5[e]	1.5[e]	3[k]	3[k]	1.5[e]	1.5[e]
Metabolism constants							
Urinary clearance elimination (mL/hour)	CLURI	4.17E-08[l]	4.17E-08[l]	0.09[i]	0.09[i]	0.01[j]	0.01[j]
Clearance—transfer from mother to fetus (mL/hour)	CLPLA_FET	N/A	16[e]	N/A	0.17[i]	N/A	0.17[i]
Liver (biliary elimination and metabolism; $hour^{-1}$)	KBILE_LI	Inducible	Inducible	Inducible	Inducible	Inducible	Inducible
Interspecies constant ($hour^{-1}$)	KELV	0.0011[i]	0.0011[i]	0.4[i]	0.4[i]	0.15[e]	0.15[e]
AhR							
Affinity constant in liver (nmol/mL)	KDLI	0.1[e]	0.1[e]	0.0001[e]	0.0001[e]	0.0001[e]	0.0001[e]
Binding capacity in liver (nmol/mL)	LIBMAX	0.35[e]	0.35[e]	0.00035[e]	0.00035[e]	0.00035[e]	0.00035[e]
Placenta binding capacity (nmol/mL)	PLABMAX	N/A	0.2[j]	N/A	0.0002[j]	N/A	0.0002[j]
Affinity constant protein (AhR) in placenta (nmol/mL)	KDPLA	N/A	0.1[j]	N/A	0.0001[j]	N/A	0.0001[j]
CYP1A2 induction parameters							
Dissociation constant CYP1A2 (nmol/mL)	KDLI2	40[j]	40[j]	0.02[i]	0.02[i]	0.04[j]	0.04[j]
Degradation process CYP1A2 (nmol/mL)	CYP1A2_1OUTZ	1,600[e]	1,600[e]	1.6[e]	1.6[e]	1.6[e]	1.6[e]
Dissociation constant during induction (nmol/mL)	CYP1A2_1EC50	130[e]	130[e]	0.13[e]	0.13[e]	0.13[e]	0.13[e]
Basal concentration of CYP1A2 (nmol/mL)	CYP1A2_1A2	1,600[e]	1,600[e]	1.5[k]	1.5[k]	1.6[e]	1.6[e]
First-order rate of degradation ($hour^{-1}$)	CYP1A2_1KOUT	0.1[e]	0.1[e]	0.1[e]	0.1[e]	0.1[e]	0.1[e]
Time delay before induction process (hour)	CYP1A2_1TAU	0.25[e]	0.25[e]	1.5[k]	1.5[k]	0.25[e]	0.25[e]
Maximal induction of CYP1A2 (unitless)	CYP1A2_1EMAX	9,300[i]	9,300[i]	600[e]	600[e]	600[e]	600[e]

Table 3-8. Parameters of the PBPK model for TCDD (continued)

Parameter description	Symbol	Parameter values					
		Human nongestational[a]	Human gestational[a]	Mouse nongestational	Mouse gestational	Rat nongestational	Rat gestational
Other constants							
Oral absorption constant ($hour^{-1}$)	KABS	0.06[i]	0.06[i]	0.48[i]	0.48[i]	0.48[e]	0.48[e]
Gastric nonabsorption constant ($hour^{-1}$)	KST	0.01[m]	0.01[m]	0.30[i]	0.30[i]	0.36[e]	0.36[e]

[a]Units for human nongestational parameters are L rather than mL and kg rather than g where applicable.
[b]Body weight varies by study (Emond et al., 2006; Emond et al., 2005; Emond et al., 2004).
[c]Krishnan and Andersen (1991).
[d]Units are L/kg/hr.
[e]Wang et al. (1997).
[f]ILSI (1994).
[g]Fixed.
[h]Leung et al. (1990).
[i]Optimized.
[j]Emond et al. (2006; 2005; 2004).
[k]Wang et al. (2000).
[l]Lawrence and Gobas (1997).
[m]Calculated to estimate 87% bioavailability of TCDD in humans (Poiger and Schlatter, 1986).

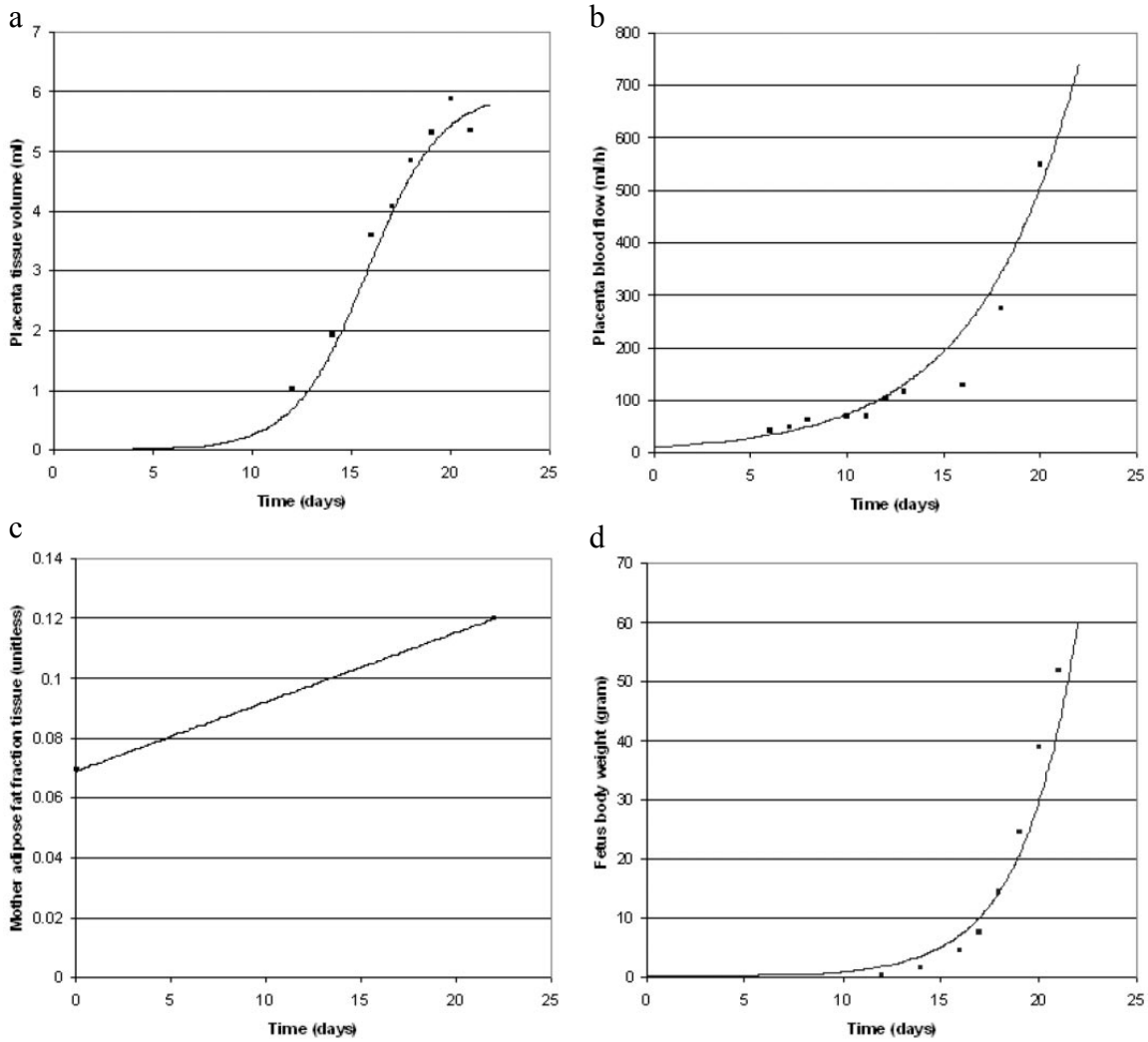


Figure 3-13. Growth rates for physiological changes occurring during gestation.
(a) Placental growth during gestation (calculated for n = 10 placenta). Experimental data from Sikov (1970). (b) Blood flow rate in placental compartment during gestation. Experimental data from Buelke-Sam et al. (1982a; 1982b). (c) Fat fraction of body weight during gestation. Experimental data came from Fisher et al. (1989), and (d) Fetal growth during gestation. Experimental data obtained from Sikov (1970).

For the gestational rat model, the parameters describing the growth of the placental and fetal compartments as well as temporal change in blood flow during gestation were incorporated based on existing data. Exponential equations for the growing compartments were used (see Figure 3-13), except for adipose tissue, for which a linear growth increment based on literature data was specified. All relevant physiological parameters for the pregnant rat were obtained from the literature while remaining input parameters were set equal to that of the nonpregnant rat

(obtained from Wang et al., 1997); see Table 3-8. The current version of the rat gestational model contains parameters for variable elimination from Emond et al. (2006; Table 3-8) and still provides essentially the same predictions as the original publication (Emond et al., 2004).

3.3.4.3.2.4. ***Model performance and degree of evaluation***

The PBPK model of Emond et al. (2006; 2005; 2004) had parameters estimated by fitting to dose and time-course data, so that the resulting model consistently reproduced available kinetic data. The same model structure with a single set of species-specific parameters could reproduce the kinetics of TCDD following various doses and exposure scenarios not only in the rat but also in humans. The simulations of the PBPK model of Emond et al. (2006) have been compared with two sets of previously published rat data: blood pharmacokinetics following a single dose of 10 µg/kg (the dose corresponding to the mean effective dose for induction of CYP1A2) (Santostefano et al., 1998) (see Figure 3-14); and hepatic TCDD concentrations following chronic exposure to average daily exposures of 3.5 to 125 ng/kg (Walker et al., 1999) (see Figure 3-15). It is relevant to note that the PBPK model of Emond et al. (2006, 2004) is essentially a reduced version of the Wang et al. (1997) model, and it, therefore, provides simulations of liver and fat concentrations of TCDD that deviated by not more than 10−15% of those of Wang et al. (1997). The nongestational model of Emond et al. (2004) was calibrated against kinetic data in liver, fat, blood, and rest of body of female Sprague-Dawley rats given a single dose of 10 µg TCDD/kg (data from Santostefano et al., 1996) and in liver and fat of male Wistar rats treated with a loading dose of 25 ng/kg followed by a weekly maintenance dose of 5 ng TCDD/kg by gavage (data from Krowke et al., 1989).

The gestational rat PBPK model was calibrated against the following kinetic data sets (Emond et al., 2004):

- TCDD concentration in blood, fat, liver, placenta, and fetus of female Long−Evans rats given 1, 10, or 30 ng/kg, 5 days/week, for 13 weeks prior to mating followed by daily exposure through parturition (Hurst et al., 2000b);
- TCDD concentration in tissues (liver, fat), blood, placenta and fetus determined on gestation day (GD) 16 and GD 21 following a single dose of 0.05, 0.8, or 1 µg/kg given on GD 15 to pregnant Long-Evans rat (Hurst et al., 2000a);

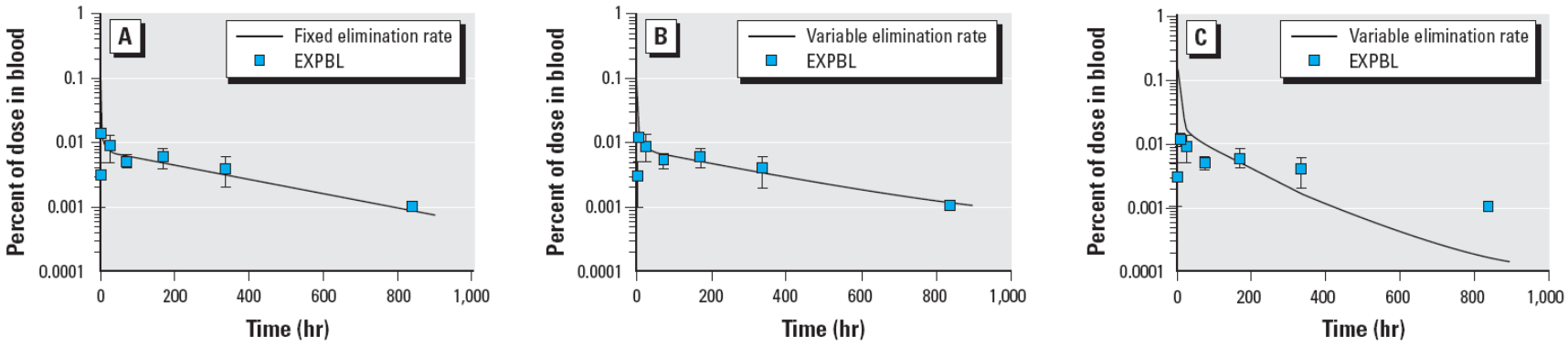


Figure 3-14. Comparisons of model predictions to experimental data using a fixed elimination rate model with hepatic sequestration (A) and an inducible elimination rate model with (B) and without (C) hepatic sequestration.

EXBL, experimental blood levels. Model predictions were compared with the data of Santostefano et al. (1998), where female rats were exposed to a single oral dose of 10 µg of TCDD/kg BW. Error bars are ∟SD.

Source: Emond et al. (2006).

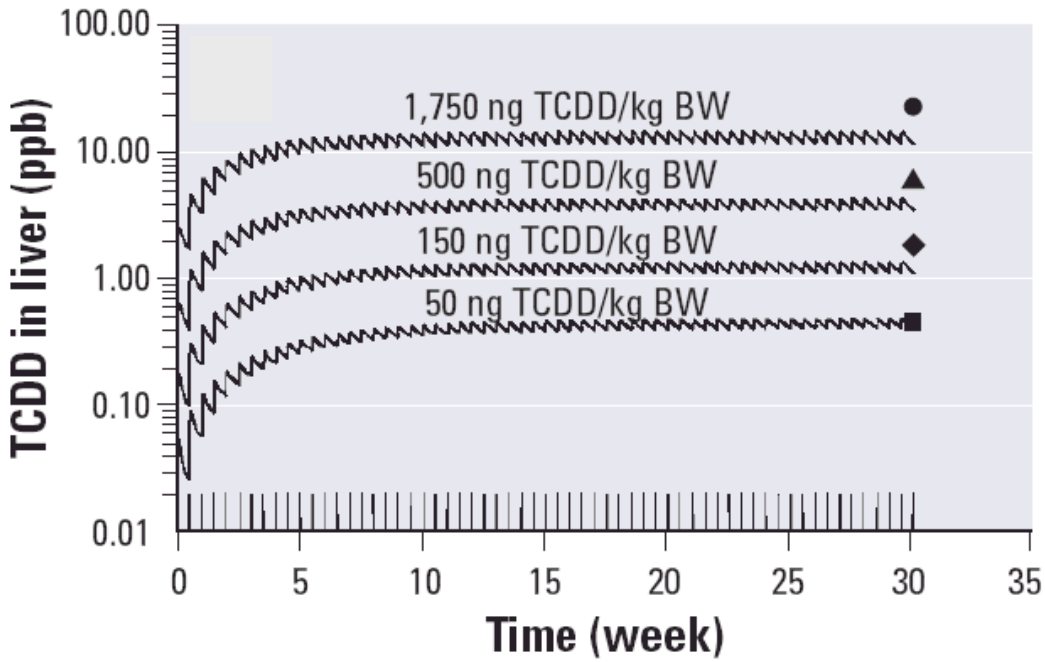


Figure 3-15. PBPK model simulation of hepatic TCDD concentration (ppb) during chronic exposure to TCDD at 50, 150, 500, or 1,750 ng TCDD/BW using the inducible elimination rate model compared with the experimental data measured at the end of exposure.

Source: Emond et al. (2006).

- Maternal and fetal tissue concentrations on GD 9, GD 16, and GD 21 after a single dose of 1.15 µg TCDD/kg given to Long−Evans rats on GD 9 or GD 15 (Hurst et al., 1998); and
- Fetal TCDD concentrations determined on GD 19 and GD 21 in rats exposed to 5.6 µg TCDD/kg on GD 18 (Li et al., 2006).

Furthermore, the scaled rat model was shown to be capable of simulating human data (see Figures 3-16 and 3-17). In this regard, it is useful to note that the computational version of the PBPK model of Emond et al. (2006; 2005) also contained the necessary equation to transform the model output of blood concentration into serum lipid-adjusted concentration of TCDD. This conversion is calculated by dividing the estimated total blood TCDD levels with the product of two constants, the serum portion of total blood and the lipid content in serum. The human model of Emond et al. (2005; Emond model) has advantages for improving the TCDD dosimetry used in existing human epidemiologic studies because the model predicts the

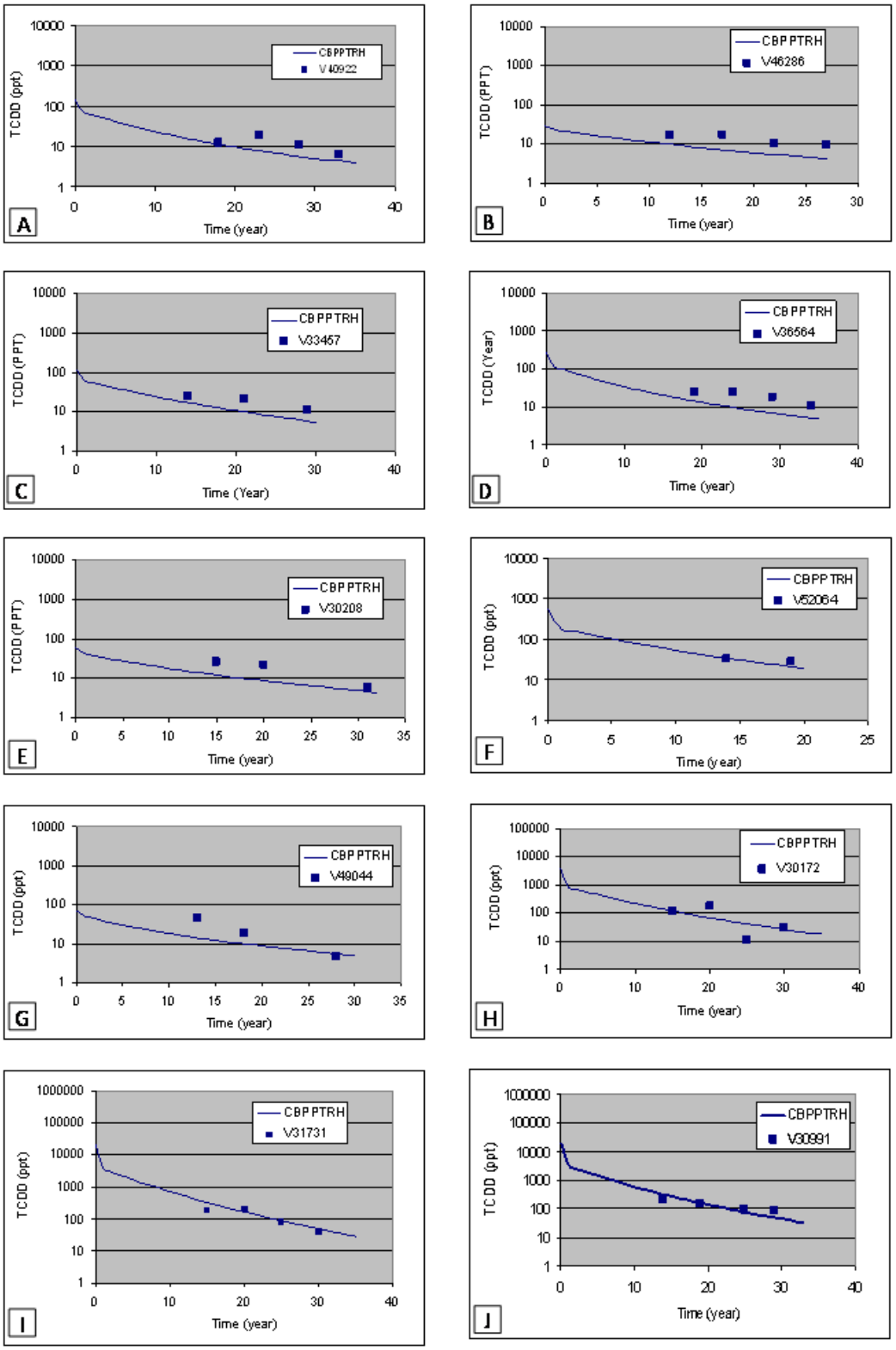


Figure 3-16. Model predictions of TCDD blood concentration in 10 veterans (A−J) from Ranch Hand Cohort.
Source: Emond et al. (2005).

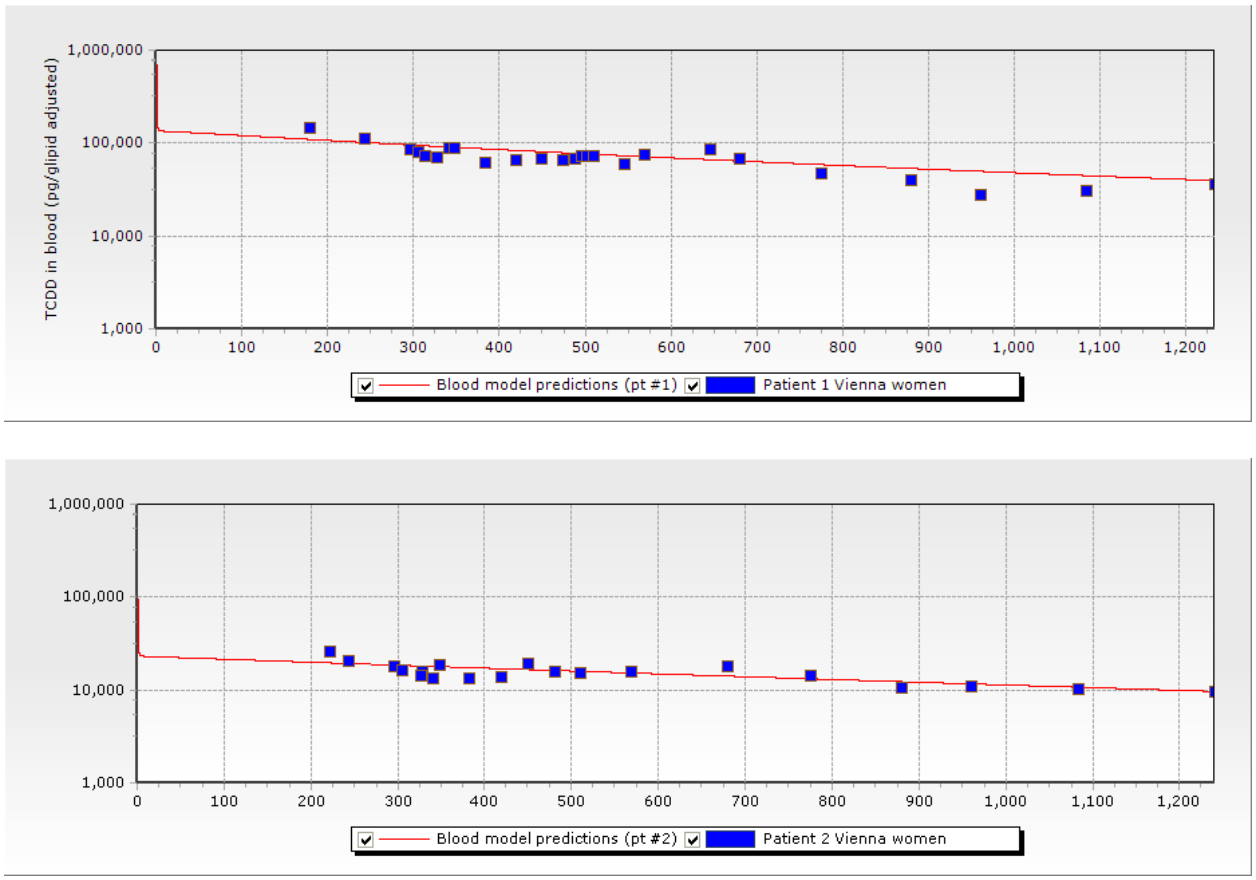


Figure 3-17. Time course of TCDD in blood (pg/g lipid adjusted) for two highly exposed Austrian women (patients 1 and 2).
Symbols represent measured concentrations, and lines represent model predictions. These data were used as part of the model evaluation (Geusau et al., 2002).

Source: Emond et al. (2005).

redistribution of TCDD within the body (to stores in fat and liver) based on physiological principles. However, because the dose-dependency of metabolic elimination in the Emond model was not calibrated to human data, it is important to review the predictions of this model using a database of human observations that is as extensive as possible and a spread of internal TCDD concentrations that is as wide as possible. Thus, presented below is a juxtaposition of modeled elimination rates from the Emond model with observations for two highly exposed Austrian patients (severe intoxication of ~~"u~~nknown origin" (Geusau et al., 2001) and 9 of

10 Ranch Hand veterans[28] used for the original ―validation" comparisons presented in the Emond et al. (2005)).

Figure 3-18 shows the time course of the declines in TCDD serum concentrations in two highly exposed Austrian subjects compared with the Emond model results. The comparison in Figures 3-17 and 3-18 indicates that the Emond model adequately describes the rate of TCDD elimination for the more highly exposed Austrian patients but predicts a somewhat faster rate of decline than that observed for the less heavily exposed patient.

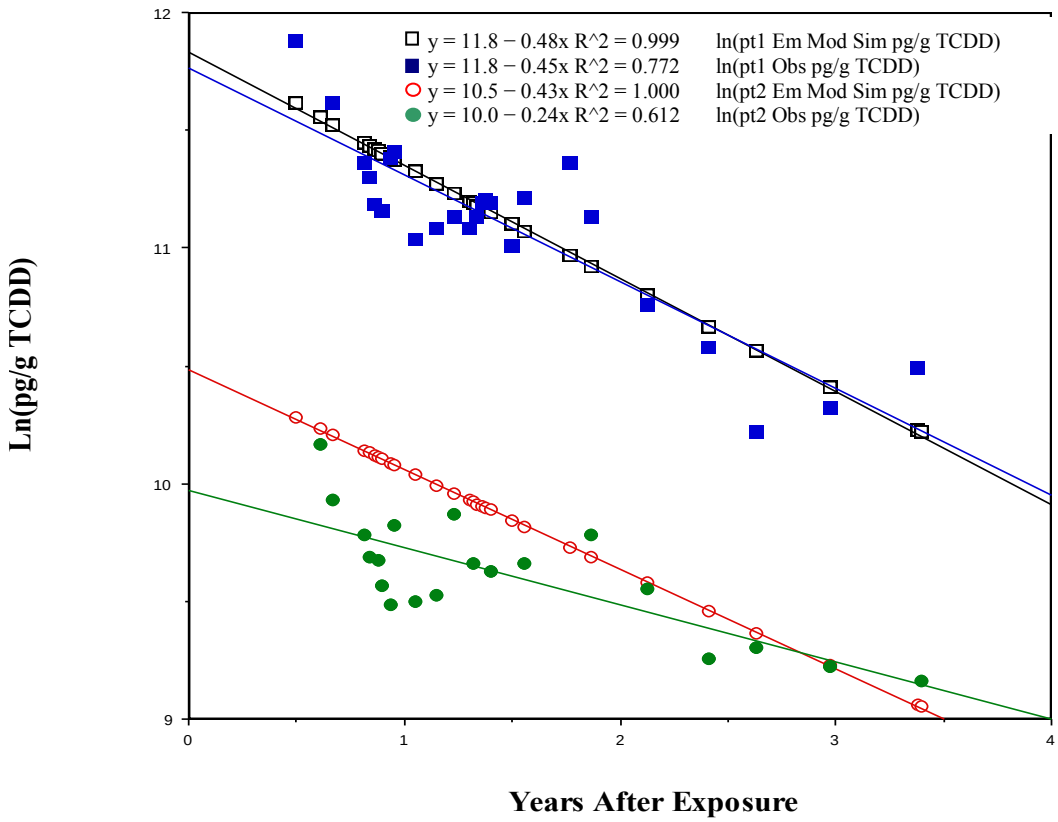


Figure 3-18. Observed vs. Emond et al. (2005) model simulated serum TCDD concentrations (pg/g lipid) over time (ln = natural log) in two Austrian women.

Data from Geusau et al. (2002).

[28] In preliminary comparisons, the simulation run for the 10th Ranch Hand veteran appeared anomalous and was, therefore, excluded from this summary.

Figure 3-19 shows the results of combining the simulated and observed rates of loss for a group of Austrian and Ranch Hand subjects evaluated by Emond et al. (2005), counting only one data point per person. The X-axis in this figure is the TCDD serum concentration at the midpoint of the observations for each subject. The error bars in the figure represent ±1 standard error. The results of this figure illustrate two points: (1) the Emond model simulation (open squares) are generally very close to the actual data (solid circles) for the nine Ranch Hand subjects (clustered toward lower left corner) and one of the two Austrian patients (upper right corner); and (2) both the Emond model simulation results and the actual data show a linear trend, and linear regression lines were plotted, respectively, as shown in Figure 3-19.

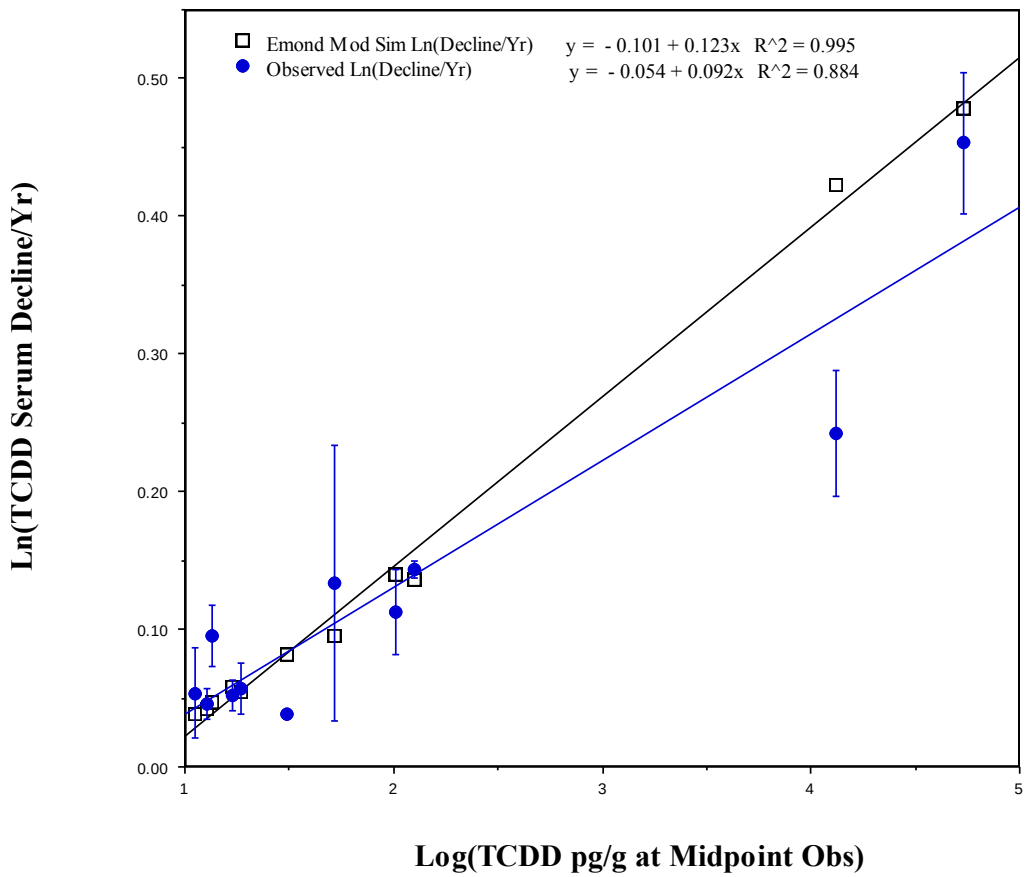


Figure 3-19. Comparison of the dose dependency of TCDD elimination in the Emond model vs. observations of nine Ranch Hand veterans and two highly exposed Austrian patients.
Circles are observed data.

Table 3-9 presents the results of regression analyses of the observed rates of decline in relation to the estimated TCDD serum levels at the midpoint of the observations for each subject in the Ranch Hand study (see Figure 3-19). These results indicate that some appreciable dose dependency of TCDD elimination is unequivocally supported. However, the central estimate of the slope of the relationship between the log of the TCDD elimination rate and the log of the TCDD level is only about 75% of that expected under the Emond et al. PBPK model (i.e., 0.092 ÷ 0.123 = 0.748).

Table 3-9. Regression analysis results for the relationship between $\log_{10}$ serum TCDD at the midpoint of observations and the $\log_{10}$ of the rate constant for decline of TCDD levels using Ranch Hand data

Item	Aspect	Value
Summary of fit	RSquare	0.894
	RsquareAdj	0.871
	Root mean square error	0.044
	Mean responses	0.130
	Observations (or sum weights)	11
Parameter estimates	Intercept	
	Estimate	−0.054
	Standard deviation	0.026
	t ratio	−2.07
	Prob>|t|	0.0679
	Log (TCDDpg/g)	
	Estimate	0.092
	Standard error	0.011
	t ratio	8.28
	Prob>|t|	<0.0001

Overall, the conclusion from the above analysis is that the Emond model is reasonable to use, but the model might be improved by (1) including the two dose-independent pathways of elimination documented in the Geusau papers (GI elimination via the feces and loss via the sloughing of skin cells), and (2) reducing the extent of loss via the dose-dependent metabolism pathway from the liver (Harrad et al., 2003; Geusau et al., 2002) so that overall loss rates for the average elimination rates from the Ranch Hand veterans are maintained.

3.3.4.3.2.5. ***Sensitivity analysis of the physiologically based pharmacokinetic (PBPK) model***

A sensitivity analysis was performed on each of the animal and human Emond PBPK models to determine the most sensitive variables. In each case, all input variables in each model were included in the analysis. For equations where the parameter value varies with age according to an equation (body weight in all models, liver and adipose tissue fractions in the human models, and fetal weight, placental weight, and placental perfusion in the gestational models), a constant multiplier of 1.0 was included in each equation; then, for the sensitivity analysis, this value was varied by a fixed percentage to determine the relative effect of changing the compartmental weight fractions.

To perform the analysis, a representative dosing protocol was selected for each model to ensure the analysis was performed in dose ranges that were applicable to the overall health assessment. For each study modeled, multiple doses were used to investigate model sensitivity across a dosing range. Table 3-10 shows the dosing protocols selected for each model. For the human models, doses in the range of the identified reference dose and POD dose discussed in Section 4 were used in the analysis.

To perform the sensitivity analysis, variable values were varied by fixed percentages one at a time to determine the associated change in the average whole blood concentration. The blood concentration averages were calculated in each study in the same manner as in the main health assessment, as detailed in Appendix E and repeated for convenience in Table 3-10. To determine the local sensitivity of the whole blood concentration to each variable, the variable values were increased and decreased from the standard model configuration by 5%. This local analysis shows the effects of changing the variables by relatively small amounts to account for a theoretical level of uncertainty in the input parameters. To determine a more global sensitivity of the whole blood concentrations to each variable, the variable values were increased and decreased by 50%. In some cases, such a wide change may overestimate the actual uncertainty in the variable value in the literature; however, such a change is useful in helping to determine how the model sensitivity may change across large portions of the variable parameter space.

Table 3-10. Dosing protocols for human and animal models

Model	Study	Low dose	High dose	Averaging period
Rat	NTP (2006b); 105 weeks	3 ng/kg 5 days per week (2.14 ng/kg-day adjusted dose)	100 ng/kg 5 days per week (71.4 ng/kg-day adjusted dose)	105 weeks
Mouse	NTP (1982a); male mouse, 2-year duration	5 ng/kg biweekly (1.4 ng/kg-day adjusted dose)	200 ng/kg biweekly (71 ng/kg-day adjusted dose)	2 years
Rat gestational	Markowski et al. (2001)	20 ng/kg, single dose	180 ng/kg, single dose	Single day
Mouse gestational	Li et al. (2006)	2 ng/kg-day for GDs 1−3	100 ng/kg-day for GDs 1−3	3 days
Human	Standard lifetime scenario (daily intake for 70 years)	7×10^{-4} ng/kg-day	0.02 ng/kg-day	70 years
Human gestational	Standard gestational scenario (daily intake, pregnancy at age 45)	7×10^{-4} ng/kg-day	0.02 ng/kg-day	9 months of pregnancy

For each percentage change in the variable, the associated percentage change in the average whole blood concentration was recorded. Then, the elasticity was calculated as the percent change in the average whole blood concentration divided by the percent change in the variable value. Thus, variables where the magnitude of the elasticity is greater than 1 will induce a change of greater than 5% in the whole blood concentration when the variable value is changed by 5%. The sign of the elasticity indicates whether the whole blood concentration is positively or negatively correlated with the variable. The elasticities were examined, and a value of 0.1 was selected as a threshold to determine the most sensitive variables in each model. This value tended to represent a limit, with a cluster of variables having higher magnitude elasticities and the remaining variables having much lower elasticities. Variables were then ranked according to the magnitude of the elasticity in the case where the variables were increased by 5% for presentation.

Table 3-11 shows the most sensitive variables for the rat and mouse nongestational models and rat and mouse gestational models for the low and high doses when variables were increased by +5%. The associated elasticities are shown in each case. The only variable with elasticity above one is the Hill coefficient (*h* in Eq. 3-20). The other most sensitive variables are

Table 3-11. Most sensitive variables for the rat and mouse nongestational and gestational models

Variable	Variable description	Rat, low dose, +5% elasticity	Rat, high dose, +5% elasticity	Mouse, low dose, +5% elasticity	Mouse, high dose, +5% elasticity
Nongestational					
HILL	Hill coefficient	3.3	3.0	3.4	2.8
CYP1A2_1OUTZ	Induction concentration in degradation process (nmol/L)	−0.8	−0.8	−0.8	−0.7
CYP1A2_1A2	Induction basal concentration of 1A2 (nmol/L)	0.8	0.8	0.9	0.7
WLI0	Fractional liver weight (unitless)	−0.6	−0.7	−0.6	−0.6
CYP1A2_1EMAX	Maximum induction over basal effect (unitless)	−0.5	−0.7	−0.5	−0.6
KELV	Interspecies constant (hr^−1)	−0.3	−0.7	−0.5	−0.6
LIBMAX	Liver binding capacity (nmol/l)	−0.4	−0.4	−0.3	−0.3
CYP1A2_1EC50	Induction disassociation constant for 1A2 (nmol/L)	0.4	0.4	0.3	0.4
KDLI	Liver affinity proteins AhR (nmol/L)	0.3	0.2	0.3	0.3
KABS	Intestinal excretion and absorption constant (hr^−1)	0.3	0.3	0.3	0.3
KST	Gastric excretion and absorption constant (hr^−1)	−0.3	−0.3	−0.3	−0.3
Gestational					
HILL	Hill coefficient	1.2	1.4	0.6	1.4
WLI0	Fractional liver weight (unitless)	−0.4	−0.4	−0.2	−0.4
KABS	Intestinal excretion and absorption constant (hr^−1)	0.4	0.4	0.4	0.3
CYP1A2_1OUTZ	Induction concentration in degradation process (nmol/L)	−0.4	−0.4	−0.3	−0.4
KDLI2	Liver affinity proteins 1A2 (nmol/L)	0.4	0.4	0.2	0.3
KST	Gastric excretion and absorption constant (hr^−1)	−0.4	−0.3	−0.3	−0.3

Table 3-11. Most sensitive variables for the rat and mouse nongestational and gestational models (continued)

Variable	Variable description	Rat, low dose, +5% elasticity	Rat, high dose, +5% elasticity	Mouse, low dose, +5% elasticity	Mouse, high dose, +5% Elasticity
QCCAR	Cardiac output (l/kg-hr)	−0.3	−0.3	−0.4	−0.3
QFF	Adipose tissue blood flow fraction of cardiac output (unitless)	−0.2	−0.2	−0.4	−0.2
CYP1A2_1EMAX	Maximum induction over basal effect (unitless)	−0.2	−0.3	−0.1	−0.3
PAFF	Adipose diffusional permeability fraction (unitless)	−0.2	−0.2	−0.4	−0.2
LIBMAX	Liver binding capacity (nmol/L)	−0.1	−0.2	−0.1	−0.2
KDLI	Liver affinity proteins AhR (nmol/L)	0.1	0.1	0.1	0.2
CYP1A2_1EC50	Induction disassociation constant for 1A2 (nmol/L)	0.1	0.2	0.1	0.2
CYP1A2_1KOUT	Induction first-order rate of degradation (hr^−1)	−0.1	−0.2	0.0	0.0

associated with the overall dioxin elimination/sequestration rate, including the CYP1A2 induction rates, the liver weight, the binding capacity and affinity, and the gastric and intestinal excretion rates. For the gestational model dosing protocols, the Hill coefficient remains the most sensitive variable, but the elasticity decreases compared with the nongestational analysis. Otherwise, many of the most sensitive variables remain those associated with elimination. Additional parameters related to the adipose tissue blood flow and with the adipose diffusional permeability fraction are also relatively sensitive.

Table 3-12 shows the most sensitive variables for the human nongestational and gestational models. The additional variables associated with the adipose compartment partition coefficient, the body weight, and the fractional adipose tissue volume are also relatively sensitive variables at the reference dose and POD dose compared with the animal models. For all models, the elasticities are relatively similar across the different doses evaluated.

Table 3-12. Most sensitive variables for the human nongestational and gestational models

Variable	Variable description	Human nongestational, POD dose +50% elasticity	Human nongestational, POD dose +5% elasticity	Human gestational, POD dose +50% elasticity	Human gestational, POD dose +5% elasticity
HILL	Hill coefficient	5.35	3.56	5.75	3.75
CYP1A2_1OUTZ	Induction concentration in degradation process (nmol/L)	−0.44	−0.58	−0.45	−0.61
CYP1A2_1A2	Induction basal concentration of 1A2 (nmol/L)	0.46	0.53	0.52	0.59
CYP1A2_1EMAX	Maximum induction over basal effect (unitless)	−0.42	−0.56	−0.44	−0.596
SA_CHNGELI	Fraction liver-weight multiplier for sensitivity analysis (unitless)	−0.43	−0.57	−0.44	−0.59
KELV	Interspecies constant (hr^−1)	−0.39	−0.50	−0.43	−0.56
CYP1A2_1EC50	Induction disassociation constant for 1A2 (nmol/L)	0.30	0.34	0.32	0.36
KDLI	Liver affinity proteins AhR (nmol/L)	0.30	0.34	0.31	0.35
LIBMAX	Liver binding capacity (nmol/L)	−0.27	−0.31	−0.28	−0.34
SA_CHNGEBW	Body-weight multiplier for sensitivity analysis (unitless)	0.31	0.01	0.47	0.09
PF	Adipose tissue:blood partition coefficient (unitless)	−0.07	−0.06	−0.04	−0.03
SA_CHNGEF	Fraction adipose-weight multiplier for sensitivity analysis (unitless)	−0.06	−0.07	−0.03	−0.03
KABS	Intestinal excretion and absorption constant (hr^−1)	0.07	0.09	0.06	0.09
KST	Gastric excretion and absorption constant (hr^−1)	−0.09	−0.09	−0.09	−0.09
KDLI2	Liver affinity proteins 1A2 (nmol/L)	0.05	0.07	0.03	0.03

In order to observe the difference between the local and global elasticities, Figures 3-20 and 3-21 show the elasticities for the most sensitive variables in the human nongestational model for the POD dose and reference dose, respectively. In general, the elasticities are similar across the different percentage changes in variable values that were tested. Changes in variables by −50% tend to lead to the greatest elasticities. Changing the variable values by +5% and −5% lead to almost the same elasticities for nearly all the variables. These same conclusions hold for all the other models and doses as well.

Of the variables to which the blood concentrations are most sensitive, most of the variables are either derived from Wang et al. (1997) or are optimized (see Table 3-8). For the human model, parameters set equal to values in the rat model may be subject to particular uncertainty. In particular, the AhR and CYP1A2 induction parameters typically were based on the rat model parameters. The exception is CYP1A2_1EMAX, the maximum induction of CYP1A2, which is an optimized parameter. The variable elimination rate, *kelv*, and the intestinal excretion, KST, are also both optimized against data. For variables that are optimized, a sensitivity analysis that varies each parameter one at a time may overestimate the associated model uncertainty associated with the variable. A change in KST, for example, would necessitate a commensurate change in the other optimized variables in order to suitably capture the comparison data, and the overall changes in the blood concentrations might be small.

The most sensitive variable in all the models is the Hill parameter. The elasticity is high in part because the Hill parameter is an exponent; thus, small changes in the value can lead to larger changes in the whole blood concentration. However, as stated above, any change in the Hill parameter would also necessitate changes in optimized variables in order to maintain an adequate fit with the data. The next section explores the effect of changing the Hill parameter and the effect of changing the CYP1A2 induction parameters on the model fits to literature data.

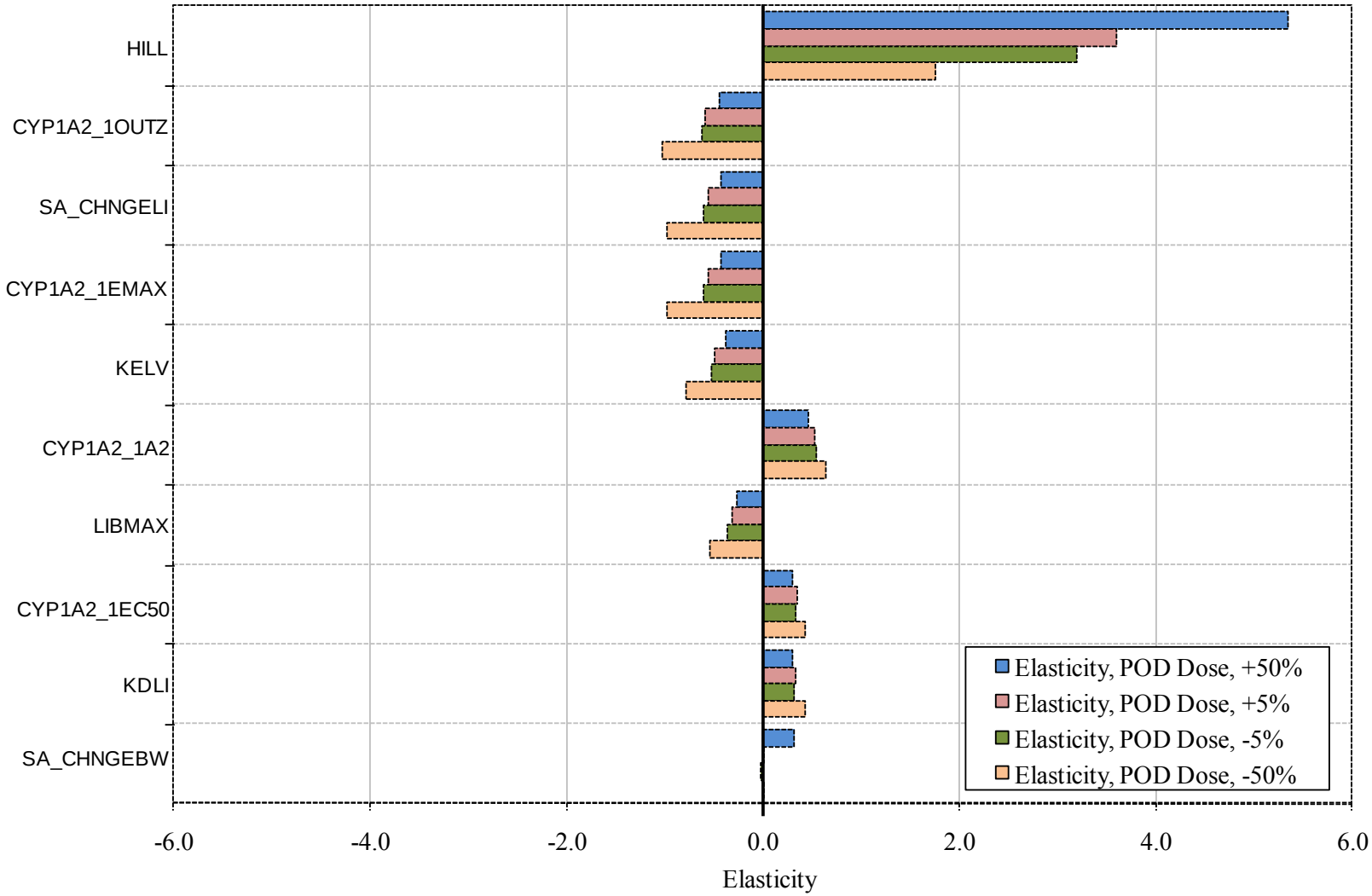


Figure 3-20. Elasticities in the nongestational human model, POD dose.

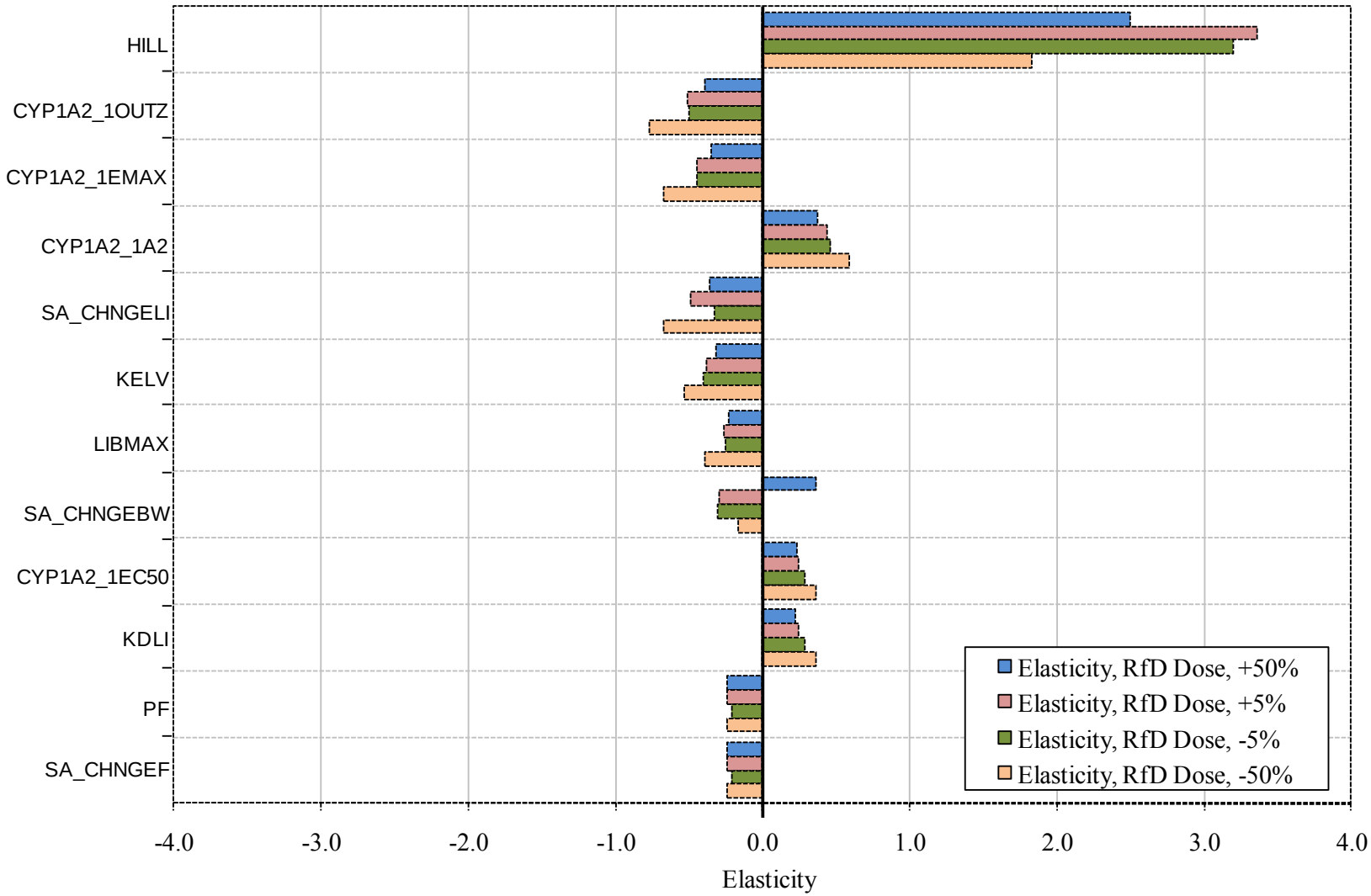


Figure 3-21. Elasticities in the nongestational human model, RfD dose.

3.3.4.3.2.6. ***Further uncertainty analysis of the Hill coefficient and CYP1A2 induction parameters***

As illustrated by the sensitivity analysis of the PBPK model, the predicted TCDD blood concentrations are very sensitive to the Hill coefficient (*h*) as described in Eq. 3-20. This parameter is included in the mathematical description for the induction of the CYP1A2. Therefore, the best type of data needed to estimate an in vivo value for this constant would be time-course levels of hepatic CYP1A2 in response to TCDD exposure. This type of data is only available in experiments conducted in animals. The PBPK model adopted a value of 0.6 for this parameter based on the earlier reported models by Wang et al. (2000) and Santostefano et al. (1998). In both cases, the value of 0.6 used for the Hill coefficient (the model parameter *Hill*) in the model was fit to describe the temporal relationship between TCDD exposure and CYP1A2-induction levels in animals. Note that the value of 0.6 for *Hill* indicates supralinear behavior at low exposure levels, which translates to a supralinear relationship between oral intake and blood TCDD concentrations.

For humans, the only data available to calibrate the in vivo model parameters are blood levels of TCDD. Predicted TCDD blood levels are influenced by the Hill coefficient when it is implicitly included in the description for the hepatic elimination of TCDD by induced levels of CYP1A2 as described in Eq. 3-21. However, as was illustrated earlier, the elimination of TCDD by the liver is also influenced by the numerical optimization of the *kelv* constant in the same equation. Therefore, estimation of the Hill coefficient using human blood data is highly dependent on the simultaneous estimation of *kelv*.

In order to estimate the interdependence of *Hill* and *kelv* and to investigate the behavior of the Emond human PBPK model in the absence of supralinearity, EPA calibrated the model to several human data sets after setting *Hill* to 1 and varying *kelv*. A Hill coefficient of 1 results in low-dose linearity, where supralinear behavior is first eliminated. However, EPA does not consider a *Hill* value of 1 necessarily to be a plausible replacement for the model variable of 0.6; it is just being used to investigate the behavior of the model as a sensitivity analysis. The data sets are TCDD serum concentrations (lipid-adjusted serum concentration [LASC]) over time for four individuals: two Austrian adult females (Geusau et al., 2002) (1996) and two Italian (Seveso) males—a 6-year-old and a 50-year-old (Needham et al., 1997); the data are presented in Tables 3-13 and 3-14. The results of Hill coefficient sensitivity analysis simulations are shown in Figure 3-22 and Table 3-15. For each data set, the simulation was run four times—once with

Table 3-13. TCDD serum measurements over time for two Austrian women exposed to TCDD in 1997[a]

Austrian woman 1		Austrian woman 2	
Day	**TCDD LASC (ppt)**	**Day**	**TCDD LASC (ppt)**
0	144,000	0	26,000
63	111,000	53	20,500
116	85,600	63	16,100
126	80,900	77	15,900
135	72,200	84	14,300
147	70,200	98	13,200
161	87,700	105	18,500
168	89,900	140	13,300
203	62,100	177	13,700
240	65,100	207	19,300
270	68,300	238	15,700
295	64,900	267	15,200
309	68,100	326	15,700
316	72,600	437	17,700
323	73,700	533	14,100
330	72,500	637	10,500
366	60,300	718	11,000
389	73,900	841	10,100
466	85,600	998	9,500
500	68,100		
596	47,100		
700	39,300		
781	27,400		
904	30,300		
1,054	35,900		

[a]Source of data: (Geusau et al., 2001).

Table 3-14. TCDD serum measurements over time for two Seveso males exposed to TCDD in 1976[a]

Seveso male (6 years old)		Seveso male (50 years old)	
Day	**TCDD LASC (ppt)**	**Day**	**TCDD LASC (ppt)**
0	15,900	0	1,770
826	4,350	92	807
1,522	2,269	981	1,069
2,193	580	1,218	809
5,867	324	1,921	680
		6,011	807

[a]Source of data: Needham et al. (1997).

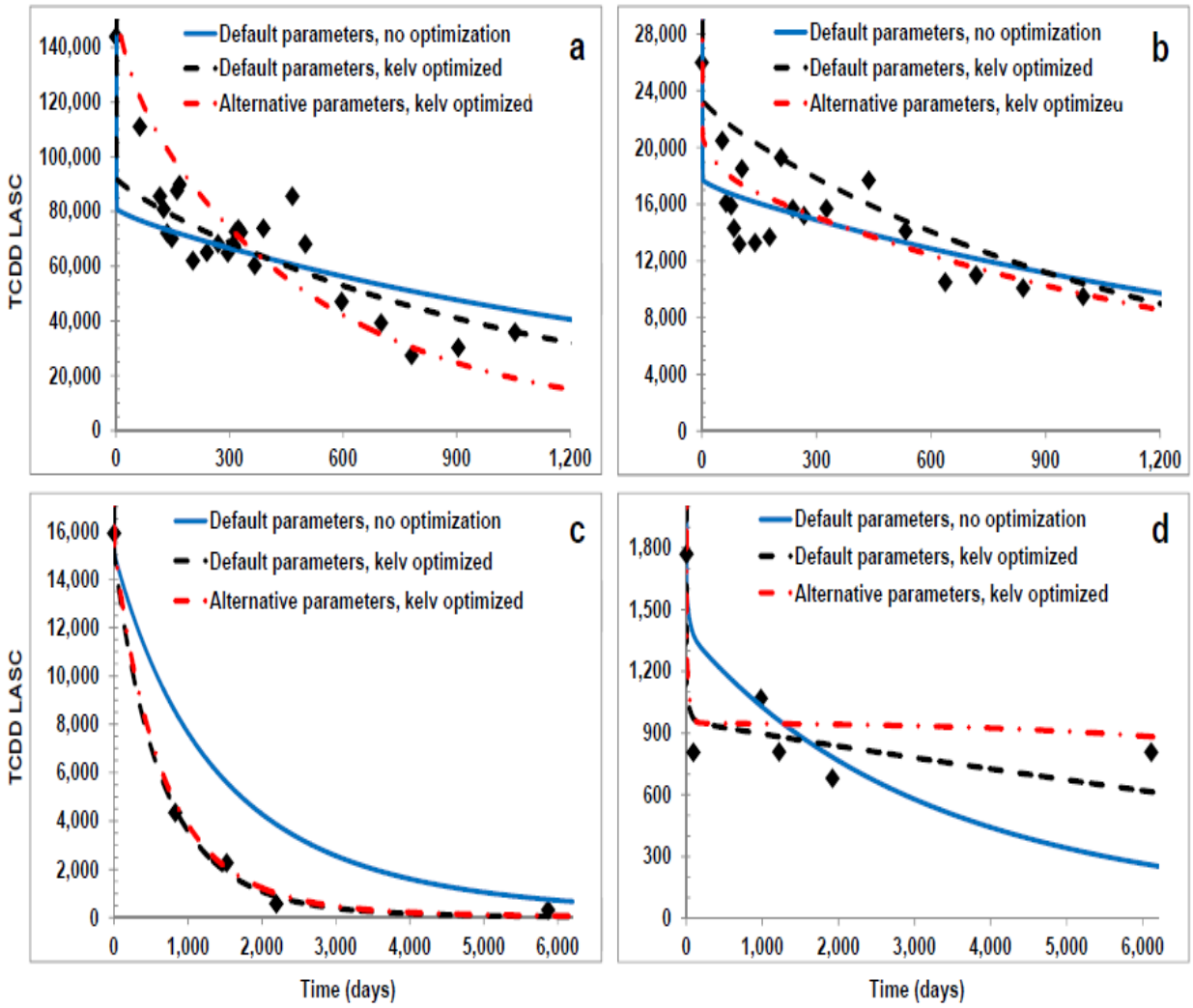


Figure 3-22. Hill coefficient sensitivity analysis.
Calibration of Emond human PBPK model for 2 values of *Hill* for four human data sets: (a) Austrian Woman 1, (b) Austrian Woman 2, (c) Seveso 6-year-old male, (d) Seveso 50-year-old male; see text for source of data. Values for *kelv* other than the standard model value of 0.0011 are optimized.

Table 3-15. Results of Hill coefficient sensitivity analysis simulations with Emond human PBPK model

	Hill = 0.6 *kelv* = default *doseiv* optimized	**Hill = 1 *kelv* = default *doseiv* optimized**	**Hill = 0.6 *kelv* and *doseiv* optimized**	**Hill = 1 *kelv* and *doseiv* optimized**
Hill				
	0.6	1.0	0.6	1.0
kelv				
Austrian 1	0.0011	0.0011	1.73E-03	5.74E-03
Austrian 2			1.79E-03	4.89E-03
Seveso 6			0.00300	0.00490
Seveso 50			2.94E-04	4.79E-03
doseiv				
Austrian 1	7.00E+04	1.20E+04	8.00E+04	1.98E+04
Austrian 2	1.30E+04	2.40E+03	1.80E+04	3.40E+03
Seveso 6	1.10E+04	3.48E+02	1.10E+04	9.98E+02
Seveso 50	4.98E+02	9.76E+01	2.98E+02	1.37E+02

the default model parameters (*Hill* = 0.6, *kelv* = 0.0011), once with *Hill* = 1.0 and *kelv* unchanged, once with *Hill* = 0.6 and *kelv* optimized for best fit to the data, and once with *Hill* = 1.0 and *kelv* optimized. In each case, the initial dose (model parameter *doseiv*), assuming a single instantaneous exposure at the time of first serum measurement, was optimized for best fit; the exposure in this case would be a simulation of the body burden at the time, as the actual exposure scenario is unknown. In all cases, simply changing the value of *Hill* resulted in poor fits. Optimizing *kelv* with *Hill* set to either to 0.6 or 1 yields much better fits, as would be expected, with both values fitting the data equally well when the inter-related parameter, *kelv,* is optimized.

EPA also investigated the impact of alternate values for other model parameters related to the CYP1A2 induction algorithm. Budinsky et al. (2010) reported an in vitro temporal relationship between CYP1A2 induction and TCDD levels in human and rat primary hepatocytes. Budinsky et al. (2010) used the CYP1A2 induction data to estimate Hill function constants, such as baseline, fold, and maximal CYP1A2 mRNA inductions. Using their data, an

estimate for the human in vivo baseline, fold, and maximal response of CYP1A2 induction can be approximated as illustrated in Eq. 3-22 and 3-23:

$$\frac{Tj\ a\ \ R!\ basr\}human_{invitro}}{Tj\ a\ \ R!\ basr\}anima\}_{invitro}}\Bigg)\Bigg(\ Tj\ a\ \ R!\ srsr\}rnz\sim r\}_{invivo}\ \ Tj\ a\ \ R2_basal_{yu\sim rn\ vquz[r\}vntpzn[z[o} \qquad \text{(Eq. 3-22)}$$

and

$$\frac{Tj\ a\ \ R!\ \wedge r]\ human_{invitro}}{Tj\ a\ \ R!\ \wedge r]\ anima\}_{invitro}}\Bigg)\Bigg(\ Tj\ a\ \ R!\ \wedge r\ ]\ rnz\sim r\}_{invivo}\ \ Tj\ a\ \ R!p\wedge\ \ r]\ _{yu\sim rn\ vquz[r\}vntpzn[z[o} \qquad \text{(Eq. 3-23)}$$

The values used in these equations are shown in Table 3-16.

Table 3-16. Alternative CYP1A2 parameter estimates for sensitivity analysis of Emond human PBPK model

	Budinsky et al. (2010) values		Emond model value		Alternative scaled value[a]
	Human	**Rat**	**Human**	**Rat**	**Human**
CYP1A2 Basal	11.6	22.4	1,600	1.6	**829**
CYP1A2 Max	12,900	322	9,300	600	**24,037**
EC_{50}/KDLI	0.329	0.0628	130	0.04	**209**

[a]Emond model rat value multiplied by the ratio of the corresponding human:rat parameter values from Budinsky et al. (2010).

The calculated in vivo human CYP1A2 baseline, fold, and maximal induction response, with their corresponding minimum and maximum values, are then used in the PBPK model to estimate mean, minimum, and maximum blood levels in comparison to data for two Austrian cases, and the Seveso cohort. This analysis was done with *Hill* set to 0.6 and optimizing *kelv* and *doseiv* for the data sets in Tables 3-13 and 3-14. Results of the simulations are shown in Figure 3-23 and Table 3-17.

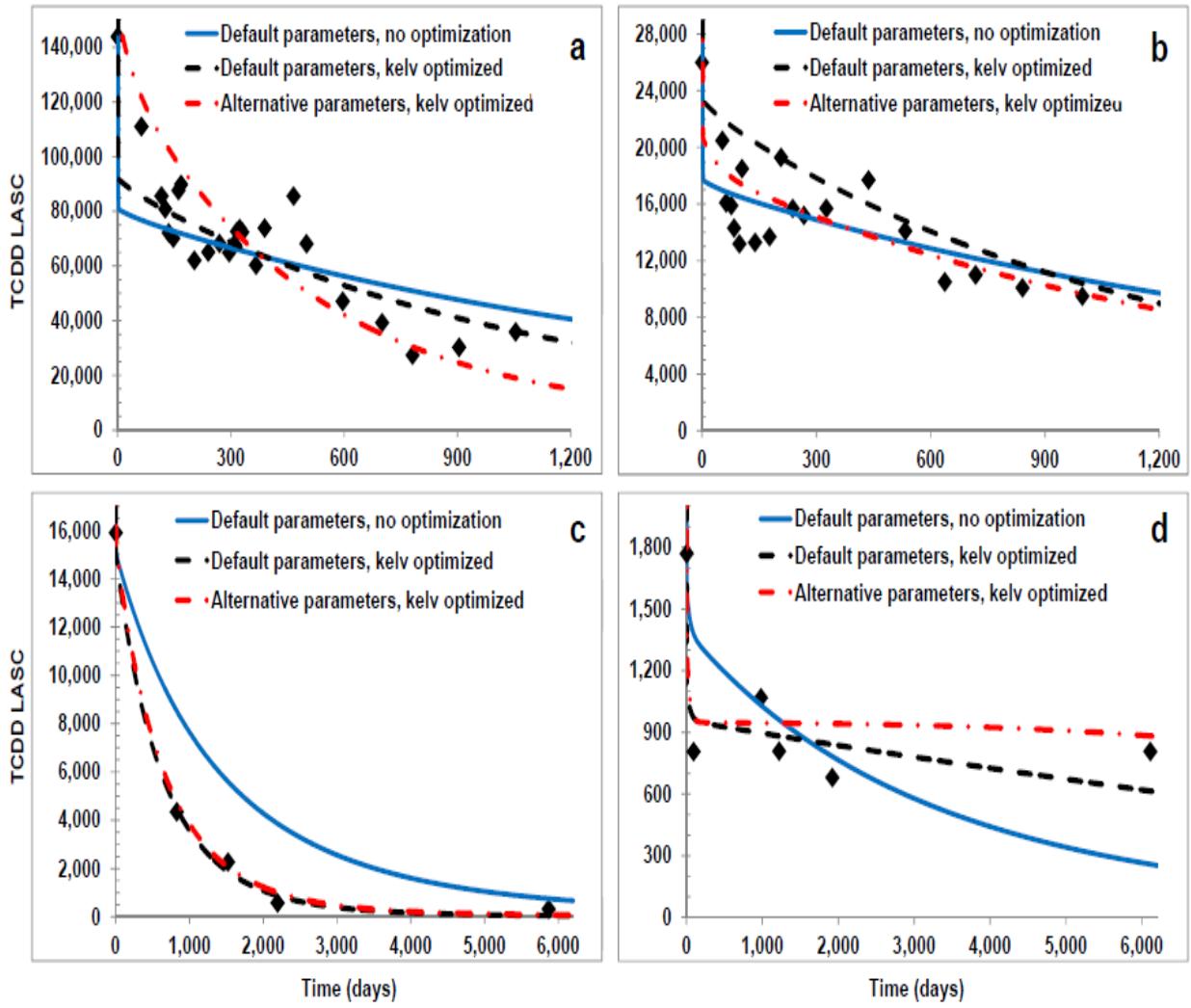


Figure 3-23. CYP1A2 parameter sensitivity analysis.
Calibration of Emond human PBPK model for alternate values of CYP1A2 parameters other than *Hill* for four human data sets: (a) Austrian Woman 1, (b) Austrian Woman 2, (c) Seveso 6-year-old male, (d) Seveso 50-year-old male; see text for source of data. Alternate parameters were estimated from data presented in Budinsky et al. (2010).

Table 3-17. Results of CYP1A2 parameter sensitivity analysis simulations with Emond human PBPK model

	Hill = 0.6 *kelv* = default *doseiv* optimized	Hill = 0.6 *kelv* and *doseiv* optimized	*Hill* = 0.6, Alternative parameters,[a] *kelv* and *doseiv* optimized
kelv			
Austrian 1	0.0011	1.73E-03	4.36E-04
Austrian 2		1.79E-03	1.67E-04
Seveso 6		0.00300	0.00030
Seveso 50		2.94E-04	9.68E-06
doseiv			
Austrian 1	7.00E+04	8.00E+04	6.98E+04
Austrian 2	1.30E+04	1.80E+04	8.00E+03
Seveso 6	1.10E+04	1.10E+04	5.98E+03
Seveso 50	4.98E+02	2.98E+02	1.97E+02

[a]Alternative scaled values from Table 3-16.

An attempt to directly use the in vitro values of the Hill function estimated in the Budinsky et al. (2010) in the PBPK model was not successful in simulating blood levels in Figure 3-23. The failure in using these values directly may be a result of the usual in vitro-to-in vivo extrapolation complications such as in vitro cellular competency to exhibit toxicological response comparable to the in vivo ones, and TCDD media to cell sequestration. It is also important to note that the in vitro preparations in the Budinsky et al. (2010) came from a limited set of five female subjects. Average and standard variation levels obtained from this set of human subjects cannot be representative of overall human population.

It is clear from the results shown in Figures 3-22 and 3-23, that several different combinations of *CYP1A2* induction parameters can be used to simulate the data well. This process illustrates the interdependencies of these parameters when in vivo blood levels in humans are the only source of data to estimate them.

The impact of varying these parameters on model predictions of human oral intakes corresponding to a range of lifetime average serum concentrations is shown in Table 3-18. The range of concentrations was chosen to be representative of human intakes of interest for the RfD

Table 3-18. Results of Emond human PBPK model parameter sensitivity analysis simulations. Comparison of modeled human oral intakes for a range of lifetime average TCDD serum concentrations for alternative parameter values.

Lifetime average TCDD LASC[a] (ppt)	Standard model configuration Hill = 0.6 kelv = 0.0011 CYP1A2_1A1 = 1,600 CYP1A2_1EMAX = 9,300 CYP1A2_1EC50 = 130 PF = 100	Alternative Hill Hill = 1 kelv = 0.0011 CYP1A2_1A1 = 1,600 CYP1A2_1EMAX = 9,300 CYP1A2_1EC50 = 130 PF = 100	Standard Hill, optimized elimination Hill = 0.6 kelv = 0.0017 CYP1A2_1A1 = 1,600 CYP1A2_1EMAX = 9,300 CYP1A2_1EC50 = 130 PF = 100	Alternative Hill, optimized elimination Hill = 1 kelv = 0.0050 CYP1A2_1A1 = 1,600 CYP1A2_1EMAX = 9,300 CYP1A2_1EC50 = 130 PF = 100	Alternative induction parameters[b] optimized elimination Hill = 0.6 kelv = 0.0002 CYP1A2_1A1 = 829 CYP1A2_1EMAX = 24,037 CYP1A2_1EC50 = 209 PF = 100
30	1.0E−03	3.8E−04	1.3E−03	3.9E−04	7.7E−04
100	5.7E−03	1.3E−03	8.0E−03	1.5E−03	4.1E−03
300	3.0E−02	4.2E−03	4.3E−02	5.9E−03	1.9E−02
1,000	1.9E−01	1.8E−02	2.8E−01	3.7E−02	1.2E−01
3,000	9.6E−01	8.1E−02	1.4E+00	2.3E−01	5.8E−01

[a]From lifetime female model.
[b]Estimated from Budinksy et al. (2010).

derivation in Section 4. Comparing the optimized simulations for the alternative *Hill* values shows that, for these data sets, changing *Hill* to 1 decreases the modeled intakes for the TCDD serum concentrations in this range by about 70−85%. Using the alternative parameters estimated from Budinsky et al. (2010) results in 40−60% lower intakes than for the standard parameters (optimized *kelv*). Thus, it would appear that, although the *Hill* value of 0.6 results in a supralinear relationship between TCDD intake and serum concentrations in the Emond model, eliminating the supralinear behavior does not result in higher predicted intakes for lower TCDD serum concentrations, as might be expected. However, strong conclusions cannot be made from these results because the data used for the optimization are not ideal in at least two respects: (1) they only address CYP1A2 dynamics indirectly, and (2) there are only four data sets, and they are not necessarily representative of the entire population. In Section 4.5.1.1.1, a sensitivity analysis is presented that illustrates the predicted change in the point of departure when the *Hill* value is changed to 1.

3.3.4.3.2.7. ***Confidence in physiologically based pharmacokinetic (PBPK) model predictions of dose metrics***

The PBPK model facilitates prediction of absorbed dose, body burden, and blood concentration of TCDD for oral exposures in adult humans and rats (adult and developing) with high confidence (see Table 3-19). The model output of blood concentration can be normalized to lipid content representative of the study group (species, sex, age, lifestage, and diet). However, the PBPK model of Emond et al. (2006; 2005; 2004) does not simulate plasma and erythrocyte TCDD concentrations separately, and it predicts tissue concentrations on the basis of tissue:whole blood partition coefficients and not on the basis of serum lipid-normalized values.

The reliability of this model for simulating the liver concentration of TCDD in rats is considered to be high, but it is considered to be medium for humans. Although empirical data on bound or free concentrations were not used to evaluate model performance in humans, the biological phenomena (consistent with available data) related to the hepatic sequestration, enzyme induction, and dose-dependent elimination are described in the model. This is one of the situations where PBPK models are uniquely useful; that is, they permit the prediction of system behavior based on understanding of the mechanistic determinants, even though the required data cannot be directly obtained in the system (e.g., bound concentrations in the liver of exposed humans). For these dose measures (i.e., bound concentration and total liver concentration), the

Table 3-19. Confidence in the PBPK model simulations of TCDD dose metrics

Dose metric	Human model	Rat model	Mouse model
Administered dose	N/A	N/A	N/A
Absorbed dose	H	H	M
Body burden	H	H	M
Serum (blood) concentration	H	H	M
Total liver concentration	M/L	H	M
Receptor occupancy (bound concentration)	L	L	L

H = high, M = medium, L = low, N/A = not applicable.

level of confidence can be further improved or diminished by the outcome of sensitivity analysis. In this regard, the results of a focused sensitivity analysis indicate that the most sensitive parameters of the human model are among the most uncertain (i.e., those parameters for which estimates were not obtained in humans) with respect to prediction of liver TCDD concentration, contrary to the animal model (see Section 3.3.5).

With respect to the mouse model, however, the level of confidence is low to medium, given that it has not been verified extensively with blood, body burden, or tissue concentration, time-course, or dose-response data. However, the mouse PBPK model, based on the rat model that has been evaluated with several PK data sets, has been shown to reproduce well the limited mouse liver kinetic data (see Figures 3-24 through 3-31; Boverhoff et al., 2005). The same model structure has been used for simulating kinetics of TCDD in humans successfully. Overall, the adult mouse model, given its biological basis combined with its ability to simulate TCDD kinetics in multiple species, is considered to exhibit a medium level of confidence for simulating dose metrics for use in high to low dose extrapolation and interspecies (mouse to human) extrapolation. Even though similar considerations are applicable to gestational model in mice, the confidence level is considered to be low because very limited comparison with empirical data has been conducted (see Figure 3-31). Despite the uncertainty in these predictions, the scaled rat gestational model, given its biological and mechanistic basis, might be of use in predicting dose metrics in these groups that might form the basis of PODs in certain key studies.

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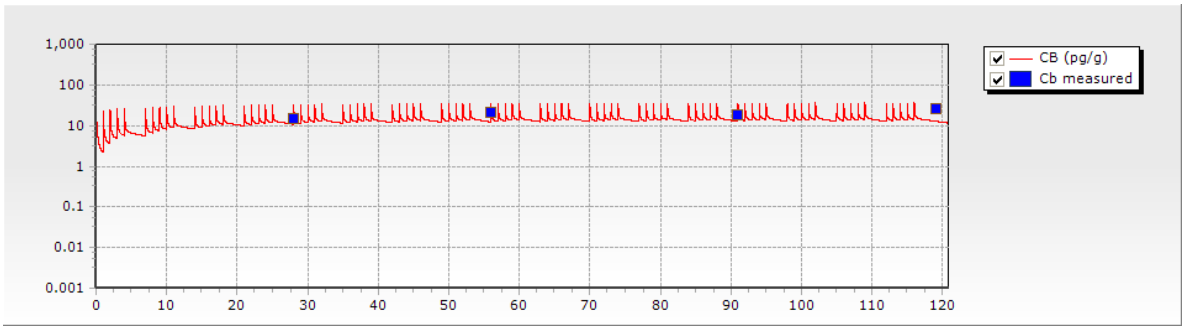

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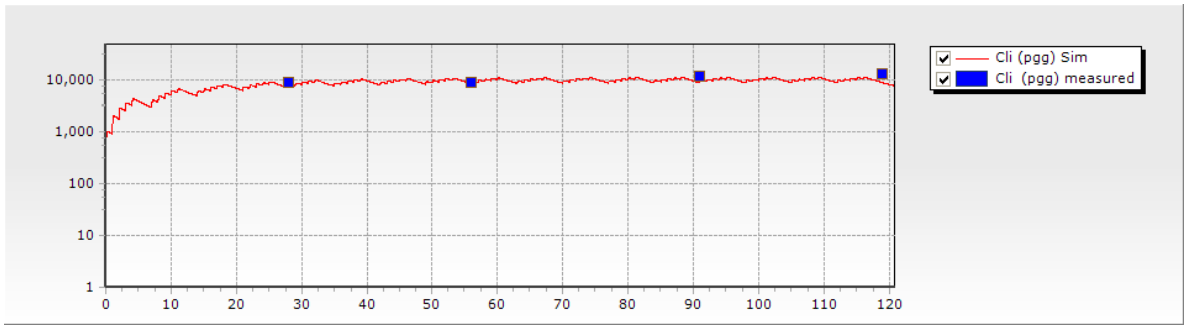

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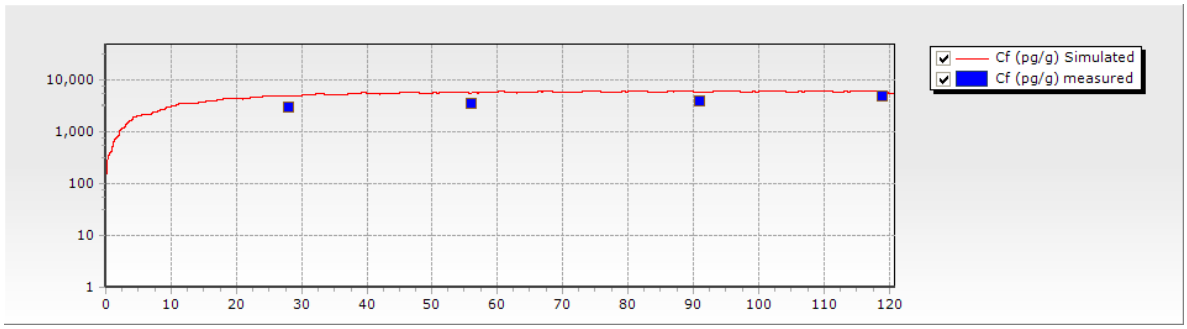

Figure 3-24. Experimental data (symbols) and model simulations (solid lines) of (A) blood, (B) liver, and (C) adipose tissue concentrations of TCDD after oral exposure to 150 ng/kg-day, 5 days/week, for 17 weeks in mice.
Y-axis represents concentration in pg/g, and X-axis represents time in days.

Source: Experimental data were obtained from Diliberto et al. (2001).

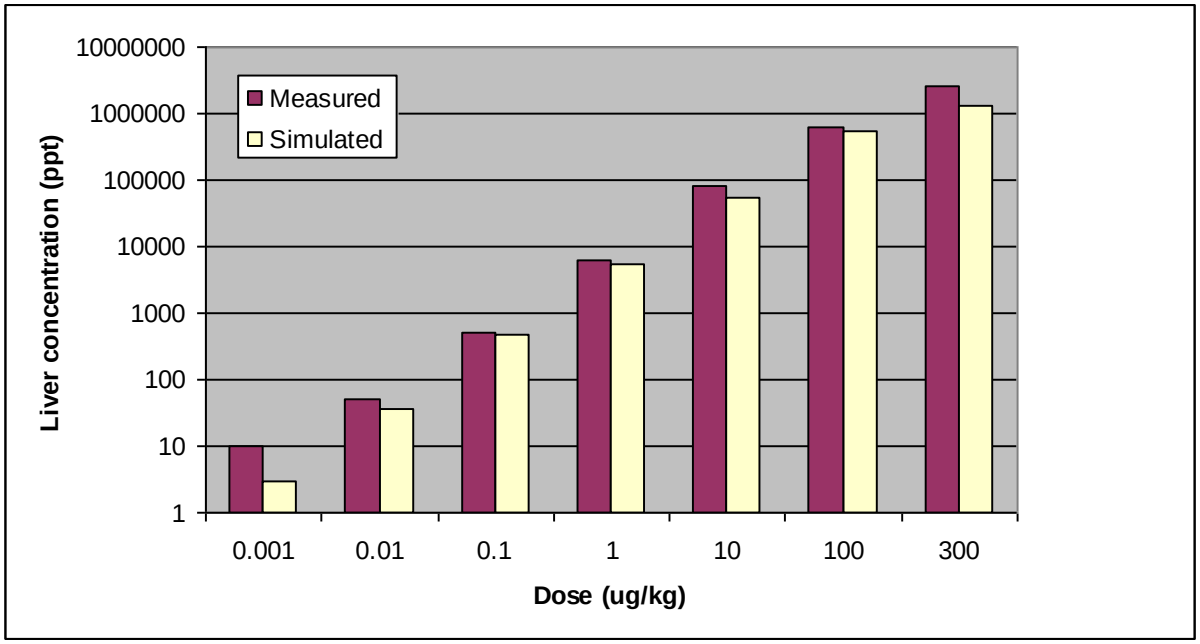

Figure 3-25. Comparison of PBPK model simulations with experimental data on liver concentrations in mice administered a single oral dose of 0.001−300 µg TCDD/kg.

The simulations and experimental data were obtained 24 hour post-exposure.

Source: Data obtained from Boverhoff et al. (2005).

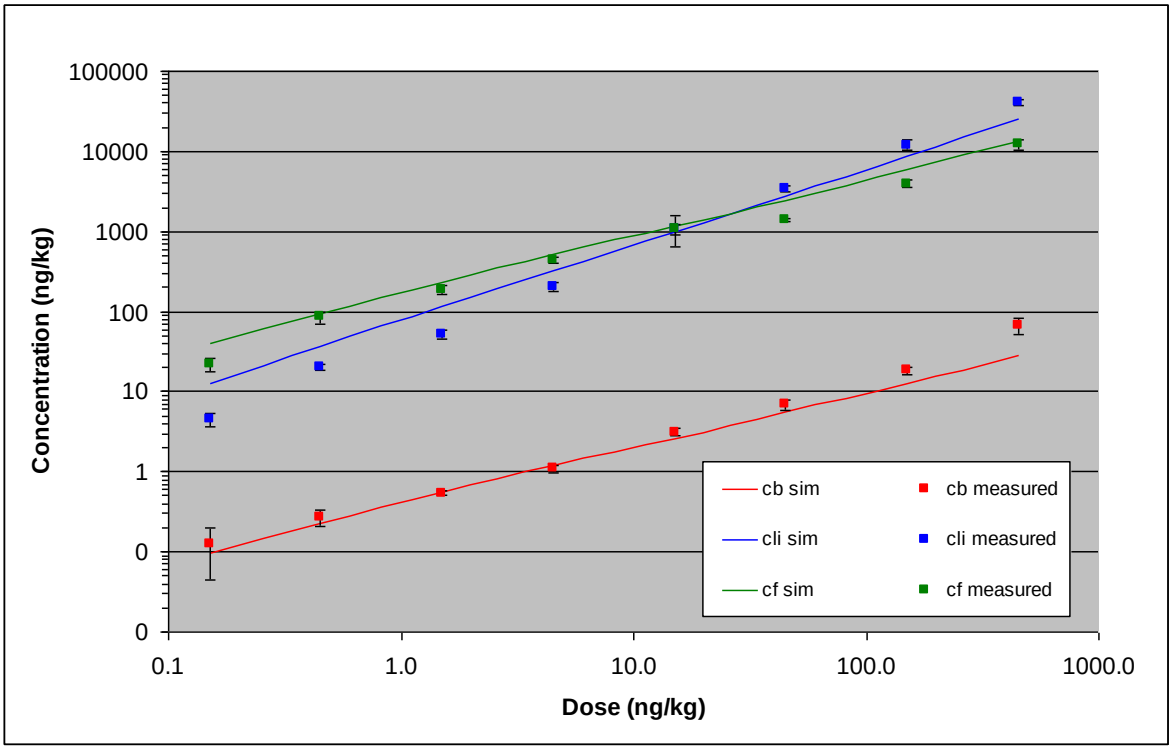

Figure 3-26. Comparison of model simulations (solid lines) with experimental data (symbols) on the effect of dose on blood (cb), liver (cli), and fat (cf) concentrations following repetitive exposure to 0.1−450 ng TCDD/kg, 5 days/week, for 13 weeks in mice.

Source: Data obtained from Diliberto et al. (2001).

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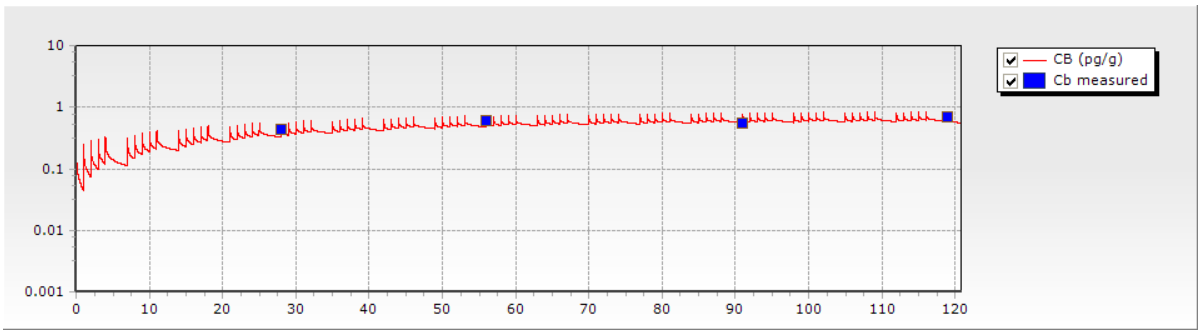

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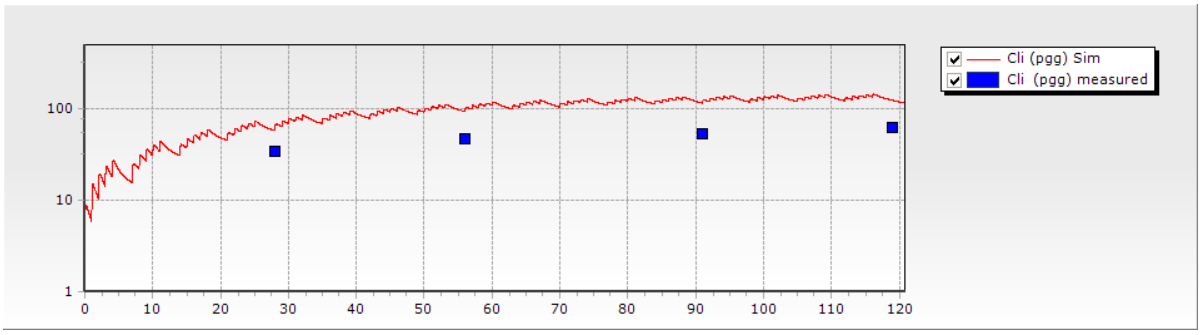

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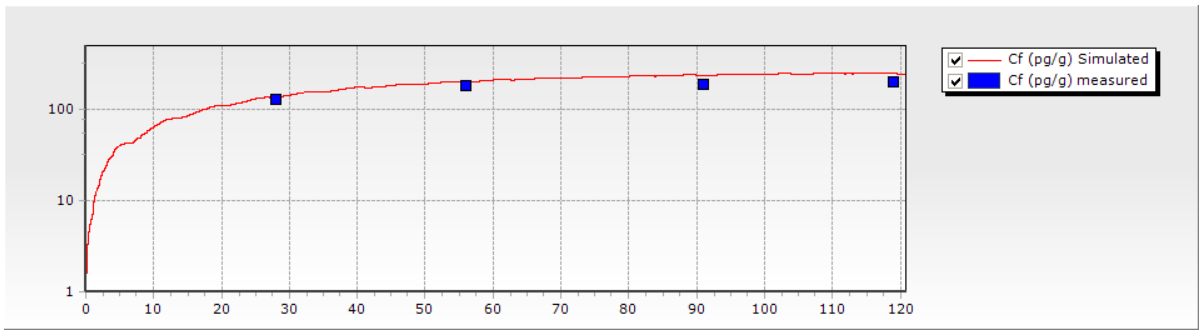
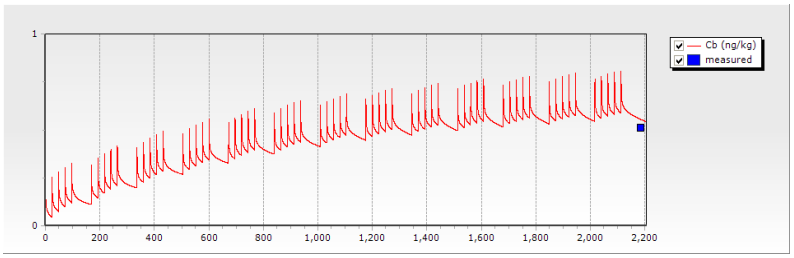


Figure 3-27. Comparison of experimental data (symbols) and model predictions (solid lines) of (A) blood, (B) liver, and (C) adipose tissue concentrations of TCDD after oral exposure to 1.5 ng/kg-day, 5 days/week, for 17 weeks in mice.

Y-axis represents concentration in pg/g, and X-axis represents time in days.

Source: Experimental data were obtained from Diliberto et al. (2001).

A



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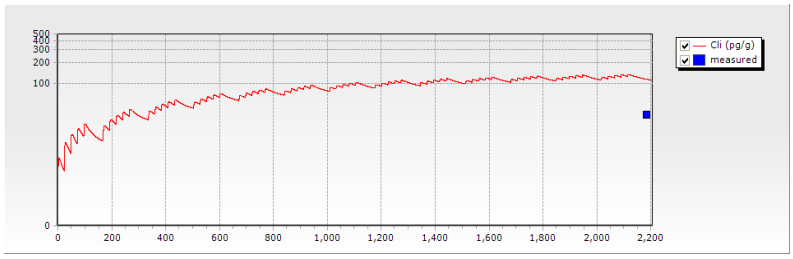

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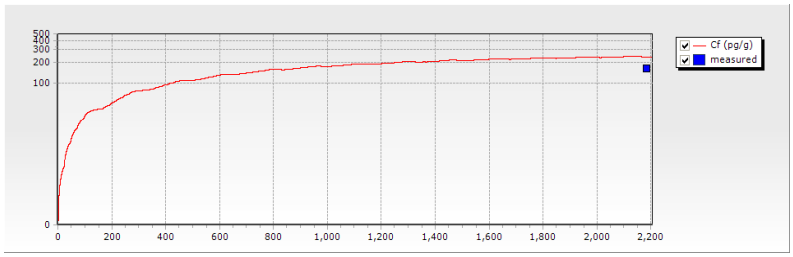

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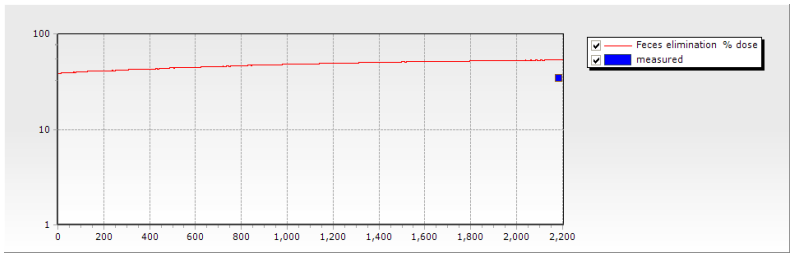

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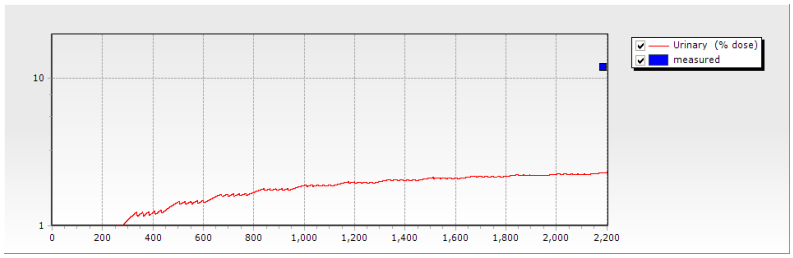
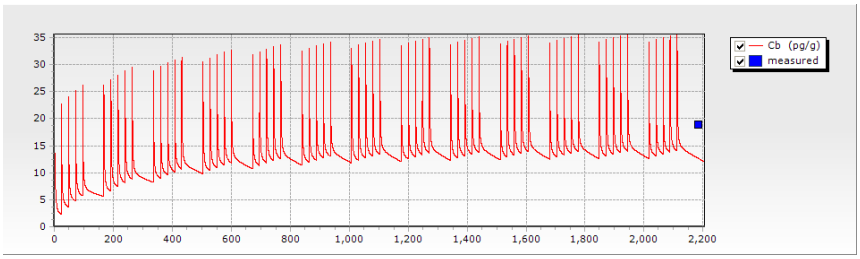


Figure 3-28. Comparison of experimental data (symbols) and model predictions (solid lines) of (A) blood concentration, (B) liver concentration, (C) adipose tissue concentration, (D) feces excretion (% dose), and (E) urinary elimination (% dose) of TCDD after oral exposure to 1.5 ng/kg-day, 5 days/week, for 13 weeks in mice.

Y-axis represents concentration in pg/g, and X-axis represents time in days.

Source: Experimental data were obtained from Diliberto et al. (2001).

A



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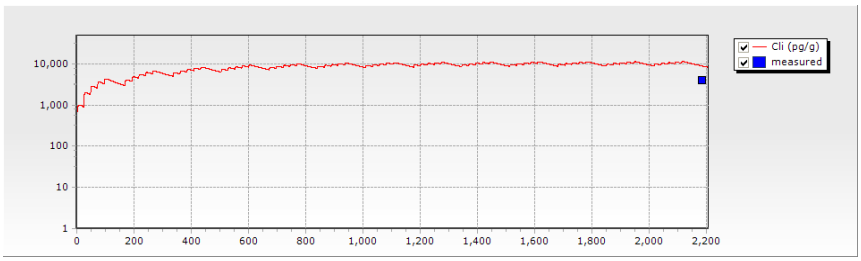

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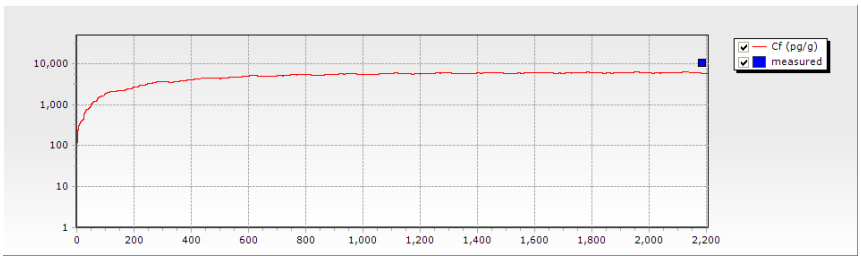

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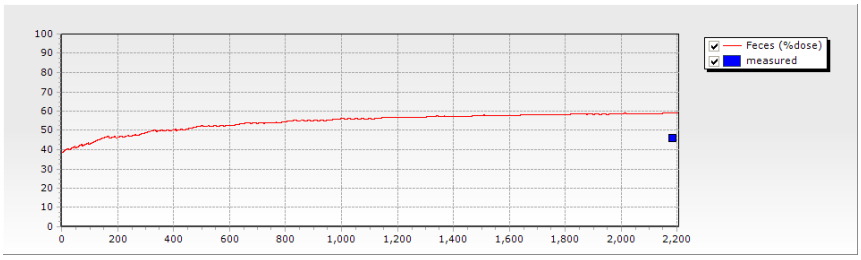

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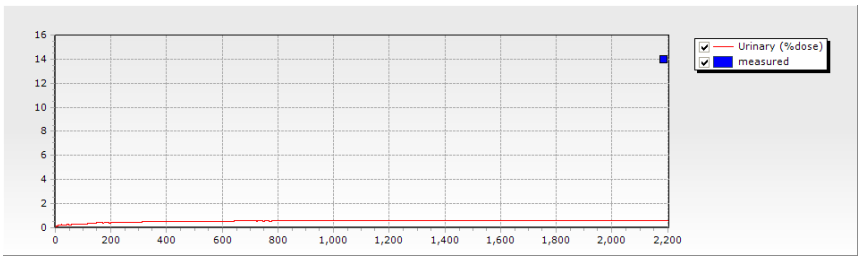
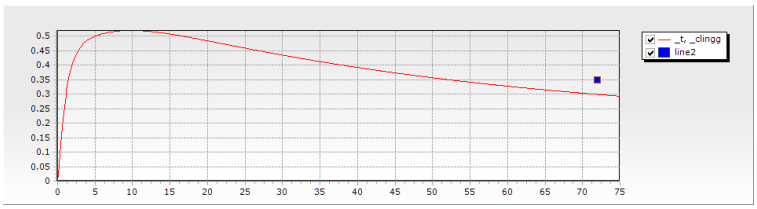


Figure 3-29. Comparison of experimental data (symbols) and model predictions (solid lines) of (A) blood concentration, (B) liver concentration, (C) adipose tissue concentration, (D) feces excretion (% dose), and (E) urinary elimination (% dose) of TCDD after oral exposure to 150 ng/kg-day, 5 days/week, for 13 weeks in mice.

Y-axis represents concentration in pg/g, and X-axis represents time in days.

Source: Experimental data were obtained from Diliberto et al. (2001).

A



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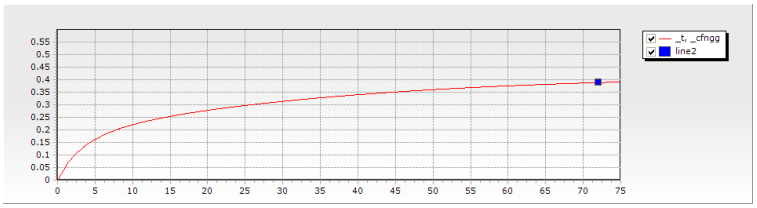

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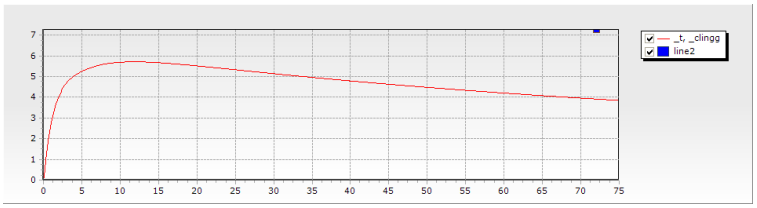

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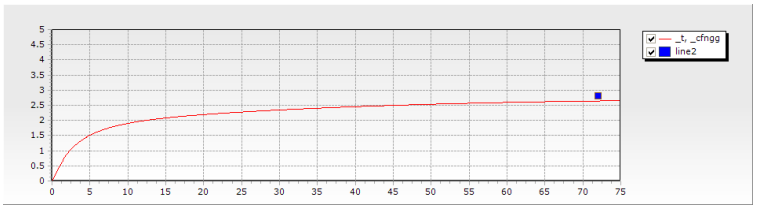

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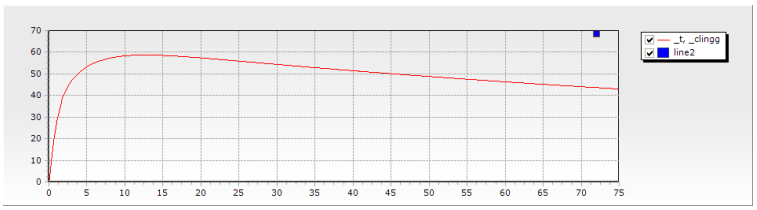

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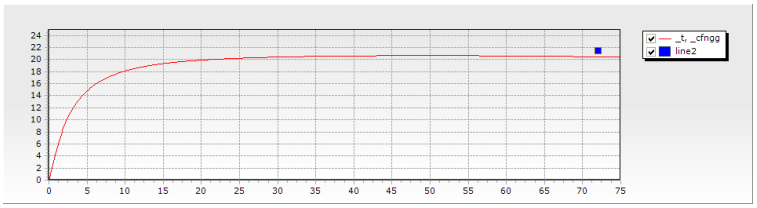
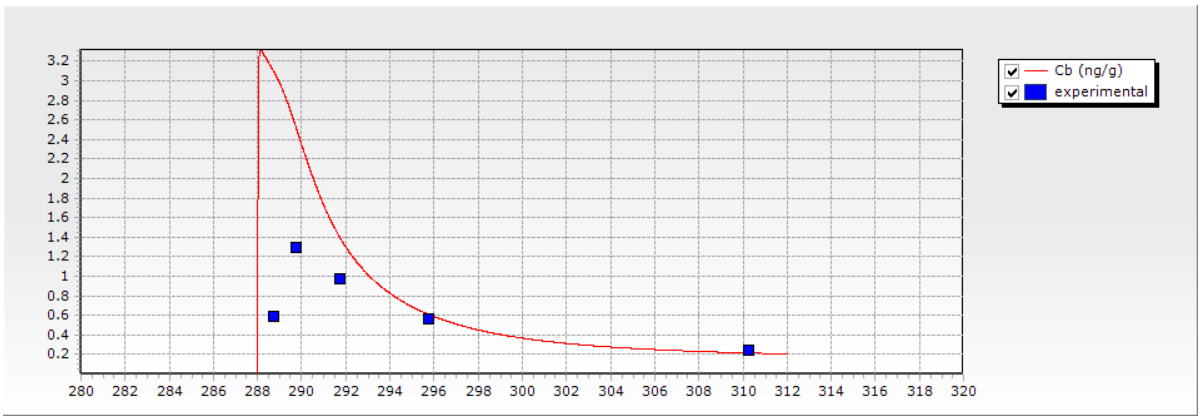


Figure 3-30. PBPK model simulations (solid lines) vs. experimental data (symbols) on the distribution of TCDD after a single acute oral exposure to A−B) 0.1, C−D) 1.0, and E−F) 10 µg of TCDD/kg of body weight in mice.
Liver and adipose concentration for each dose was measured after 72 hours. Y-axis represents the concentration in tissues (ng/g); insets A, C, and E represent liver tissue, whereas B, D, and F correspond to adipose tissue. X-axis represents the time in hours.

Source: Experimental data were obtained from Santostefano et al. (1996).

A



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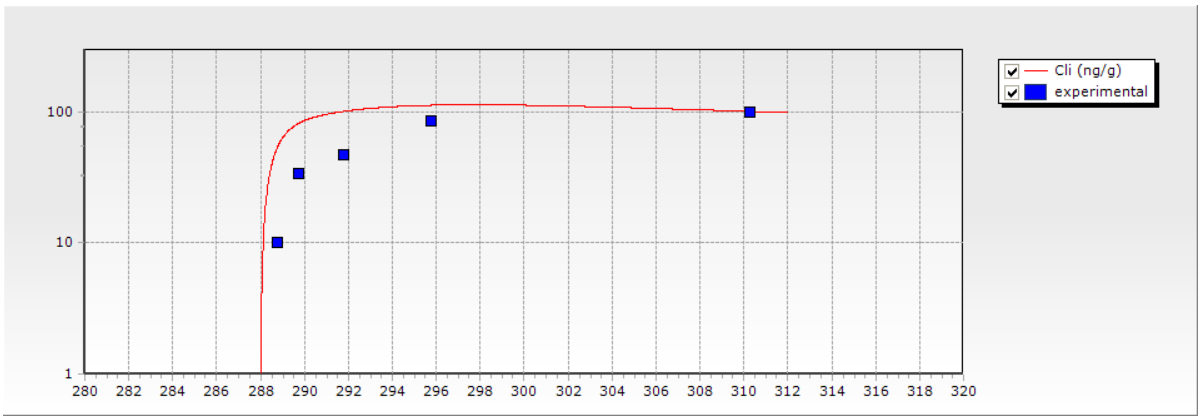

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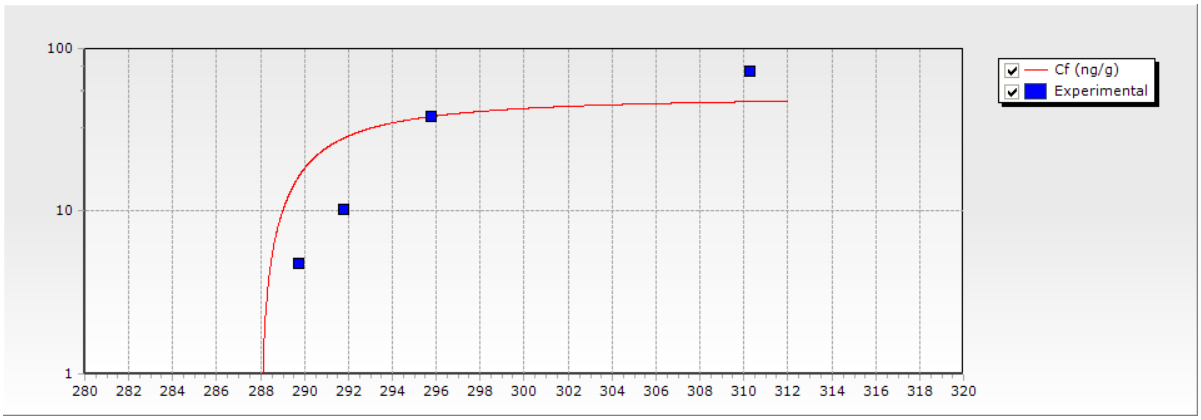

Figure 3-31. PBPK model simulation (solid lines) vs. experimental data (symbols) on the distribution of TCDD after a single dose of 24 µg/kg BW on GD 12 in mice.

Concentrations expressed as ng TCDD/g tissue. (A) maternal blood, (B) maternal liver, and (C) maternal adipose tissue. Y-axis represents the tissue concentration, whereas X-axis represents the time in hours.

Source: Experimental data were obtained from Abbott et al. (1996).

3.3.4.4. *Applicability of Pharmacokinetics (PK) Models to Derive Dose Metrics for Dose-Response Modeling of TCDD: Confidence and Limitations*

Both the CADM and PBPK models describe the kinetics of TCDD following oral exposure to adult animals and humans by accounting for the key processes affecting kinetics, including hepatic sequestration phenomena, induction, and nonlinearity in elimination, and distribution in adipose tissue and liver. Both models can be used for estimating body burdens and serum lipid adjusted concentrations of TCDD. However, there are several differences between these two models. The PBPK model calculates the free and bound concentrations of TCDD in the intracellular subcompartment of tissues. The total or receptor-bound concentrations in liver are unambiguous and more easily interpretable with the PBPK model than with the CADM model. In addition, the PBPK model computes bound and total concentrations as a function of the free concentration in the intracellular compartment of the tissue. By contrast, the CADM model simulates the total concentration based on empirical consideration of hepatic processes. Consequently, the amount of TCDD bound to AhR or CYP1A2 cannot be simulated with the CADM model. The CADM model computes only the total TCDD concentration in liver and describes TCDD elimination through partitioning from circulating lipids across the lumen of the large intestine into the feces, while the PBPK model accounts for this process empirically within its hepatic elimination constant. Elimination of TCDD via skin, a minor process, is not described by either model. Thus, dose-response modeling based on body burden of TCDD in adult animals and humans can be conducted with either of the models, provided the duration of the experiment is at least 1 month, due to limitations in the CADM model. As shown in Figure 3-32, the predicted slope and body burden over a large dose range are quite comparable (generally within a factor of two).

Results of simulations of serum lipid concentrations or liver concentrations vary for the two models to a larger extent (up to a factor of 7), particularly for simulations of short duration. These differences reflect two characteristics of the PBPK model: first, quasi-steady-state is not assumed in the PBPK model; second, the serum lipid composition used in the model is not the same as the adipose tissue lipids. The CADM model does not account for differential solubility of TCDD in serum lipids and adipose tissue lipids, nor does it account for the diffusion-limited uptake by adipose tissue. Therefore, the PBPK model would appear to be superior to the CADM model with respect to the ability to simulate serum lipid and tissue concentrations during exposures that do not lead to the onset of steady-state condition in the exposed organism.

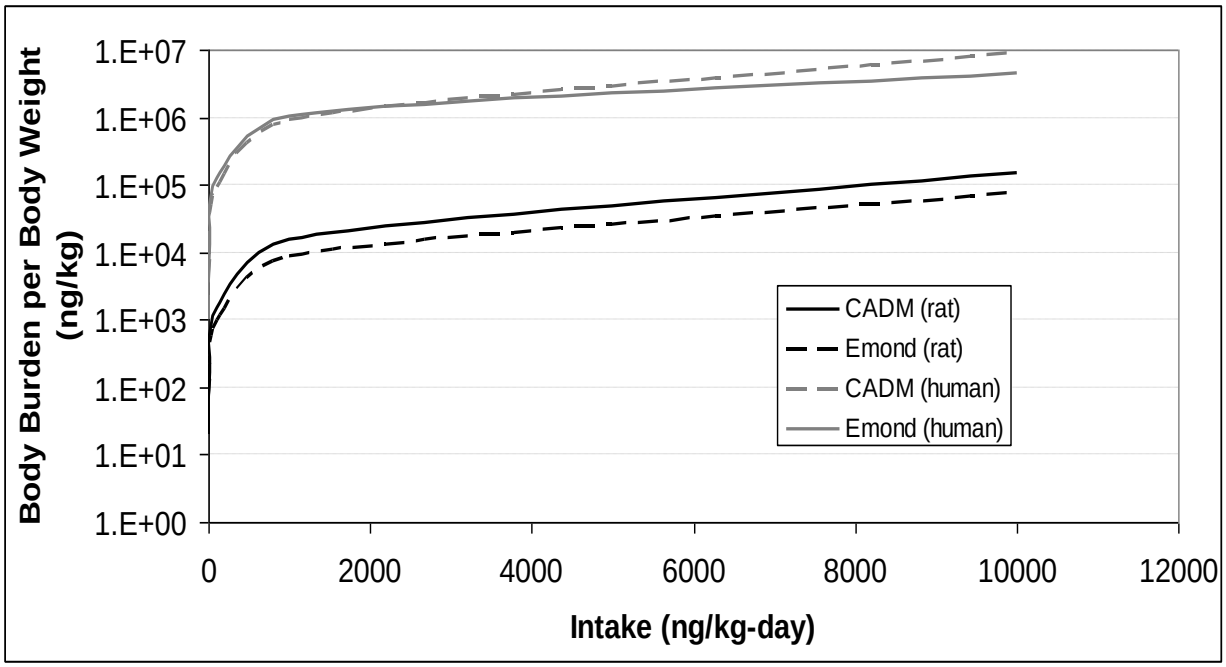

Figure 3-32. Comparison of the near-steady-state body burden simulated with CADM and Emond models for a daily dose ranging from 0 to 10,000 ng/kg-day in rats and humans.
The rat model was run for 13 weeks, and the human model was run from ages 20 to 30. The time-averaged concentration was used for each.

The CADM model is less complex than the PBPK model and has fewer parameters. Because the CADM model is constructed by fitting to data, its performance is likely to be reliable for the range of exposure doses, species, and life stages from which the parameter estimates were obtained. On the other hand, the PBPK model structure and parameters are biologically based and can be adapted for each species and life stage. Accordingly, the PBPK model has been adapted to simulate the kinetics of TCDD in the human fetus and in pregnant rats, as well as in adult humans and rats (Emond et al., 2006; Emond et al., 2005; Emond et al., 2004). The time step for calculation and dosing in the CADM model corresponds to 1 month. This requirement represents a constraint in terms of the use of this model to simulate a variety of dosing protocols used in animal toxicity studies. This requirement, however, is not a constraint with the PBPK models. So, either model would appear to be useful when simulating the body burden and serum lipid concentrations following a longer duration of exposure; but the PBPK model would be preferred for simulating alternative dose metrics of TCDD (e.g., blood concentration, total tissue concentration, bound concentration) for various exposure scenarios

(including single dose studies), routes, and life stages in the species of relevance, to TCDD dose-response assessment, particularly, mice, rats, and humans.

Two minor modifications, to enhance the biological basis, were made to the PBPK model of Emond et al. (2006), before its use in the computation of dose metrics for TCDD. The first one involved the recalculation of the volume of the rest of the body as follows:

$$WRE0 = (0.91 - (WLIB0 \times WLI0 + WFB0 \times WF0 + WLI0 + WF0)/(1 + WREB0)) \qquad \text{(Eq. 3-24)}$$

where

$WRE0$	= weight of cellular component of rest of body compartment (as fraction of body weight);
$WLI0$	= weight of cellular component of liver compartment (as fraction of body weight);
$WF0$	= weight of cellular component of fat compartment (as fraction of body weight);
$WREB0$	= weight of the tissue blood component of the rest of body compartment (as fraction of body weight);
$WLIB0$	= weight of the tissue blood component of the liver compartment (as fraction of body weight); and
$WFB0$	= weight of the tissue blood component of the fat compartment (as fraction of body weight).

In the original code, the weight of the rest of body compartment was calculated as the difference between 91% of body weight and the sum total of the fractional volumes of blood, liver tissue (intracellular component), and adipose tissue (intracellular component). The blood compartment in the PBPK model is not explicitly characterized with a volume; as a result, the total volume of the compartments is less than 91%. The recalculations shown above were used to address this problem. Given the very low affinity of TCDD for blood and rest of the body, reparameterizing the model resulted in less than a 1% change in output compared to the published version of the PBPK model for chronic exposure scenarios (Emond et al., 2006).

The second minor modification related to the calculation of the rate of TCDD excreted via urine. The original model code computed the rate of excretion by multiplying the urinary clearance parameter with the concentration in the rest of the body compartment. Instead, the

code was modified to use the blood concentration in this equation. This resulted in the reestimation of the urinary clearance value in the rat and human models, but it did not result in any significant change in the fit and performance of the original model. In addition to the minor modifications in the model structure, a recalibration of the gastric nonabsorption constant of the PBPK model was conducted to match human oral bioavailability data (Poiger and Schlatter, 1986).

The revised parameter estimates of the rat, mouse, and human models are captured in Table 3-8 with a footnote.

3.3.4.5. *Recommended Dose Metrics for Key Studies*

The selection of dose metrics for the dose-response modeling of key studies is largely the result of (1) the relevance of a dose metric on the basis of current knowledge of TCDD's mechanism of action for critical endpoints and (2) the feasibility and reliability of obtaining the dose metric with available PK models. Secondarily, the goodness-of-fit of the dose-response models (which reflects the relationship of the selected internal dose measures to the response) can be used to inform selection of the most appropriate dose metric for use in deriving TCDD toxicity values.

Body burden—even though this metric is based on mechanistic considerations—is a somewhat distant measure of dose with respect to target tissue dose, and this metric represents the ―overall" average concentration of TCDD in the body. However, a benefit of body burden is that this metric represents a dose measure for which the available PK models can provide highly certain estimates. Thus, the overall confidence associated with the use of body burden in TCDD assessment is categorized as medium.

The confidence in the ability of PK models to simulate blood concentration as a dose metric is high, given that the models have been shown to consistently reproduce whole blood (or serum lipid-normalized) TCDD concentration profiles in both humans and rats. Considering the facts that the PBPK models simulate whole blood rather than the serum lipid-normalized concentrations of TCDD and that the study-specific values of serum lipid content are not known with certainty, it is preferable to rely on TCDD blood concentrations as the dose metric. The blood concentrations, if intended, can be normalized on the basis of appropriate total lipid levels. However, based on mechanistic considerations, the confidence in their use would be somewhat

lower for hepatic effects. This conclusion reflects the concern regarding the inconsistent relationship between the two variables with increasing dose levels and the fraction of steady-state attained at the time of observation. For other systemic effects related to tissue concentrations, the confidence in the use of TCDD serum or blood concentration is high, particularly for chronic exposures, given the absence of data on organ-specific nonlinear mechanisms. In general, the tissue concentration typically cannot be calculated as a reliable dose metric with either the CADM or the Emond models. One exception is the use of the Emond PBPK models to estimate levels in liver, a metric that is relevant based on MOA considerations. However, it is noted that the hepatic TCDD level encompasses free and bound TCDD, and it is a highly complex entity for dose metric considerations. Finally, the AhR-bound concentration may be evaluated for receptor-mediated effects. This dose metric can be obtained by PBPK models, although uncertainties associated with the lack of data for this dose metric render it to be of low confidence (see Table 3-19). The alternative dose metrics for dose-response modeling of TCDD selected on the basis of MOA and PK modeling considerations are summarized in Tables 3-20 and 3-21.

Table 3-20. Overall confidence associated with alternative dose metrics for noncancer dose-response modeling for TCDD using rat PBPK model

End point	Body burden	Blood or serum concentration	Liver concentration	Bound concentration in liver
Liver effects	M		H	M/L
Nonhepatic effects	M	H		M/L

H = high, M = medium, L = low.

Table 3-21. Overall confidence associated with alternative dose metrics for noncancer dose-response modeling for TCDD using mouse PBPK model

End point	Body burden	Blood or serum concentration	Liver concentration	Bound concentration in liver
Liver effects	M		M	L
Nonhepatic effects	M	M		L

H = high, M = medium, L = low.

These measures of internal dose can be obtained as peak, average, integral (AUC), or terminal values. For chronic exposures in rodents (ca. 2 years), the terminal and average values would be fairly comparable under steady-state conditions. For less-than lifetime exposures, however, the terminal and average values will differ, and, therefore, an overall average or integrated value (AUC) would be more appropriate. Similarly, for developmental exposures, these alternative dose metrics can be obtained with reference to the known or hypothesized exposure window of susceptibility.

3.3.5. Uncertainty in Dose Estimates

3.3.5.1. *Sources of Uncertainty in Dose Metric Predictions*

3.3.5.1.1. *Limitations of available pharmacokinetics (PK) data*

3.3.5.1.1.1. ***Animal data***

The available animal data relate to blood, liver, and adipose tissue concentrations for certain exposure doses and scenarios. Although these data are informative regarding the dose- and time-dependency of TCDD kinetics for the range covered by the specific studies (see Section 3.3.2), they do not provide the peak, average, terminal, or lipid-normalized values of dose metrics associated with the key studies selected for this assessment. The limited available animal PK data are useful, however, in the evaluation of the pharmacokinetic models (see Section 3.3.4).

3.3.5.1.1.2. ***Human data***

The human data on potential dose metrics are restricted to the serum lipid-adjusted TCDD concentrations associated with mostly uncharacterized exposures (see Sections 3.3.2 and 3.3.3). While these data are useful in estimating half-lives in exposed human individuals, they do not provide estimates of hepatic clearance or reflect target organ exposure. Some autopsy data have been used to infer the partition coefficients; however, these data were collected without quantification of the temporal nature of TCDD uptake (see Section 3.2). Despite the limitations associated with the available human data, there has been some success in using these data to infer the half-lives and elimination rates in humans using pharmacokinetic models (Emond et al., 2006; Aylward et al., 2005b; Carrier et al., 1995a).

3.3.5.1.2. *Uncertainties associated with model specification*

Uncertainty associated with model specification should be viewed as a function of the specific application, such as interspecies extrapolation, intraspecies variability, or high-dose-to-low-dose extrapolation. Because the use of pharmacokinetic models in this assessment is limited to interspecies extrapolation and high-dose-to-low-dose extrapolation, it is essential to evaluate the confidence in predicted dose metrics for these specific purposes. For interspecies extrapolation, the PBPK and CADM models calculate differences in dose metric between an average adult animal and an average adult human. Both models have a biologically and mechanistically relevant structure along with a set of parameters with reasonable biological basis and reproduce a variety of pharmacokinetic data on TCDD in both rodents and humans. These models possess low uncertainty with respect to body burden, blood, and TCDD/serum (lipid) concentration for the purpose of conducting rat to human extrapolation. However, for other dose metrics, such as free, total, or bound hepatic concentrations, the uncertainty is higher in the CADM model compared to the PBPK model due to model specification differences related to the mechanisms of sequestration and induction in the liver (see Section 3.3.3).

For the purpose of high-dose-to-low-dose extrapolation in experimental animals, confidence in both models is high with respect to a variety of dose metrics (see previous discussion). The high confidence results from the use of the PBPK models to reproduce a number of data sets covering a wide range of dose levels in rodents (i.e., rats, mice) including the dose ranges of most of the key toxicological studies. Given that the TCDD levels during and at the end of exposures were not measured in most of the key studies, use of the PBPK models is preferred because these models account for dose-dependent elimination, induction, and sequestration. Despite the empirical nature of the specification of these key processes in PBPK models, they essentially reproduce the dose-dependent behavior in rodents, supporting their use in deriving dose metrics for dose-response modeling of TCDD. Overall, the confidence in the use of the alternative dose metrics (identified in Table 3-19) is greater than the confidence in the use of administered dose for TCDD, for relating to the concentration within tissues to produce an effect. The administered dose does not take into account interspecies differences in the volume of distribution and clearance or the complex nonlinear processes determining the internal dose.

The PBPK model of Emond et al. (2006) could benefit from further refinement and validation, including a more explicit consideration of dose-independent elimination pathways.

As indicated in Section 4, there is some uncertainty associated with the way the elimination of TCDD is described in the existing human PBPK model. The current model essentially treats all TCDD elimination as related to dose-dependent metabolism in the liver. In this regard, the classical and more recent PK data on TCDD may be useful in further improving the confidence in their predictions. However, it is likely that there is dose-independent elimination of TCDD via feces and, to a lesser extent, skin; juxtaposition of available elimination rate data with the PBPK model predictions suggests that the current PBPK model modestly overestimates the dose-dependency of overall TCDD elimination. (The central estimate of the slope of the relationship between the log of the TCDD elimination rate and the log of the TCDD level is only about three−fourths of that expected using the unmodified PBPK model). Emond et al. (2005) acknowledge that the model did not describe the elimination of TCDD from the blood into the intestines, but it indirectly accounted for this phenomenon with the use of the optimized elimination rate.

3.3.5.1.3. *Impact of human interindividual variability*

The sources and extent of human variability suggested by the available data are presented in Section 3.3.3, although there is some discussion of the impact of individual differences in body fat content. The CADM model facilitates the simulation of body burden and serum lipid concentrations on the basis of BMI and tissue weights of people, and the PBPK model simulates alternative dose metrics in the fetus and in pregnant animals in addition to adult animals and humans. However, neither of these models has been parameterized for simulation of population kinetics and distribution of TCDD dose metrics. Therefore, at the present time, a quantitative evaluation of the impact of human variability on the dose metrics of TCDD is not feasible, and dose metric-based replacement of the default interindividual factor has not been attempted.

3.3.5.2. *Qualitative Discussion of Uncertainty in Dose Metrics*

The usefulness of the CADM and PBPK models for conducting dose-response modeling (rodent bioassays), interspecies (rodent to human) and intraspecies (high-dose to low-dose) extrapolations is determined by their reliability in predicting the desired dose metrics. The confidence in the model predictions of dose metrics is dictated by the extent to which the model has been verified with empirical data relevant to the dose metric, supplemented by sensitivity

and uncertainty analyses. Analysis of sensitivity or uncertainty has not been conducted with the CADM model. For the PBPK model, Emond et al. (2006) published the initial results from sensitivity analyses of acute exposure modeling (see Section 3.3.3). One of the objectives of a sensitivity analysis that is of highest relevance to this assessment is the identification of the most critical model parameters with respect to the model output (i.e., dose metric).

If the model simulations have only been compared to entities that do not correspond to the moiety representing the dose metric, or if the comparisons have only been done for some but not all relevant dose levels, routes, and species, then the reliability in the predictions of dose metric can be an issue. The extent to which model results are uncertain will depend largely upon the extent to which the dose metric is measurable (e.g., serum concentrations of TCDD) or inferred (e.g., AhR-bound TCDD concentration).

With respect to TCDD body burden, whole-liver and blood concentration predictions in the rat model, which are well-calibrated with measured data, uncertainty is relatively low. Therefore, the need for sensitivity and uncertainty analysis is less critical, and confidence in these dose metrics is high. For those dose metrics that are not directly measurable or are less easily verified by available calibration methods, such as free-liver and AhR-bound concentrations, sensitivity and uncertainty analyses are crucial for assessing the reliability of model predictions, and confidence is low. For the human model, calibration is largely dependent on blood (LASC) TCDD measurements, which are much less extensive than for the rat model. Because the blood measurements are reported as LASC, uncertainty and variability in serum:blood and fat:serum ratios also are a factor when evaluating the adequacy of the whole-blood TCDD metric. Furthermore, the human data are mostly representative of much higher exposures than the environmental exposures of interest to the EPA. Because of these additional uncertainties, only medium confidence can be held in the human model whole-blood TCDD concentration predictions at higher exposures (observed effect range) and low-to-medium confidence at lower exposures (background exposure range).

Sensitivity analysis for the Emond rat PBPK model predictions of liver TCDD concentration indicated that hepatic CYP1A2 concentration is the most sensitive parameter (Emond et al., 2006). For the Emond human PBPK model, the absorption parameters, basal concentration of CYP1A2, and adipose tissue:blood partition coefficients were identified as highly sensitive parameters.

Confidence in the Emond rat and human PBPK models at high exposures is medium for the purpose of rat-to-human extrapolation based on blood concentrations, given that the key human model parameters are both sensitive and uncertain; confidence is low for lower exposures. Conversely, confidence in the use of AhR-bound TCDD is low because of the large uncertainty in the fraction of AhR-bound TCDD in the liver.

With regard to the predictability of body burden, the absorption and excretion parameters were among the sensitive parameters in the rat. Several other parameters were also identified as being sensitive in humans. Despite the sensitivity to these parameters and the uncertainty associated with individual parameter estimates, the overall confidence in the model predictions of body burden appears to be high given the reproducibility of empirical data on tissue burdens and blood concentrations of TCDD in various experiments by both models. Similar conclusions can be drawn for blood concentration of TCDD predicted by the PBPK model, except that the assigned value of blood (serum) lipid content will have additional impact on this dose metric to the extent that the calibration data were in terms of LASC. Variability of total lipid levels and variability of the contribution of phospholipids and neutral lipids to the total lipid pool across species, lifestage, and study groups is to be expected (Bernert et al., 2007; Poulin and Theil, 2001).

Both conceptual (biological) relevance and prediction uncertainty are important in the choice of dose metric for dose-response modeling and interspecies extrapolation. Conceptual relevance has to do with how ―close" the metric is to the observed effect, taking into account both the target tissue and the MOA. In this context, a greater degree of confidence is held for dose metrics that are more proximate to the event (i.e., specific effect). Prediction uncertainty reflects the lack of confidence in the model predictions of dose metrics. Tables 3-22 and 3-23 provide a qualitative ranking of the importance and magnitude of each dose metric with respect to these two sources of uncertainty. Conceptual relevance is low for the use of administered dose in dose-response modeling because known (nonlinear) physiological processes are ignored; conversely, conceptual uncertainty is much lower for use of internal dose metrics more proximal to the affected organs.

Table 3-22. Contributors to the overall confidence in the selection and use of dose metrics in the dose-response modeling of TCDD based on rat and human PBPK models[a]

Dose metric	Conceptual relevance	Prediction uncertainty	Overall confidence
Administered dose	L	NA	L
Body burden	M	M	M−L
Blood concentration	M	L	M
Liver concentration	L	M	L
Receptor (AhR) occupancy	H	H	L

[a]Using professional judgment, EPA ranked its confidence in the CADM model as low, medium, or high (or not applicable) based on model simulations of administered dose, absorbed dose, body burden, serum lipid concentration, total tissue (liver) concentration, and receptor occupancy.
H = high, M = medium, L = low, NA = not applicable.

Table 3-23. Contributors to the overall uncertainty in the selection and use of dose metrics in the dose-response modeling of TCDD based on mouse and human PBPK models

Dose metric	Conceptual uncertainty	Prediction uncertainty
Administered dose	H	NA
Absorbed dose	H	L
Body burden	M	M
Blood or serum concentration	M	M
Tissue concentration	L	M/H
Receptor occupancy	L	H

H = high, M = medium, L = low, NA = not applicable

Table 3-22 presents a cross-walk of relevance, uncertainty, and overall confidence associated with the use of various dose metrics for dose-response modeling of TCDD. Using professional judgment, EPA ranked its confidence in PBPK models as low, medium, or high (or not applicable) based on model simulations of administered dose, absorbed dose, body burden, serum lipid concentration, total tissue (liver) concentration, and receptor occupancy. As shown in Table 3-22, blood/serum concentrations have the highest overall confidence (medium), followed by body burden (medium to low) for application in dose-response modeling. When using the mouse PBPK model along with the human model (see Table 3-23), the contribution of

the prediction uncertainty to the overall uncertainty increases due to the limited comparison of the mouse model simulations with empirical data.

3.3.6. Use of the Emond Pysiologically Based Pharmacokinetic (PBPK) Models for Dose Extrapolation from Rodents to Humans

EPA has selected the Emond et al. (2006; 2005; 2004) PBPK models, as modified by EPA for this assessment, for establishing toxicokinetically equivalent exposures in rodents and humans.[29] The 2003 Reassessment (U.S. EPA, 2003) presented a strong argument for using the relevant tissue concentration as the effective dose metric. However, no models exist for estimation of all relevant tissue concentrations. Therefore, EPA has decided to use the concentration of TCDD in blood as a surrogate for tissue concentrations, assuming that tissue concentrations are proportional to blood concentrations. Furthermore, because the RfD is necessarily expressed in terms of average daily exposure, the blood concentrations are expressed as averages over the relevant period of exposure for each endpoint. Specifically, blood concentrations in the model simulations are averaged from the administration of the first dose to the administration of the last dose plus one dosing interval (time) unit in order to capture the peaks and valleys for each administered dose. That is, for daily dosing, 24 hours of TCDD elimination following the last dose is included in the average (the modeling time interval is 1 hour); for a weekly dosing protocol, a full week is included. In addition, because of the accumulation of TCDD in fat and the large differences in elimination kinetics between rodent species and humans, exposure duration plays a much larger role in TK extrapolation across species than for rapidly eliminated compounds. Because of these factors, EPA is using discrete exposure scenarios that relate human and rodent exposure durations. The use of discrete exposure scenarios was introduced previously in Section 3.4.4.2 describing first-order kinetic modeling and is further described in the following paragraphs. This section concludes with a quantitative evaluation of the impact of exposure duration on the rodent-to-human TK extrapolation from both the human and rodent —ends" of the process.

Figure 3-33 shows the TCDD blood concentration-time profile for continuous exposure at 0.01 ng/kg-day, as predicted by the Emond human PBPK model, and the target TCDD concentrations corresponding to the three discrete exposure scenarios used by EPA in this

[29] The models will be referred to hereafter as the —Emond human PBPK model" and the —Emond rodent PBPK model," with variations when referring to individual species or components (e.g., gestational).

document. The target concentrations are those that would be identified in the animal bioassay studies that correspond to a particular POD (no-observed-adverse-effect level, lowest-observed-adverse-effect level, or benchmark dose lower confidence bound) established for that bioassay. That is, the target concentrations represent the toxicokinetically equivalent internal exposure to be translated into an equivalent human intake (or HED).

For the lifetime exposure scenario, the HED is —matched" to the lifetime average TCDD blood concentration from a lifetime animal bioassay result by determining the continuous daily intake that would result in that average blood concentration for humans over 70 years. A table for converting lifetime-average blood concentrations and other internal dose metrics to daily human TCDD intake rates is presented in Appendix E.4.

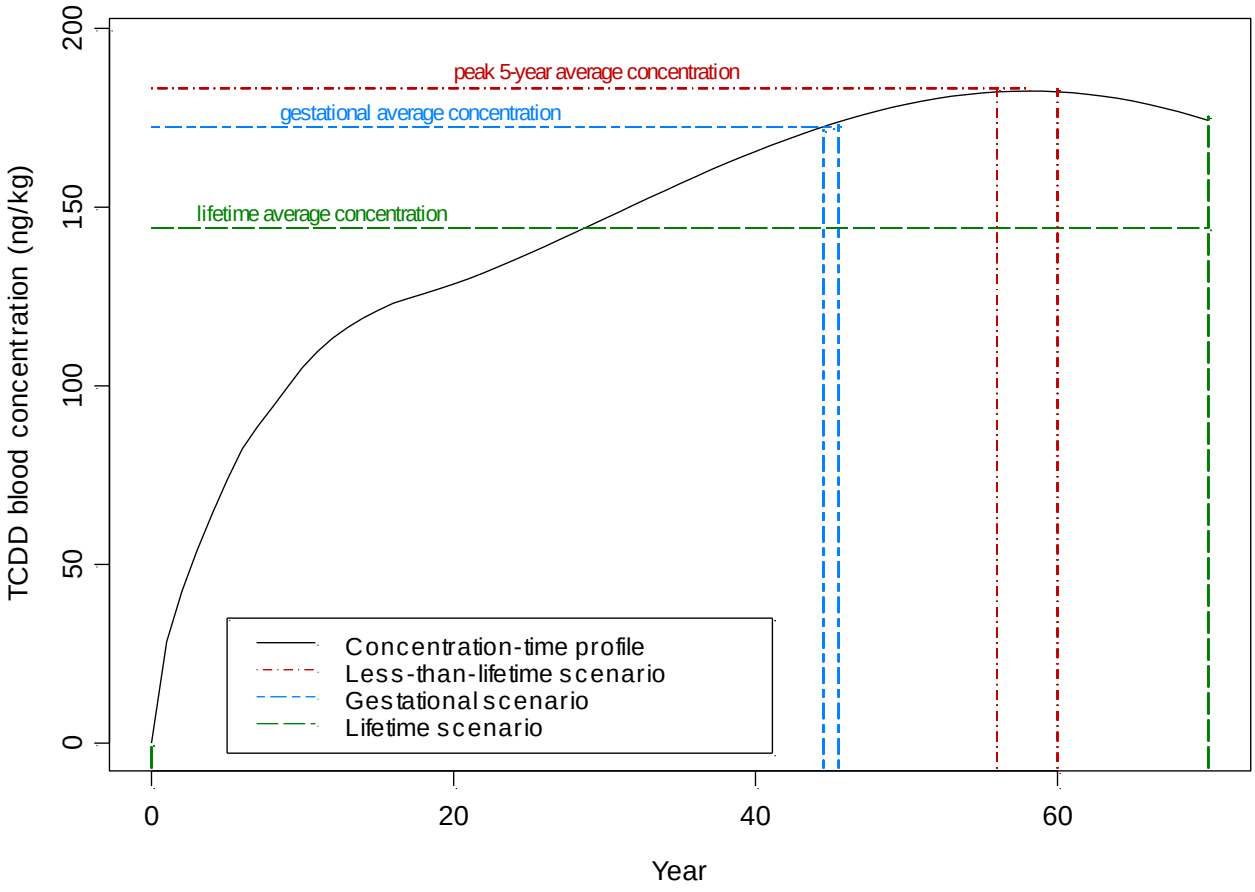

Figure 3-33. TCDD serum concentration-time profile for lifetime, less-than-lifetime, and gestational exposure scenarios, with target concentrations shown for each; profiles generated with Emond human PBPK model.

For the gestational exposure scenario, the effective TCDD blood concentration (usually the peak) determined for the particular POD in a particular developmental study is matched to the average TCDD blood concentration over the gestational portion of the human gestational exposure scenario. The HED is determined as the continuous daily intake, starting from birth that would result in that average blood concentration over the 9-month gestational period for a pregnancy beginning at 45 years of age. The choice of 45 years as the beginning age of pregnancy is conservative in that the daily exposure achieving the target blood concentration is smaller than for pregnancies occurring earlier in life (e.g., a pregnancy beginning at 30 years of age). A table for converting average gestational blood concentrations and other internal dose metrics to human intake for the 45-year-old pregnancy scenario is presented in Appendix E.4. Also, a comparison of the 45-year old pregnancy scenario to one beginning at age 25 is presented in Table 3-24. Using the 25 year-old pregnancy scenario increases the HED by 30 to 60% for typical animal bioassay PODs (3 to 30 ng/kg).

Table 3-24. Comparison of human equivalent doses from the Emond human PBPK model for the 45-year-old and 25-year-old gestational exposure scenarios

Animal bioassay POD (ng/kg-day)	Species	TCDD blood concentration[a]	HED 45 year-old	HED 25 year-old	25-yr:45-yr ratio
3	Mouse	8.800E−02	6.79E−04	1.03E−03	1.5
	Rat	1.815E−01	1.87E−03	2.98E−03	1.6
30	Mouse	7.115E−01	1.51E−02	2.07E−02	1.4
	Rat	1.367E+00	4.22E−02	5.41E−02	1.3

[a]Determined from the Emond rodent PBPK models assuming a single exposure on GD 13.

For a less-than-lifetime exposure, the average TCDD blood concentration over the exposure period in the animal bioassay associated with the POD is matched to the average over the 5-year period that includes the peak concentration (58 years for an intake of 0.01 ng/kg-day). The HED is determined as the continuous daily intake that would result in the target concentration over peak 5-year period. The use of the peak is analogous to the approach in the 2003 Reassessment, where the terminal steady-state body burden played the same role. The 5-year average over the peak is taken to smooth out sharp peaks and more closely approximate a

plateau. The choice of peak is health protective because humans of any age must be protected for short-term exposures, and the daily intake achieving a given TCDD blood concentration is smallest when matched to the peak exposure as opposed to an average over shorter durations. Thus, target concentrations for any exposure duration of less-than-lifetime must be averaged backwards from the end of the lifetime scenario, rather than from the beginning. The only exception would be if the short-term endpoints evaluated in the animal bioassay were associated with a specific life stage (such as for the gestational scenario). Note that this scenario lumps all exposures from 1 day to over 1 year in rodents into the same less-than-lifetime category. Conceptually, duration-specific scenarios could be constructed by defining equivalent rodent and human exposure durations. However, for the most part, defining duration equivalents across species is a somewhat arbitrary exercise, not generally based on physiologic or toxicological processes, but relying primarily on fraction-of-lifetime conversions. EPA defines "lifetime" exposure as 2 years and 70 years for rodents and humans, respectively. So, a half-lifetime equivalence of 1 year in rodents and 35 years in humans is defined easily. Also, considering a subchronic exposure to be 10–15% of lifetime, leads to an equivalence of 90 days in rodents and 7–10 years in humans. However, in the practical sense with respect to the Emond human PBPK model predictions, the differences in the dose-to-target-concentration ratios are not significantly dissimilar from the peak 5-year average scenario, differing by less than 5%. A table for converting less-than-lifetime average blood concentrations and other internal dose metrics to human intake is presented in Appendix E.4.

The net effect of using three different scenarios for estimating the HED from rodent exposures is that, for the same target concentration, the ratio of administered dose (to the rodent) to HED will be larger for short-term exposures than for chronic exposures. Figure 3-34 is similar to Figure 3-33, except that it shows the relationship of daily intake to a fixed target TCDD blood concentration level. Figure 3-34 shows that, for human intakes of approximately 0.01 ng/kg-day, the difference in the defined scenarios is 40% or less, with a lifetime-scenario daily intake of 0.014 ng/kg-day required to reach the same target concentration for a shorter-term exposure of 0.01 ng/kg-day. The corresponding daily intake for the gestational scenario is 0.011 ng/kg-day. Because of the nonlinearities in the Emond human PBPK model, the magnitude of the difference between the lifetime and less-than-lifetime exposure scenarios increases at lower intake levels, but not to a substantial degree.

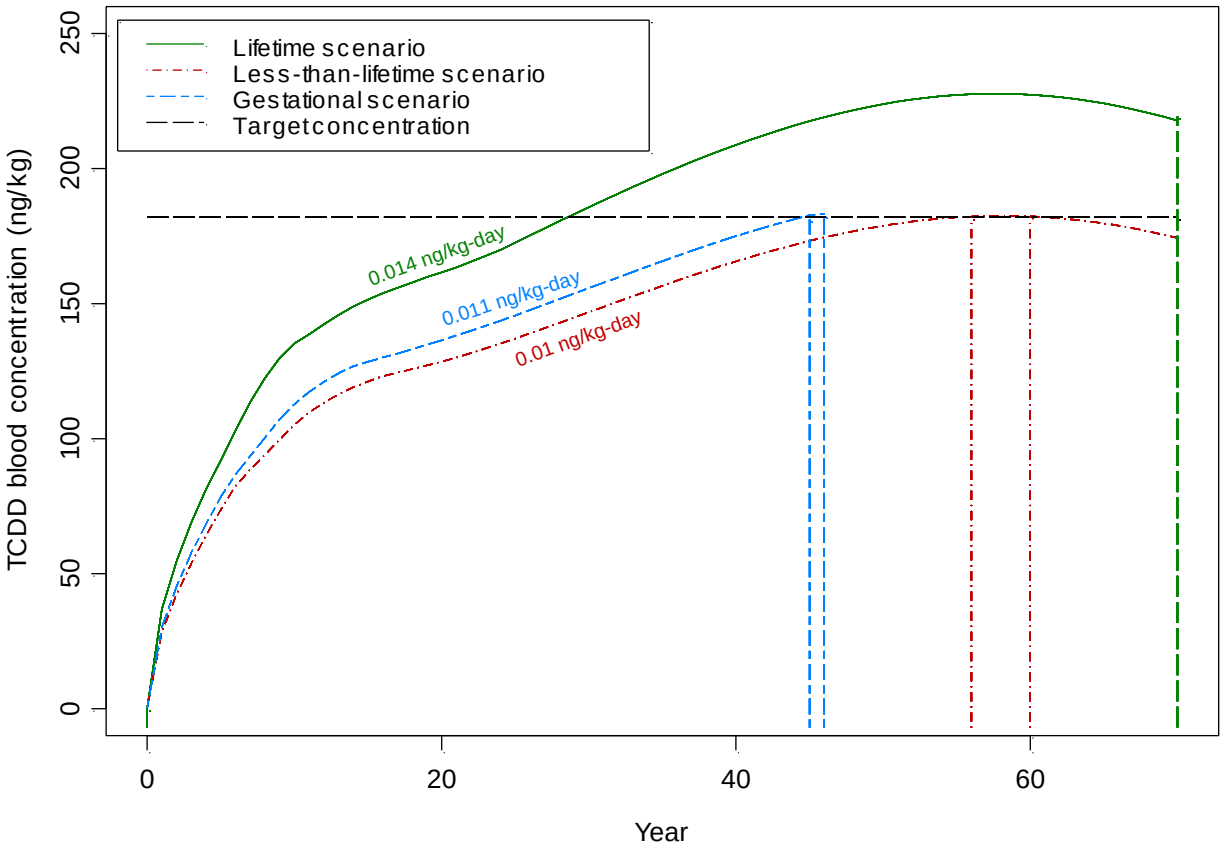

Figure 3-34. TCDD serum concentration-time profile for lifetime, less-than-lifetime and gestational exposure scenarios, showing continuous intake levels to fixed target concentration; profiles generated with Emond human PBPK model.

The differential effect of short- and long-term exposures is much more accentuated at the rodent end of the exposure kinetic modeling. Analogous to the processes described in the previous section for first-order body burden (see Section 3.3.4.2), the TCDD blood concentration for single exposures is essentially the immediate absorbed fraction of the administered dose, which will be somewhat lower than the administered dose, while for chronic exposure, the TCDD blood concentration will reflect the long-term accumulation from daily exposure, which will be very much larger than the administered dose (expressed as a daily intake). Table 3-25 shows the overall impact of TK modeling on the extrapolation of administered dose to HED, comparing the Emond PBPK and first-order body burden models. For comparison purposes, the

administered dose is fixed at 1 ng/kg-day for all model runs. Large animal-to-human TK extrapolation factors (TK_{EF}) are evident for short-term mouse studies, decreasing in magnitude with increasing exposure duration. The only exception is the slightly lower extrapolation factor for the mouse 1-day exposure, which is the result of the relatively short TCDD half-life (10 days) in mice and the use of the peak TCDD blood concentration as representative of single exposures, compared to the average TCDD blood concentration over the exposure period used for multiple exposures. The TK_{EF}s are lower for rats because of the slower elimination of TCDD in rats compared to mice. Also, because of the nonlinear kinetics inherent in the Emond PBPK model, the span of the HED (13-fold for mice) across these exposure durations is greater than the span of the LASC (fourfold for mice). Because of the dose-dependence of TCDD elimination in the Emond model, the TK_{EF} becomes smaller with decreasing intake. The result of this nonlinearity is that, although Table 3-25 shows much lower TK_{EF}s for the Emond PBPK model than for the first-order body burden metric, at much lower HED levels, the predictions of the two models are much closer.

Table 3-25. Impact of toxicokinetic modeling on the extrapolation of administered dose to HED, comparing the Emond PBPK and first-order body burden models (administered dose = 1 ng/kg-day)

Exposure duration (days)	1st-order BB		Emond PBPK		
	HED[a] (ng/kg−day)	TK_{EF}[b]	LASC[c] (ng/kg)	HED (ng/kg−day)	TK_{EF}
Mouse					
1	2.57E−4	3,882	75.5	9.49E−4	1,054
14	1.47E−3	681	64.4	8.17E−4	1,224
90	3.25E−3	307	173	3.83E−3	261
365	3.70E−3	270	248	6.66E−3	150
730	4.43E−3	226	263	1.08E−2	93
Rat					
1	2.63E−4	3,802	110	1.87E−3	535
14	1.76E−3	569	208	5.22E−3	192
90	6.13E−3	163	599	2.81E−2	36
365	8.68E−3	115	811	4.52E−2	22
730	1.07E−2	93	853	6.47E−2	15

[a]Human-equivalent doses.
[b]Rodent-to-human toxicokinetic extrapolation factor.
[c]Lipid-adjusted serum concentration.

4. ORAL REFERENCE DOSE

This section presents U.S. EPA's response to the NAS recommendations that EPA discuss more explicitly the modeling of noncancer endpoints and develop a RfD to address noncancer effects associated with oral 2,3,7,8-TCDD exposures. Section 2 details the selection of the animal bioassays with the lowest TCDD doses associated with the development of adverse noncancer effects and the selection of relevant epidemiologic studies of adverse noncancer health effects. Section 3 discusses the kinetic modeling and estimation of human equivalent daily oral doses that are used in TCDD RfD development in this section. This section discusses the modeling of noncancer health effects data associated with TCDD exposure and the derivation of an RfD. Specifically, Section 4.1 summarizes the NAS comments on TCDD dose-response modeling and EPA's response, including justification of selected noncancer effects and statistical characterization of modeling results. Section 4.2 presents the TCDD dose-response modeling undertaken for identification of candidate PODs for derivation of an RfD. In Section 4.3, EPA derives an RfD for TCDD. Section 4.4 describes the qualitative uncertainties in the RfD. Finally, Section 4.5 presents two separate focused quantitative analyses of uncertainty for the TCDD RfD. The first focuses on three data sets (from two epidemiologic studies and one animal bioassay) and quantifies the consequences of alternative decisions in the development of PODs based on these studies. The second develops POD estimates for several studies, some of which did not qualify for consideration for RfD derivation in the study selection process, but could be considered in the context of investigating uncertainty limits for the RfD.

4.1. NAS COMMENTS AND EPA'S RESPONSE ON IDENTIFYING NONCANCER EFFECTS OBSERVED AT LOWEST DOSES

The NAS recommended that EPA identify the noncancer effects associated with low-dose TCDD exposures and discuss its strategy for identifying and selecting PODs for noncancer endpoints, including biological significance of the effects.

> With respect to noncancer end points, the committee notes that EPA does not use a rigorous approach for evaluating evidence from studies... (p. 47 NAS, 2006b)

The Reassessment should describe clearly the following aspects:

1. The effects seen at the lowest body burdens that are the primary focus for any risk assessment—the ~~"~~critical effects."

2. The modeling strategy used for each noncancer effect, paying particular attention to the critical effects, and the selection of a point of comparison based on the biological significance of the effect; if the ED_{01} is retained, then the biological significance of the response should be defined and the precision of the estimate given... (p. 187, NAS, 2006b).

In this document, EPA has developed a strategy for identifying the noncancer data sets and PODs that represent the most sensitive and toxicologically-relevant endpoints for derivation of an RfD for TCDD. EPA began this process by using the animal bioassays and epidemiologic studies that met its study inclusion criteria as sources of these data sets.

For all noncancer epidemiologic studies that were identified as suitable for further quantitative dose-response analyses in Section 2.4.1, EPA has chosen to use NOAELs and LOAELs to identify PODs; BMD modeling was not feasible given the nature of the data presented in these studies. Figure 4-1 shows EPA's process for determination of PODs from these key epidemiologic studies. EPA first evaluated the dose-response information in the study to determine whether it provided an estimate of TCDD exposure and an observed health outcome that was toxicologically relevant[30] for RfD derivation. If such data were available, EPA identified a NOAEL or LOAEL as a POD. For each of these, EPA applied a toxicokinetic model to estimate the continuous oral daily intake associated with the POD that could be used in the derivation of an RfD (see Section 4.2.3). If all of this information was available, the result was included as a POD for derivation of a candidate RfD.

Figures 4-2 and 4-3 together present the strategy EPA used to evaluate the study/endpoint combinations found in the noncancer animal bioassays that met EPA's study inclusion criteria in Section 2,4.2, estimate PODs, and develop a final set of candidate RfDs for TCDD. Figure 4-2 summarizes the disposition of the 78 animal noncancer studies selected for TCDD dose-response analyses. Of these studies, 16 were eliminated because EPA determined that they contained no toxicologically-relevant endpoints that could be used to derive a candidate RfD (discussed

[30] RfDs are based on health endpoints that are inherently adverse or clearly linked to downstream functional or pathological alterations (U.S. EPA, 2002).

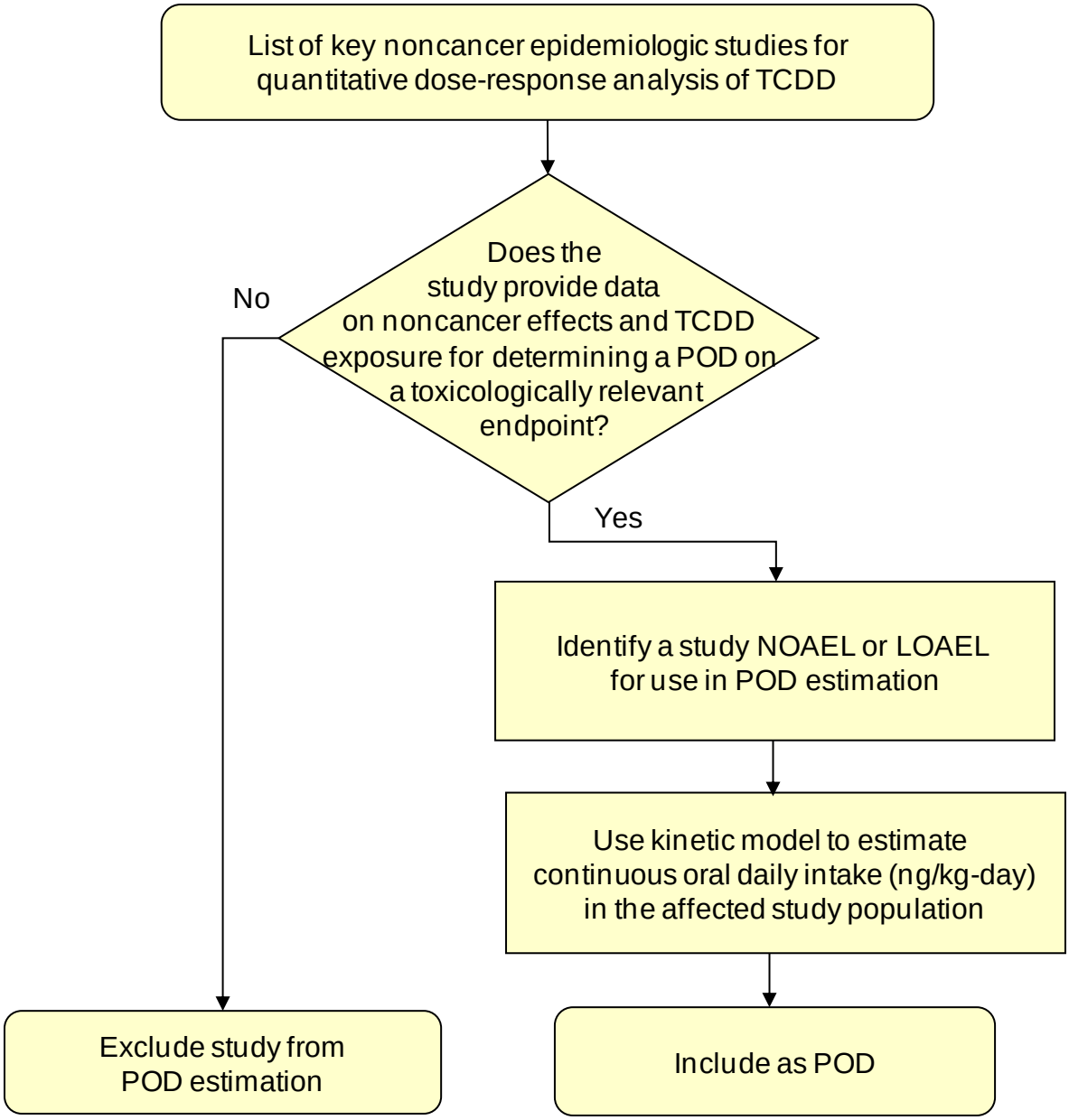

Figure 4-1. EPA's process to identify and estimate PODs from key epidemiologic studies for use in noncancer dose-response analysis of TCDD. For each noncancer study that qualified using the study inclusion criteria, EPA evaluated the dose-response information developed by the study authors to evaluate whether the study provided noncancer effects and TCDD dose data for a toxicologically relevant endpoint. If such data were available, EPA identified a NOAEL or LOAEL as a POD. Then, EPA used a human kinetic model to estimate the continuous oral daily intake (ng/kg-day) for the POD that could be used in the derivation of a candidate RfD based on the study data. If all of this information was available, then the result was included as a POD.

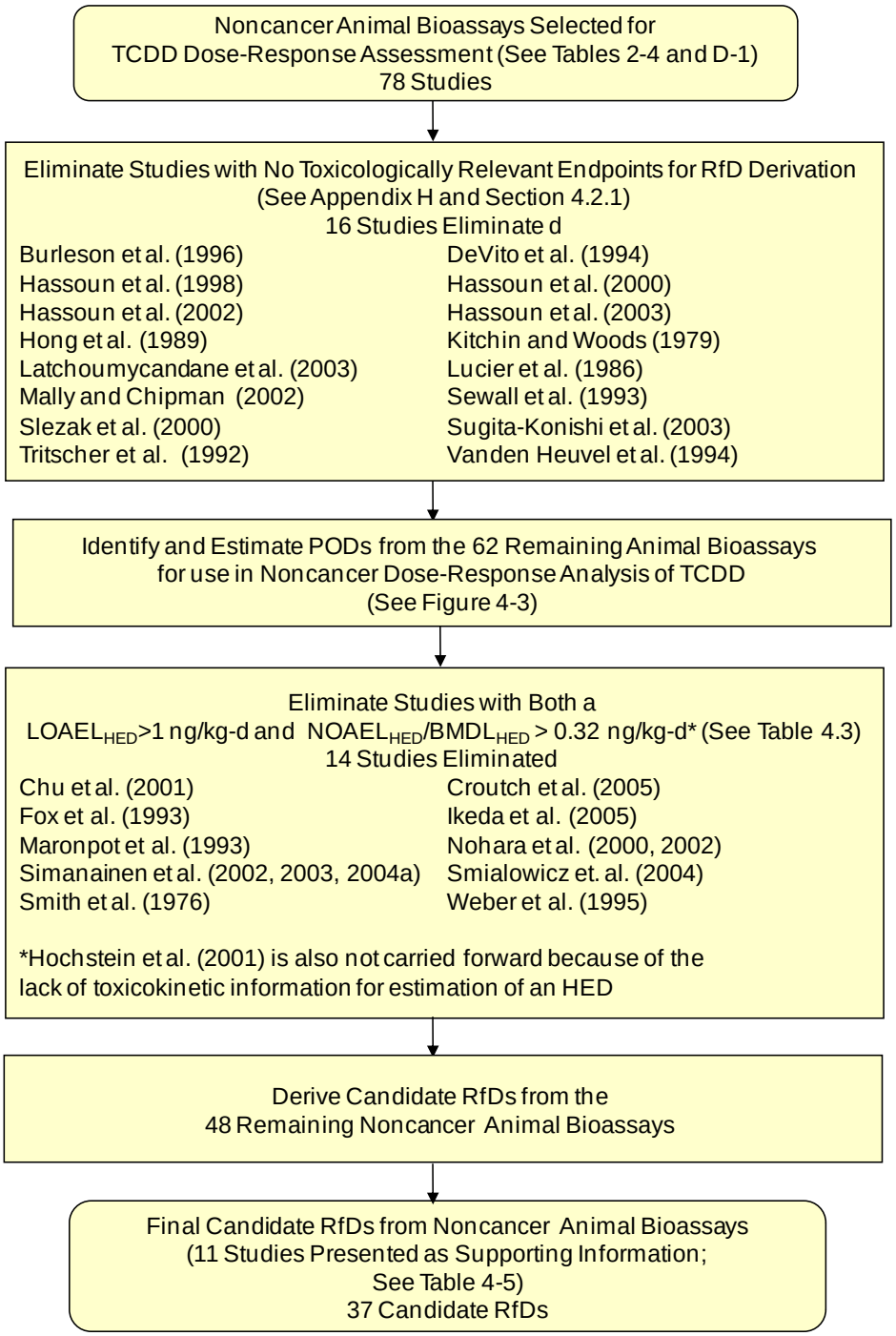

Figure 4-2. Disposition of noncancer animal bioassays selected for TCDD dose-response analysis.

EPA evaluated each noncancer endpoint found in the 78 studies that passed the study inclusion criteria. From this evaluation, EPA eliminated 16 studies that contained no toxicologically relevant endpoints for RfD derivation. Then, as detailed in Figure 4-3, EPA selected and identified PODs for use in deriving candidate RfDs. EPA then eliminated 13 studies based on dose limits for the PODs' HEDs; one study was also not carried forward because of the lack of toxicokinetic information for estimation of an HED. Of the remaining 48 studies, EPA derived 37 RfD candidates, with 11 studies presented as supporting information.

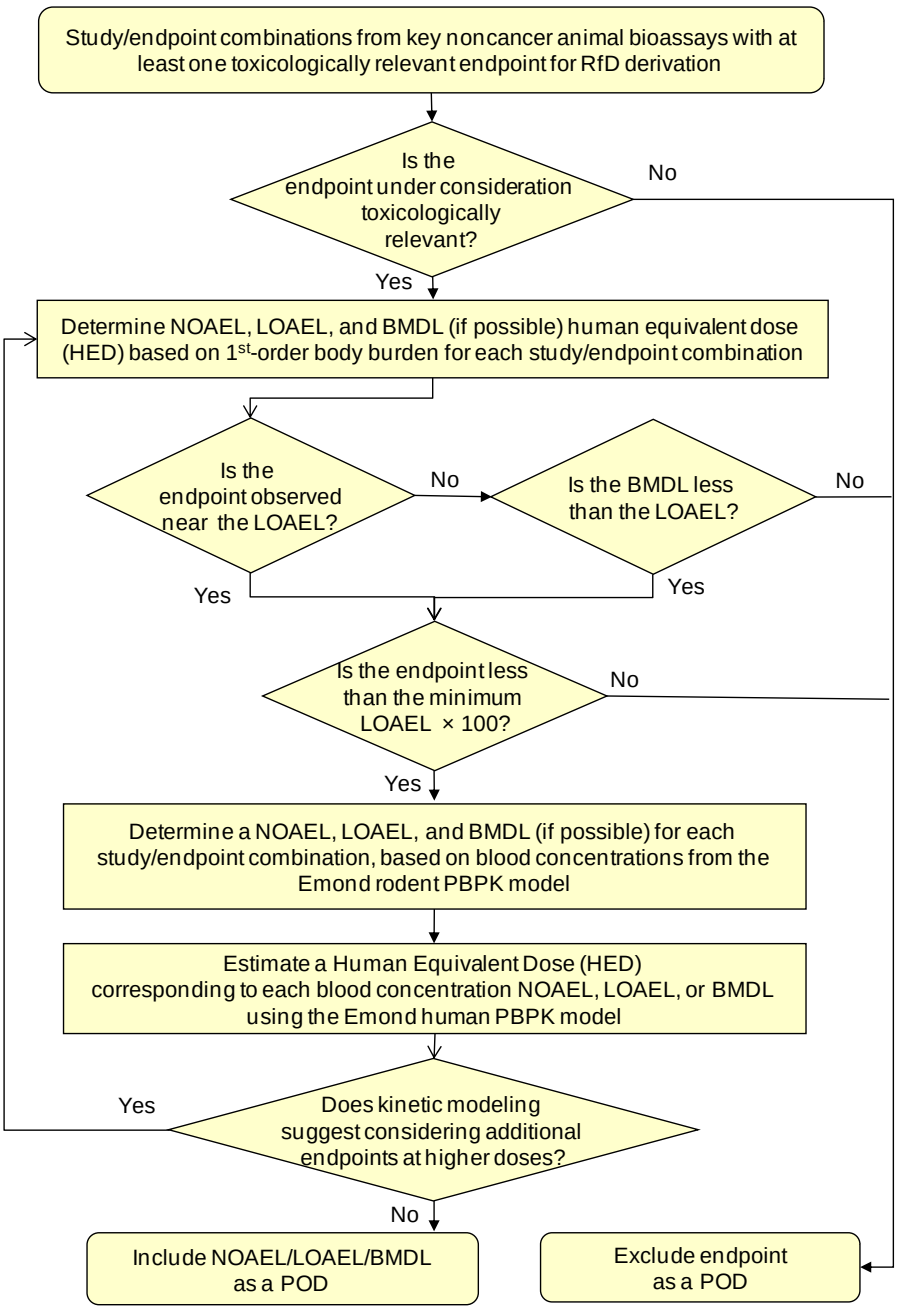

Figure 4-3. EPA's process to identify and estimate PODs from key animal bioassays for use in noncancer dose-response analysis of TCDD.
For the studies with at least one toxicologically relevant endpoint, EPA first determined if each endpoint was toxicologically relevant. If so, EPA determined the NOAEL, LOAEL, and BMDL HED based on 1st-order body burdens for each endpoint. Within each study, these potential PODs were included when the endpoint was observed near the LOAEL and if the BMDL was less than the LOAEL. Then, if the endpoint was less than the minimum LOAEL × 100 across all studies, EPA calculated PODs based on blood concentrations from the Emond rodent PBPK model and, for all of the PODs, HEDs were estimated using the Emond human PBPK model. If the kinetic modeling results suggested considering additional endpoints at higher doses, the process was repeated. Finally, the lowest group of the toxicologically relevant PODs was selected for final use in derivation of candidate RfDs.

further in Section 4.2.1). EPA then identified PODs from the remaining bioassays; at this point, Figure 4-2 refers to Figure 4-3, which is a flow chart of the iterative process used to estimate PODs and compare them within and across the remaining studies to arrive at a final set of PODs from these bioassays (see additional details below). From this final set of PODs, Figure 4-2 shows that EPA then eliminated 13 studies from further analysis because both of the following conditions were met: HED $LOAEL_{HED}$ (HED estimate based on LOAELs) >1 ng/kg-day and $NOAEL_{HED}$ or $BMDL_{HED}$ >0.32 ng/kg-day (see Table 4-3). One additional study was also not carried forward because of the lack of toxicokinetic information for estimation of an HED. These dose limits were imposed to limit the size of the analysis yet ensure representation of all important health effects associated with TCDD exposure. From the final list of 48 studies, EPA derived 37 candidate RfDs, with 11 studies presented as supporting information.

Figure 4-3 summarizes the strategy employed for identifying and estimating PODs from the 62 animal bioassays with at least one toxicologically relevant endpoint for RfD derivation. For the noncancer endpoints within these studies, EPA first evaluated the toxicological relevance of each endpoint, rejecting those judged not to be relevant for RfD derivation. Next, initial PODs based on the first-order body burden metric (see Section 3.3.4.2) and expressed as HEDs (i.e., $NOAEL_{HED}$, $LOAEL_{HED}$, $BMDL_{HED}$) were determined for all relevant endpoints (summarized in Table 4-3). Because there were very few NOAELs and BMDL modeling was largely unsuccessful due to data limitations (see Section 4.2), the next stage of evaluation was carried out using LOAELs only. Within each study, effects not observed at the LOAEL (i.e., reported at higher doses) with $BMDL_{HED}$s greater than the $LOAEL_{HED}$ were eliminated from further analysis, as they would not be considered as candidates for the final POD on either a BMDL or NOAEL/LOAEL basis (i.e., the POD would be higher than the PODs of other relevant endpoints). In addition, all endpoints with $LOAEL_{HED}$ estimates beyond a 100-fold range of the lowest identified $LOAEL_{HED}$ across all studies were (temporarily) eliminated from further consideration, as they would not be POD candidates either (i.e., the POD would be higher than the PODs of other relevant endpoints). For the remaining endpoints, EPA then determined final potential PODs based on TCDD whole-blood concentrations obtained from the Emond rodent PBPK models. HEDs were then estimated for each of these PODs using the Emond human PBPK model. At this point, if the PBPK modeling results suggested considering additional endpoints at higher doses, the process was repeated. From the final set of HEDs, a POD was

selected[31] for each study, to which appropriate UFs were applied following EPA guidance (see Section 4.3.3 following). The resulting candidate RfDs were then considered in the final selection process for the RfD. Other endpoints occurring at slightly higher doses representing additional effects associated with TCDD exposure (beyond the 100-fold $LOAEL_{HED}$ range) were evaluated, modeled, and included in the final candidate RfD array[32] to examine endpoints not evaluated by studies with lower PODs. In addition, BMD modeling based on administered dose was performed on all endpoints for comparison purposes. The final array of selected endpoints is shown in Table 4-4 (summary of BMD analysis) and Table 4-5 (candidate RfDs).

The NAS recommended that EPA better justify the selection of response levels for endpoints used to develop risk estimates. The NAS commented on EPA's decision to estimate an ED_{01} for noncancer bioassay/data set combinations as a comparative tool across studies, suggesting that EPA identify and evaluate the levels of change associated with adverse effects to define the BMR level for continuous noncancer endpoints.

> The committee notes that the choice of the 1% response level as the POD substantially affects … the noncancer analyses…. The committee recommends that the Reassessment use levels of change that represent clinical adverse effects to define the BMR level for noncancer continuous end points as the basis for an appropriate POD in the assessment of noncancer effects (p. 72, NAS, 2006b).

> The committee concludes that EPA did not adequately justify the use of the 1% response level (the ED_{01}) as the POD for analyzing epidemiological or animal bioassay data for … noncancer effects (p. 18, NAS, 2006b).

In the 2003 Reassessment (U.S. EPA, 2003), EPA was not attempting to derive an RfD when it conducted TCDD dose-response modeling. The 2003 Reassessment developed ED_{01} estimates for noncancer effects in an attempt to compare disparate endpoints on a consistent response scale. Importantly, the 2003 Reassessment defined the ED_{01} as 1% of the maximal response for a given endpoint, not as a 1% change from control. Because RfD derivation is the primary goal of noncancer health effects assessment in this document, the noncancer modeling effort undertaken here differs substantially from the modeling in the 2003 Reassessment.

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[31] In the standard order of consideration: BMDL, NOAEL, and LOAEL.

[32] However, studies with a lowest dose tested greater than 30 ng/kg-day were not included in the expanded evaluation.

The NAS committee was concerned with the statistical power to determine the shape of the dose-response curve at doses far below observed dose-response information. EPA agrees that the shape of the dose-response curve in the low-dose region cannot be determined confidently when based on higher-dose information. An observed response above background near (or below) the BMR level is needed for discrimination of the shape of the curve and for accurate estimation of an ED_x or BMDL. Although many of the ED_{01}s presented in the 2003 Reassessment were near the lowest dose tested, responses at the lowest doses were often high and much greater than a 1% response (i.e., 1% of the maximum response). The lack of an observed response near the BMR level is often a problem in interpretation of BMD modeling results.

In this document, EPA has used a 10% BMR for dichotomous data for all endpoints; there were no developmental studies that accounted for litter effects, for which a 5% BMR would be used (U.S. EPA, 2000). For continuous endpoints in this document, EPA has used a BMR of 1 standard deviation from the control mean whenever a specific toxicologically-relevant BMR could not be defined. For the vast majority of continuous endpoints, EPA could not establish unambiguous levels of change representative of adversity, which EPA defines as ~~"a~~ biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism, or reduces an organism's ability to respond to an additional environmental challenge" (U.S. EPA, 2012). For body and organ weight change, EPA has previously established a BMR of 10% change, which also is used in this document.

The NAS commented on EPA's development of ED_{01} estimates for numerous study/data set combinations in the 2003 Reassessment, suggesting that EPA had not appropriately characterized the statistical confidence around such model predictions in the low-response region of the model.

> It is critical that the model used for determining a POD fits the data well, especially at the lower end of the observed responses. Whenever feasible, mechanistic and statistical information should be used to estimate the shape of the dose-response curve at lower doses. At a minimum, EPA should use rigorous statistical methods to assess model fit and to control and reduce the uncertainty of the POD caused by a poorly fitted model. The overall quality of the study design is also a critical element in deciding which data sets to use for quantitative modeling (NAS, 2006b, p. 18).

> EPA should … assess goodness-of-fit of dose-response models for data sets and provide both upper and lower bounds on central estimates for all statistical estimates. When quantitation is not possible, EPA should clearly state it and explain what would be required to achieve quantitation (NAS, 2006b, p. 10).

The NAS also commented that EPA report information describing the adequacy of dose-response model fits, particularly in the low response region. For those cases where biostatistical modeling was not possible, NAS recommended that EPA identify the reasons.

> The Reassessment should also explicitly address the importance of statistical assessment of model fit at the lower end and the difficulties in such assessments, particularly when using summary data from the literature instead of the raw data, although estimates of the impacts of different choices of models would provide valuable information about the role of this uncertainty in driving the risk estimates (NAS, 2006b, p. 73).

To address this concern, in this document EPA has reported the standard suite of goodness-of-fit measures from the benchmark dose modeling software (BMDS 2.1). These include chi-square *p*-values, Akaike's Information Criterion (AIC), scaled residuals at each dose level, and plots of the fitted models. For the multistage model, when restricted lower-order coefficients hit the lower bound (zero), EPA used likelihood ratio tests to evaluate whether the improvement in fit afforded by estimating successively higher-order coefficients could be justified. Goodness-of-fit measures are reported for all key data sets in Appendix G. (Section 4.2.4.2 discusses the BMD modeling criteria for model evaluation.)

4.2. NONCANCER DOSE-RESPONSE ASSESSMENT OF TCDD

This section describes EPA's evaluation of TCDD dose response for noncancer endpoints from studies that met the study inclusion criteria. Discussions include BMD modeling procedures, kinetic modeling, and development of PODs for derivation of the RfD. Section 4.2.1 discusses the types of endpoints that are considered relevant by EPA for derivation of toxicity values (U.S. EPA, 2005a, b, 1998, 1996, 1994, 1991) and lists the study/endpoint combinations that were not considered for the TCDD RfD derivation, with supporting text in Appendix H. Section 4.2.2 describes how EPA has used PBPK modeling to estimate effective internal exposures as an alternative to using administered doses or body burdens based on first-order

kinetics. Section 4.2.3 details the dose-response analysis of the epidemiologic data, with supporting information on kinetic modeling in Appendix F. Section 4.2.4 details the dose-response analysis for the animal bioassay data, with supporting information on kinetic modeling in Appendix E; Appendix G provides the BMDS input tables (see Section G.1) and output for all modeling, including blood concentrations (see Section G.2) and administered dose (see Section G.3).

4.2.1. Determination of Toxicologically Relevant Endpoints

The NAS committee commented on the low-dose model predictions and the need to discuss the biological significance of the noncancer health effects modeled in the 2003 Reassessment. In selecting POD candidates from the animal bioassays for derivation of the candidate RfDs, EPA considered the toxicological relevance of the identified endpoint(s) from any given study. Some endpoints/effects may be sensitive, but lack general toxicological significance because of lack of inherent adversity,[33] being an adaptive response, or not being clearly linked to downstream functional or pathological alterations. Endpoints not considered to be toxicologically relevant for TCDD include CYP induction, oxidative stress measures, mRNA induction, protein phosphorylation, certain immune system responses, gap junction disruption, and epidermal growth factor signaling. As an example, CYP induction alone is not considered a significant toxicological effect given that CYPs are induced as part of the normal hepatic metabolism of xenobiotic agents. Additionally, the role of CYP induction in the noncancer toxicity of TCDD is unknown, thus, due to the lack of obvious pathological significance, TCDD-induced CYP induction is not considered a relevant endpoint for RfD derivation. Another example is oxidative stress. As an example, TCDD has been shown to induce changes in oxidative stress markers, but no other indicators of brain pathology were assessed (Hassoun et al., 2003; 2000; 1998). In this case, it is impracticable to link the markers of oxidative stress to a toxicological outcome in the brain; thus, this endpoint is not considered relevant for RfD derivation. Studies otherwise meeting the study inclusion criteria, but with no toxicologically-relevant endpoints that were considered suitable for derivation of a candidate RfD are listed in Figure 4-2, and described and discussed in Appendix H.

[33] An adverse effect is defined in EPA's Integrated Risk Information System glossary as ~~"a~~ biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism, or reduces an organism's ability to respond to an additional environmental challenge" (U.S. EPA, 2012).

4.2.2. Use of Toxicokinetic Modeling for TCDD Dose-Response Assessment

Because relevant toxicokinetic models for TCDD disposition in rodents and humans are available, EPA has not applied the standard uncertainty factor approach in the derivation of the TCDD RfD. In addition, because of the much slower elimination of TCDD in rodents than in humans, EPA has determined that the standard uncertainty factor approach can underestimate the interspecies toxicokinetic extrapolation factor by an order of magnitude or more (U.S. EPA, 2003). The toxicokinetic models chosen by EPA are the rodent and human PBPK models described by Emond et al. (2006; 2005; 2004)[34] and modified by EPA for this assessment as described in Section 3.3.4 (hereafter referred to as the "Emond [rodent or human] PBPK model"). Both the rodent and human models have a gestational component, which allow for more relevant exposure comparisons between general adult exposures and the numerous gestational exposure studies. Ideally, a relevant tissue concentration for each effect would be estimated. However, at present, no models exist for estimation of all relevant tissue concentrations. As virtually all TCDD is found in the adipose fraction of tissues, or bound to specific proteins, a preferred approach to developing a dose metric would be to account for the fat fraction of each tissue and protein binding; however, EPA has decided that the modeling of such estimates is too uncertain, and EPA has not found sufficient data to implement this approach. Therefore, EPA has decided to use the concentration of TCDD in whole blood as a surrogate for tissue concentrations, assuming that tissue concentrations are proportional to whole-blood concentrations. Furthermore, because the RfD is necessarily expressed in terms of average daily exposure, the blood concentrations are expressed as averages over the relevant period of exposure for each endpoint. For the animal bioassays, the relevant period of exposure is the duration of dosing, starting at the age of the animals at the beginning of the study. For humans, the relevant period of exposure is generally a lifetime, which is defined as 70 years. However, EPA varied the averaging time for the equivalent human blood concentrations to correspond to the test-animal exposure duration in the following manner.

- For correspondence with animal chronic exposures,[35] the human-equivalent TCDD blood concentration is assumed to be the 70-year average.

[34] The Emond PBPK models are three-compartment dynamic models.
[35] Assumed to be ≥75% of nominal lifetime, or about 550 days in rodents.

- For correspondence with animal gestational exposures, the human-equivalent TCDD blood concentration is assumed to be the average over 45 years for a female, beginning at birth, plus 9 months of gestational exposure.[36] Forty five years of age is considered here as an upper limit on the age at which a typical human female can conceive and bear a child.
- For correspondence with any other animal exposure duration, the human-equivalent TCDD blood concentration is assumed to be the average over the equivalent human exposure duration calculated backward from the peak exposure plateau at or near the end of the 70-year scenario. The average is determined from the terminal end of the human exposure period to be protective of less-than-lifetime exposures occurring at any time in a lifetime; the daily oral intake achieving the target blood concentration is smaller than for the same exposure period beginning at birth. The determination of equivalent exposure durations across species is problematic and somewhat arbitrary, so EPA uses the average peak blood concentration as the human equivalent for all less-than-chronic animal exposures (other than gestational).[37] For the first-order kinetics model, the average peak exposure is close to the theoretical steady-state asymptote (see Section 3.3.4.2). However, for the Emond human PBPK model used by EPA in this assessment, the timing of the peak exposure is dose-dependent and tends to decline after 60 years in some cases. Therefore, the 5-year average TCDD blood concentration that includes the peak (~~"5~~-year peak") is used as the relevant dose-metric for the PBPK model applications (see Section 3.3.6 and Figure 3-33).

4.2.3. Noncancer Dose-Response Assessment of Epidemiologic Data

The following four epidemiologic studies describing noncancer endpoints were identified in Section 2.4.1 as studies to be evaluated for development of PODs for derivation of candidate RfDs: Baccarelli et al. (2008), Mocarelli et al. (2008), Alaluusua et al. (2004), and Eskenazi et al. (2002b). Each of these studies described effects observed in the Seveso cohort (see detailed study summaries in Appendix C and Table 2-2). Each study reported individual-level human exposure measures and provided information from which EPA could determine a ~~"c~~ritical exposure window" (see Text Box 2-2) of susceptibility over which the effective TCDD exposures could be quantified for dose-response assessment. For studies that reported grouped data by TCDD exposure ranges, the representative values for the ranges were determined by

[36] See Section 3.3.6 for a discussion of this issue, including a comparison of the 45-year old pregnancy scenario to one beginning at age 25 in Table 3-24.

[37] By comparison to a half-lifetime equivalent (1 year in rodents, 35 years in humans), in the 1st-order kinetic model the ratio of body burden to oral intake does not differ significantly from the average-peak scenario; all shorter-term scenarios differ even less (see Section 3.3.4.2). These relationships, with respect to the 5-year peak, hold for the PBPK model results, as well (see Section 3).

taking the geometric mean of the range limits, assuming that the TCDD concentration distribution in the population is more likely to be skewed (e.g., lognormal) than symmetrical (e.g., normal or uniform). A sufficient number of significant digits are carried through intermediate results to avoid round-off error in the final value. EPA used toxicokinetic modeling (Emond human PBPK model) to estimate daily TCDD intake rates for the exposure groups presented in these studies (see Appendix F for details). The exposure scenario in all of these studies, except Baccarelli et al. (2008), entailed an initial high pulse exposure at the time of the plant explosion followed by low-level background exposure over a period of several years across the critical exposure window, resulting in internal exposure profiles characterized by a 5 to 10-fold difference in initial and final TCDD serum concentrations (as LASC). For these scenarios, EPA modeled both the peak TCDD LASC and the average LASC over the critical window, then estimated daily average continuous TCDD intakes over the critical-window duration corresponding to each of the peak and critical-window average serum concentrations. Estimation of LASC and intakes was accomplished using the Emond human PBPK model. EPA considered the critical-window average exposures to be important, although they were much lower than the peak exposures, because the relatively slow elimination of TCDD engenders concerns for an ongoing contribution of residual TCDD body burdens to the adverse health outcomes during the period of susceptibility. However, the overall average exposure does not reflect the influence of the much higher peak exposure, which may be a significant factor in TCDD toxicity (Kim et al., 2003).[38] That is, EPA is uncertain as to whether the health outcomes, often observed many years beyond the period of susceptibility, are a result of permanent damage from the initial high exposure or more gradual impairment from longer-term ongoing exposure. For these reasons, EPA derived the PODs for RfD consideration by averaging the TCDD intakes for the peak exposure and critical-window exposure average, essentially treating each as equally likely. EPA focused on identifying NOAELs and LOAELs for these studies. EPA did not conduct BMD modeling because the covariates identified by the study authors could not be incorporated by modeling the grouped response data. EPA's development of PODs for these studies is described in this section, with kinetic modeling details provided in Appendix F; the results are shown in Table 4-1.

[38] Kim et al. (2003) found a significantly higher fraction of altered hepatic foci in rats treated with a single high TCDD dose than those administered a continuous dose over 15 weeks, yielding similar terminal liver TCDD concentrations.

Table 4-1. PODs for epidemiologic studies of TCDD

Study	POD (ng/kg-day)	Critical effects
Alaluusua et al. (2004)	0.0406[a] (NOAEL)	Dental effects in adults exposed to TCDD in childhood
Baccarelli et al. (2008)	0.020[b] (LOAEL)	Elevated TSH in neonates
Mocarelli et al. (2008)	0.020[c] (LOAEL)	Decreased sperm count and motility in men exposed to TCDD in childhood

[a]Mean of peak exposure (0.0655 ng/kg-day) and average exposure over 10-year critical window (0.0156 ng/kg-day).
[b]Maternal exposure corresponding to neonatal TSH concentration exceeding 5 µU/mL.
[c]Mean of peak exposure (0.032 ng/kg-day) and average exposure over 10-year critical window (0.0080 ng/kg-day).

4.2.3.1. *Baccarelli et al. (2008)*

For Baccarelli et al. (2008), EPA was able to define a LOAEL in terms of the maternal TCDD serum levels corresponding to neonatal TSH level above 5 µ-Units TSH per mL of serum (5 µU/mL) (see Appendix C, Section C.1.2.1.5.7, and Table 2-2 for study details). The adversity benchmark of 5 µU/mL is based on the WHO (1994) indicator for follow up examination for potential hypothyroidism (see following discussion in Section 4.3.4.1). Baccarelli et al. (2008) performed regression modeling of neonatal TSH against maternal TCDD LASC but did not estimate the equivalent oral intake. The regression model related the level of TSH in 3-day-old neonates to TCDD concentrations in maternal plasma at birth (given as LASC). The authors extrapolated maternal plasma concentrations from previous measurements using a simple first-order pharmacokinetic model. The study authors also reported group average neonatal TCDD serum levels for infants above and below the 5 µU/mL limit. However, because there is limited information regarding the relationship between maternal and neonatal serum TCDD levels, EPA determined that there was too much uncertainty in estimating maternal intake from neonatal TCDD serum concentrations directly. Therefore, EPA determined the maternal intake at the LOAEL from the maternal serum-TCDD/TSH regression model by finding the maternal TCDD LASC at which neonatal TSH exceeded 5 µU/mL. EPA then used the Emond PBPK model under the human gestational scenario (see Section 4.2.2) to estimate the continuous daily oral TCDD intake that would result in a TCDD LASC corresponding to a neonatal TSH of 5 µU/mL at the end of gestation; EPA established the resulting maternal intake (0.020 ng/kg-day) as the LOAEL, shown in Table 4-1 as a POD for derivation of candidate RfDs (PBPK modeling details are shown in Appendix F).

4.2.3.2. *Mocarelli et al. (2008)*

Mocarelli et al. (2008) reported decreased sperm concentrations (21−33%) and decreased motile sperm counts (12−25%) in men who were 1−9 years old in 1976 at the time of the accident (initial TCDD exposure event) (see Appendix C, Section C.1.2.1.5.8, and Table 2-2 for study details). Men who were 10−17 years old in 1976 were not adversely affected. Serum (LASC) TCDD levels were measured within 1 year of the initial exposure. Serum TCDD levels and corresponding responses were reported by quartile, with a reference group of less-exposed individuals assigned a TCDD LASC value of 15 ppt (which was the mean of individuals outside the contaminated area). The lowest exposed group median was 68 ppt (1st quartile). Because sperm effects were detected only among boys under the age of 10, EPA assumes there is a maximum 10-year critical exposure window for elicitation of these effects.[39] However, for the exposure profile, with a high initial pulse followed by an extended period of elimination with only background exposure, the estimation of an average exposure resulting in the effect is somewhat complicated. EPA implemented a procedure for the estimation of the continuous daily TCDD intake associated with the LOAEL in the Mocarelli et al. (2008) study using the following 5-step process:

1. Using the Emond human PBPK model, the initial (peak) serum TCDD concentrations (LASC) associated with the accident were back-calculated based on the time that had elapsed between the explosion and the serum collection. As serum measurements were taken within 1 year after the event, a lag time to measurement of 0.5 years was assumed. The group average peak serum concentration for the 1st quartile was estimated to be 249 ppt.
2. The oral exposure associated with the peak serum TCDD concentration (peak exposure) was calculated using the Emond PBPK model.
3. Starting with the peak exposure and accounting for background TCDD intake, the average daily serum TCDD concentration experienced by a representative individual in the susceptible lifestage (boys under 10 years old) was estimated using the Emond PBPK model. The average subject age at the time of the event was 6.2 years. Consequently, a critical exposure window for the cohort was estimated to be, on average, 3.8 years (i.e., a boy aged 6.2 years would remain in this exposure window for 3.8 more years until he was 10 years of age). The critical window average serum concentration for the 1st quartile group was estimated to be 57.7 ppt (45 ppt at 10 years).

[39] Neither the study authors nor EPA assume 10 years to be the age of puberty onset; 10 years is the age that the study authors used to divide their study population by magnitude of effect.

4. Using the Emond PBPK model, the average daily TCDD intake rate needed to attain the 3.8-year average serum TCDD concentration in a boy 10 years old was calculated.
5. The LOAEL POD was calculated as the average of the peak exposure intake (0.032 ng/kg-day) and the 3.8-year average exposure intake (0.0080 ng/kg-day), resulting in LOAEL of 0.020 ng/kg-day, shown in Table 4-1 as a POD for derivation of a candidate RfD.

The PBPK modeling details are shown in Appendix F.

4.2.3.3. *Alaluusua et al. (2004)*

For Alaluusua et al. (2004), the approach for estimation of daily oral TCDD intake is virtually identical to the approach used for the Mocarelli et al. (2008) data. (see Appendix C, Section C.1.2.1.5.5, and Table 2-2 for study details.) Alaluusua et al. (2004) reported dental effects in male and female adults who were less than 5 years of age at the time of the initial exposure (1976). For the 75 boys and girls who were less than 5 years old at the time of the accident, 25 (33%) were subsequently diagnosed with some form of dental enamel defect. For the 38 individuals who were older than 5, only 2 (5.3%) suffered dental enamel defects at a later date. In addition, the incidence of missing permanent teeth (lateral incisors and second premolars) was 3 times as prevalent in zone ABR subjects compared with zone non-ABR residents. A window of susceptibility of approximately 5 years is assumed. Serum measurements for this cohort were taken within a year of the accident. Serum TCDD levels and corresponding responses were reported by tertile, with a reference group of less-exposed individuals assigned a TCDD LASC value of 15 ppt (ng/kg); the tertile group geometric means were 72.1, 365.4, and 4,266 ppt. The incidence of dental effects for the reference group was 26% (10/39). The incidence of dental effects in the 1st, 2nd, and 3rd tertile exposure groups was 10% (1/10), 45% (5/11), and 60% (9/15), respectively. EPA judged that the NOAEL and LOAEL were 72.1 and 365.4 ppt TCDD in serum (LASC), in the 1st tertile and 2nd tertile, respectively. Following the same procedure used for the Mocarelli et al. (2008) study (see Section 4.2.3.2), EPA estimated the continuous daily human oral TCDD intake associated with each of the tertiles for both peak and average exposure across the critical exposure window, assuming that the average age of the susceptible cohort at the time of the accident was 2.5 years. Separate estimates for boys and girls were developed based on both the peak intake and average intake across the critical exposure window (PBPK modeling details are shown in Appendix F).

The estimated averaged daily oral intakes for the tertiles, averaged for boys and girls, are 0.0655, 1.65, and 111 ng/kg-day for the peak exposure and 0.0156, 0.149, and 4.81 ng/kg-day for the critical exposure window average. The LOAEL for this study was determined to be 0.897 ng/kg-day, which is the average of the peak exposure and window average exposure for the second tertile. A study NOAEL of 0.0406 ng/kg-day for the first tertile was determined similarly and serves as a POD for derivation of a candidate RfD in Table 4-1.

4.2.3.4. *Eskenazi et al. (2002b)*

The approach used to estimate daily TCDD intake in Eskenazi et al. (2002b) combines the approaches EPA used for Baccarelli et al. (2008), Mocarelli et al. (2008), and Alaluusua et al. (2004). Eskenazi et al. (2002b) reported menstrual effects in female adults who were premenarcheal in 1976 at the time of the initial exposure (see Appendix C, Section C.1.2.1.4.1 and Table 2-2 for study details). In Rigon et al. (2010) , the median age at menarche was shown to be 12.4 in Italian females with intergenerational decreases in age at menarche. Thus, EPA established a window of susceptibility of approximately 13 years for this analysis. The average age of the premenarcheal girls at the time of the initial exposure in 1976 was 6.8 years, establishing an average critical-window exposure duration of 6.2 years for this cohort. Serum samples were collected within a year of the accident from this cohort. However, serum TCDD levels and corresponding responses were not reported by percentile, and no internal reference group was identified. As for Baccarelli et al. (2008), Eskenazi et al. (2002b) developed a regression model relating menstrual cycle length to plasma TCDD concentrations (LASC) measured in 1976. The model estimated that menstrual cycle length was increased 0.93 days for each 10-fold increase in TCDD LASC, with a 95% confidence interval of −0.01 to 1.86 days. The determination of a LOAEL is somewhat arbitrary, with no independent measure of an adversity threshold to establish the toxicological significance of a given increase in menstrual cycle length. The study authors did not present data for unexposed premenarcheal girls (in 1976), so an appropriate reference population is not available. EPA did not conduct BMD modeling because of the lack of a reference population and the inability to include the covariates considered by the study authors in their analysis. However, an approximate LOAEL can be estimated from Figure 1 in Eskenazi et al. (2002b), noting that both the length of the menstrual cycle and its variance increases above TCDD concentrations of about 1,000 ppt. The highest

measured concentration is 16,500 ppt. Consistent with the previously established method for determining representative values for age limits (see Sections 4.2.3.2 and 4.2.3.3), the geometric mean of 4,060 ppt for this range is assigned as a LOAEL. The lower range of TCDD concentrations is too large to treat as a single group for estimating a NOAEL, but using the study authors' regression model, a TCDD LASC of approximately 50 ppt corresponds to a menstrual cycle length of 28 days, generally considered to be the average normal length. These two (1976) serum levels were then modeled by EPA using the Emond human PBPK model in the same manner as for Mocarelli et al. (2008) and Alaluusua et al. (2004), but with a 6.2-year exposure window for the premenarcheal girls. The resulting peak and window-average TCDD intakes for the 50 ppt exposure are 0.0168 and 0.00364 ng/kg-day, respectively; the average of the two intakes is 0.0102 ng/kg-day. The peak and window-average TCDD intakes for the LOAEL exposure (4,060 ppt) are 60.0 and 1.52 ng/kg-day, respectively; the average of the two intakes of 30.8 ng/kg-day defines the LOAEL POD. Further details of the PBPK modeling can be found in Appendix F. Although 0.0102 ng/kg-day could be considered to be a NOAEL, there is too much uncertainty in the upper end of the NOAEL range, given the very large (3,000-fold) difference between it and the LOAEL, for using it as a NOAEL POD. The LOAEL of 30.8 ng/kg-day, also uncertain in magnitude and toxicological significance, is 1,540-fold higher than the LOAEL PODs for Mocarelli et al. (2008) and Baccarelli et al. (2008), and will not be a factor in the derivation of the RfD. Therefore, the LOAEL for this study is not considered further in this assessment except in the context of the RfD uncertainty analysis presented in Section 4.5.

4.2.4. Noncancer Dose-Response Assessment of Animal Bioassay Data

EPA followed the strategy illustrated in Figures 4-2 and 4-3 to evaluate the animal bioassay data for TCDD dose response. For the administered average daily doses (ng/kg-day) in each animal bioassay, EPA identified NOAELs and/or LOAELs based on the original data presented by the study author. Section 2.4.2 identifies these values in Table 2-4 and in the study summaries found in Appendix D. These became PODs for consideration in the derivation of an RfD for TCDD. The candidate RfD values associated with these PODs are presented in Table 4-5. All PODs were converted to HEDs using the Emond PBPK models, with whole-blood TCDD concentration as the effective dose metric. The remainder of this section

describes the steps in this process and concludes with the PODs from the animal bioassay data that were considered for derivation of the RfD.

4.2.4.1. *Use of Kinetic Modeling for Animal Bioassay Data*

Whole-blood TCDD concentrations corresponding to the administered doses in each mouse or rat bioassay qualifying as a final RfD POD were estimated using the appropriate Emond rodent PBPK model. In each case, the simulation was performed using the exposure durations, body weights, and average daily doses from the original studies. For all multiple-exposure protocols, the time-weighted average blood TCDD concentrations over the exposure period were used as the relevant dose metric. For single (gestational and nongestational) exposures, the initial peak blood TCDD concentrations were considered to be the most relevant exposure metric. Gestational exposures were modeled using the species-specific gestational component of the Emond rodent PBPK model. Bioassays employing exposure protocols spanning gestational and postpartum life stages were modeled by sequential application of the gestational and nongestational models.

The Emond PBPK models do not contain a lactation component, so exposure during lactation was not modeled explicitly. Only one bioassay (Shi et al., 2007) considered as a POD for RfD derivation included exposure during lactation. In Shi et al. (2007), pregnant animals were exposed weekly to TCDD throughout gestation and lactation. Exposure was continued in the offspring following weaning for 10 months. For assessment of maternal effects, the Emond gestational model was used, terminating at parturition. For assessment of long-term exposure in the offspring, the Emond nongestational model was used, ignoring prior gestational and lactational exposure, with the assumption that the total exposure during these periods was small relative to exposure in the following 10 months. The assumption is conservative in that effects observed in the offspring would be attributed entirely to adult exposure, which is somewhat less than the actual total exposure.

The model code, input files, and PBPK modeling results for each bioassay are reported in Appendix E. The modeled TCDD blood concentrations were used for BMD modeling of bioassay response data and determination of NOAELs and LOAELs. BMD modeling was performed, as described in Section 3.3.6, by substituting the modeled blood concentrations for the administered doses and calculating the corresponding BMDL. For each of these LOAEL,

NOAEL, or BMDL blood-concentration equivalents, corresponding HEDs were estimated using the Emond human PBPK model for the appropriate gestational or nongestational scenario as described previously (see Section 4.2.2).

4.2.4.2. *Benchmark Dose Modeling of the Animal Bioassay Data*

BMD modeling was performed for each study/endpoint combination using BMDS 2.1 to determine BMDs and BMDLs. The input data tables for these noncancer studies are shown in Appendix G, Section G.1, including both administered doses (ng/kg-day) and blood concentrations (ng/kg [ppt])[40] and either incidence data for the dichotomous endpoints or mean and standard deviations for the continuous endpoints (see Section 4.2.4.1 and Sections 3.3.4 and 3.3.5 for a description of the development of TCDD blood concentrations using kinetic modeling).

Evaluation of BMD modeling performance, goodness-of-fit, dose-response data, and resulting BMD and BMDL estimates included statistical criteria as well as professional judgment of their statistical and toxicological properties. For the continuous endpoints, all available models were run separately using both the assumption of constant variance and the assumption of modeled variance. Saturated (0 degrees of freedom) model fits were rejected from consideration. Parameters in models with power or slope parameters were constrained to prevent supralinear fits, which EPA considers not to be biologically plausible and which often have undesirable statistical properties (i.e., the BMDL converges on zero). Table 4-2 shows each model and any restrictions imposed.

[40] Units of ng/kg will be used exclusively for oral intakes in this section. Blood and tissue concentrations will be expressed in ppt units.

Table 4-2. Models run for each study/endpoint combination in the animal bioassay BMD modeling

Model	Restrictions imposed
Continuous models	
Exponential M2−M5, not grouped	Adverse direction specified according to the response data; power ≥1
Hill	Adverse direction is automatic; $n > 1$
Linear	Adverse direction is automatic; degree of polynomial = 1
Polynomial	Adverse direction is automatic; degree of polynomial unrestricted; restrict the sign of the power to nonnegative or nonpositive, depending on the direction of the responses
Power	Adverse direction is automatic; power ≥1
Dichotomous models	
Gamma	Power ≥1
Logistic	None
Log-Logistic	Slope ≥1
Log-Probit	None
Multistage	Beta ≥0, 2nd degree polynomial
Probit	None
Weibull	Power ≥1

For the quantal/dichotomous endpoints, all primary BMDS dichotomous models were run. The alternative dichotomous models were fit to several data sets, but the results were very sensitive to the assumed independent background response and the fits were not accepted. The confidence level was set to 95%, and all initial parameter values were set to their defaults in BMDS. For the continuous endpoints, 1 standard deviation was chosen as the default for the BMR when a specific toxicologically-relevant BMR could not be defined. For the dichotomous endpoints, a BMR of 10% extra risk was used for all endpoints.[41]

The model output tables in Appendix G show all of the models that were run, both restricted and unrestricted, goodness-of-fit statistics, BMD and BMDL estimates, and whether bounds were hit for constrained parameters. After all models were run, the one giving the best fit was selected using the selection criteria in the draft BMD Technical Guidance (U.S. EPA, 2000). Acceptable model fits were those with chi-square goodness-of-fit *p*-values greater than 0.1. For continuous endpoints, the preference was for models with an asymptote term (plateau for high-dose response) because continuous measures do not continue to rise (or fall) with dose forever; this phenomenon is particularly evident for TCDD. Unbounded models, such as the

[41] There were no developmental studies that accounted for litter effects, for which a 5% BMR would be used.

power model, must account for the plateauing effect entirely in the shape parameter, generally resulting in a supralinear fit. Also, for the continuous endpoints, the *p*-value for the homogenous variance test (Test 2) was used to determine whether constant variance ($p > 0.1$) or modeled variance ($p < 0.1$) should be used. As BMDS offers only one variance model, model fits for modeled variance models were not necessarily rejected if the variance model did not fit well (Test 3 *p*-value < 0.05). Within the group of models with acceptable fits, the selected model was generally the one with the lowest AIC. If the AICs were similar, the model with the lowest BMDL was selected. However, particularly for continuous models, the fit of the model to the control-group response and in the lower response range was assessed. Models with higher BMDLs or AICs but much better fit to the lower response data were often chosen over the nominally best-fitting model.

For many data sets, no models satisfied the acceptance criteria, and no clear BMD/BMDL selection could be made. In these cases, model fits were examined on an individual basis to determine the reasons for the poor fits. On occasion, high doses were dropped, and the models were refit. Also, if a poor fit to the control mean was evident, the model was refit to the data after fixing the control mean by specifying the relevant parameter in BMDS. However, these techniques rarely resulted in better fits. If the fit was still not acceptable, the NOAEL/LOAEL approach was applied to the study/data set combination. Most of the problems with BMD modeling were a consequence of lack of response data near the BMR; many of the TCDD data sets failed to show a response near the BMR, whether it was a 10% dichotomous relative change or a continuous 1 standard deviation change. Responses at the lowest doses were generally much higher than the BMR, resulting in a lack of ~~"a~~nchoring" at the critical response levels of interest, resulting in insufficient information for precise numerical estimation of BMDLs.

4.2.4.3. *Points of Departure (PODs) from Animal Bioassays Based on Human Equivalent Dose (HED) and Benchmark Dose (BMD) Modeling Results*

Table 4-3 summarizes the PODs that EPA estimated for each key animal study included for TCDD noncancer dose-response modeling that also contained toxicologically relevant endpoints (see Section 4.2.1 and Appendix H for excluded studies). After estimating the blood TCDD concentration associated with a particular toxicity measure (NOAEL, LOAEL, or BMDL) obtained from a rodent bioassay, EPA estimated a corresponding HED using the Emond

human PBPK model (described in Section 3). Table 4-3 summarizes the NOAEL, LOAEL, or BMDL based on the administered animal doses for each key bioassay/data set combination. Table 4-3 also summarizes the continuous daily HED corresponding to these administered doses as 1st order body burdens and as whole-blood concentrations. The doses in Table 4-3 are defined as follows, all in units of ng/kg-day:

- Administered Dose NOAEL: Average daily dose defining the NOAEL for the test species in the animal bioassay
- Administered Dose LOAEL: Average daily dose defining the LOAEL for the test species in the animal bioassay
- Administered Dose BMDL: BMDL for the test species based on modeling of the administered doses from the animal bioassay
- First-Order Body Burden HED NOAEL: Average daily dose defining the NOAEL for humans derived from the animal bioassay using the first-order kinetics body-burden model
- First-Order Body Burden HED LOAEL: Average daily dose defining the LOAEL for humans derived from the animal bioassay using the first-order kinetics body-burden model
- First-Order Body Burden HED BMDL: Human-equivalent BMDL from BMD modeling of the animal bioassay data using first-order body burdens
- Blood Concentration HED NOAEL: Average daily dose defining the NOAEL for humans derived from the animal bioassay using the Emond human PBPK model
- Blood Concentration HED LOAEL: Average daily dose defining the LOAEL for humans derived from the animal bioassay using the Emond human PBPK model
- Blood Concentration HED BMDL: Human-equivalent BMDL from BMD modeling of the animal bioassay data using the Emond human PBPK model

An evaluation of key BMD analyses is presented in Table 4-4. Tables showing the best model fit for each study/endpoint combination and the associated BMD/BMDL are shown in Appendix G. As described in Section 4.2.4.2, the BMD modeling was largely unsuccessful, primarily because of a lack of response data near the BMR, poor modeled representation of control values, or nonmonotonic responses yielding poor fits. The comments column in Table 4-4 lists reasons for poor results.

Table 4-3. Summary of key animal study PODs (ng/kg-day) based on three different dose metrics: administered dose, first-order body burden HED, and blood concentration

Study	Endpoint	Administered dose[a]			1st-order body burden HED[b]			Blood concentration HED[c]		
		NOAEL	LOAEL	BMDL[d]	NOAEL	LOAEL	BMDL[d]	NOAEL	LOAEL	BMDL[d]
Amin et al. (2000)	Saccharin preference ratio, female	–	2.50E+01	–[e]	–	2.49E−02	–[e]	–	1.71E−01	–[e]
Bell et al. (2007b)	Balano-preputial separation in male pups	–	2.40E+00	2.87E+00	–	1.26E−02	1.50E−02	–	8.85E−02	4.34E−02
Bowman et al.(1989a; 1989b); Schantz and Bowman (1989); Schantz et al. (1986); Schantz et al. (1992)	Neurobehavioral effects	–	1.20E−01	–	–	8.22E−03	–	–	–	–
Cantoni et al. (1981)	Urinary coproporhyrins	–	1.43E+00	–[e]	–	1.24E−02	–[e]	–	6.37E−02	–[e]
Chu et al. (2001)	Tissue-weight changes	2.50E+02	1.00E+03	–	7.55E−01	3.02E+00	–	7.03E+00	2.96E+01	–
Chu et al. (2007)	Liver lesions	2.50E+00	2.50E+01	–	7.55E−03	7.55E−02	–	3.49E−02	5.63E−01	–
Crofton et al. (2005)	Serum T4	3.00E+01	1.00E+02	–[e]	1.92E−02	6.40E−02	–[e]	1.69E−01	7.43E−01	–[e]
Croutch et al. (2005)	Decreased body weight	5.43E+01	2.17E+02	–	2.22E−01	8.89E−01	–	7.81E−01	3.57E+00	–
DeCaprio et al. (1986)	Decreased body weight, organ-weight changes	6.10E−01	4.90E+00	–	4.11E−03	3.30E−02	–	–	–	–
Fattore et al. (2000)	Decreased hepatic retinol	–	2.00E+01	–	–	1.23E−01	–	–	7.82E−01	–
Fox et al. (1993)	Increased liver weight	5.70E−01	3.27E+02	–	1.42E−03	8.12E−01	–	8.08E−04	3.05E+00	–
Franc et al. (2001)	Organ-weight changes	1.00E+01	3.00E+01	1.34E+01	6.62E−02	1.99E−01	8.87E−02	4.49E−01	1.41E+00	2.61E−01
Franczak et al. (2006)	Abnormal estrous cycle	–	7.14E+00	–	–	5.95E−02	–	–	3.18E−01	–
Hojo et al. (2002)[f]	DRL response per minute	–	2.00E+01	–[e]	–	5.26E−03	–[e]	–	5.51E−02	–[e]
Hochstein et al. (2001)[g]	Kit mortality at 6 weeks	–	2.65E+00	–	–	–	–	–	–	–

Table 4-3. Summary of key animal study points of departure (PODs) (ng/kg-day) based on three different dose metrics: administered dose, 1st-order body burden human equivalent dose (HED) and blood concentration HED (continued)

Study	Endpoint	Administered dose[a]			1st-order body burden HED[b]			Blood concentration HED[c]		
		NOAEL	LOAEL	BMDL[d]	NOAEL	LOAEL	BMDL[d]	NOAEL	LOAEL	BMDL[d]
Hutt et al. (2008)	Embyrotoxicity	–	7.14E+00	–	–	4.67E−02	–	–	2.52E−01	–
Ikeda et al. (2005)	Sex ratio	–	1.65E+01	–	–	1.05E−01	–	–	2.75E+00	–
Ishihara et al. (2007)	Sex ratio	1.00E−01	1.00E+02	–	3.18E−04	3.18E−01	–	4.91E−05	4.96E−01	–
Kattainen et al. (2001)	3rd molar length	–	3.00E+01	–[e]	–	7.89E−03	–[e]	–	9.01E−02	–[e]
Keller et al. (2008a; 2008b; 2007)	Missing mandibular molars	–	1.00E+01	–[e]	–	2.58E−03	–[e]	–	9.88E−03	–[e]
Kociba et al. (1976)	Liver and hematologic effects and body-weight changes	7.14E+00	7.14E+01	–	4.53E−02	4.53E−01	–	2.62E−01	3.03E+00	–
Kociba et al. (1978)	Liver and lung lesions, increased urinary porphyrins	1.00E+00	1.00E+01	–[e]	1.07E−02	1.07E−01	–[e]	6.33E−02	6.34E−01	–[e]
Kuchiiwa et al. (2002)	Immunoreactive neurons	–	7.00E−01	–	–	3.11E−03	–	–	2.75E−03	–[e]
Latchoumycandane and Mathur (2002)[h]	Sperm production	–	1.00E+00	–[e]	–	3.87E−03	–[e]	–	1.62E−02	–[e]
Li et al. (1997)	Increased serum FSH	3.00E+00	1.00E+01	–[e]	7.89E−04	2.63E−03	–[e]	2.90E−03	1.67E−02	–[e]
Li et al. (2006)	Hormone levels (serum estradiol)	–	2.00E+00	–[e]	–	9.85E−04	–[e]	–	1.58E−03	–[e]
Markowski et al. (2001)	FR2 revolutions	–	2.00E+01	–[e]	–	6.25E−03	–[e]	–	5.15E−02	–[e]
Maronpot et al. (1993)	Increased relative liver weight	1.07E+01	3.50E+01	–	8.97E−02	2.93E−01	–	5.03E−01	1.71E+00	–
Miettinen et al. (2006)	Cariogenic lesions in pups	–	3.00E+01	–[e]	–	7.89E−03	–[e]	–	8.95E−02	–[e]
Murray et al. (1979)	Fertility index in F2 generation	1.00E+00	1.00E+01	–[e]	9.43E−03	9.43E−02	–[e]	2.89E−02	3.79E−01	–[e]
NTP (1982b)	Liver lesions	–	1.39E+00	–[e]	–	6.47E−03	–[e]	–	2.16E−02	–[e]
NTP (2006a)	Liver and lung lesions	–	2.14E+00	–[e]	–	2.34E−02	–[e]	–	1.36E−01	–[e]
Nohara et al. (2000)	Decreased spleen cellularity	8.00E+02	–	–	2.10E−01	–	–	5.34E+00	–	–
Nohara et al. (2002)	Mortality from influenza virus-A challenge	5.00E+02	–	–	1.29E−01	–	–	1.37E+00	–	–
Ohsako et al. (2001)	Anogenital distance in pups	1.25E+01	5.00E+01	–[e]	3.29E−03	1.32E−02	–[e]	2.74E−02	1.78E−01	–[e]

Table 4-3. Summary of key animal study points of departure (PODs) (ng/kg-day) based on three different dose metrics: administered dose, 1st-order body burden human equivalent dose (HED) and blood concentration HED (continued)

Study	Endpoint	Administered dose[a]			1st-order body burden HED[b]			Blood concentration HED[c]		
		NOAEL	LOAEL	BMDL[d]	NOAEL	LOAEL	BMDL[d]	NOAEL	LOAEL	BMDL[d]
Schantz et al. (1996)	Maze errors	–	2.50E+01	–[e]	–	–[e]	4.55E−02	–	1.71E−01	–[e]
Seo et al. (1995)	Decreased thymus weight	2.50E+01	1.00E+02	–	2.49E−02	9.96E−02	–	1.67E−01	9.15E−01	–
Sewall et al. (1995)	Serum T4	1.07E+01	3.50E+01	5.16E+00	8.97E−02	2.93E−01	4.33E−02	5.03E−01	1.71E+00	1.80E−01
Shi et al. (2007)	Serum estradiol in female pups	1.43E−01	7.14E−01	2.24E−01	1.23E−03	6.13E−03	1.92E−03	4.47E−03	2.69E−02	4.74E−03
Simanainen et al. (2002)	Decreased serum T4	1.00E+02	3.00E+02	–	2.63E−02	7.89E−02	–	4.26E−01	1.67E+00	–
Simanainen et al. (2003)	Decreased thymus weight and change in EROD activity	1.00E+02	3.00E+02	–	2.63E−02	7.89E−02	–	4.26E−01	1.67E+00	–
Simanainen et al. (2004)	Decreased daily sperm production	1.00E+02	3.00E+02	–	2.63E−02	7.89E−02	–	4.26E−01	1.67E+00	–
Smialowicz et al. (2004)	Decreased antibody response to SRBCs	3.00E+02	1.00E+03	–	7.73E−02	2.58E−01	–	7.23E−01	3.28E+00	–
Smialowicz et al. (2008)	PFC per 10^6 cells	–	1.07E+00	–[e]	–	5.00E−03	–[e]	–	6.26E−03	–[e]
Smith et al. (1976)	Cleft palate in pups	1.00E+02	1.00E+03	1.84E+02	1.59E−01	1.59E+00	2.93E−01	5.24E−01	7.61E+00	9.46E−01
Sparschu et al. (1971)	Decreased fetal body weight	3.00E+01	1.25E+02	–[e]	5.45E−02	2.27E−01	–	3.18E−01	1.73E+00	–[e]
Toth et al. (1979)	Skin lesions	–	1.00E+00	–[e]	–	3.70E−03	–[e]	–	9.91E−03	–[e]
VanBirgelen et al. (1995a)[i]	Decreased liver retinyl palmitate	–	1.35E+01	–[e]	–	8.32E−02	–[e]	–	5.14E−01	–[e]
Vos et al. (1973)	Decreased delayed-type hypersensitivity response to tuberculin	1.14E+00	5.71E+00	–	6.43E−03	3.22E−02	–	–	–	–
Weber et al. (1995)	Increased liver weight	1.00E+03	3.00E+03	–	3.51E−01	1.05E+00	–	3.27E+00	1.18E+01	–
White et al. (1986)	Decreased serum complement	–	1.00E+01	–[e]	–	2.23E−02	–[e]	–	2.77E−02	–[e]
Yang et al. (2000)	Increased endometrial implant survival	1.79E+01	–	–	6.74E−01	–	–	–	–	–

Table 4-3. Summary of key animal study PODs (ng/kg-day) based on three different dose metrics: administered dose, 1st-order body burden HED and blood concentration HED (continued)

[a]Average administered daily dose over the experimental exposure period.
[b]HED based on 1st-order body burden model described in Section 3.3.4.2.
[c]HED based on Emond rodent and human PBPK models described in Section 3.3.6.
[d]BMR = 0.1 for quantal endpoints and 1 standard deviation control mean for continuous endpoints, except for body and organ weights, where BMR = 10% relative deviation from control mean.
[e]BMD modeling unsuccessful (see Table 4-4 and Appendix G for details).
[f]Zareba et al. (2002) is considered to be the same study but report effects at doses above the LOAEL that are not considered further; this study is not carried forward for determination of an RfD POD but is included in the RfD uncertainty analysis presented in Section 4.4.
[g]Hochstein et al. (2001) is not carried forward because of the lack of toxicokinetic information for estimation of an HED.
[h]Latchoumycandane et al. (2002a; 2002b) are considered to be the same study but report effects (not toxicologically relevant) at doses above the LOAEL that are not considered further; these two studies are not carried forward.
[i]Van Birgelen et al. (1995b) is considered to be the same study but reports effects at doses above the LOAEL that are not considered further; this study in not carried forward for determination of an RfD POD but is included in the RfD uncertainty analysis presented in Section 4.4.

− value not established or not modeled; DRL = differential reinforcement of low rate.

Table 4-4. TCDD analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ppt)[a]

Study[b,c]	NOAEL/ LOAEL	Endpoint	Control response	First response[d]	Max response[e]	Model fit detail	BMD/ BMDL	Comments
Amin et al. (2000) (rat)	– 3.38E+00	Saccharin consumed, female, (0.25%) (n = 10)	–	22% ↓ (0.3 SD)	66% ↓	Continuous linear, modeled variance (p = 0.55)	9.15E+00 6.09E+00	BMDL > LOAEL; restricted power model, constrained parameter hit lower bound
						Continuous power, modeled variance, unrestricted (p = NA)	8.37E+00 3.42E+00	Saturated model; supralinear fit (power = 0.74)
		Saccharin consumed, female (0.50%) (n = 10)	–	49% ↓ (0.7 SD)	80% ↓	Continuous linear, modeled variance (p = 0.06)	1.02E+01 6.57E+00	Restricted power model, constrained parameter hit lower bound
						Continuous power, modeled variance, unrestricted (p = NA)	6.57E+00 1.15E+00	Saturated model; supralinear fit (power = 0.40)
		Saccharin preference ratio, female (0.25%) (n = 10)	–	29% ↓ (1.8 SD)	33% ↓	Continuous linear, modeled variance (p = 0.002)	1.16E+01 5.57E+00	BMDL > LOAEL; no response near BMR; near maximal response at LOAEL
		Saccharin preference ratio, female (0.50%) (n = 10)	–	39% ↓ (1.1 SD)	54% ↓	Continuous linear, constant variance (p = 0.14)	8.14E+00 5.11E+00	BMDL > LOAEL; near maximal response at LOAEL; restricted power model, constrained parameter hit lower bound
						Continuous power, constant variance, unrestricted (p = NA)	2.60E+00 1.06E−14	Saturated model; supralinear fit (power = 0.28)

Table 4-4. TCDD BMD analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ppt)[a] (continued)

Study[b,c]	NOAEL/ LOAEL	Endpoint	Control response	First response[d]	Max response[e]	Model fit detail	BMD/ BMDL	Comments
Bell et al. (2007b) (rat)	– 2.20E+00	Balano-preputial separation in male pups (n = 30 [dams])	1/30	5/30	15/30	Dichotomous log-logistic, restricted (p = 0.78)	2.25E+00 1.39E+00	Adequate fit; constrained parameter bound hit; not litter based; selected
						Dichotomous log-logistic, unrestricted (p = 0.50)	2.00E+00 2.80E−01	Supralinear fit (slope = 0.93); selected
Cantoni et al. (1981) (rat)	– 1.85E+00	Urinary uroporhyrins (n = 4)	–	2.4-fold ↑ (5.7 SD)	87-fold ↑	Continuous exponential (M2), modeled variance (p = 0.0003)	3.76E+00 2.76E+00	No response near BMR; poor fits for all modeled variance models; constant variance poor representation of control SD; BMDL > LOAEL
		Urinary coproporhyrins (n = 4)	–	2.4-fold ↑ (3.1 SD)	4.0-fold ↑	Continuous exponential (M4), modeled variance (p = 0.49)	5.34E−01 1.80E−01	No response near BMR
						Continuous power, modeled variance, unrestricted (p = 0.61)	2.77E−02 2.03E−05	Supralinear fit (n = 0.30); poor model choice for plateau effect
Crofton et al. (2005) (rat)	3.46E+00 9.26E+00	Serum T4, (n = 4−14)	–	29% ↓ (1.9 SD)	51% ↓	Continuous exponential (M4), constant variance (p = 0.94)	5.19E+00 3.03E+00	No response near BMR

Table 4-4. TCDD BMD analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ppt)[a] (continued)

Study[b,c]	NOAEL/ LOAEL	Endpoint	Control response	First response[d]	Max response[e]	Model fit detail	BMD/ BMDL	Comments
Franc et al. (2001) (rat)	6.59E+00 1.45E+01	S-D Rats, Relative Liver Weight	–	8.1% ↑ (0.58 SD)	55% ↑	Continuous power, constant variance (p = 0.84)	9.47E+00 4.59E+00	Acceptable fit; selected
		L-E Rats, Relative Liver Weight	–	6.3% ↑ (0.63 SD)	22% ↑	Continuous Hill, modeled variance, restricted (p = 0.83)	7.72E+00 1.22E+00	Constrained parameter hit lower bound; poor fit for variance model
						Continuous Hill, modeled variance, unrestricted (p = N/A)	7.22E+00 1.15E+00	Supralinear fit (power = 0.55)
		S-D Rats, Relative Thymus Weight	–	9.0% ↓ (0.11 SD)	77% ↓	Continuous exponential (M4), modeled variance (p = 0.72)	1.88E+00 9.22E−01	Poor fit for responses in controls and lowest exposure group
						Continuous polynomial, modeled variance (p = 0.40)	4.78E+00 3.89E+00	No response near BMR; otherwise acceptable fit
		L-E Rats, Relative Thymus Weight	–	7.7% ↓ (0.15 SD)	66% ↓	Continuous exponential (M4), constant variance (p = 0.23)	2.08E+00 5.93E−01	Poor fit for responses in controls and lowest exposure group; dose-response relationship not significant
		H-W Rats, Relative Thymus Weight	–	3.7% ↓ (0.10 SD)	51% ↓	Continuous exponential (M2), constant variance (p = 0.70)	5.09E+00 3.13E+00	No response near BMR; otherwise acceptable fit

Table 4-4. TCDD BMD analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ppt)[a] (continued)

Study[b,c]	NOAEL/ LOAEL	Endpoint	Control response	First response[d]	Max response[e]	Model fit detail	BMD/ BMDL	Comments
Hojo et al. (2002) (rat)	– 1.62E+00	DRL reinforce per minute (n = 12)	–	55% ↑ (1.0 SD)	80% ↑	Continuous exponential (M4), constant variance (p = 0.054)	1.32E+00 2.37E−03	Poor fit; near maximal response at lowest dose, BMD/BMDL ratio >100
		DRL response per minute (n = 12)	–	105% ↓ (2.4 SD)	105% ↓	Continuous exponential (M4), constant variance (p = 0.48)	3.81E−01 1.55E−02	No response data near BMR; maximal response at lowest dose, BMD/BMDL ratio »20
Kattainen et al. (2001) (rat)	– 2.23E+00	3rd molar length in pups (n = 4−8)	–	15% ↓ (4.2 SD)	27% ↓	Continuous Hill, modeled variance, restricted (p = 0.02)	3.13E−01 1.68E−01	No response data near BMR; Constrained parameter lower bound hit
						Continuous Hill, modeled variance, unrestricted (p < 0.001)	1.21E−02 –	BMDL could not be calculated
		3rd molar eruption in pups (n = 4−8)	1/16	3/17	13/19	Dichotomous log-logistic, restricted (p = 0.98)	2.40E+00 1.33E+00	Constrained parameter lower bound hit
						Dichotomous log-logistic, unrestricted (p = 0.95)	1.93E+00 1.84E−01	Supralinear fit (slope = 0.91)
Keller et al. (2008a; 2008b; 2007) (mouse)	– 5.37E−01	Missing molars (n = 23−36)	0/29	2/23	30/30	Dichotomous 1° multistage (p = 0.26)	1.09E+00 7.62E−01	Poor fit at first response level; not most sensitive endpoint; other endpoints not amenable to BMD modeling

Table 4-4. TCDD BMD analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ppt)[a] (continued)

Study[b,c]	NOAEL/ LOAEL	Endpoint	Control response	First response[d]	Max response[e]	Model fit detail	BMD/ BMDL	Comments
Kociba et al. (1978) (rat)	1.55E+00 7.15E+00	Uroporphyrin per creatinine, females (n = 5)	–	15% ↑ (0.48 SD)	89% ↑	Continuous linear, constant variance (p = 0.79)	1.31E+01 9.29E+00	BMDL > LOAEL; otherwise adequate fit
		Urinary coproporphyrins, females (n = 5)	–	67% ↑ (5.1 SD)	78% ↑	Continuous exponential (M4), modeled variance (p = 0.01)	1.57E+00 7.18E−01	Poor fit; no response near BMR
		Liver lesions (n = 50)						No data presented
		Lung lesions (n = 50)						No data presented
Kuchiiwa et al. (2002) (mouse)	1.42E+02 –	Immunoreactive Neurons in Dorsalis, males (n = 6)	–	42% ↓ (3.5 SD)	64% ↓	Continuous linear, constant variance (p = NA, insufficient degrees of freedom)	6.04E−02 4.27E−02	No response near BMR
		Immunoreactive Neurons in Medianus, males (n = 6)	–	63% ↓ (4.8 SD)	75% ↓	Continuous linear, modeled variance (p = NA, insufficient degrees of freedom)	4.93E−02 3.23E−02	No response near BMR
		Immunoreactive Neurons in B9, males (n = 6)	–	69% ↓ (6.6 SD)	87% ↓	Continuous linear, constant variance (p = NA, insufficient degrees of freedom)	4.17E−02 3.01E−02	No response near BMR
		Immunoreactive Neurons in Magnus, males (n = 6)	–	55% ↓ (7.0 SD)	75% ↓	Continuous linear, modeled variance (p = NA, insufficient degrees of freedom)	3.35E−02 2.05E−02	No response near BMR

Table 4-4. TCDD BMD analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ppt)[a] (continued)

Study[b,c]	NOAEL/ LOAEL	Endpoint	Control response	First response[d]	Max response[e]	Model fit detail	BMD/ BMDL	Comments
Latchoumy-candane and Mathur (2002) (rat)	– 7.85E−01	Daily sperm production (n = 6)	–	29% ↓ (1.0 SD)	41% ↓	Continuous Hill, constant variance, restricted (p = 0.96)	1.17E−01 1.32E−02	Near maximal response at LOAEL; constrained parameter bound hit; standard deviations given in paper interpreted as standard errors
						Continuous Hill, constant variance, unrestricted (p = N/A)	9.96E−02 1.23E−09	Slightly supralinear fit (n = 0.92)
Li et al. (1997) (rat)	2.66E−01 7.99E−01	FSH in female rats (n = 10)	–	3.6-fold ↑ (2.0 SD)	19-fold ↑	Continuous power, modeled variance, restricted (p < 0.01)	2.00E+02 1.36E+02	Power hit lower bound
						Continuous power, modeled variance, unrestricted (p = 0.003)	1.96E−01 2.48E−02	Supralinear fit (power = 0.31)
Li et al. (2006) (mouse)	– 1.59E−01	Serum estradiol (n = 10)	–	2.0-fold ↑ (0.8 SD)	2.4-fold ↑	Continuous linear, constant variance (p = 0.16)	1.61E+01 5.38E+00	BMDL > LOAEL; high control coefficient variation (CV) (1.25); near maximal response at low dose; nonmonotonic response; other model fits are step-function-like
		Serum progesterone (n = 10)	–	33% ↓ (2.0 SD)	61% ↓	Continuous Hill, modeled variance (p = 0.39)	9.46E−04 8.01E−11	No response data near BMR; large CVs (>1) for treatment groups; poor fit for variance model; Hill coefficient at lower bound (step-function)

Table 4-4. TCDD BMD analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ppt)[a] (continued)

Study[b,c]	NOAEL/ LOAEL	Endpoint	Control response	First response[d]	Max response[e]	Model fit detail	BMD/ BMDL	Comments
Markowski et al. (2001) (rat)	– 1.56E+00	FR5 run opportunities (n = 4–7)	–	10% ↓ (0.21 SD)	51% ↓	Continuous Hill, constant variance (p = 0.94)	1.72E+00 9.08E−01	Constrained parameter upper bound hit
						Continuous power, constant variance, unrestricted (p = 0.13)	2.67E+00 1.03E−14	Saturated model; supralinear fit (power = 0.39); BMD/BMDL ratio »100
		FR2 revolutions (n = 4–7)	–	9% ↓ (0.15 SD)	43% ↓	Continuous Hill, constant variance (p = 0.65)	1.84E+00 5.99E−01	Constrained parameter bound hit (upper bound)
						Continuous power, constant variance, unrestricted (p = 0.16)	5.74E+00 1.03E−14	Supralinear fit (power = 0.32)
		FR10 run opportunities (n = 4–7)	–	15% ↓ (0.24 SD)	57% ↓	Continuous exponential (M2) , constant variance (p = 0.30)	8.57E+00 2.89E+00	BMDL > LOAEL
Miettinen et al. (2006) (rat)	– 2.22E+00	Cariogenic lesions in pups (n = 4–8)	25/42	23/29	29/32	Dichotomous log-logistic, restricted (p = 0.60)	1.43E+00 5.17E−01	Constrained parameter lower bound hit; near maximal response at LOAEL; high control response
						Dichotomous log-logistic, unrestricted (p = 0.73)	4.94E−02 –	Supralinear fit (slope = 0.47); BMDL could not be calculated
Murray et al. (1979) (rat)	1.12E+00 5.88E+00	Fertility in F2 gen. (no litters) (n = 20)	4/32	0/20	9/20	Dichotomous multistage (p = 0.08)	2.73E+00 1.37E+00	Poor fit; nonmonotonic response; no response data near BMR
NTP (1982b) (mouse)	– 7.67E−01	Toxic hepatitis; males (n = 50)	1/73	5/49	44/50	Dichotomous multistage (p = 0.04)	2.78E+00 1.34E+00	No acceptable model fits; lowest BMDL shown

Table 4-4. TCDD BMD analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ppt)[a] (continued)

Study[b,c]	NOAEL/ LOAEL	Endpoint	Control response	First response[d]	Max response[e]	Model fit detail	BMD/ BMDL	Comments
NTP (2006a) (rat)	– 2.56E+00	Hepatocyte hypertrophy (n = 53–54)	0/53	19/54	52/53	Dichotomous multistage (p = 0.02)	9.27E−01 7.91E−01	Poor fits for all models
		Alveolar metaplasia (n = 52–54)	2/53	19/54	46/52	Dichotomous log-logistic (p = 0.72)	6.50E−01 3.75E−01	No response near BMR
		Oval cell hyperplasia (n = 53–54)	0/53	4/54	53/53	Dichotomous probit (p = 0.23)	5.67E+00 4.79E+00	Relatively poor fit for control and low-dose groups; negative response intercept (same for logistic); BMDL > LOAEL
						Dichotomous Weibull (p = 0.08)	5.72E+00 4.09E+00	Marginal fit; BMDL > LOAEL
		Gingival hyperplasia (n = 53–54)	1/53	7/54	16/53	Dichotomous log-logistic, restricted (p = 0.06)	5.85E+00 3.73E+00	Poor fit; constrained parameter bound hit; BMDL > LOAEL
						Dichotomous log-logistic, unrestricted (p = 0.66)	7.05E−01 1.26E−05	Supralinear fit (slope = 0.37)
		Eosinophilic focus, multiple (n = 53–54)	3/53	8/54	42/53	Dichotomous probit (p = 0.46)	5.58E+00 4.86E+00	Relatively poor fit to control response; BMDL > LOAEL
		Liver fatty change, diffuse (n = 53–54)	0/53	2/54	48/53	Dichotomous Weibull (p = 0.72)	3.92E+00 2.86E+00	BMDL > LOAEL; otherwise adequate fit
NTP (2006a) (rat) (continued)	– 2.56E+00 (continued)	Liver necrosis (n = 53–54)	1/53	4/54	17/53	Dichotomous log-probit, unrestricted (p = 0.80)	7.50E+00 3.50E+00	Adequate fit; slightly supralinear; BMDL > LOAEL
		Liver pigmentation (n = 53–54)	4/53	9/54	53/53	Dichotomous log-probit (p = 0.96)	2.46E+00 1.89E+00	Adequate fit
		Toxic hepatopathy (n = 53–54)	0/53	2/54	53/53	Dichotomous multistage (p = 0.69)	3.98E+00 3.06E+00	BMDL > LOAEL; otherwise adequate fit

Table 4-4. TCDD BMD analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ppt)[a] (continued)

Study[b,c]	NOAEL/ LOAEL	Endpoint	Control response	First response[d]	Max response[e]	Model fit detail	BMD/ BMDL	Comments
Ohsako et al. (2001) (rat)	1.04E+00 3.47E+00	Anogenital distance in male pups (n = 5)	–	12% ↓ (1.0 SD)	17% ↓	Continuous Hill, constant variance, restricted (p = 0.15)	2.88E+00 8.03E−01	Constrained parameter lower bound hit; near maximal response at LOAEL
						Continuous Hill, constant variance, unrestricted (p = 0.056)	3.49E+00 3.05E−01	Supralinear fit (n = 0.59)
Schantz et al. (1996)	- 3.38E+00	Facilitory effect on radial arm maze learning (n = 10)	–	22% ↓ (1.2 SD)	34% ↓	Continuous linear, constant variance (p = 0.16)	7.00E+00 4.60E+00	BMDL > LOAEL; otherwise adequate fit
Sewall et al. (1995) (rat)	7.11E+00 1.66E+01	Serum T4 (n = 9)	–	9.1% ↓ (0.6 SD)	40% ↓	Continuous Hill, constant variance, restricted (p = 0.90)	1.03E+01 3.60E+00	Constrained parameter hit lower bound; otherwise acceptable fit; selected
						Continuous Hill, constant variance, unrestricted (p = 0.86)	9.71E+00 1.97E+00	Supralinear fit (power = 0.57)
Shi et al. (2007) (rat)	3.42E−01 1.07E+00	Serum estradiol in female pups (n = 10)	–	38% ↓ (0.4 SD)	62% ↓	Continuous exponential (M4), modeled variance (p = 0.69)	8.07E−01 3.54E−01	Adequate fit; selected
Smialowicz et al. (2008) (mouse)	– 4.38E−01	PFC per spleen (n = 15)	–	24% ↓ (0.5 SD)	89% ↓	Continuous power, unrestricted, modeled variance (p = 0.27)	1.19E+01 3.76E+00	BMDL > LOAEL; fit at control and low dose inconsistent with data; constrained parameters in other models hit lower bounds
		PFC per 10^6 cells (n = 8−15)	–	24% ↓ (0.5 SD)	9.3-fold ↓	Continuous power unrestricted, constant variance (p = 0.48)	1.90E+00 2.16E−01	Constant variance test failed; observed control variance underestimated by 35%; poor fits for all modeled variance models

Table 4-4. TCDD BMD analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ppt)[a] (continued)

Study[b,c]	NOAEL/ LOAEL	Endpoint	Control response	First response[d]	Max response[e]	Model fit detail	BMD/ BMDL	Comments
Smith et al. (1976) (mouse)	7.11E+00 5.06E+01	Cleft palate in pups (n = 14−41)	0/34	2/41	10/14	Dichotomous log-logistic, restricted (p = 0.42)	3.52E+01 1.06E+01	Adequate fit; selected
Sparschu et al. (2008; 1971) (rats)	5.09E+00 1.63E+01	Male fetus weight (n = 3−117)	–	2.7% ↑ (0.1 SD)	33% ↓	Continuous exponential (M5), modeled variance (p < 0.0001)	5.46E+02 1.30E+02	BMDL > LOAEL; variance not captured by either variance model; poor fit in region surrounding NOAEL and LOAEL
		Female fetus weight (n = 4−129)	–	2.3% ↑ (0.06 SD)	30% ↓	Continuous exponential (M2), modeled variance (p < 0.028)	1.03E+03 6.48E+02	BMDL > LOAEL; variance not captured by either variance model; poor fit in region surrounding NOAEL and LOAEL
Toth et al. (1979) (mouse)	– 5.73E−01	Skin lesions (n = 38−44)	0/38	5/44	25/43	Dichotomous log-logistic, restricted (p = 0.08)	6.41E+00 4.02E+00	Constrained parameter lower bound hit
						Dichotomous log-logistic, unrestricted (p = 0.74)	5.97E−01 6.77E−02	Supralinear fit (slope = 0.48)
	– 5.73E−01 (cont.)	Dermal amyloidosis (n = 38−44)	0/38	5/44	17/43	Dichotomous log-logistic, restricted (p = 0.05)	1.50E+01 8.75E+00	Poor fit; constrained parameter lower bound hit; BMDL > LOAEL
						Dichotomous log-logistic, unrestricted (p = 0.90)	4.84E−01 5.31E−03	Supralinear fit (slope = 0.33)
Van Birgelen et al. (1995a) (rat)	– 7.20E+00	Hepatitic retinol (n = 8)	–	44% ↓ (0.74 SD)	96% ↓	Continuous exponential (M4), modeled variance (p < 0.01)	2.49E+01 3.36E+00	Poor fit
						Continuous power, modeled variance, unrestricted (p = 0.01)	3.80E−01 1.39E−02	Poor fit; supralinear fit (power = 0.14)

Table 4-4. TCDD BMD analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ppt)[a] (continued)

Study[b,c]	NOAEL/ LOAEL	Endpoint	Control response	First response[d]	Max response[e]	Model fit detail	BMD/ BMDL	Comments
		Hepatitic retinyl palmitate (n = 8)	–	80% ↓ (1.4 SD)	99% ↓	Continuous exponential (M4), modeled variance (p < 0.01)	1.42E+02 3.65E+01	Poor fit; no response near BMR
						Continuous power, modeled variance, unrestricted (p = 0.24)	5.26E−02 5.89E−05	Supralinear fit (power = 0.06)
White et al. (1986) (mouse)	– 1.09E+00	Total hemolytic complement activity (CH50) (n = 8)	–	41% ↓ (2.6 SD)	81% ↓	Continuous Hill, modeled variance, restricted (p = 0.002)	8.63E+00 1.50E+00	Poor fit; no response near BMR; constrained parameter bound hit; BMDL > LOAEL
						Continuous Hill, modeled variance, unrestricted (p = 0.07)	1.48E−01 4.35E−03	Supralinear fit (n = 0.25)

[a]Animal whole blood concentrations were used to determine the HEDs in Table 4-3 and Table 4-5.
[b]The following studies previously presented in Table 4-3 are not presented in Table 4-4 because toxicokinetic models for guinea pigs, minks, or monkeys, and were not found: DeCaprio et al. (1986); Hochstein et al (2001); Vos et al. (1973); Yang et al. (2000).
[c]The following studies previously presented in Table 4-3 are not presented in Table 4-4 because the data were not amenable to BMD modeling: Chu et al. (2001); Chu et al. (2007); Croutch et al. (2005); Fattore et al. (2000); Fox et al. (1993); Franczak et al. (2006); Hutt et al. (2008); Ikeda et al. (2005); Ishihara et al. (2007); Kociba et al. (1976); Maronpot et al. (1993); Nohara et al. (2000); Nohara et al. (2002); Seo et al. (1995); Simanainen et al. (2002); Simanainen et al. (2003); Simanainen et al. (2004); Smialowicz et al. (2004); Weber et al. (1995).
[d]Magnitude of response at first dose where response differs from control value (in the adverse direction); continuous response magnitudes given as relative to control plus change relative to control standard deviation; quantal response given as number affected/total number.
[e]Magnitude of response maximally differing from control value (in the adverse direction).
SD = standard deviation; S-D = Sprague-Dawley; L-E = Long-Evans; H-W = Han-Wistar; DRL = differential reinforcement of low rate.

4.3. REFERENCE DOSE (RfD) DERIVATION

Table 4-5 lists all the studies and endpoints considered for derivation of the RfD in order of candidate RfD from lowest to highest (The selection process was previously described in Section 4.1). The range of studies includes three of the four human studies.[42] Figure 4-4 (exposure-response array) shows all of the endpoints listed in Table 4-5 graphically in terms of PODs in human-equivalent intake units (ng/kg-day). The human study endpoints are shown at the far left of the figure, and the animal bioassay endpoints are arranged by category to the right. Figure 4-5 demonstrates the same endpoints, arrayed by RfD value, showing the POD, applicable UFs, and candidate RfD.

Table 4-5 illustrates the study, species, strain and sex, study protocol, and toxicological endpoints observed at the lowest TCDD doses. The table also identifies the human-equivalent BMDLs (when applicable), NOAELs, and LOAELs, as well as the composite uncertainty factor (UF) that applies to the specific endpoint and the corresponding candidate RfD.[43] The NOAELs, LOAELs, and BMDLs are presented as HEDs, based on the assumption that whole-blood concentration is the toxicokinetically equivalent TCDD dose metric across species and serves as a surrogate for tissue concentration.[44] For rats and mice, these estimates relied on the two Emond PBPK models—one for the relevant rodent species and one for the human—as described previously (see Section 3.3.4.3). The guinea pig and monkey studies that are included in Table 4-5 are given in HED units based on the first-order body burden model (described in Section 3.3.4.2) because there are no published PBPK models to estimate TCDD disposition in guinea pigs and monkeys. The values listed for guinea pigs and monkeys are not directly comparable to those for rats and mice but are probably biased low, as first-order body burden HED estimates for rats and mice are generally two to fivefold lower than the corresponding PBPK model estimates. The LOAELs for the human studies also rely on the Emond PBPK model, as described in Sections 4.2.2 and 4.2.3.

[42] The RfD derived from the study of Eskenazi et al. (2002b) was outside the RfD range presented in Table 4-5.
[43] Extra digits are retained for transparency and comparison prior to rounding to one significant digit for the final RfD.
[44] The procedures for estimating HEDs based on TCDD blood concentration are described in the preceding section.

Table 4-5. Candidate RfDs for TCDD using blood-concentration-based human equivalent doses

Study	Species, strain (sex, if not both)	Protocol	Endpoint	$NOAEL_{HED}$ (N) or $BMDL_{HED}$ (B) (ng/kg-day)	$LOAEL_{HED}$ (ng/kg-day)	UF[a]	RfD (mg/kg-day)
Li et al. (2006)	Mouse, NIH (F)	Gavage GDs 1−3; n = 10	Hormone levels in pregnant dams (decreased progesterone, increased estradiol)	−	1.6E−03	300	5.3E−12
Kuchiiwa et al. (2002)	Mouse, ddY	Maternal 8 week-gavage prior to mating; n = 3	Decreased serotonin-immunoreactive neurons in raphe nuclei of male offspring (F1)	−	2.7E−03	300	9.2E−12
Smialowicz et al. (2008)	Mouse, $B6C3F_1$ (F)	90-day gavage; n = 8−15	Decreased SRBC response	−	6.3E−03	300	2.1E−11
Bowman et al.(1989a; 1989b); others[b]	Rhesus Monkey (F)	Daily dietary exposure, 3.5−4 years n = 3−7	Neurobehavioral effects	−	8.2E−03[c]	300	2.7E−11
Keller et al. (2008a; 2008b; 2007)[d]	Mouse, CBA/J and C3H/HeJ	Gavage GD 13; n = 23−36 (pups)	Missing molars, mandibular shape changes in pups	−	9.9E−03	300	3.3E−11
Toth et al. (1979)	Mouse, Swiss/ H/Riop (M)	1-year gavage; n = 38−44	Dermal amyloidosis, skin lesions	−	9.9E−03	300	3.3E−11
Latchoumy-candane and Mathur (2002); others[e]	Rat, Wistar (M)	45-day oral pipetting; n = 6	Decreased sperm production	−	1.6E−02	300	5.4E−11
NTP (1982b)	Mouse, $B6C3F_1$ (M)	2-year gavage; n = 50	Liver lesions	−	2.2E−02	300	7.2E−11
White et al. (1986)	Mouse, $B6C3F_1$ (F)	14-day gavage; n = 6−8	Decreased serum complement	−	2.8E−02	300	9.2E−11
Li et al. (1997)	Rat, S-D (F, 22 day-old)	Single gavage; n = 10	Increased serum FSH	2.9E−03 (N)	1.7E−02	30[f]	9.7E−11
DeCaprio et al. (1986)	Guinea pig, Hartley	90-day dietary; n = 10	Decreased body weight, organ weight changes (liver, kidney, thymus, brain)	4.1E−03[c] (N)	3.3E−02[c]	30[f]	1.4E−10
Shi et al. (2007)	Rat, S-D (F)	11-month gavage; n = 10	Decreased serum estradiol	4.5E−03 (N) 4.7E−03 (B)	2.7E−02	30[f]	1.6E−10
Markowski et al. (2001)	Rat, Holtzman	Gavage GD 18; n = 4−7	Neurobehavioral effects in pups (running, lever press, wheel spinning)	−	5.2E−02	300	1.7E−10

Table 4-5. Candidate RfDs for TCDD using blood-concentration-based human equivalent doses (continued)

Study	Species, strain (sex, if not both)	Protocol	Endpoint	$NOAEL_{HED}$ (N) or $BMDL_{HED}$ (B) (ng/kg-day)	$LOAEL_{HED}$ (ng/kg-day)	UF[a]	RfD (mg/kg-day)
Hojo et al. (2002); Zareba et al. (2002)	Rat, S-D	Gavage GD 8; *n* = 12	Food-reinforced operant behavior in pups	–	5.5E−02	300	1.8E−10
Cantoni et al. (1981)	Rat, CD-COBS (F)	45-week gavage; *n* = 4	Increased urinary porhyrins	–	6.4E−02	300	2.1E−10
Vos et al. (1973)	Guinea pig, Hartley (F)	8-week gavage; *n* = 10	Decreased delayed-type hypersensitivity response to tuberculin	6.4E−03[c] (N)	3.2E−02[c]	30[f]	2.1E−10
Miettinen et al. (2006)	Rat, Line C	Gavage GD 15; *n* = 3−10	Cariogenic lesions in pups	–	8.9E−02	300	3.0E−10
Kattainen et al. (2001)	Rat, Line C	Gavage GD 15; *n* = 4−8	Inhibited molar development in pups	–	9.0E−02	300	3.0E−10
NTP (2006a)	Rat, S-D (F)	2-year gavage; *n* = 53	Liver and lung lesions	–	1.4E−01	300	4.5E−10
Amin et al. (2000)	Rat, S-D	Gavage GDs 10−16; *n* = 10	Reduced saccharin consumption and preference	–	1.7E−01	300	5.7E−10
Schantz et al. (1996)	Rat, S-D (F)	Gavage GDs 10-16; *n* = 80−88	Maze errors (facilitatory effect)	–	1.7E−01	300	5.7E−10
Mocarelli et al. (2008)	**Human (M)**	**Childhood exposure; *n* = 157**	**Decreased sperm concentration and sperm motility, as adults**	**–**	**2.0E−02[g]**	**30[h]**	**6.7E−10**
Baccarelli et al. (2008)	**Human infants**	**Gestational exposure; *n* = 51**	**Increased TSH in newborn infants**	**–**	**2.0E−02[i]**	**30[h]**	**6.7E−10**
Hutt et al. (2008)	Rat, S-D (F)	13-week dietary; *n* = 3	Embryotoxicity	–	2.5E−01	300	8.4E−10
Ohsako et al. (2001)	Rat, Holtzman	Gavage GD 15; *n* = 5	Decreased anogenital distance in male pups	2.7E−02 (N)	1.8E−01	30[f]	9.1E−10
Murray et al. (1979)	Rat, S-D	3-generation dietary	Reduced fertility and neonatal survival (F0 and F1)	2.9E−02 (N)	3.8E−01	30[f]	9.6E−10
Franczak et al. (2006)	Rat, S-D (F)	Gavage GD 14, 21, PND 7, 14; *n* = 7	Abnormal estrous cycle	–	3.2E−01	300	1.1E−09
Chu et al. (2007)	Rat, S-D (F)	28-day gavage, *n* = 5	Liver lesions	3.5E−02 (N)	5.6E−01	30[f]	1.2E−09
Bell et al. (2007b)	Rat, CRL:WI (Han) (M)	17-week dietary; *n* = 30	Delay in onset of puberty	4.3E−02 (B)	8.9E−02	30[f]	1.4E−09

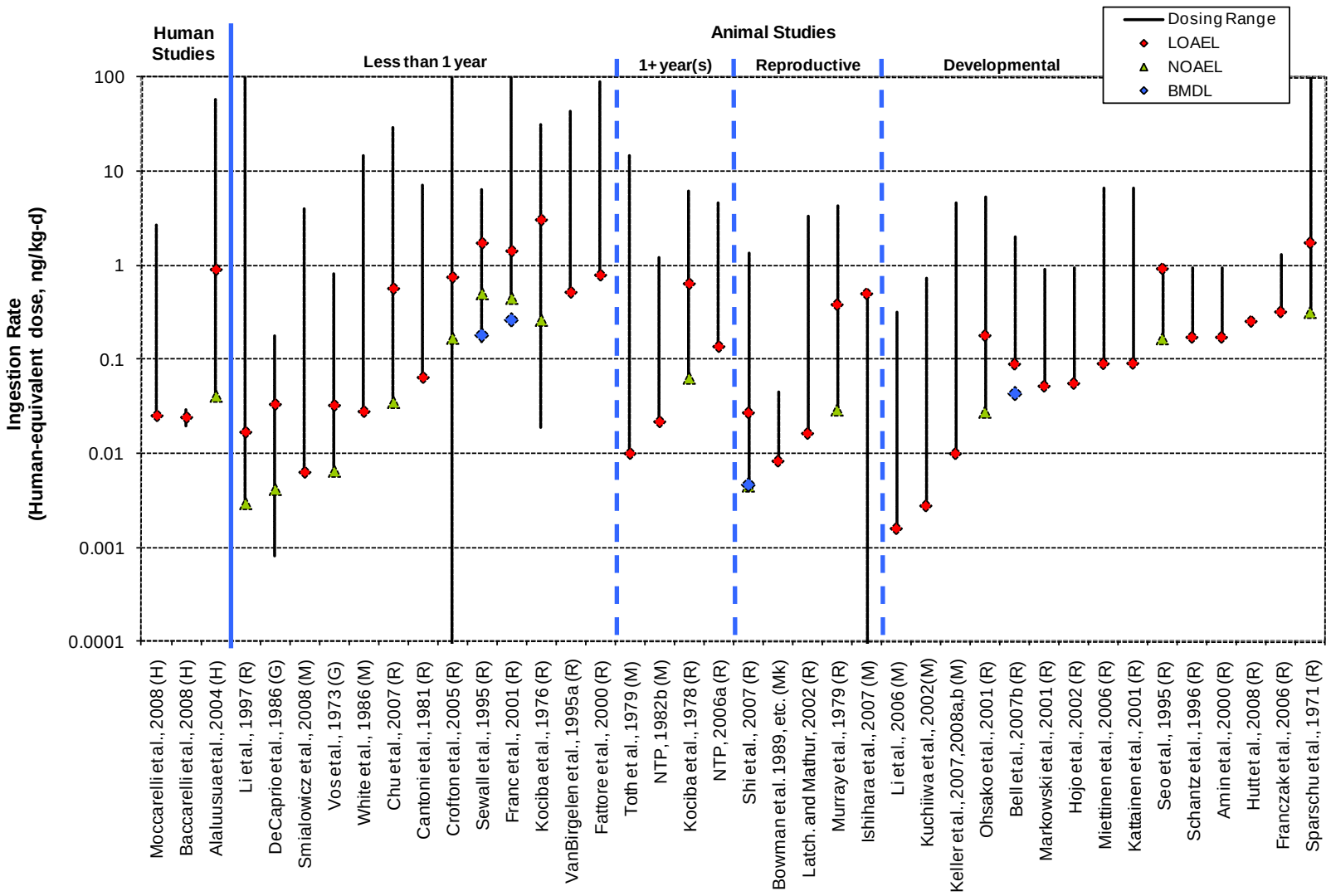
Table 4-5. Candidate RfDs for TCDD using blood-concentration-based human equivalent doses (continued)

Study	Species, strain (sex, if not both)	Protocol	Endpoint	$NOAEL_{HED}$ (N) or $BMDL_{HED}$ (B) (ng/kg-day)	$LOAEL_{HED}$ (ng/kg-day)	UF[a]	RfD (mg/kg-day)
Ishihara et al., (2007)	Mouse, ICR (M)	Weekly gavage for 5 weeks; *n* = 42−43	Decreased male/female sex ratio	–[j]	5.0E−01	300	1.7E−09
VanBirgelen et al. (1995a)[k]	Rat, S-D (F)	13-week dietary; *n* = 8	Decreased liver retinyl palmitate	–	5.1E−01	300	1.7E−09
Kociba et al. (1978)	Rat, S-D (F)	2-year dietary; *n* = 50	Liver and lung lesions, increased urinary porhyrins	6.3E−02 (N)	6.3E−01	30[f]	2.1E−09
Fattore et al. (2000)	Rat, S-D	13-week dietary; *n* = 6	Decreased hepatic retinol	–	7.8E−01	300	2.6E−09
Seo et al. (1995)	Rat, S-D	Gavage GDs 10−16; *n* = 10	Decreased serum T4 and thymus weight	1.7E−01 (N)	9.1E−01	30[f]	5.6E−09
Crofton et al. (2005)	Rat, Long-Evans (F)	4-day gavage; *n* = 4−14	Decreased serum T4	1.7E−01 (N)	7.4E−01	30[f]	5.6E−09
Sewall et al. (1995)	Rat, S-D (F)	30-week gavage; *n* = 9	Decreased serum T4	5.0E−01 (N) 1.8E−01 (B)	1.7E+00	30[f]	6.0E−09
Franc et al. (2001)	Rat, Long-Evans (F)	22-week gavage; *n* = 8	Increased relative liver weight; decreased relative thymus weight	4.5E−01 (N) 2.6E−01 (B)	1.4E+00	30[f]	8.7E−09
Kociba et al. (1976)	Rat, S-D	5-days/week gavage for 13 weeks; *n* = 12	Liver and lung lesions, increased urinary porphyrins	2.6E−01 (N)	3.0E+00	30[f]	8.7E−09
Sparschu et al. (1971)	Rat, S-D (F)	Gavage GD 6−15; *n* = 4−129	Decreased fetal body weight	3.2E−01 (N)	1.7E+00	30[f]	1.1E−08
Alaluusua et al. (2004)	Human	Childhood exposure; *n* = 48	Dental defects	4.1E−02[l] (N)	9.0E−01[m]	3[n]	1.4E−08

[a]Except where indicated, $UF_A = 3$ (for dynamics), $UF_H = 10$, $UF_L = 10$.
[b]Schantz and Bowman (1989); Schantz et al. (1986); Schantz et al. (1986).
[c]HED determined from 1st-order body burden model; no PBPK model available for guinea pigs or monkeys; Hochstein et al. (2001) was not presented in the table because no PBPK model exists for minks and 1st-order body burden could not be calculated because a TCDD half-life could not be determined.
[d]Results from three separate studies with identical designs combined.
[e]Latchoumycandane et al. (2002a; 2002b).
[f]$UF_L = 1$ (NOAEL or BMDL).
[g]Mean of peak exposure (0.0321 ng/kg-day) and average exposure over 10-year critical window (0.0080 ng/kg-day).
[h]$UF_H = 3$, $UF_L = 10$.
[i]Maternal exposure corresponding to neonatal TSH concentration exceeding 5 μU/mL.
[j]The NOAEL of 4.9E−5 was excluded from consideration because of the large dose spacing in the study.

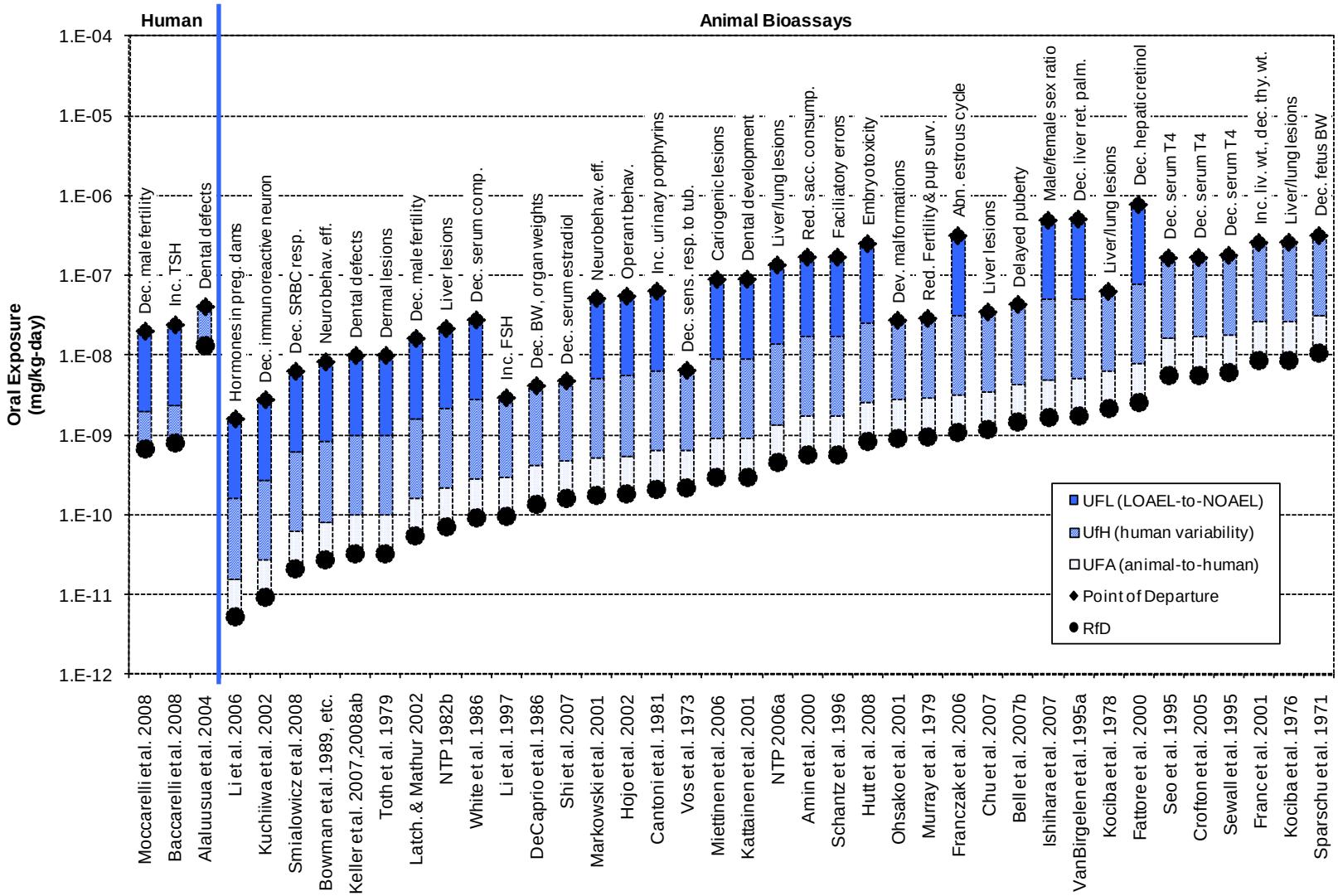
Table 4-5. Candidate RfDs for TCDD using blood-concentration-based human equivalent doses (continued)

[k]Van Birgelen et al. (1995b) is considered to be the same study but reports effects at doses above the LOAEL that are not considered further; this study in not carried forward for determination of an RfD POD but is included in the RfD uncertainty analysis presented in Section 4.4.
[l]Mean of peak exposure (0.0655 ng/kg-day) and average exposure over 10-year critical window (0.0156 ng/kg-day).
[m]Mean of peak exposure (1.65 ng/kg-day) and average exposure over 10-year critical window (0.149 ng/kg-day).
[n]$UF_H = 3$.
S-D = Sprague-Dawley.



G = guinea pig; H = human; M = mouse; Mk = monkey; R = rat

Figure 4-4. Exposure-response array for ingestion exposures to TCDD.



Dec = decreasing effect; Inc = increasing effect

Figure 4-5. Candidate RfD array.

As is evident from Table 4-5, very few NOAELs and even fewer BMDLs have been established for low-dose TCDD studies. BMD modeling was unsuccessful for all of the endpoints without a NOAEL, primarily because of the lack of dose-response data near the BMR (see discussion in Section 4.2). Therefore, the RfD assessment rests largely on evaluation of LOAELs to determine the POD.

4.3.1. Toxicological Endpoints

As can be seen in Table 4-5, a wide array of toxicological endpoints has been observed following TCDD exposure, ranging from subtle developmental effects to overt toxicity. Developmental effects in rodents include embryotoxicity, neonatal mortality, dental defects, delayed puberty in males, and several neurobehavioral effects. Reproductive effects reported in rodents include altered hormone levels in females and decreased sperm production in males. Immunotoxicity endpoints, such as decreased response to SRBC challenge in mice and decreased delayed-type hypersensitivity response in guinea pigs, are also observed. Longer durations of TCDD exposure in rodents are associated with organ and body weight changes, renal toxicity, hepatotoxicity, and lung lesions. Adverse effects in human studies are also observed, which include both male and female reproductive effects, increased TSH in neonates, and dental defects in children. Other outcomes including diabetes (Michalek and Pavuk, 2008) and hepatic effects (Michalek et al., 2001b) have also been associated with adult human TCDD exposures, but EPA was unable to quantify the exposure-response relationship (see Appendix C). All but three of the study/endpoint combinations from animal bioassays listed in Table 4-5 are on TCDD-induced toxicity observed in mice and rats; the other three study/endpoint combinations are effects in guinea pigs and monkeys. Although the effects of TCDD also have been investigated in hamsters and mink, those studies were not included for final POD consideration because the effect levels were greater than those in Table 4-5, or because effective oral intakes could not be estimated.

Three human studies were also included for final POD consideration in the derivation of an RfD and are presented in Table 4-5 as candidate RfDs. All three human study/endpoint combinations are from studies on the Seveso cohort. The developmental effects observed in these studies were associated with TCDD exposures either in utero or in early childhood between 1 and 10 years of age. Baccarelli et al. (2008) reported increased levels of TSH in newborns

exposed to TCDD in utero, indicating a possible dysregulation of thyroid hormone metabolism. Mocarelli et al. (2008) reported decreased sperm concentrations and decreased motile sperm counts in men who were 1−9 years of age in 1976 at the time of the Seveso accident (initial TCDD exposure event). Alaluusua et al. (2004) reported dental effects in adults who were less than 5 years of age at the time of the initial exposure (1976).

4.3.2. Exposure Protocols of Points of Depature (PODs)

The studies in Table 4-5 represent a wide variety of exposure protocols, involving different methods of administration and exposure patterns across virtually all exposure durations and life stages. Both dietary and gavage administration have been used in rodent studies, with gavage being the predominant method. Gavage dosing protocols vary quite widely and include single gestational exposures, multiple daily exposures (for up to 2 weeks, intermittent schedules that include 5 days/week, once weekly, or once every 2 weeks), and loading/maintenance dose protocols, in which a relatively high dose is initially administered followed by lower weekly doses. The intermittent dosing schedules require dose-averaging over time periods as long as 2 weeks, which introduces uncertainty in the effective exposures. In other words, the high unit dose may be more of a factor in eliciting the effect than the average TCDD tissue levels over time. Although the loading/maintenance dose protocols are designed to maintain a constant internal exposure, these protocols are somewhat inconsistent with the constant daily TCDD dietary exposures associated with human ingestion patterns.

The epidemiologic studies conducted in the Seveso cohort represent exposures over different life stages including gestation, childhood, and young adulthood. The Seveso exposure profile is essentially a high initial pulse TCDD exposure followed by a 20−30 year period of elimination with only background exposures to TCDD and DLCs.[45] While the exposures were measured soon after the initial pulse, health outcomes were realized, or measured, 10−20 years following the initial exposure; the biologically-relevant critical exposure window for susceptibility varies with effect and may be unknown. Therefore, the effective exposure profiles for the Seveso cohort studies vary considerably. For the Mocarelli et al. (2008) and Alaluusua et al. (2004) studies, where early childhood exposures proximate to the initial event are associated with the outcomes, there is some uncertainty as to the magnitude of the effective

[45] In Section 4 the DLC term is exclusive of TCDD.

doses. Although the effects are associated with TCDD exposure in the first 10 years of life, it is not clear to what extent the initial peak exposure is primarily responsible for the effects. It is also not clear if averaging exposure over the critical window is appropriate given the fairly large (sixfold) difference between initial TCDD body burden and body burden at the end of the critical exposure window. Because of the uncertainty in the influence of the peak exposure relative to the average exposure over the entire window of susceptibility, the LOAELs for both Mocarelli et al. (2008) and Alaluusua et al. (2004) are calculated as the average of the peak exposure and average exposure across the critical exposure window (see Section 4.2 for details).

For the gestational exposure study (Baccarelli et al., 2008), the critical exposure window is strictly defined and relatively short (9 months) and occurs long after the initial maternal exposure (18−29 years).[46] The maternal serum TCDD concentrations were measured 16−22 years after the initial exposure when internal exposures were falling off less steeply; consequently, there is less uncertainty in the toxicokinetic extrapolation between time of measurement and time of birth. The narrow critical exposure window at a much later time than the initial exposure (where the TCDD elimination curve is flattening) is assumed to lead to a relatively steady-state exposure over the critical time period with much less uncertainty in the magnitude of the effective dose. With the exception of Eskenazi et al. (2002b) (see Section 4.2.4), the effective exposures for other effects reported for the Seveso cohort (see Section C.1.1.1.4) have not been quantified for consideration as an RfD POD. These exposures and effects are not represented in Table 4-5 because either critical exposure windows cannot be identified, unequivocal adverse effect levels cannot be determined, or individual exposure estimates were not reported. Several of these studies, however, are included in the uncertainty analysis presented in Section 4.5.

4.3.3. Uncertainty Factors

Based on U.S. EPA (2002), UFs address five areas of uncertainty. Table 4-5 summarizes the composite (total) UF applied to the POD for each endpoint.

For the PODs based on animal bioassays, the following UFs were applied:

[46] The Sevesso accident occurred on July 10, 1976 and the subjects evaluated in the Baccarelli et al., (2008) study were born between January 1, 1994 and June 30, 2005.

- *Interspecies extrapolation (UF_A).* A factor of 3 ($10^{0.5}$) was applied for interspecies extrapolation. The factor of 3 represents the residual uncertainty for toxicodynamics after accounting for toxicokinetic differences with kinetic modeling. Although there are in vitro studies (Budinsky et al., 2010; Silkworth et al., 2005) that report higher rodent sensitivities than humans for AhR-dependent enzyme induction, EPA believes that there is insufficient information on subsequent toxicological processes to conclude that rodents are more sensitive than humans for downstream adverse effects.
- *Human interindividual variability (UF_H).* A factor of 10 was applied to account for human interindividual variability in susceptibility to TCDD because there is insufficient information on sensitive populations to justify a lower value.
- *LOAEL-to-NOAEL (UF_L).* For all PODs based on the animal bioassay endpoints lacking a NOAEL, a factor of 10 was applied to account for LOAEL-to-NOAEL uncertainty. The factor of 10 is the standard value in the absence of information suggesting a lower value; the magnitude of the effects for most of the LOAELs is relatively high compared to controls.
- *Subchronic-to-chronic (UF_S).* A UF for study duration was not applied, because chronic effects for animal bioassays are well represented in the database.
- *Database factor (UF_D).* A UF for database deficiencies was not applied because the database for TCDD contains an extensive range of human and animal studies that examine a comprehensive set of endpoints. There is no evidence to suggest that additional data would result in a lower RfD.

For the PODs based on epidemiologic studies, the following UFs were applied:

- *UF_A.* A UF for interspecies extrapolation was not applied because human data were utilized for derivation of the RfD.
- *UF_H.* A factor of 3 was selected for interindividual variability to account for human-to-human variability in susceptibility. The individuals evaluated in the two principal studies included infants (exposed in utero) and adults who were exposed when they were less than 10 years of age, groups that are considered to represent sensitive lifestages. These studies considered together associate TCDD exposures with health effects in potentially vulnerable lifestage subgroups. A UF of 1 was not applied because the sample sizes for the lifestages studied were relatively small, which, combined with uncertainty in exposure estimation, may not fully capture the range of interindividual variability. In addition, potential chronic effects were not fully elucidated for humans and could possibly be more sensitive.
- *UF_L.* A factor of 10 was applied to account for LOAEL-to-NOAEL uncertainty. The factor of 10 for UF_L is the standard value in the absence of information suggesting a lower value.

- UF_S. A UF for study duration was not applied, because, although chronic effect levels are not well defined for humans, animal bioassays indicate that duration of exposure is not likely to be a determining factor in toxicological outcomes. Developmental effects and other short-term effects occur at doses similar to effects noted in chronic studies.
- UF_D. A UF for database deficiencies was not applied because the database for TCDD contains an extensive range of human and animal studies that examine a comprehensive set of endpoints. There is no evidence to suggest that additional data would result in a lower RfD.

4.3.4. Choice of Human Studies for Reference Dose (RfD) Derivation

For selection of the POD, the human studies are preferred, as EPA favors human data over animal data of comparable quality. The human studies included in Table 4-5 (Baccarelli et al., 2008; Mocarelli et al., 2008; Alaluusua et al., 2004) each evaluate a segment of the Seveso civilian population (i.e., not an occupational cohort) exposed directly to TCDD released from an industrial accident. (The identification of PODs from these studies is detailed in Sections 4.3.4.1, 4.3.4.2, and 4.3.4.3.) Thus, exposures were primarily to TCDD, with apparently minimal DLC exposures beyond those associated with background intake,[47] qualifying these studies for use in RfD derivation for TCDD. In addition, health effects associated with TCDD exposures were observed in humans, eliminating the uncertainty associated with interspecies extrapolation. The cohort members who were evaluated included infants (exposed in utero) and adults who were exposed when they were less than 10 years of age. These studies considered together associate TCDD exposures with health effects in potentially vulnerable lifestages. Finally, the two virtually identical RfDs from different endpoints in different studies provide an additional level of confidence in the use of these data for derivation of the RfD for TCDD.

Although the human data are preferred, Table 4-5 presents a number of animal studies with RfDs that are lower than the human RfDs. Two of the rat bioassays among this group of studies—Bell et al. (2007b) (RfD = 1.4E−9 mg/kg-day based on delay in the onset of puberty) and NTP (2006a) (RfD = 4.5E−10 mg/kg-day based on liver and lung lesions)—are of particular note. Both studies were recently conducted. Both were very well designed and conducted, using 30 or more animals per dose group (see Table 4-6 for a discussion of these studies' strengths and

[47] As an example, note the lack of statistically significant effects reported by Baccarelli et al. (2008) (Figures 2C and D) in regression models based on either maternal plasma levels of noncoplanar PCBs or total TEQ on neonatal TSH levels.

weaknesses); both also are consistent with and, in part, have helped to define the current state of practice in the field. Bell et al. (2007b) evaluated several reproductive and developmental endpoints, initiating TCDD exposures well before mating and continuing through gestation. NTP (2006a) is the most comprehensive evaluation of TCDD chronic toxicity in rodents to date, evaluating dozens of endpoints at several time points in all major tissues. Thus, proximity of the RfDs derived from these two recent high-quality studies provides additional support for the use of the human data for RfD derivation.

There are several animal bioassay candidate RfDs at the lower end of the RfD range in Table 4-5 that are more than 10-fold below the human-based RfDs. Two of these studies report effects that are analogous to the endpoints reported in the three human studies and support the RfDs based on human data. Specifically, decreased sperm production in Latchoumycandane and Mathur (2002) is consistent with the decreased sperm counts and other sperm effects in Baccarelli et al. (2008), and missing molars in Keller et al. (2008a; 2008b; 2007) are similar to the dental defects seen in Alaluusua et al. (2004). Thus, because these endpoints have been associated with TCDD exposures in humans, these animal studies were not selected for RfD derivation in preference to human data showing the same effects.

Another characteristic of the remaining studies in the lower end of the candidate RfD distribution is that they are dominated by mouse studies (comprising 7 of the 9 lowest candidate RfDs). EPA has less confidence in the candidate RfD estimates based on mouse data than those based on either the rat or human data. EPA has less confidence in the use of the Emond mouse PBPK model to estimate the PODs because of the lack of key mouse-specific data, particularly for the gestational component (see Section 3.3.4.3.2.5). The toxicokinetic interspecies extrapolation factors used for mice are very large, introducing a potential for large errors. The ratio of administered dose to HED (D_a:HED) ranges from 65 to 1,227 depending on the duration of exposure. The D_a:HED for mice is, on average, about four times larger than that used for rats.

Table 4-6. Qualitative analysis of the strengths and limitations/uncertainties associated with animal bioassays providing PODs for the TCDD RfD

Study	Strengths	Limitations	Remarks
Bell et al. (2007b)	• Large sample size of both rat dams and offspring/dose employed • Several developmental effects tested	• Batch-to-batch variation of up to 30% in TCDD concentration in the diet • Longer-term dosing of dams does not accurately define gestational period when fetus is especially sensitive to TCDD-induced toxicity	Study is a significant addition to a substantial database on the developmental toxicity of TCDD in laboratory animals
Cantoni et al. (1981)	• Experiments were designed to test qualitative and quantitative composition and the course of urinary excretion in TCDD-induced porphyria	• Small sample size of rats/dose employed (n = 4) • Concurrent histological changes with tissue porphyrin levels were not examined • TCDD used for dosing was of unknown purity	Early study on porphyrogenic effects of TCDD
DeCaprio et al. (1986)	• Subchronic oral dosing duration up to 90 days • Male and female guinea pigs tested	• Relatively small sample size of guinea pigs/dose employed (n = 10) • No histopathological analyses performed • TCDD used for dosing was of unknown purity	Limited subchronic study; PBPK model not available for estimation of HED
Franc et al. (2001)	• Three different rat strains with varying sensitivities to TCDD were utilized (Sprague-Dawley, Long Evans, Han/Wistar) • Longer-term oral dosing up to 22 weeks	• Relatively small sample size of rats/dose employed (n = 8) • Only female rats were tested • Concurrent liver histopathological changes with liver-weight changes were not examined • Gavage exposure was only biweekly	Limited subchronic study
Hojo et al. (2002)	• Low TCDD dose levels used allowed for subtle behavioral deficits to be identified in rat offspring • Preliminary training sessions in operant chamber apparatuses were extensive • Neurobehavioral effects are exposure-related and cannot be attributed to presence of learning or discrimination deficits	• Relatively small sample size of rat dams/dose employed (n = 12) • Small sample size of rat offspring/dose evaluated (n = 5−6) • Neurobehavioral effects induced by TCDD at earlier or later gestational dosing dates are unknown because of single gavage administration on GD 8 • Although BMD analysis was conducted, the model parameters were not constrained according to EPA guidance, so the results cannot be used	One of a few neurobehavioral toxicity studies; somewhat limited study size

Table 4-6. Qualitative analysis of the strengths and limitations/uncertainties associated with animal bioassays possessing candidate PODs for the TCDD RfD (continued)

Study	Strengths	Limitations	Remarks
Keller et al. (2008a; 2008b; 2007)	• Six different inbred mouse strains were utilized • Large sample size of mouse offspring/dose/strain evaluated • Low TCDD dose levels used compared to typical mouse studies allowed for identification of subtle sensitivity differences in presence of absence of third molars, variant molar morphology, and mandible structure in offspring	• Unknown sample size of mouse dams/dose/strain employed • All inbred strains possessed sensitive *b* allele at the *Ahr* locus (i.e., a potentially resistant subpopulation was not evaluated for comparison purposes) • Morphological dental and mandibular changes induced by TCDD at earlier or later gestational dosing dates are unknown because of single gavage administration on GD 13 • Difficulties breeding A/J mice led to abandonment of that strain in the analysis (Keller et al., 2008a; 2008b)	Endpoint similar to effects observed at higher exposure levels in humans; HED highly uncertain using mouse PBPK model
Latchoumy-candane and Mathur (2002)	• Compared to epididymal sperm counts, the testicular spermatid head count provides better quantitation of acute changes in sperm production and can indicate pathology	• Small sample size of rats/dose employed (n = 6) • Oral pipette administration of TCDD may be a less efficient dosing method than gavage	Endpoint has human relevance, similar to critical effects in principal human study for RfD
Li et al. (2006)	• Female reproductive effects (i.e., early embryo loss and changes in serum progesterone and estradiol) were tested at multiple exposure times—early gestation, preimplantation, and peri- to postimplantation	• Small sample size of dams/dose (n = 10) • Large dose-spacing interval (25-fold at lowest 2 doses)	Endpoint has human relevance but HED highly uncertain using mouse PBPK model
Markowski et al. (2001)	• Low TCDD dose levels used allowed for subtle behavioral deficits to be identified in rat offspring • Several training sessions on wheel apparatuses were extensive • Neurobehavioral effects are exposure-related and cannot be attributed to motor or sensory deficits	• Unknown sample size of rat dams/dose employed • Small sample size of rat offspring/dose evaluated (n = 4−7) • TCDD used for dosing was of unknown purity and origin • Only two treatment levels • Neurobehavioral effects induced by TCDD at earlier or later gestational dosing dates are unknown because of single gavage administration on GD 18	One of a few neurobehavioral toxicity studies; somewhat limited study size
NTP (1982b)	• Large sample size of mice and rats/dose employed • Comprehensive 2-year bioassay that assessed body weights, clinical signs, and pathological changes in multiple tissues and organs	• Elevated background levels of hepatocellular tumors in untreated male mice • Gavage exposure was only 2 days/week • Only two treatment levels	Comprehensive chronic toxicity evaluations of TCDD in rodents; HED highly uncertain using mouse PBPK model

Table 4-6. Qualitative analysis of the strengths and limitations/uncertainties associated with animal bioassays possessing candidate PODs for the TCDD RfD (continued)

Study	Strengths	Limitations	Remarks
NTP (2006a)	• Chronic exposure duration with several interim sacrifices • Large number of dose groups with close spacing • Large number of animals per dose group • Comprehensive suite of endpoints evaluated • Comprehensive biochemical, clinical, and histopathological tests and measures • Detailed reporting of results, with individual animal data presented as well as group summaries	• Single species, strain, and sex • Lowest dose tested too high for establishing NOAEL	Study is the most comprehensive chronic TCDD toxicity evaluation in rats to date
Shi et al. (2007)	• Study design evaluated TCDD effects on aging female reproductive system (i.e., exposure began in utero and spanned across reproductive lifespan) • Several female reproductive endpoints were evaluated, including cyclicity, endocrinology, serum hormone levels, and follicular reserves	• Relatively small sample size of rats/dose employed (n = 10)	Endpoint similar to effects observed at higher exposure levels in humans
Smialowicz et al. (2008)	• SRBC plaque forming cell assay is highly sensitive and reproducible across laboratories when examining TCDD	• Small sample size of animals/dose (n = 8) • Only female mice were tested • Thymus and spleen weights were only other immune response-related endpoints tested	Limited immunotoxicity study
Toth et al. (1979)	• Large sample size of mice/dose employed • Chronic exposure duration	• Reporting of findings is terse and lacks sufficient detail (e.g., materials and methods, thorough description of pathological findings, etc.) • Limited number of endpoints examined • Only male mice were tested	Limited chronic study; HED highly uncertain using mouse PBPK model
Vos et al. (1973)	• Three different animal species tested (guinea pigs, mice, and rats) • Effects of TCDD tested on both cell-mediated and humoral immunity	• Small sample size of animals/dose employed in each experiment (n = 5−10) • Only female guinea pigs and rats were tested, and only male mice were tested • Only one experimental assay was utilized to assess cell-mediated or humoral immunity; humoral immunity was only investigated in guinea pigs • TCDD used for dosing was of unknown purity	Endpoints relevant to humans but study size limited; PBPK model not available for estimation of HED

Table 4-6. Qualitative analysis of the strengths and limitations/uncertainties associated with animal bioassays possessing candidate PODs for the TCDD RfD (continued)

Study	Strengths	Limitations	Remarks
White et al. (1986)	• Total hemolytic complement (CH50) is representative functional assay of the complete complement sequence	• Small sample size of rats/dose employed (n = 6−8) • Individual complement factors may be significantly depleted without affecting CH50 activity (only C3 is measured) • TCDD used for dosing was of unknown purity	Endpoint similar to effects observed at higher exposure levels in humans; HED highly uncertain using mouse PBPK model

In addition, each one of the mouse studies has other qualitative limitations and uncertainties (discussed above and in Table 4-6) that further reduce confidence in using them as the basis for the RfD.

4.3.4.1. *Identification of Point of Departure (POD) from Baccarelli et al. (2008)*

Baccarelli et al. (2008) reported increased levels of TSH in newborns exposed to TCDD in utero, indicating a possible dysregulation of thyroid hormone metabolism. The study authors related TCDD concentrations in maternal plasma to neonatal TSH levels using a multivariate linear regression model adjusting for a number of covariates (gender, birth weight, birth order, maternal age, hospital, and type of delivery). Based on this regression modeling, EPA has defined the LOAEL for Baccarelli et al. (2008) to be the maternal TCDD LASC of 235 ppt (at delivery) corresponding to a neonatal TSH level of 5 μU/mL.

The WHO (1994) established the 5 μU/mL standard as an indicator of potential iodine deficiency and potential thyroid problems in neonates. Increased TSH levels are indicative of decreased thyroid hormone (T4 and/or T3) levels. The 5 μU/mL limit for TSH measurements in neonates was recommended by WHO (1994) for use in population surveillance programs as an indicator of iodine deficiency disease (IDD). In explaining this recommendation, WHO (1994) stated that

> While further study of iodine replete populations is needed, a limit of 5μU/ml whole blood… may be appropriate for epidemiological studies of IDD [iodine deficiency disease.] Populations with a substantial number of newborns with TSH levels above the limit could indicate a significant IDD problem.

For TCDD, the toxicological concern is not likely to be iodine uptake inhibition, but rather increased metabolism and clearance of T4, as evidenced in a number of animal studies (see discussion in Section 4.3.6.1). Baccarelli et al. (2008) discount iodine status in the population as a confounder, as exposed and referent populations all lived in a relatively small geographical area. It is unlikely that there was iodine deficiency in one population and not in the other population based on iodine levels in the soil.

Clinically, a TSH level of >4 μU/mL in a pregnant woman is followed up by an assessment of free T4, and treatment with L-thyroxine is prescribed if T4 levels are low (Glinoer

and Delange, 2000). This is to ensure a sufficient supply of T4 for the fetus, which relies on maternal T4 exclusively during the 1st half of pregnancy (Chan et al., 2005); (Calvo et al., 2002; Morreale de Escobar et al., 2000). Adequate levels of thyroid hormone also are essential in the newborn and young infant as this is a period of active brain development (Zoeller and Rovet, 2004; Glinoer and Delange, 2000). Smaller reserves, higher demand, and shorter half-life of thyroid hormones in newborns and young infants also could make this lifestage more susceptible to the impact of insufficient levels of T4 (Savin et al., 2003; Greer et al., 2002; Van Den Hove et al., 1999). Thyroid hormone disruption during pregnancy and in the neonatal period can lead to neurological deficiencies, particularly in the attention and memory domains (Oerbeck et al., 2005). While such altered hormone levels are associated with decreased intelligence quotient (IQ) scores (e.g., 2009) report such associations among adolescents), the exact relationship between TSH increases and adverse neurodevelopmental outcome is not well defined. A TSH level above 20 µU/L in a newborn infant is cause for immediate intervention to prevent mental retardation, often caused by a malformed or ectopic thyroid gland in the newborn (WHO, 2007; Rovet, 2002; Glinoer and Delange, 2000). Recent epidemiologic data indicate concern for even lower level thyroid hormone perturbations during pregnancy. For example, Haddow et al. (1999) reported that women with subclinical hypothyroidism, with a mean TSH of 13.2 µU/L had children with IQ deficits of up to 4 IQ points on the Wechsler IQ scale. Neonatal TSH within the first 72 hours of birth [as was evaluated by Baccarelli et al. (2008)] is a sensitive indicator of both neonatal and maternal thyroid status (Delange et al., 1983). Animal models have recently indicated that very modest perturbations in thyroid status for even a relatively short period of time can lead to altered brain development (Sharlin et al., 2010; Royland et al., 2008; Sharlin et al., 2008; Ausó et al., 2004; Lavado-Autric et al., 2003). Rodent bioassay results also suggest that elevated TSH levels in neonates can affect sperm development as adults (Anbalagan et al., 2010); this study also reported reduced fertility among adult males and females with increased neonatal TSH levels.

EPA has defined the LOAEL for Baccarelli et al. (2008) to be the maternal TCDD LASC of 235 ppt corresponding to a neonatal TSH level of 5 µU/mL, determined by the regression modeling performed by the study authors. Using the Emond human PBPK model, the daily oral intake at the LOAEL is estimated to be 0.020 ng/kg-day (see Section 4.2.3.1). A NOAEL is not

defined because it is not clear what maternal intake should be assigned to the group below 5 μU/mL.

4.3.4.2. *Identification of Point of Departure (POD) from Mocarelli et al. (2008)*

Mocarelli et al. (2008) reported decreased sperm concentrations and decreased motile sperm counts in men who were 1−9 years old in 1976 at the time of the Seveso accident (initial TCDD exposure event). The sperm concentrations and motile sperm counts of men who were 10−17 years old in 1976 were not decreased. Serum (LASC) TCDD levels were measured in samples collected within 1 year of the initial exposure. Serum TCDD levels and corresponding responses were reported by quartile, with a reference group of less-exposed individuals assigned a TCDD LASC value of 15 ppt (which was the mean of the TCDD LASC reported in individuals outside the contaminated area). In the reference group, mean sperm concentrations and percent motile sperm counts were approximately 73 million sperm/mL and 41%, respectively. The lowest exposed group (1st-quartile) TCDD LASC median was 68 ppt. In the 1st quartile, mean sperm concentrations of approximately 55 million sperm/mL[48] and motile sperm counts of approximately 36% were reduced about 24 and 12%, respectively, from the reference group. Further decrease in these measures in the groups exposed to more than 68 ppt was minimal. Relative to the reference population, the percent decreases in sperm concentrations were approximately 25, 21, and 33% in the 2nd, 3rd, and 4th quartiles, respectively, and the percent decreases in progressive sperm motility were approximately 20, 25, and 22% in the 2nd, 3rd, and 4th quartiles, respectively.

Mocarelli et al. (2008) also conducted a separate analysis of all the 22−31 year-old men (combining all quartiles of the men exposed when they were 1−9 years of age). In the exposed men, the mean total sperm concentration was reported by Mocarelli et al. (2008) to be 53.6 million/mL, with a value of 21.8 million/mL at 1 standard deviation below the mean. In the comparison group that consisted of men not exposed to TCDD by the Seveso explosion and of the same age as the exposed men, the mean total sperm concentration was 72.5 million/mL (31.7 million/mL at 1 standard deviation below the mean).

There is no clear adverse effect level indicating male fertility problems for either of these sperm effects. As sperm concentration decreases, the probability of pregnancy from a single

[48] This estimate is based on Figure 3 in Mocarelli et al. (2008).

ejaculation also decreases; infertile conditions arise when the number of normal sperm per ejaculate is consistently and sufficiently low. Previously, the incidence of male infertility was considered increased at sperm concentrations less than 20 million sperm/mL (WHO, 1980). More recently, Cooper et al. (2010) suggested that the 5th percentile for sperm concentration (15 million/mL) could be used as a limit by clinicians to indicate needed follow-up for potential infertility. Skakkeback (2010) suggests the following two limits for human sperm concentrations: 15 million sperm/mL, based on Cooper et al. (2010) and 40 million sperm/mL. Skakkeback justifies the upper level of 40 million sperm/mL citing a study by Bonde et al. (1998) of couples planning to become pregnant for the first time; in the Bonde study, pregnancy rates declined when sperm concentrations were below 40 million sperm/mL. Skakkeback suggests that 15 million sperm/mL may be too low of a limit off for normal fertility and that sperm concentrations between 15 million sperm/mL and 40 million sperm/mL may indicate a range of reduced fertility. For fertile men, between 50% and 60% of sperm are motile (Swan et al., 2003; Slama et al., 2002; Wijchman et al., 2001). Any impacts on these reported levels could become functionally significant, leading to reduced fertility. Low sperm counts are typically accompanied by poor sperm quality with respect to morphology and motility (Slama et al., 2002).

EPA judged that the impact on sperm concentration and quality reported by Mocarelli et al. (2008) is biologically significant given the potential for functional impairment. Although a decrease in sperm concentration of 25% likely would not have clinical significance for a typical individual, EPA's concern with the reported decreases in sperm concentration and total number of motile sperm (relative to the comparison group) is that such decreases associated with TCDD exposures could lead to shifts in the distributions of these measures in the general population. Because male fertility is susceptible to reductions in both the number and quality of sperm produced, such shifts in the population could result in decreased fertility in men at the low ends of these population distributions. Further, in the group exposed due to the Seveso accident, individuals 1 standard deviation below the mean had sperm concentrations of 21.8 million/mL; this concentration falls near the low end of the range of reduced fertility (15 million and 40 million sperm/mL) suggested by (Skakkebaek, 2010); the corresponding concentration of 31.7 million/mL for the comparison group at one standard deviation below the mean is slightly more than twice the lower end of that range.

EPA has designated the lowest exposure group (68 ppt) as a LOAEL, which translates to a continuous daily oral intake of 0.020 ng/kg-day (see Section 4.2.3.2). The reference group is not designated as a NOAEL because the serum levels were not measured for this group, directly, and background exposures to DLCs are relatively large by comparison to TCDD in this group, introducing too much uncertainty in quantifying the full NOAEL exposure (see discussion in Section 4.5). Also, there is no clear zero-exposure measurement for any of these endpoints, complicating the interpretation of the reference group response as a true ―control" response (see discussion in Section 4.4). However, males less than 10 years old can be designated as a sensitive lifestage as compared to older males who were not affected.

4.3.4.3. *Identification of Point of Departure (POD) from Alaluusua et al. (2004)*

Alaluusua et al. (2004) reported dental enamel defects and missing permanent teeth in male and female adults who were less than 5 years of age, but not older, at the time of the initial exposure (1976) in Seveso. EPA used the same approach to estimate daily TCDD intake as was used for the Mocarelli et al. (2008) data; a window of susceptibility of about 5 years was established. Serum measurements for this cohort were taken within a year of the accident. Serum TCDD levels and corresponding responses were reported by tertile, with a reference group of less-exposed individuals assigned a TCDD LASC value of 15 ppt (ng/kg); the tertile group means were 130, 383, and 1,830 ppt. Both a NOAEL and LOAEL can be defined for this study. The NOAEL is 0.12 ng/kg-day, corresponding to the TCDD LASC of 130 ppt at the first tertile. The LOAEL is 0.93 ng/kg-day at the second tertile. The children in this cohort less than 5 years old can be designated as a sensitive lifestage as compared to older individuals who were not affected relative to the reference group.

4.3.5. Derivation of the Reference Dose (RfD)

The two human studies, Baccarelli et al. (2008) and Mocarelli et al. (2008), have identical LOAELs of 0.020 ng/kg-day. Together, these two studies define the most sensitive health effects in the epidemiologic literature and constitute the best foundation for establishing a POD for the RfD, and are designated as coprincipal studies. Therefore, increased neonatal TSH levels in Baccarelli et al. (2008) and male reproductive effects (decreased sperm count and motility) in Mocarelli et al. (2008) are designated as cocritical effects. A composite UF of 30 is applied to

the LOAEL of 0.020 ng/kg-day to account for lack of a NOAEL ($UF_L = 10$) and human interindividual variability ($UF_H = 3$); the resulting RfD in standard units is 7×10^{-10} mg/kg-day. Table 4-7 presents the details of the RfD derivation.

4.3.6. Studies Reporting Outcomes Comparable to the Principal Studies Used to Derive the Reference Dose (RfD)

Other animal and human epidemiologic studies report associations between TCDD exposures and effects similar to those reported by Baccarelli et al. (2008) and Mocarelli et al. (2008).

4.3.6.1. *Dysregulation of Thyroid Hormone Metabolism Associated with Dioxin Exposure in Neonates*

One of the principal studies for the dioxin noncancer RfD, Baccarelli et al. (2008), reported increased levels of TSH in newborns exposed to TCDD in utero, indicating a possible dysregulation of thyroid hormone metabolism. No other human studies that met the selection criteria of this analysis reported similar effects.

However, based on an analysis of over 20 epidemiology studies, Goodman et al. (2010) concluded that DLC exposures were not clearly or consistently correlated with differences in thyroid hormone levels in neonates and children less than 12 years of age. Focusing on neonatal TSH for direct comparison to Baccarelli et al. (2008), Goodman et al. (2010), in Table 3 of their analysis, identify 13 different studies, including Baccarelli et al. (2008), which measured infant TSH levels within 1 week of birth. Of these studies, only Baccarelli et al. (2008) was TCDD-specific and evaluated exposures well above ambient exposure levels. The other studies examined total TEQ or individual DLCs near background exposure levels. The LOAEL derived by EPA from Baccarelli et al. (2008) is approximately sixfold higher than the ambient total TEQ exposure levels at the time of the exposures for the general Seveso population[49] and more than 30-fold above an estimate of current TEQ levels (Lorber et al., 2009). In the other studies, the exposures appear to have been largely to DLCs, with TCDD as a minor component. Because the equivalent TCDD exposure for DLCs is derived from TEF methodology, which is conservative in nature (TEFs are higher than the median), the total TEQ concentrations would likely be over-estimated (relative to TCDD) and uncertain. In addition, only 2 of the other 12 studies evaluated

[49] Estimated by EPA to be 3.5×10^{-3} ng/kg-day on a total TEQ basis (see Section 4.5.1.1.1 and Appendix F).

Table 4-7. Basis and derivation of the TCDD RfD

Principal study detail		
Study	**POD (ng/kg-day)**	**Critical effects**
Mocarelli et al. (2008)	0.020 (LOAEL)	Decreased sperm count (20%) and motility (11%) in men exposed to TCDD during childhood
Baccarelli et al. (2008)	0.020 (LOAEL)	Elevated TSH (>5 μU/mL) in neonates

RfD derivation	
POD	0.020 ng/kg-day (2.0E−8 mg/kg-day)
UF	30 (UF_L = 10, UF_H = 3)
RfD	7×10^{-10} (7E−10) mg/kg-day (2.0E−8 , 30)

Uncertainty factors		
LOAEL-to-NOAEL (UF_L)	10	No NOAEL established; cannot quantify lower exposure group in Baccarelli et al. (2008); magnitude of effects at LOAEL sufficient to require a 10-fold factor.
Human interindividual variability (UF_H)	3	A factor of 3 ($10^{0.5}$) is used because the effects were elicited in sensitive lifestages. A further reduction to 1 was not made because the sample sizes were relatively small, which, combined with uncertainty in exposure estimation, may not fully capture the range of interindividual variability. In addition, chronic effects are levels are not fully elucidated for humans and could possibly be more sensitive.
Interspecies extrapolation (UF_A)	1	Human study.
Subchronic-to-chronic (UF_S)	1	Chronic effect levels are not well defined for humans; however, animal bioassays indicate that duration of exposure does not seem to be a determining factor in toxicological outcomes. Developmental effects and other short-term effects occur at doses similar to effects noted in chronic studies. Considering that exposure in the principal studies encompasses the critical window of susceptibility associated with development, a UF to account for exposure duration is not used.
Database sufficiency (UF_D)	1	The database for TCDD contains an extensive range of human and animal studies that examine a comprehensive set of endpoints. There is no evidence to suggest that additional data would result in a lower RfD.

by Goodman et al. (2010) reported TSH measures 3 days after birth, which is an international standard and would be most comparable to those in Baccarelli et al. (2008). TSH levels generally peak about 2 hours after birth then decline rapidly to typical long-term levels over the next few days (Steinmaus et al., 2010). Several of the studies included in Table 3 of Goodman et al. (2010) evaluated cord-blood TSH measurements, which represent early high TSH concentrations and are not directly comparable to 3-day measurements. Given these considerations, particularly the relatively low ambient exposures and differences in the timing of TSH measures, it would be unlikely that any consistent pattern would be detected across these studies.

Several animal studies that met the selection criteria evaluated the effects of TCDD on the thyroid or thyroid hormone levels. Overall, this set of studies show that TCDD affects thyroid hormone levels and the thyroid gland. The studies of Sewall et al. (1995), Seo et al. (1995), Van Birgelen et al. (1995a; 1995b), Crofton et al. (2005), and NTP (2006a) each reported decreases in T4 levels. In response to TCDD treatment, NTP (2006a) reported increases in total T3 concentrations, and both NTP (2006a) and Sewall et al. (1995) reported increased TSH concentrations. Sewall et al. (1995) and Chu et al. (2007) reported reductions in thyroid follicles, with Chu et al. (2007) noting that, of the health effects observed in their study, thyroid effects were the most sensitive to TCDD exposures. Although none of these studies address in utero or neonatal exposure, they show that TCDD can affect the level of thyroid hormones and the thyroid organ in adult animals.

4.3.6.2. *Male Reproductive Effects associated with Dioxin Exposures*

The other principal study for the dioxin noncancer RfD, Mocarelli et al. (2008), reported decreased sperm concentrations and decreased motile sperm counts in men who were aged 1−9 years at the time of the Seveso accident (initial TCDD exposure event). The sperm concentrations and motile sperm counts of men who were 10−17 years old in 1976 were not adversely affected. While no other human studies that met the selection criteria of this analysis reported similar effects, a newly published study, Mocarelli et al. (2011), also reports male reproductive effects. Several animal studies that met the study selection criteria also reported male reproductive effects.

Mocarelli et al. (2011) examined the relationship between maternal serum TCDD levels and semen quality in male offspring. Analyses were based on 39 of the 78 men aged 18−26 years born to women residing in the areas most heavily polluted by dioxin after the explosion in Seveso, Italy, in 1976 and age-matched controls (58 out of 123 recruited) born to women residing in noncontaminated areas of Italy. In the exposed group of women, pregnancies occurred between 9 months and 6 years after the accident (March 1977−January 1984). The male offspring of these women were categorized based on whether they were breastfed (n = 21, born to 20 mothers) or formula-fed (n = 18, born to 17 mothers) as infants. In the comparison group, 36 were breastfed, and 22 were formula-fed. Sons born to dioxin-exposed women whose spouses were also exposed to TCDD, as well as all men with reported diseases, were excluded.

TCDD exposures were based on estimated maternal serum concentration at conception. To estimate these levels in the exposed group, the authors relied on maternal serum measures, all of which were collected shortly after the accident in 1976−1977, and a biokinetic model (Kreuzer et al., 1997) that estimated TCDD elimination from the time of the accident to conception for individual women (average half-life = 4 years). Mothers of sons in the comparison group were assumed to be exposed to average background TCDD levels of 10 ppt based on measurements reported in Eskenazi et al. (2004).

Semen samples were collected from all participants. These samples were maintained at 37°C and examined within an hour of ejaculation. For serum inhibin B and follicle stimulating hormone (FSH) analyses, fasting blood samples were obtained the morning of semen collection. Statistical analyses were performed on sperm properties, serum hormone levels, and TCDD levels using a "general linear model" (Mocarelli et al., 2011). Model covariates included age, duration of abstinence prior to semen collection, smoking status, exposures to organic solvents, adhesives or paints, BMI, alcohol use, educational level, and employment status.

Relative to the comparison group, men born to exposed mothers had decreased sperm concentration (46 million vs. 81 million sperm/mL; p = 0.01), total sperm count (144 million vs. 231 million sperm; p = 0.03), and total number of motile sperm (51 million vs. 91 million; p = 0.05). Relative to the breastfed comparison group, breastfed sons born to exposed mothers exhibited decreased sperm concentrations (36 million vs. 86 million sperm/mL; p = 0.002), total sperm counts (117 million vs. 231 million sperm; p = 0.02), and motile sperm counts (39 million vs. 98 million; p = 0.01). Relative to the breastfed comparison group, breastfed sons born to

exposed mothers also exhibited increased FSH concentrations (4.1 vs. 2.6 IU/L; $p = 0.03$) and decreased inhibin B levels (70.2 million vs. 101.8 pg/mL; $p = 0.01$). The formula-fed exposed and comparison groups were not significantly different by any of these measures.

This study was well-designed with well-characterized exposures (for the exposed group), which relied on measured sera TCDD concentrations and a peer-reviewed TCDD elimination model to estimate maternal serum TCDD levels at the time of conception. Exposures in the comparison group relied on estimates from other studies. The study excluded sons of fathers that were likely highly exposed to TCDD, to limit potential influences from highly exposed fathers. The study relies on self-reported recollection of infant feeding (i.e., breastfed vs. formula-fed), which may lead to some misclassification based on recall error. Statistically significant associations were evident for both the exposed men and their comparison group and breastfed men and the breastfed comparison group.

In this study, elevated TCDD exposures during and after pregnancy (via breast-feeding) led to long-term decrements in male reproductive endpoints. These effects included changes in levels of hormones that affect spermatogenesis; they also include decreases in sperm concentration and sperm motility.

In addition, two rodent bioassays also report sperm effects associated with dioxin treatment. Latchoumycandane and Mathur (2002) reported decreased daily sperm production and decreased reproductive organ weights in male albino Wistar rats given daily oral doses of TCDD for 45 days. The LOAEL was 1.0 ng/kg-day, which corresponds to a $LOAEL_{HED}$ of 0.016 ng/kg-day (see Table 4-5); a NOAEL was not identified. Simanainen et al. (2004) reported a reduction in daily sperm production and cauda epididymal sperm reserves in male rat pups born to dams exposed to 300 ng/kg TCDD or higher on GD 15 by oral gavage. In this case a NOAEL of 100 ng/kg was identified, which corresponds to a $NOAEL_{HED}$ of 0.426 ng/kg-day, with a $LOAEL_{HED}$ of 1.7 ng/kg-day (see Table 4-3). Detailed descriptions of these studies can be found in Appendix D.

4.4. QUALITATIVE UNCERTAINTIES IN THE REFERENCE DOSE (RfD)

Exposure assessment is a key limitation of the epidemiologic studies (of the Seveso cohort) used to derive the RfD. The Seveso cohort exposure profile consists of an initial high

TCDD exposure[50] followed by a drop in body burden to background levels over a period of about 20 years, at which time the effects were observed. This exposure scenario is inconsistent with the constant daily intake scenario addressed by the RfD methodology. The determination of an effective average daily dose from the Seveso exposure scenario requires a consideration of the biologically-relevant critical time-window of susceptibility and the influence of the peak exposure on the occurrence of the observed effects, particularly when the peak exposure is high relative to the average exposure over the critical exposure window (see Text Box 2-2). For one of the principal studies (Mocarelli et al., 2008), a maximum susceptibility exposure window can be identified based on the age of the population at risk. However, the influence of the peak exposure on the effects observed 20 years later is unknown, and the biological significance of averaging the exposure over several years, with internal exposure measures spanning a 5.5-fold range, is unknown. EPA has not developed guidance for large interval averaging. Furthermore, because there is an assumption of a threshold level of exposure below which noncancer effects are not expected to occur, averaging over large intervals could include exposures that are below a threshold. The process used by EPA to estimate the LOAEL exposure for the Mocarelli et al. (2008) study is a compromise between the most- and least-conservative alternatives; as such, there is some uncertainty in the estimate, perhaps in the range of 3- to 10-fold in either direction. This uncertainty also applies to the LOAEL determined for the developmental dental effects reported in Alaluusua et al. (2004) and the increased menstrual cycle length reported in Eskenazi et al. (2002b) (see Section 4.2.3.4); in both of those studies, the uncertainty is greater, as the difference between peak and average internal exposures is an order of magnitude or more. The LOAEL for increased TSH in neonates (Baccarelli et al., 2008), however, is less uncertain because the critical exposure window is much narrower (9 months), and the developmental exposures occurred 20 to 30 years after the initial exposure, when internal TCDD concentrations for the pregnant women likely were leveling off; that is, exposure over the critical window was more constant and estimation of the relevant exposures was less uncertain. However, there is some uncertainty in the magnitude of the exposures because they were estimated from

[50] Mocarelli (2001) reported the release from the Seveso plant to contain a mixture of TCDD, ethylene glycol, and sodium hydroxide. Because these chemicals are not thought to persist in the environment or in the body, coexposure to these additional contaminants along with TCDD would not have a significant impact on longer-term TCDD dose-response. For acute exposure, male reproductive or thyroid hormone effects are not evident for ethylene glycol (U.S. EPA, 2012). It is unlikely that sodium hydroxide, being primarily a caustic agent, would cause these effects.

measurements in sera taken several years prior to pregnancy and do not take into account changing patterns of exposure during pregnancy.

Another source of uncertainty using human epidemiologic data is the lack of completely unexposed populations. The available TCDD epidemiologic data were obtained by comparing populations that experienced elevated TCDD exposures to populations that experienced lower exposures, rather than to a population with no TCDD exposure. An additional complicating factor is coexposure to DLCs, which can act toxicologically in the same way as TCDD. Although the accidental exposure to the Seveso women's cohort was primarily to TCDD, background exposure was largely to DLCs. Eskenazi et al. (2004) reported that TCDD comprised only 20% of the total TEQ in the serum of the reference group that was not exposed as a result of the Seveso factory explosion, which implies that the effective background TEQ exposure was approximately fivefold higher than exposure to TCDD. WHO (1998) estimated that TCDD may comprise only 5−20% of background exposures to dioxin and DLCs. The higher background exposure could be significant at the lower TCDD exposure levels, with the effect diminishing as TCDD exposure increased. For dose-response modeling, the effect of a higher background dose (i.e., total TEQ), if included, would be to shift the response curve to the right, with responses now being associated with higher exposures. Adding a constant to all exposures would also reduce the proportional spread of the exposures, which would tend to alter the shape of the dose-response curve towards sublinear. Both the right shift and the more sublinear shape would result in higher POD estimates. In addition, the response in the reference population is not a true zero-exposure (TEQ-free) response. The actual magnitude of the impact of the DLC background exposure is impossible to assess without knowing the zero-exposure background response. The (TEQ-free) background response cannot be assessed as no TEQ-free population exists. Ideally, an independent absolute measure of adversity in terms of the response variable, such as the 5 μU/mL neonatal TSH benchmark, is needed for dose-response modeling.

As part of the uncertainty analysis for the TCDD RfD, the possible influence of different background DLC exposure assumptions on the POD estimates derived from the two principal studies, Baccarelli et al. (2008) and Mocarelli et al. (2008), and one comprehensive animal bioassay, NTP (2006a), is examined quantitatively in Section 4.5. In addition, the range of possible PODs for other epidemiologic studies that did not pass all the selection criteria in comparison to the principal studies is presented in Section 4.5.

A primary strength of the TCDD database is that analogous effects have been observed in animal bioassays for most of the human endpoints, increasing the overall confidence in the relevance to humans of the effects reported in rodents and the association of TCDD exposure with the health outcomes reported in humans. Table 4-5 shows that low-dose TCDD exposures are associated with a wide array of toxicological endpoints in rodents including developmental effects, reproductive effects, immunotoxicity, and chronic toxicity. Effects reported in human studies are similar, including male reproductive effects, increased TSH in neonates, and dental defects in children; other human health effects such as female reproductive effects and chloracne have been observed at higher exposures (see Appendix C). Severe liver toxicity, which is a consistently reported effect in rodents, has not been observed in humans; Michalek et al.(2001c), however, reported slightly elevated liver enzyme levels in serum and other nonspecific liver effects for the Ranch Hand cohort, suggestive of mild liver toxicity. Overt immunological endpoints, reported in the rodent bioassays, also have not been reported in human studies. However, with respect to immunological effects, Baccarelli et al. (2004; 2002) evaluated immunoglobin and complement levels in the sera of TCDD-exposed individuals from the Seveso cohort and found reduced immunoglobulin in the highest exposure groups but no effect on other immunoglobulins or on C3 or C4 complement levels and no indication of compromised immune response. The latter finding indicates that at least one immunological measure in humans is not a sensitive endpoint, as it is for mice, with large reductions in serum complement at low exposure levels (White et al., 1986).

Although there is a substantial amount of qualitative concordance of effects between rodents and humans, quantitative concordance is not as strong, with reference to Table 4-5. The differential sensitivity of mice and humans for the serum complement endpoint is one example. Other examples of differential sensitivity are developmental dental effects and thyroid hormonal dysregulation. Developmental dental defects are relatively sensitive effects in rodents, appearing at exposure levels in mice (Keller et al., 2008a; 2008b; 2007) more than an order of magnitude lower than effect levels in humans (Alaluusua et al., 2004). In contrast, thyroid hormone effects are seen in rats (Crofton et al., 2005) at 30-fold higher exposures than for humans (Baccarelli et al., 2008). Male reproductive effects (sperm production) occur in rats (Latchoumycandane and Mathur, 2002) and humans (Mocarelli et al., 2008) at about the same dose. To what extent these differential sensitivities depend on specifics of the comparison, such as species (mouse vs. rat),

life-stage (e.g., fetal vs. adult), endpoint measure (e.g., T4 vs. TSH), or magnitude of the lowest dose tested, cannot be determined, so strong conclusions about quantitative concordance cannot be made.

A more detailed tabular and graphical presentation of qualitative and quantitative cross-species comparisons of selected toxicological endpoints for all the animal and human studies that met the EPA selection criteria is given in Appendix D.3. The endpoints include male and female reproductive effects, thyroid hormone levels, and developmental dental effects, all of which have been reported for humans. In addition, immunological and neurological effects are shown because they are sensitive effects in experimental animal studies, although not evident in humans. Hepatic effects, which are not shown in Appendix D.3, are evident in virtually all rodent studies that looked for them and are often severe, but are not severe in humans. The analysis presented in Appendix D.3 supports the conclusion that there is a substantial amount of qualitative concordance of effects between rodents and humans, but a much lower quantitative concordance. However, there are no endpoints in the selected animal bioassays that address diabetes or glucose metabolism. There may be other animal studies showing effects of interest at higher doses in those studies that did not meet the dose limit selection criterion.

A number of qualitative strengths and limitations/uncertainties are associated with the animal bioassays listed in Table 4-5, as articulated in Table 4-6. Considering the issue of lowest tested dose, the general lack of NOAELs and acceptable BMDLs is a primary weakness of the rodent bioassay database. None of the eight most sensitive rodent studies in Table 4-5, spanning an 18-fold range of LOAELs, had defined NOAELs or BMDLs. NOAELs or BMDLs were established for only 4 of the next 13 rodent studies. In addition, many of these LOAELs are characterized by relatively high responses with respect to the control population, so it is not certain that a 10-fold lower dose (based on the application of UF_L of 10) would be approximately equivalent to a NOAEL. A major reason for the failure of BMD modeling was that the responses were not ―anchored" at the low end (i.e., first response levels were far from the BMR [see Table 4-4]). Another major problem with the animal bioassay data was nonmonotone and flat response profiles. The small dose-group sizes and large dose intervals probably contributed to many of these response characteristics that prevented successful BMD modeling. Larger study sizes with narrower dose intervals at lower doses are still needed to clarify rodent response to TCDD.

Lower TCDD doses have been tested in rodents but almost entirely for investigation of specialized biochemical endpoints[51] that EPA does not consider to be toxicologically relevant for the derivation of a noncancer RfD (see Appendix H). There is, however, a fundamental limit to the lowest dose of TCDD that can be tested meaningfully, as TCDD is present in feed stock and accumulates in unexposed animals prior to the start of any study. This issue is illustrated by the presence of TCDD in tissues of unexposed control animals, often at significant levels relative to the lowest tested dose in low-dose studies (Bell et al., 2007b; Ohsako et al., 2001; Vanden Heuvel et al., 1994a; 1994b) (see Text Box 4-1). Some DLCs also have been measured in animal feeds (Bell et al., 2007b; NTP, 2006a) and are anticipated to accumulate in unexposed test animals, further complicating the interpretation of low-dose studies.

Text Box 4-1. Background levels of TCDD in Control Group Animals

TCDD tissue levels in control animals are rarely reported either explicitly or implicitly. Vanden Heuvel et al. (1994)however, reported TCDD concentrations in livers of control animals (10-week-old female Sprague-Dawley rats) of 0.43 ppt (ng/kg) compared to 0.49 ppt in the livers of animals given a single oral TCDD dose of 0.1 ng/kg. Assuming proportionality of liver concentration to total body burden, the body burden of untreated animals was 87.8% of that of treated animals at the lowest dose. The equivalent (single) administered dose for untreated animals (d_0) can be calculated as equal to $0.878 \times (0.1 + d_0)$, assuming proportionality of body burden to administered dose and that all animals started with the same TCDD body burdens. The calculation yields a value of 0.72 ng/kg for d_0, which represents the accumulated TCDD from all sources in these animals prior to being put on and during test. This value would raise the nominal 0.1 ng/kg TCDD dose 8-fold to 0.82 ng/kg. The next higher dose of 1 ng/kg would be nearly doubled to 1.72 g/kg. The impact on higher doses would be negligible, because the ratio of treatment dose to apparent background exposure levels increases with higher treatment levels. Bell et al. (2007) reported slightly higher levels (0.66 ppt) in the livers of slightly older untreated pregnant female Sprague-Dawley rats (mated at 16−18 weeks of age and tested 17 days later).

Ohsako et al. (2001) reported TCDD concentrations in the fat of offspring of untreated pregnant Holtzman rats that were 46% of the TCDD fat concentrations in animals exposed in utero to 12.5 ng/kg (single exposure on GD 15). This level of TCDD would imply a very large background exposure, but quantitation based on simple kinetic assumptions probably would not reflect the more complicated indirect exposure scenario.

Bell et al. (2007) also reported concentrations of 0.1 and 0.6 ppt TCDD measured in two samples of feed stock. Assuming that the average of 0.35 ppt is representative of the entire supply of feed stock and a food consumption factor of 10% of body weight per day, the average daily oral exposure from feed to these animals would be 0.035 ng/kg. Discrimination of outcomes from longer-term repeated exposures might be problematic at exposure levels around 0.1 ng/kg-day. Background exposure was not much of an issue for Bell et al. (2007), as the lowest TCDD exposure level was 2.4 ng/kg-day (28-day dietary exposure).

NTP (2006b) reported TCDD concentrations in the liver and fat of untreated female Sprague-Dawley rats after 2 years on test that were 1% and 2.5% of the levels in the liver and fat of the low-dose TCDD treatment group (2.14 ng/kg-day) (NTP, 2006a), respectively. Assuming proportionality of fat concentration and oral intake, control animal exposure would have been approximately 0.05 ng/kg-day, similar to the estimate from Bell et al. (2007). As for the latter study, background intake for the NTP (2006a) study animals would not have a large effect on the dose-response assessment given the lowest exposure level of 2.14 ng/kg-day.

In all of these studies, except the 28-day exposure in Bell et al. (2007), control animals were gavaged with corn oil vehicle. TCDD concentrations in corn oil were not reported in any of the studies.

[51] Enzyme induction, oxidative stress indicators, mRNA levels, etc.

4.5. QUANTITATIVE UNCERTAINTY IN THE REFERENCE DOSE (RfD)

The development of each candidate RfD in Sections 4.1 through 4.3 required the analysis of numerous kinetic, toxicologic, and epidemiologic data sets. These analyses included interpretive decisions that were made considering different sources of uncertainty in each study and EPA's methods for developing RfDs. This section quantifies the impacts of some sources of uncertainty encountered in the development of candidate RfDs (Sections 1.1 and 1.3 describe the NAS and SAB comments pertaining to uncertainty analysis for the RfD). In Section 4.5.1, the impacts of some sources of uncertainty encountered in the development of candidate RfDs based on Baccarelli et al. (2008), Mocarelli et al. (2008) and NTP (2006a) are elucidated using ―*variable* sensitivity" trees depicting the sensitivity of the POD value to choices made for PBPK model variables and inputs. In Section 4.5.2, an additional range of potential PODs is presented as a bounding analysis considering background DLC exposures and several epidemiologic studies, some of which did not qualify for RfD consideration, but for which limiting NOAEL and LOAEL values can be estimated for purposes of comparison. All modeling for the analyses in Sections 4.5.1.1 and 4.5.2 was carried out using the Emond human PBPK model (see Appendix F). Modeling of the NTP (2006a) data in Section 4.5.1.2 was carried out using the Emond and CADM rodent PBPK models and the Emond human PBPK model (see Appendix E).

In the analyses in Sections 4.5.1 and 4.5.2, EPA has terminated the sensitivity analysis results at the POD level (human daily oral intake in ng/kg-day), as the PODs provide a comparable measure across interpretive decisions. To extend these analyses further, candidate RfDs can be estimated by converting the POD values EPA has generated to mg/kg-day and then dividing by the appropriate uncertainty factors.

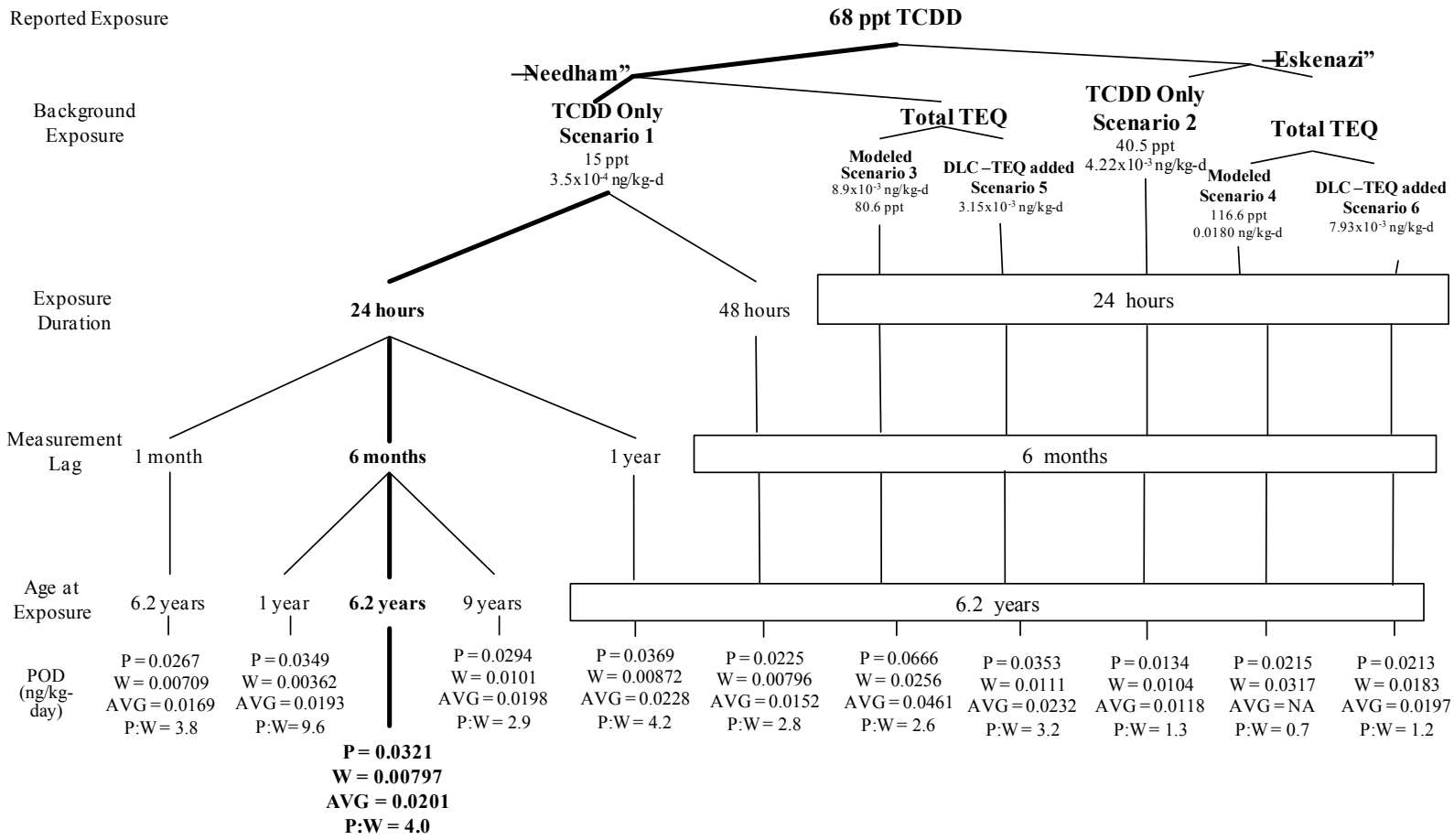
4.5.1. Development of Variable Sensitivity Trees for the Principal Epidemiologic Studies that were the basis of the Reference Dose (RfD) and for the NTP (2006a) Rodent Bioassay

In this section, the impacts of some sources of uncertainty encountered in the development of candidate RfDs based on Baccarelli et al. (2008), Mocarelli et al. (2008) and NTP (2006a) are elucidated using ―variable sensitivity" trees depicting the sensitivity of the POD value to choices made for PBPK model variables and inputs. These studies were chosen for this analysis because Baccarelli et al. (2008) and Mocarelli et al. (2008) are the principal studies used to develop the RfD, and NTP (2006a) is among the most recent and comprehensive rodent

bioassay studies of TCDD. For each of the three PODs used to develop candidate RfDs from these studies, EPA generated plausible alternative interpretations of the information used to define judgment-based inputs for specific model variables. The goal of this analysis is to provide quantitative insights on critical uncertainties encountered in the development of the RfD by illustrating the consequences (quantified as alternative PODs at the end of each branch in each tree) of plausible alternative interpretations of these key data sets.

Previously, in their examination of low-dose carcinogenicity associated with formaldehyde and chloroform exposures, Evans et al. (1994a; 1994b) assigned subjective weights to each branch of a probability tree and calculated probability masses for population risks associated with alternate interpretations of toxicological and pharmacokinetic data and exposure information.[52] In the examination of uncertainty undertaken in this section, EPA utilizes the development of sensitivity trees; subjective probability weights are not developed for any of the branches, and there is no propagation of probabilities across branches. Further, these trees do not present a comprehensive analysis of quantitative uncertainty of the three candidate RfDs; rather, EPA has focused on the impacts of key interpretive decisions largely dealing with exposure and kinetic modeling uncertainties. However, it should be noted that because POD values do not vary greatly across each of the three trees (less than a factor of 3 or 4 in either direction; see Figures 4-6 through 4-8), it is unlikely that the distribution of probability mass resulting from specific probability assignments would result in a significant amount of mass away from the chosen PODs. In this analysis, the structure of the decisions and the resulting POD estimates are presented as sensitivity trees in graphical form (see Figures 4-6 through 4-8). In these figures, the left-hand columns depict the variables considered in the sensitivity analysis. For each variable in a column, alternative values are presented in the row to its right. Beginning with the top row of a tree, the pathway for a single POD calculation is represented by the series of lines that moves down through specific values on subsequent rows and ends with a POD. The series of bolded lines in each figure represents the primary POD estimation that was used to develop the RfD for that study in Section 4.3, termed hereafter the ―standard pathway". For all other POD calculations, alternative values for each variable were assessed one at a time, while

[52] Small (2008) discusses other studies of distributional approaches in risk assessment by Sielken and collaborators that are similar to those of Evans and colleagues. These include the following: Sielken (1993, 1990), Holland and Sielken (1993), Sielken and Valdez Flores (1999, 1996), and Sielken et al. (1995).



W = critical window average, P = peak exposure, AVG = average of P and W, P:W = ratio of peak to window-average exposure
NA = not applicable (see description of Scenario 4 in text)

Figure 4-6. Sensitivity tree showing TCDD exposure-variable uncertainty for Mocarelli et al. (2008).

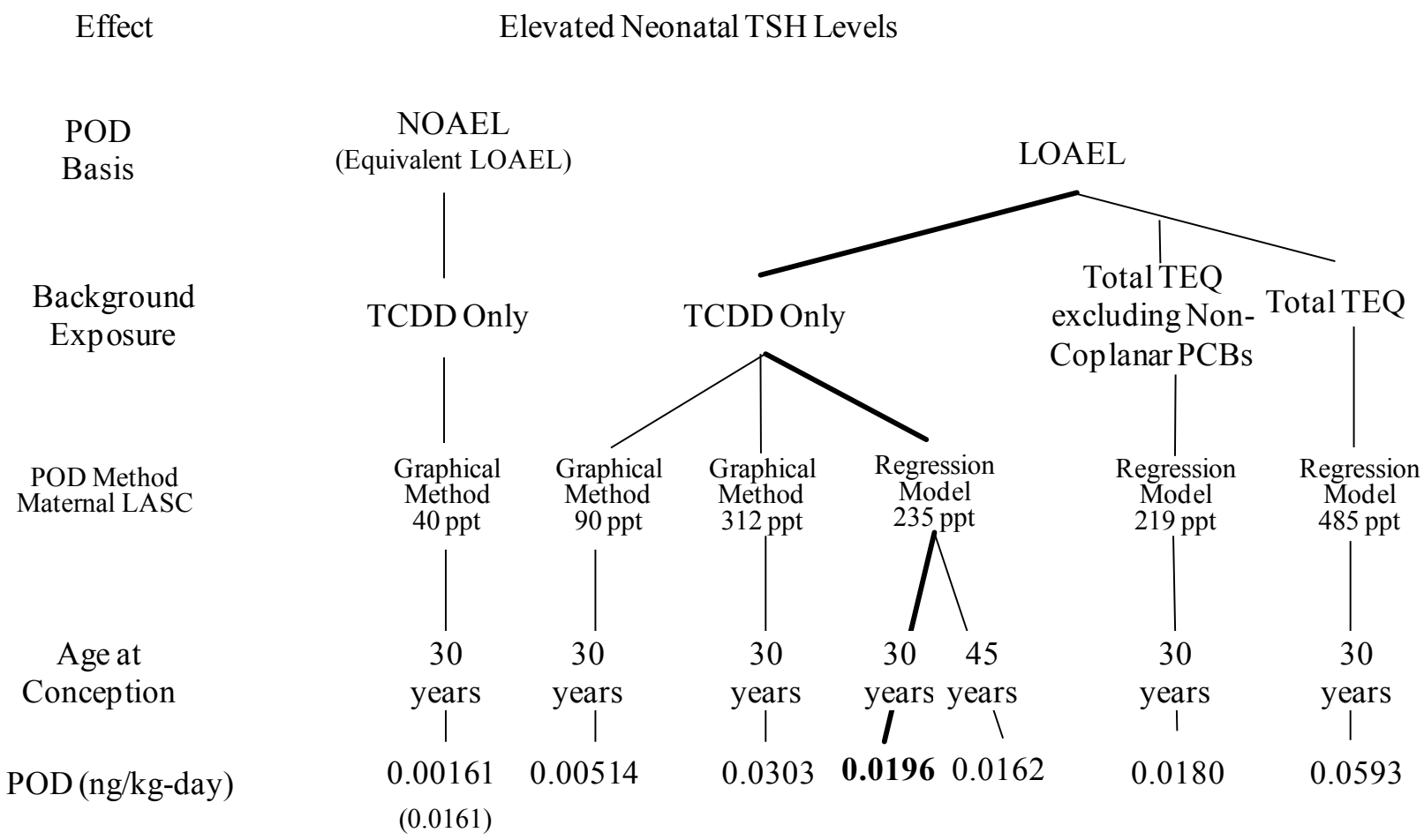


Figure 4-7. Sensitivity tree showing TCDD exposure-variable uncertainty for Baccarelli et al. (2008).

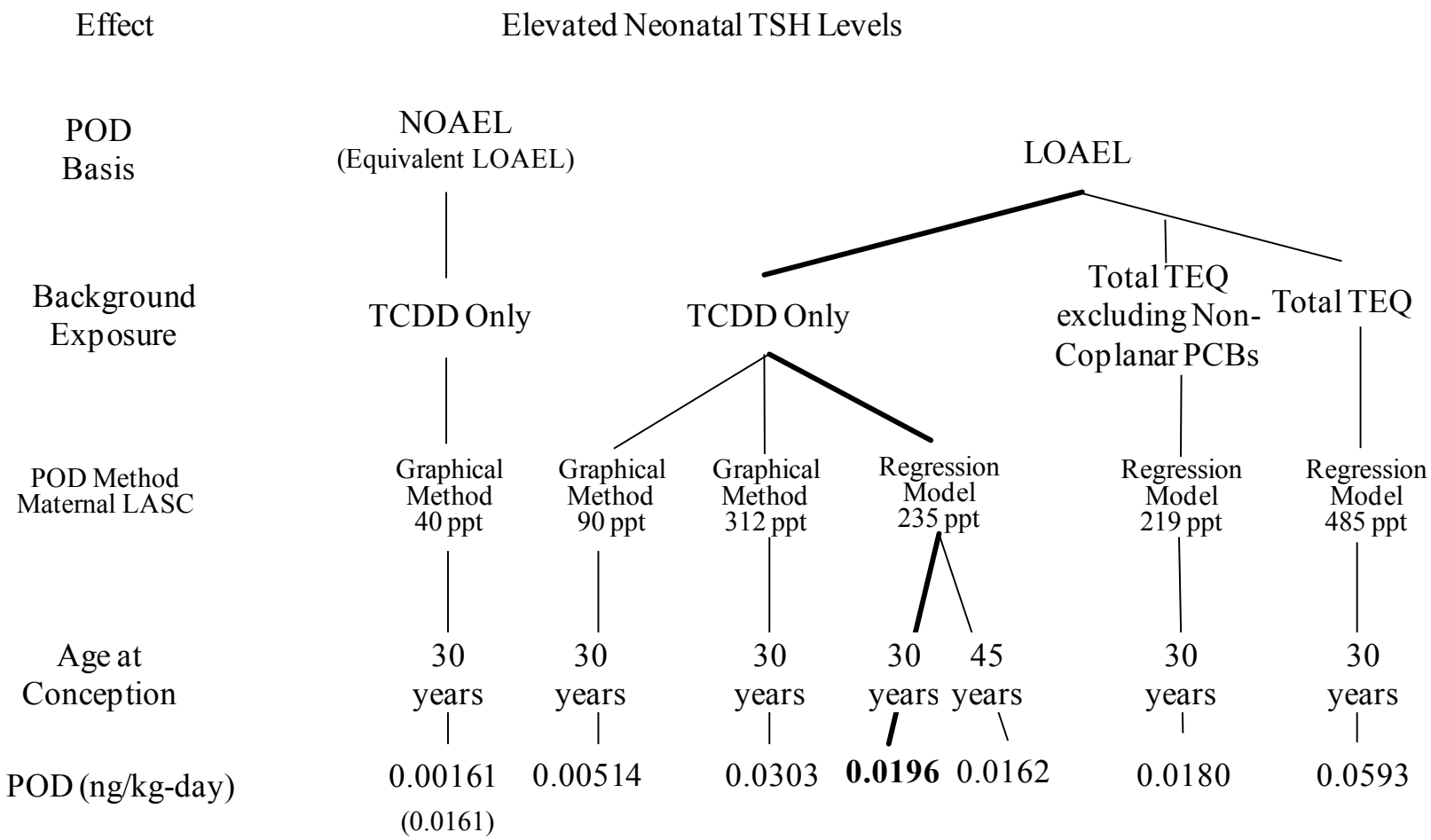


Figure 4-8. Sensitivity tree showing TCDD exposure-variable uncertainty for NTP (2006a).

fixing all the other variables at the values used in standard pathway. The values used for these variables were either directly specified in the literature or were based on judgment using exposure information provided in related papers. Up to three significant digits are shown for the PODs that are presented so that differences among the PODs across analytic choices can be readily discerned.

4.5.1.1. *Epidemiologic Sensitivity Analyses*

In estimating the PODs for the principal studies for the RfD (Baccarelli et al., 2008; Mocarelli et al., 2008), a series of assumptions were made to model the exposure history of the cohorts and to estimate an intake leading to the observed effect. In this section, variable sensitivity trees highlight the effects of choosing alternative assumptions on the POD estimates for these two principal studies.

4.5.1.1.1. *Mocarelli et al. (2008)*

Mocarelli et al (2008) evaluated sperm endpoints in adult males who were exposed as children, between the ages of 1 and 9, to TCDD during the Seveso accident, which included an initial peak exposure and subsequent longer-term exposure to ambient levels (see Section C.1.2.1.5.8 for study details). To examine the impacts of potential uncertainties associated with the assumptions made in estimating the standard pathway LOAEL POD in Mocarelli et al. (2008) (see Section 4.2.3.2), EPA evaluated the impact of several alternate exposure assumptions on the oral intakes associated with the POD, as shown in Figure 4-6. The left side of the figure depicts the variables of the exposure analysis considered in the sensitivity analysis (i.e., background exposure, exposure duration, measurement lag, and age at exposure). As detailed below, the values used for these variables were not directly specified in the literature but were based on judgment of the exposure information provided in Mocarelli et al. (2008) and related papers. In addition to the variables in Figure 4-6, a discussion is also presented of the impact on the POD and RfD of changing the value of the Hill coefficient in the Emond PBPK model to 1 instead of 0.6 (see Section 3.3.4.3.2.5 for modeling details).

All of these variables are inputs to the Emond human PBPK model (see modeling code and details in Appendix F), which was used to estimate the actual exposures to the affected population and the corresponding continuous intakes for determining the RfD POD. The

sensitivity analysis begins with the reported LASC of 68 ppt TCDD in the LOAEL group. The terminal nodes at the bottom of the figure show the PODs as daily oral intakes (ng/kg-day) resulting from each alternative value for the variables examined. To address the nature of the Seveso TCDD exposures, the PODs are expressed using three different metrics as described below.

In Figure 4-6 and in the text that follows, the following abbreviations for the PODs are used:

- *—P*" identifies the intake associated with the initial peak LASC exposure estimates.
- *—W*" identifies the intake associated with the average LASC over the actual exposure window.
- *—AVG*" is the average of the intakes associated with *—P*" and *—W*." Intakes associated with either *—P*" or *—W*" conceivably could have been selected as the primary POD.
- *P:W* is the ratio of the peak intake to the window-average intake.

In the standard pathway analysis, EPA elected to use the average of the peak exposure intake (*P*) and the critical-window exposure average intake (*W*) as the basis for the POD, giving equal weight to both (see discussion in Section 4.2.3); these values are labeled as —AVG" across all terminal nodes in the tree. This was done because of the relatively large differences between peak exposures and average exposures decreasing over a relatively long time span,[53] and the uncertainty of the relative influence of acute high exposures vs. lower longer-term averages on the toxicological outcome.

Background Exposure

For Figure 4-6, background exposures in the population (labeled —Background Exposure") were estimated using six different scenarios, based on data from two different epidemiologic studies. The scenarios take into account background exposures of TCDD only, or TCDD in the presence of DLCs (i.e., total TEQ)[54]. Because DLCs are presumed to act in the same manner as TCDD (for AhR induction and subsequent effects), the magnitude of the background DLC exposure is an important concern in establishing the POD. The Emond human PBPK model was used to estimate background intakes by assuming a constant exposure from

[53] The modeled TCDD LASC decreased by a factor of 5.5 from peak exposure to the terminal value at 10 years.
[54] DLC-TEQ = non-TCDD TEQ

birth to time of serum-TCDD measurement[55] for each scenario (see Appendix F for modeling details).

Scenarios 1 and 2 consider background TCDD only, with Scenario 1 being the standard pathway defining the RfD. Scenario 2 uses a higher TCDD background estimate from a different publication than the one used by Mocarelli et al. (2008). For the remaining scenarios, the background TEQ exposures were estimated using two different methods. The first method was to model the total TEQ LASC values directly with the Emond human PBPK model, assuming that all DLCs are kinetically equivalent to TCDD. This method (—modeled TEQ") accounts for the magnitude of background DLC serum concentrations in the dose-dependent elimination mechanism in the Emond PBPK model. For the modeled-TEQ method, background DLC-TEQ LASC values at the time of blood collection (i.e., —measurement time") were estimated by EPA using measured data or by modeling with assumptions of the ratio of total TEQ to TCDD in background exposures. Total TEQ LASC values at measurement were estimated by adding the resulting DLC-TEQ LASC to the measured TCDD LASC of 68 ppt. The Emond model was then run to compute the corresponding peak and critical-window intakes, with all other model variables set to the standard-pathway values. EPA also applied a simple additive model, in which background DLC-TEQ intakes were estimated by assuming a ratio of DLC intake to TCDD intake from background sources. The background DLC intakes were then added to the modeled TCDD intakes from the first two scenarios. The DLC-TEQ intake addition method does not account for the influence of DLCs on dose-dependent elimination, but is less complicated to apply and requires fewer assumptions than the modeled-TEQ method. A limitation of both approaches, but more so for the modeled-TEQ method, is the assumption of toxicokinetic equivalence of DLCs and TCDD. The reported TEQ values are based on serum concentrations, while the TEFs, on which the TEQ values are calculated, are largely derived from oral dosing studies. The outcomes from such studies implicitly account for DLC toxicokinetics (i.e., absorption, distribution, metabolism, and elimination). Applications of TEFs to DLC serum concentrations do not account for toxicokinetics, which could be very different across DLCs.[56] In addition, because both methods use TEQ values based on nominal TEFs, the

[55] —Measurement time" is defined here as the average age (6.7 years) of the subjects studied by Mocarelli et al. (2008) when serum samples were collected, which EPA estimated as 6 months following exposure.

[56] As an example, whole body half-life estimates for the DLCs vary from about 6 months to 20 years (Ogura et al., 2004; Flesch-Janys et al., 1996). Currently, there is no human PBPK model capable of addressing toxicokinetics for

DLC contribution to total TEQ will be overestimated. The TEF methodology is designed to be health protective, in that the TEFs are not central tendency estimates but biased high by design (Van den Berg et al., 2006). Therefore, exposure estimates based on nominal TEQ values are expected to be slightly higher than actual exposure.

The following descriptions apply to the scenarios depicted in Table 4-6. Additional detail can be found in Appendix F.

- Scenario 1 (Needham TCDD scenario). The TCDD only background value used in the standard pathway analysis was based on an LASC of 15 ppt used by Mocarelli et al. (2008) in their analysis as the TCDD level in the comparison group; this value was reported by Needham et al. (1997) to be the median TCDD concentration in an unexposed reference adult population (25 years or older) (designated ―Needham" in Figure 4-6). Using the Emond PBPK model, EPA estimated a corresponding daily TCDD intake of 3.5×10^{-4} ng/kg-day from birth, assuming that 15 ppt was obtained at age 35 (see Appendix F.1.1).
- Scenario 2 (Eskenazi TCDD scenario). The alternative TCDD-only value is an age-specific background intake based on an average TCDD concentration of 40.5 ppt for girls less than 12 years of age (designated ―Eskenazi" in Figure 4-6) from Table 3 in (Eskenazi et al., 2004).[57] Assuming that background TCDD serum concentrations were similar for boys and girls in the Seveso cohort, EPA estimated an average TCDD intake of 4.22×10^{-3} ng/kg-day corresponding to the same average 40.5 ppt LASC for boys of similar age (see Appendix F.1.2).
- Scenario 3 (Needham modeled-TEQ scenario). This method models the exposure directly, by matching the ―target" total TEQ (as LASC ppt, TCDD included) at the time of measurement with the corresponding intake using the Emond model. The target total-TEQ for the 1st-quartile boys aged 6.7 years at measurement time was estimated to be 140.5 ppt TEQ. This value was obtained by adding a modeled estimate of 72.5 ppt background DLC-TEQ LASC at 6.7 years to the measured TCDD LASC of 68 ppt in Mocarelli et al. (2008). The DLC-TEQ estimate was obtained by first assuming that TCDD comprises 10% of the total background TEQ, which is approximately the proportion of TCDD to total TEQ in adult serum as reported by (Eskenazi et al., 2004) and as estimated by WHO (1998).[58] The Needham scenario TCDD background of 15 ppt was multiplied by 10 obtaining an estimate of 150 ppt total background TEQ at age 35, for which a corresponding average daily background intake from birth of 0.0180 ng/kg-

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all the DLC congeners, although both EPA (U.S. EPA, 2003) and Lorber (2002) have used DLC half-life estimates and tissue concentrations to estimate intake rates for some DLCs (excluding dioxin-like PCBs) in humans.

[57] Table 3 in Eskenazi et al. (2004) reports the results of two pools of sera collected from girls aged 0−12 years, who did not reside in areas affected by the Seveso accident and were presumably exposed only to background levels of TCDD. The 40.5 ppt estimate is the mean of the two pools (47.6 and 33.4 ppt).

[58] TCDD also is approximately 10% of the total serum TEQ as calculated by EPA from the NHANES (2001/2002) data reported by Lorber et al. (2009).

day was estimated using the Emond PBPK model. Using the background intake of 8.9 $x10^{-3}$ ng/kg-day in the Emond model, a concentration of 80.6 ppt total TEQ LASC at age 6.7 was modeled, 90% of which, or 72.5 ppt, is assumed to be DLC-TEQ. (see Appendix F.3.6 for modeling details).

- Scenario 4 (Eskenazi modeled-TEQ scenario). The method is the same as for Scenario 3. The target total TEQ for the 1st-quartile at measurement time was estimated to be 144.1 ppt TEQ, which was obtained by adding a measured value of 76.1 ppt background DLC-TEQ at 6.7 years to the measured TCDD value of 68 ppt in Mocarelli et al. (2008). The DLC-TEQ estimate was obtained by averaging the non-TCDD TEQ for the 0-12 year age group (girls) reported by Eskenazi et al. (2004); the total measured background TEQ for that group was 116.6 ppt (Table 3 in Eskenazi et al., (2004); the corresponding modeled background total TEQ intake was 0.0180 ng/kg-day. Lacking specific measurements for boys, EPA assumed that the averages for boys were the same as for girls.
- Scenario 5 (Needham DLC-TEQ intake added scenario). This method adds DLC-TEQ intakes, which are estimated by scaling the modeled TCDD intakes by the ratio of DLC:TCDD in serum for background exposures, assuming that the ratio is the same for oral intakes and serum concentrations. For Scenario 5, EPA assumes that TCDD comprises 10% of the total background TEQ, as in Scenario 3, which results in a 9:1 ratio for DLC:TCDD for background exposures. The resulting DLC-TEQ intake is 3.15×10^{-3} ng/kg-day ($9 \times 3.5 \times 10^{-4}$ ng/kg-day). The estimated DLC-TEQ intake is then added to the *P*, *W*, and *AVG* values for the standard pathway (Scenario 1).
- Scenario 6 (Eskenazi DLC-TEQ intake added scenario). The method is the same as for Scenario 5. The DLC:TCDD LASC ratio is calculated from the measured serum concentrations (TCDD = 40.5 ppt; DLC-TEQ = 76.1 ppt) reported by Eskenazi et al. (2004). The resulting DLC:TCDD LASC ratio is 1.88 (76.1 ÷ 40.5). Multiplying the corresponding TCDD background intake of 4.22×10^{-3} ng/kg-day (Scenario 2) by this factor gives a background DLC-TEQ intake of 7.93×10^{-3} ng/kg-day. The total background TEQ intake is 0.0122 ng/kg-day ($7.93 \times 10^{-3} + 4.22 \times 10^{-3}$). The estimated DLC-TEQ intake is then added to the *P*, *W*, and *AVG* values for Scenario 2.

Exposure Duration

–Exposure duration" refers to the duration of the elevated (external) TCDD exposures immediately following the Seveso accident, which is not known with certainty. In the standard pathway analysis, the –exposure duration" of the TCDD exposures due to the Seveso accident was modeled using the Emond model as a single pulse on 1 day (i.e., 24 hours). The alternative also uses the Emond model but models the exposures following the Seveso accident using pulse doses on two consecutive days (i.e., 48 hours).

Measure Lag

"Measurement lag" refers to the period of time between TCDD exposure following the Seveso accident and the collection of blood for future TCDD analyses. Within the Seveso cohort, serum samples were collected in 1976 and 1977, so in the standard pathway analysis, an average measurement lag time of 6 months was assumed for exposure to TCDD. The alternative analyses simulate lag times of 1 month and 1 year.

Age at Exposure

"Age at exposure" is the average age of the susceptible lifestage (boys, 1–9 years old) at the time of the Seveso accident. Within the cohort, the average age at exposure was reported to be 6.2 years, which was used in standard pathway analysis. The alternative analysis considers individuals who would have been 1 year or 9 years of age at the time of the Seveso accident, representing the bounds of the susceptible age range. This category is included to show the potential range of exposures across the cohort for the reported age range rather than to evaluate plausible alternatives to the mean age of 6.2 years. That is, the intakes associated with ages 1 or 9 would not be considered as PODs.

Hill Coefficient

Because the Hill coefficient is the most influential variable in the Emond PBPK model (see Section 3.3.4.3.2.5) and the value of 0.6 results in a supralinear relationship between intake and blood concentrations at low doses, EPA also evaluated the impact of changing the Hill coefficient. Based on the results of the expanded sensitivity analysis in Section 3.3.4.3.2.6, a Hill coefficient of 1 and the corresponding optimized CYP1A1 elimination constant (kelv) of 0.005 were evaluated for impact on the POD. A value of 1 was chosen because that is the lowest value where the model is no longer supralinear; otherwise the value of 1 has no biological or empirical basis. Because the relationship between TCDD serum concentrations and intake was changed for the alternative parameter specifications, a revised TCDD background exposure was modeled based on the Needham scenario. Using the revised background TCDD intake of 1.9×10^{-4} ng/kg-day, the modeled peak and window-average (TCDD-only) exposures at the LOAEL are 7.6×10^{-3} and 3.7×10^{-3} ng/kg-day, respectively. The average (i.e., *AVG*) of the peak and window intakes is 5.7×10^{-3} ng/kg-day, which is 3.5-fold lower than the LOAEL POD for the RfD.

Mocarelli et al. Sensitivity Tree Results

Overall, excluding the age-at-exposure and Hill coefficient variables, neither of which are considered to have plausible alternative values, the daily intakes (TCDD or total TEQ) based on the alternative assumptions in this tree vary between 0.0071 ng/kg-day (*W* for 1-month measurement lag) and 0.0666 ng/kg-day (*P* for modeled total TEQ, Needham background). This range spans the LOAEL POD for the standard pathway analysis of 0.020 ng/kg-day by about a factor of three on each side (2.8-fold below to 3.3-fold above). The *AVG* values, which factor in both peak and window-average exposures and are the preferred POD values[59], vary over a smaller range from 0.0118 ng/kg-day (Scenario 2: TCDD-only, Eskenazi background) to 0.0461 ng/kg-day (Scenario 3: modeled total TEQ, Needham background), bracketing the LOAEL POD for the standard pathway by about a factor of two (1.7-fold below to 2.3-fold above).

The ratio of peak intake to window-average intake (*P*:*W* ratio) is of interest in evaluating the range of exposures over which an average is taken. The *P*:*W* ratio is 4 for the standard pathway POD. In general, the higher the background exposure, the lower the peak intake and the lower the *P*:*W* ratio and the lower the impact of averaging *P* and *W*. The *P*:*W* ratio is lowest for all the Eskenazi background scenarios, decreasing to about a factor of 1 for the TEQ analyses. For the Eskenazi modeled TEQ scenario, *W* is larger than *P* because the background intake is high enough to result in a higher terminal (10-year) LASC for the target population than was experienced by the exposed population in the Seveso cohort; in this case, with a higher peak realized for the average exposure over the critical window, neither *P* nor *AVG* would be relevant and the higher *W* value would be used as the POD.

The most influential variable in either direction (above or below the standard pathway RfD LOAEL POD) is background exposure. The higher Eskenazi background exposure scenario had the largest impact on the TCDD-only intake estimates, with a 41% lower *AVG* than for the standard pathway RfD LOAEL POD, primarily because of the lower peak exposure. The 12-fold higher value for the Eskenazi TCDD background than for the Needham adult background is likely a result of higher food consumption in children and a higher average environmental concentration for the relevant childhood exposure period (1964−1976) than for the adult

[59] The *AVG* for Scenario 1 was chosen as the POD for the RfD because it accounts for both peak and window-average exposures.

exposures (ca. 1941−1976) (Lorber, 2002; Pinsky and Lorber, 1998). Also, the higher ratio of TCDD to total TEQ in children may reflect the lack of attainment of steady state for many of the DLCs relative to TCDD. The next most influential variable was exposure time, with a 24% lower *AVG* for the 48-hour exposure time than for the 24-hour scenario. However, the modeled exposures on each of the 2 days within the 48 hour period were equal when, in reality, they would be decreasing with time, such that the peak is somewhat underestimated in this analysis; longer exposure scenarios assuming constant levels would not be realistic. The largest differences in the other direction (i.e., exceeding the standard pathway RfD POD) were obtained for the modeled total TEQ scenarios, with a 2.3-fold higher *AVG* and 3.3-fold higher peak (*P*) for Scenario 3 (Needham) and a 1.6-fold higher window-average for Scenario 4 (Eskenazi). Note that any DLC background exposure estimate based on TEQ will be an over-estimate because of the conservative nature of the TEF methodology. Further, there is additional uncertainty when applying the TEF method to tissue concentrations such as LASC. All the other alternative assumptions resulted in a 16% or lower change in the *AVG* values. Although not a consideration for defining the POD, the TCDD *AVG* intakes across the susceptible age range (1−9 years) were within 5% of the standard pathway RfD POD, but with a large *P*:*W* ratio (9.6) for 1-year-olds.

In summary, the quantitative uncertainties evaluated here for the RfD LOAEL POD based on Mocarelli et al. (2008) span about a threefold range in either direction. The largest differences are those between peak and window-average exposures, which decrease when considering the alternative Eskenazi background estimates. Using the latter, the *AVG* POD is about half of the RfD POD for TCDD only (Scenario 2), but, when considering the TEQ contribution, rises to about the same value as the RfD POD with additive background DLC (Scenario 6) and to 60% higher than the RfD POD with modeled TEQ background (Scenario 4). Using the modeled-TEQ method, the Needham background DLC exposure has a larger impact on the standard RfD POD, increasing it by a factor of 2.3 (Scenario 3), but is only 16% higher than the RfD POD for the additive method (Scenario 5). Because of (1) the lack of background TEQ measures in populations from the 1970's that are directly relevant to the Mocarelli et al. (2008) study population, (2) the conservative nature of the TEF method, and (3) uncertainty in the application of the TEF method to reported human tissue concentrations, EPA cannot recommend, at this time, any particular approach for incorporating background DLC exposure directly into the POD for the RfD. Overall, given the bidirectional nature and relatively small

magnitude of the uncertainties, EPA believes that this sensitivity analysis provides support for the magnitude of the RfD.

4.5.1.1.2. *Baccarelli et al. (2008)*

Baccarelli et al evaluated thyroid-stimulating hormone levels in newborns whose mothers were exposed to TCDD during the Seveso accident (see Section C.1.2.1.5.7 for study details). To examine the impacts of potential uncertainties associated with the assumptions made in estimating the standard pathway POD for Baccarelli et al. (2008) (see Section 4.2.3.2), EPA analyzed alternate assumptions about exposure and the level of change in neonatal TSH levels associated with the designation of a LOAEL or a NOAEL from this study, as shown in Figure 4-7. The sensitivity analysis begins with elevated neonatal TSH levels. The terminal nodes at the bottom of the figure show the PODs as daily oral intakes (ng/kg-day) resulting from each alternative value for the variables examined. The left side of the figure depicts the variables considered in the sensitivity analysis (i.e., basis of the POD, background exposure, POD method of estimating material LASC, and maternal age at conception). Values for these variables are inputs to the Emond PBPK model under the human gestational scenario (see Section 4.2.2), which was used to estimate the PODs in Figure 4-7. Each POD is a continuous daily oral TCDD or TEQ intake that would result in a specified TCDD maternal LASC corresponding to a neonatal TSH of 5 μU/mL at the end of gestation (see modeling code and details in Appendix F).

POD Basis

In the standard pathway analysis, the neonatal TSH of 5 μU/mL at the end of gestation is determined to be a LOAEL. The alternative assumption evaluated in Figure 4-7 is that this value is a NOAEL. For the NOAEL in Figure 4-7, the equivalent LOAEL (by multiplying by 10)[60] is also shown for direct comparison to the LOAEL estimates. The choice of the maternal LASC value for the NOAEL is discussed below.

POD Method of Determining Maternal LASC for TCDD Only

There are several ways in which a POD could be derived from the Baccarelli et al. (2008) study. In the standard pathway RfD analysis, EPA used the study authors' regression model results from their Figure 2A (designated the "Regression Model") to determine a LOAEL based

[60] A tenfold factor is used because the LOAEL POD is divided by a UF_L of 10 in the RfD derivation. The "equivalent" LOAEL is not meant to be an alternative LOAEL but is used strictly for comparison.

on the maternal plasma concentration corresponding to neonatal TSH levels of 5 μU/mL. The advantage in using the regression model is that it was used to account for covariates that influenced the dose-response relationship. Three alternative values are examined by selecting specific points or ranges from the figures in the Baccarelli paper, without consideration of the regression modeling results (the ―graphical method"). The alternative values, therefore, do not account for the covariates. The first assumes a NOAEL of 40 ppt maternal LASC, which is essentially the highest TCDD concentration above which neonatal TSH levels are consistently above 5 μU/mL [see Figure 2A in Baccarelli et al. (2008)]. The figure (2A) shows that 5 of the 6 neonates born to women who had TCDD concentrations above 40 ppt had TSH levels above 5 μU/mL; among the 45 women who had TCDD concentrations below 40 ppt, only two had babies with TSH levels above 5 μU/mL. The second alternative assumes that the 6 neonates born to women with TCDD LASC above 40 ppt comprise a LOAEL group, with a median maternal LASC of 90 ppt. The third alternative assumes a LOAEL at the highest neonatal TSH level (8.5 μU/mL) shown in Figure 2A, which corresponds to a maternal TCDD LASC of 312 ppt.

Background Exposure

Background exposures in the population were estimated in several ways. The background TCDD exposure used in the standard pathway RfD analysis was based on continuous intake necessary to obtain 15 ppt at 30 years for females (the ―Needham" TCDD Only background in Figure 4-6); the modeled TCDD intake was 3.9×10^{-4} ng/kg-day, slightly higher than that for males. To examine the maternal TEQ exposures associated with a LOAEL based on a neonatal TSH level of 5 μU/mL, EPA relied on the regression results reported in Baccarelli et al. (2008). Baccarelli et al. (2008) reported maternal plasma TEQ concentrations in the following two ways: (1) polychlorinated dibenzo-*p*-dioxins (PCDDs), PCDFs, coplanar PCBs, without noncoplanar PCBs (see Figure 2B) and (2) PCDDs, PCDFs, coplanar PCBs, and noncoplanar PCBs, termed total TEQ (see Figure 2D). The concentrations in their Figures 2B and 2D are reported as TEQs and were modeled as TCDD for this analysis. Excluding the noncoplanar PCBs, maternal TEQ levels of 219 ppt in serum are associated with neonatal TSH level of 5 μU/mL. For the total TEQ, maternal TEQ levels of 485 ppt in serum are associated with a neonatal TSH level of 5 μU/mL. Confidence in the total TEQ estimate is lower than that

for the one without the noncoplanar PCBs because of the lower significance of the total TEQ regression coefficient ($p = 0.14$) than the one without the noncoplanar PCBs ($p = 0.005$).

Age at Conception

For the standard pathway RfD analysis, the maternal "age at conception" was set at 30 years, which was the average reported in Baccarelli et al. (2008). The alternative assumes the maternal age at conception to be 45 years of age; this is the standard gestational scenario used in estimating the human equivalent doses for the animal bioassays reporting reproductive or developmental effects and is considered to be a reasonable upper end of female fertility.

Baccarelli et al. Sensitivity Tree Results

The alternative LOAEL PODs based on this analysis of Baccarelli et al. (2008) vary between 0.005 and 0.059 ng/kg-day. These two values are roughly a factor of 4 lower and a factor of 3 larger, respectively, than the LOAEL estimate of 0.020 ng/kg-day that was the basis of the standard pathway RfD. The TCDD intake of 0.0016 ng/kg-day corresponding to the alternative NOAEL is slightly more than an order of magnitude lower than the standard pathway RfD LOAEL POD and would yield a slightly lower RfD estimate than the current RfD after eliminating the 10-fold UF_L factor. EPA has much less confidence in the NOAEL estimate than in the selected LOAEL because the NOAEL does not take into account the covariates and falls in a lower concentration range where the background DLC exposures are a much more significant component. The largest downward impact on the standard pathway LOAEL POD results from grouping the highest exposures independent of the modeling results (POD = 0.005), which decreases the LOAEL by a factor of four; however, analogous to the NOAEL alternative, the approach ignores the contribution of covariates. Using the alternative age of conception of 45 years yielded a POD of 0.0162, which is virtually the same as the standard pathway LOAEL POD of 0.0196.

The largest upward impact on the standard pathway LOAEL POD is the inclusion of modeled total TEQ (POD = 0.059), which increases the LOAEL by a factor of three. However, the model fit is poor, and the result can be compared with an analogous calculation to the additive DLC approach used for the Mocarelli analysis in Figure 4-6. An additive DLC-TEQ background of 3.5×10^{-3} ng/kg-day can be estimated for the women in the Baccarelli analysis by multiplying the TCDD background intake of 3.9×10^{-4} ng/kg-day by 9 (not shown in Figure 4-7). Adding the estimated DLC background to the standard pathway RfD LOAEL POD

of 0.0196 gives a corresponding total-TEQ intake of 0.0231 ng/kg-day. This is 1.2-fold higher than the standard pathway RfD POD but 2.6-fold lower than the modeled total-TEQ POD. Leaving out the noncoplanar PCBs greatly improves the significance of the slope, which could suggest that the noncoplanar PCBs do not contribute to the effect as much as the PCDDs and PCDFs or that there is greater uncertainty in the TEQ estimates for the noncoplanar PCBs. In either case, as for the Mocarelli analysis, any estimate of background DLC exposure based on TEQ is likely an over-estimate because of the conservative nature of TEFs; there also is uncertainty in the application of the TEF method to reported human tissue concentrations. Overall, although background DLC exposures will effectively increase the POD to some degree, EPA believes that the effect is relatively small and is in the range of the estimated standard pathway TCDD LOAEL.

In summary, the quantitative uncertainties evaluated here for the RfD POD based on Baccarelli et al. (2008) span a three to fourfold range in either direction. The alternative LOAELs at either extreme are not strong POD candidates; the lowest value (from the graphical method) does not account for covariates and there is greater uncertainty in the (total TEQ) regression model for the highest value than for the other regression models. All the other alternative LOAELs are within a factor of 1.5 of the RfD POD. Overall, as for Mocarelli et al. (2008) analysis, EPA believes that this sensitivity analysis also supports the magnitude of the RfD.

4.5.1.2. *NTP (2006a) Sensitivity Analysis*

The NTP (2006a) bioassay is a comprehensive evaluation of TCDD chronic toxicity in female Sprague-Dawley rats, evaluating dozens of endpoints at several time points in all major tissues (see Section D.1.5.8 for study details). To examine the impacts of some of the uncertainties associated with estimating the POD from the NTP (2006a) study (see Section 4.2), EPA analyzed two different approaches for estimating dose and alternate choices of rodent kinetic model and background. Figure 4-8 depicts this analysis, which relied on an approach similar to those used in characterizing some of the uncertainties in the RfDs derived from Mocarelli et al. (2008) and Baccarelli et al. (2008). The sensitivity analysis begins with the administered dose or measured tissue concentrations. The terminal nodes at the bottom of the figure show the LOAEL PODs as daily oral intakes (ng/kg-day) resulting from each alternative

value for the variables examined. The left side of the figure depicts the variables considered in the sensitivity analysis (i.e., rodent kinetic model, dose metric, background exposure, and human kinetic model). Values for these variables are inputs to the Emond or CADM rodent PBPK models and the Emond human PBPK model, which were used to estimate the PODs in Figure 4-8 (see modeling code and details in Appendix E).

The lowest administered dose of 2.14 ng/kg-day was determined to be the animal LOAEL based on liver and lung lesions in the rats. In the standard pathway candidate RfD analysis, the $LOAEL_{HED}$ was the POD.

Exposures were estimated either based on a kinetic model of the administered TCDD dose or on the measured concentrations of TCDD and DLCs in the rat adipose tissue after terminal sacrifice. NTP reported concentrations of TCDD, 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), and 3,3N,4,4N,5-pentachlorobiphenyl (PCB-126) in the adipose and liver tissues obtained from the rats after terminal sacrifice. The 2005 WHO TEF values for PeCDF and PCB-126 are 0.3 and 0.1, respectively (Van den Berg et al., 2006).

Rodent Kinetic Models

To predict average tissue concentrations based on the administered TCDD dose, EPA used both the Emond and CADM kinetic models; the Emond model was used in the standard pathway analysis. EPA also used the first-order body burden model to predict whole body TCDD concentrations; this model uses a constant half-life to simulate the elimination of TCDD from the body. Section 3 describes all of these models.

Dose Metric

EPA used several alternative dose metrics based on the modeling approach and measured tissue concentrations. The first-order body burden model estimates the TCDD concentration in the whole body. When using the Emond model to evaluate the disposition of TCDD, EPA evaluated both the whole-blood TCDD concentrations used in the standard pathway analysis and LASC. For the CADM model, EPA simulated TCDD concentrations in the adipose compartment following the administered TCDD dose. EPA also used the TCDD (see Table 13 in the NTP report) or DLC concentrations (see Tables 10 and 11 in the NTP (2006c) report) measured in the adipose tissue collected at study termination.

Background Exposure

Using the DLC concentration information, EPA estimated TEQ in two ways. In the first approach, based on an analysis of DLCs in the adipose tissue that was reported in another NTP study on DLC mixtures (NTP, 2006c), EPA initially estimated the ratio of the adipose tissue TEQ concentration to the adipose tissue TCDD concentration, then applied this ratio to the Emond whole-blood TCDD estimates assuming proportionality (resulting in a LOAEL whole blood concentration of 2.75 ppt instead of the TCDD-only concentration of 2.56 ppt used in the standard pathway analysis).

In the second approach, EPA estimated TEQ dose based on adipose tissue TCDD levels reported by NTP; the reported TCDD concentration in the fat given in the study at the lowest dose was used to estimate a LOAEL using the Emond model. Finally, using the 2005 WHO TEF values (Van den Berg et al., 2006), EPA converted the reported concentrations of TCDD, PeCDF, and PCB-126 measured in the fat of the control rats in the NTP mixtures study (NTP, 2006c) to TEQ using eq. 4-1.

$$Chemical_i(B) = \frac{Chemical_i\left(fat_{MC}\right) \times TEF_i}{TCDD\left(fat_{TCDD}\right)} \times Dose_{TCDD} \qquad \text{(Eq. 4-1)}$$

where

$Chemical_i(B)$	= estimate of background exposure to Chemical *i* in ppt units of TCDD blood concentrations at 105 weeks, for *i* = TCDD, PeCDF, and PCB126.
$Chemical_i(fat_{MC})$	= mean ppt (pg/g) of Chemical *i* in the fat tissues of the control animals at 105 weeks in mixtures study (NTP, 2006c).
$TCDD(fat_{TCDD})$	= mean pg/g of TCDD in the fat tissues of the 3 ng/kg dose group at 105 weeks in the TCDD study (NTP, 2006a).
$Dose_{TCDD}$	= 2.56 ng/kg TCDD blood concentration for the 3 ng/kg dose group in the TCDD study (NTP, 2006a).
TEF_i	= Toxicity Equivalence Factor for Chemical *i* [from Van den berg et al. (2006)].

Assuming simple proportionality of blood TCDD concentrations between controls and low-dose (2.14 ng/kg-day) animals, the TEF-adjusted ratio of each congener (Chemical *i*) in control animal fat to low-dose-animal fat is multiplied by the modeled TCDD blood concentration for the low-dose animals to obtain an equivalent background exposure in the dose

metric (ppt whole blood). For total TEQ, the estimates of all three congeners are summed. Total TEQ estimates likely are biased somewhat high because they are based on terminal (2-year) measurements rather than representing lifetime averages.

Human Kinetic Models

To estimate the final human intake LOAEL PODs in Figure 4-8, EPA used the Emond human kinetic model that was used in the standard pathway analysis; CADM does not cover all life stages needed for comparison. EPA also used first-order kinetics to estimate the LOAEL POD under the scenario that begins with first order body burden *NTP Variable Sensitivity Tree Results*

Overall, the alternative LOAEL POD estimates in this tree (see Figure 4-8) vary between 0.023 and 0.44 ng/kg-day. This range is approximately sixfold lower to threefold higher than the LOAEL POD for the standard pathway RfD of 0.14 ng/kg-day. The alternative LOAEL based on first order body burden (0.023 ng/kg-day) is the lowest value in the range, approximately 85% lower than the LOAEL based on the standard pathway approach. The difference between these two estimates is consistent with the more conservative approach used in modeling first-order TCDD body burdens. The alternative LOAEL based on the TEQ in whole blood is less than 10% greater than the LOAEL from the standard pathway RfD. The alternative candidate LOAEL based on the TCDD in lipid-adjusted serum is approximately 120% greater than the LOAEL for the standard pathway RfD. The use of the CADM model to estimate adipose tissue concentration based on administered dose resulted in a 35% increase in the LOAEL estimate relative to the LOAEL based on the standard pathway approach. The LOAELs based on measured TCDD or TEQ levels in rodent adipose tissue were greater than the LOAEL from the standard pathway RfD by approximately a factor of three. EPA believes that this sensitivity analysis is supportive of the modeling choices EPA has made in the derivation of PODs for TCDD RfD derivation.

4.5.2. Evaluation of Range of Alternative Points of Departure (PODs) for Additional Epidemiologic Endpoints

In addition to the principal studies depicted in Figures 4-6 and 4-7, EPA evaluated a number of endpoints presented in seven other Seveso cohort studies to estimate the range of potential PODs based on uncertainties in exposure duration, exposure averaging protocols, and

DLC background exposures. Included in those study/endpoint combinations are the following: two that passed all the selection criteria, developmental dental effects (Alaluusua et al., 2004) and duration of menstrual period (Eskenazi et al., 2002b); a new developmental study on semen quality (Mocarelli et al., 2011) that was published after the study selection process was completed but is useful in this uncertainty analysis of the POD ranges; and four studies that did not pass all the criteria for qualification as POD candidates (Warner et al., 2007; Eskenazi et al., 2005; Warner et al., 2004; Mocarelli, 2000) that analyzed ovarian function/progesterone, age at menopause, age at menarche, and sex ratio, respectively, but for which limiting NOAEL and LOAEL values can be estimated. Descriptions and evaluations for all of these studies, except Mocarelli et al. (2011), can be found in Appendix C. Mocarelli et al. (2011) is described earlier in this section (4.3.6.2). Tables 4-8 through 4-10 and Figure 4-9 present the exposure values modeled using the Emond human PBPK model for potential POD ranges for these 7 additional endpoints studied in the Seveso cohort. The details of the kinetic modeling for these endpoints and the corresponding background exposures can be found in Appendix F.

For most of the studies that did not pass all the criteria, the major uncertainties are the definition of the critical exposure window (see Text Box 2-2) and the corresponding relevant exposure-averaging time, and the determination of adverse effect levels. Alaluusua et al. (2004) and Eskenazi et al. (2002b) passed the selection criteria because a critical exposure window could be identified for each. Alaluusua et al. is included among the candidate RfDs in Table 4-5, but Eskenazi et al. was not carried forward because the determination of an adverse effect level for length of menstrual cycle was considered to be too arbitrary. A critical exposure window can be identified also for Warner et al. (2004) (age at menarche), but no TCDD-related adverse health outcomes were observed. However, for each of the studies considered here, with some additional assumptions, NOAELs and LOAELs at nominal group-exposure levels can be determined. When a critical window cannot be identified, the critical exposure window is assumed to be the entire duration from exposure in 1976 to time of interview (i.e., end of follow-up period). Tentative NOAELs and LOAELs are designated for those endpoints where adversity levels are difficult to define. Given these assumptions and limitations, TCDD and total TEQ intakes can be modeled but must be considered to be lower bounds on the effective exposures, given the conservative nature of the assumptions; EPA does not consider these estimates suitable for use in the derivation of the TCDD RfD.

Table 4-8. Alternative PODs for the impact of TCDD exposure during gestation and nursing on semen quality of male offspring (Mocarelli et al., 2011)

POD type	Age-at-conception scenario	Averaging protocol[a]	Maternal intake (ng/kg-day)	
			TCDD only	TCDD + DLC[b]
NOAEL	30 years	Cont. avg.	2.9×10^{-4}	2.90×10^{-3}
LOAEL			1.50×10^{-3}	4.11×10^{-3}
NOAEL	45 years	Cont. avg.	2.9×10^{-4}	2.90×10^{-3}
LOAEL			1.04×10^{-3}	3.65×10^{-3}

[a]Cont. avg. = average continuous exposure over the specified duration.
[b]Added background DLC = 2.61×10^{-3} ng/kg-day (9 × TCDD background intake at NOAEL)

Table 4-9. Alternative PODs for developmental endpoints other than increased neonatal TSH and semen quality

Population, endpoint (cite)	POD type	Averaging protocol[a]	TCDD only (ng/kg-day)		TCDD + DLC (ng/kg-day)	
			Needham	Eskenazi	Needham[b]	Eskenazi[c]
Girls, duration of menstrual cycle as women (Eskenazi et al., 2002b)	NOAEL	Cont. avg.	0.0102	3.1×10^{-3}	0.0137	0.0112
	LOAEL	Peak	61	60	61	60
		Window	1.5	1.5	1.5	1.51
		P/W avg.	31	31	31	31
Girls and boys, developmental dental effects (Alaluusua et al., 2004)	NOAEL	Peak	0.0655	0.0437	0.0688	0.0517
		Window	0.0157	0.0175	0.0190	0.0255
		P/W avg.	0.0406	0.0306	0.0439	0.0386
	LOAEL	Peak	1.65	1.51	1.65	1.52
		Window	0.149	0.151	0.152	0.159
		P/W avg.	0.897	0.841	0.900	0.849
Girls, age at menarche (Warner et al., 2004)	NOAEL	Peak	0.604	0.517	0.607	0.525
		Window	0.0394	0.0424	0.0427	0.0505
		P/W avg.	0.322	0.280	0.325	0.288

[a]Cont. avg. = average continuous daily intake over the specified duration; P = average intake for peak exposure; W = average intake for critical-window exposure; P/W avg. = average of "Peak" and "Window" intakes.
[b]Added DLC = 3.51×10^{-3} ng/kg-day for girls, 3.33×10^{-3} ng/kg-day for boy/girl average.
[c]Added DLC = 8.1×10^{-3} ng/kg-day for girls, 8.0×10^{-3} ng/kg-day for boy/girl average.

Table 4-10. Alternative PODs for adult endpoints for which critical exposure windows are undefined

Population, endpoint (cite)	POD type	Averaging protocol[a]	TCDD only (ng/kg-day)	TCDD + DLC[b] (ng/kg-day)
Men, sex ratio of offspring (Mocarelli et al., 2000)	NOAEL	Peak	0.0341	0.0373
		Window	1.58×10^{-3}	4.73×10^{-3}
		P/W avg.	0.0178	0.0210
	LOAEL	Peak	0.162	0.165
		Window	4.69×10^{-3}	7.84×10^{-3}
		P/W avg.	0.0831	0.0863
Women, age at menopause (Eskenazi et al., 2005)	NOAEL	Peak	1.6×10^{-4}–3.4×10^{-3}	1.6×10^{-3}–6.9×10^{-3}
		Window	1.6×10^{-4}–1.0×10^{-3}	1.6×10^{-3}–4.5×10^{-3}
		P/W avg.	1.6×10^{-4}–2.2×10^{-3}	1.6×10^{-3}–5.7×10^{-3}
	LOAEL	Peak	0.013–0.052	0.016–0.055
		Window	1.7×10^{-3}–3.4×10^{-3}	5.2×10^{-3}–7.0×10^{-3}
		P/W avg.	7.3×10^{-3}–0.028	0.011–0.031
Women, ovarian function, progesterone (Warner et al., 2007)	NOAEL	Peak	0.204	0.208
		Window	3.00×10^{-3}	6.51×10^{-3}
		P/W avg.	0.104	0.108

[a]Cont. avg. = average continuous daily intake over the specified duration; Peak = average intake for peak exposure; Window = average intake for critical-window exposure; P/W avg. = average of ―Peak" and ―Window" intakes.
[b]Added DLC = 3.15×10^{-3} ng/kg-day for males, 3.51×10^{-3} ng/kg-day for females, 3.33×10^{-3} ng/kg-day for male/female average.

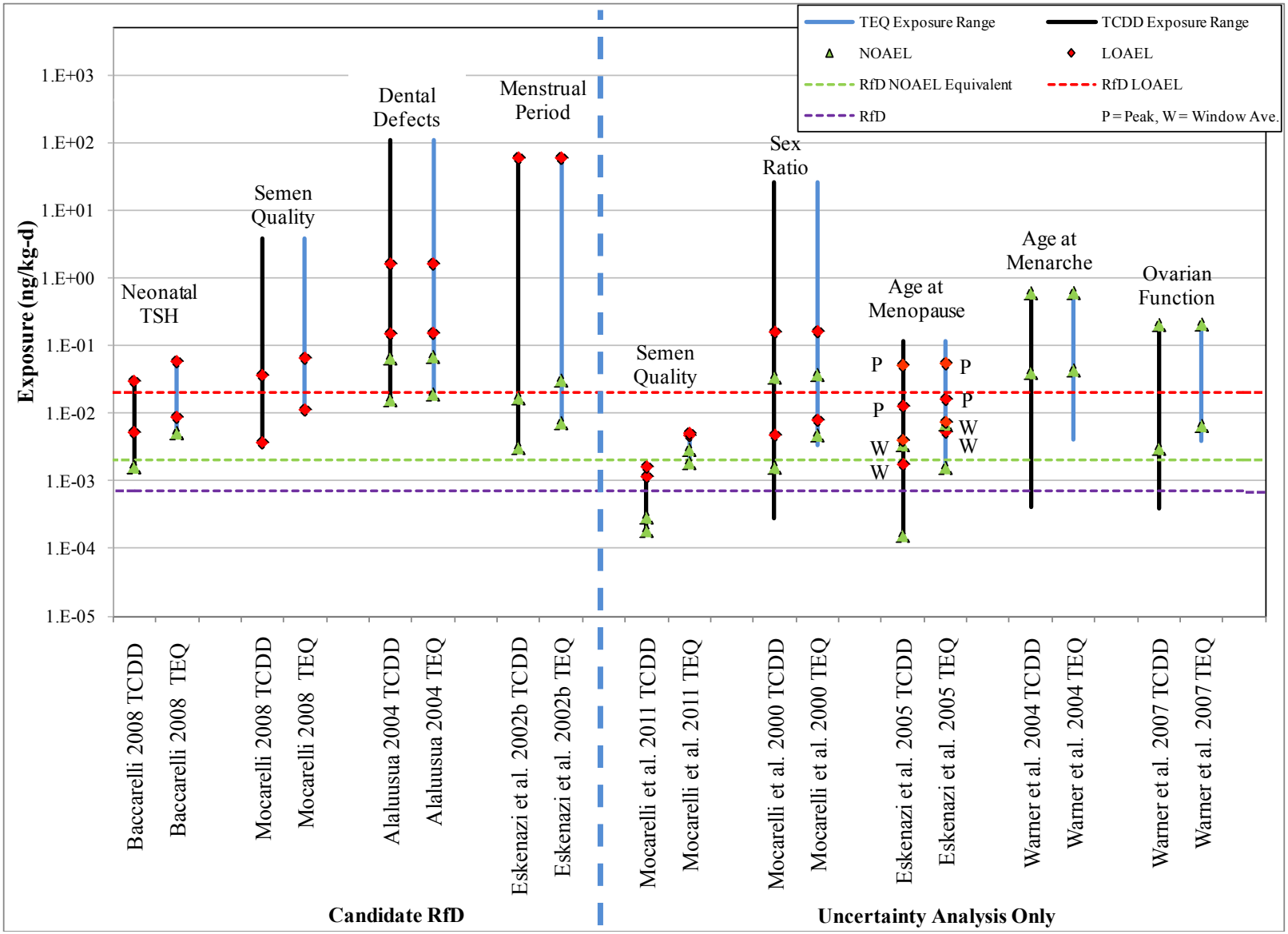

Figure 4-9. Alternative POD exposure-response array.

Additional endpoints reported in the epidemiologic literature were considered in the context of this uncertainty analysis but were excluded based on large uncertainties in defining adversity or plausible exposure profiles over time. All the Ranch Hand studies[61] were excluded because of the inability to construct effective exposure profiles with any confidence, given the 20-year lag between the actual TCDD exposures and measurement of serum levels. For the Seveso cohort, several studies[62] were eliminated from consideration because uncertainties in defining plausible NOAELs or LOAELs were too large.

For modeling of the endpoints in Tables 4-8 to 4-10, grouped exposure ranges were represented by the geometric mean of the range limits. The average daily intakes for exposures (LASC) in the background range were estimated as the continuous exposure from birth resulting in the reported serum concentrations (TCDD or total TEQ) at the average subject age at time of measurement. Peak and critical-window average exposures (as LASC) were modeled for measured LASC values greater than background using the actual exposure scenarios. Because all exposure durations were less than lifetime, average daily intakes for all modeled peak and window-average LASC were estimated using the terminal 5-year-peak average as described in Section 3.3.6. Precision is expressed to the nearest 10^{-5} ng/kg-day for all intake estimates to avoid rounding errors when adding DLC background intakes. DLC background intakes are the same as those discussed previously in this section (4.5.1.1.1). Values less than or equal to 10^{-3} are shown in scientific notation for readability.

Figure 4-9 shows the range of NOAELs and LOAELs and exposures for all of the endpoints considered in this uncertainty analysis, the endpoints on which they are based, and the study citation. The study/endpoint combinations are separated into two groups representing either those chosen for RfD POD consideration (“Candidate RfD”) or those not otherwise qualifying (“Uncertainty Analysis Only”). The NOAELS and LOAELS are indicated for each study, as appropriate, and the vertical lines through these PODs represent the range of possible PODs based on Emond PBPK results using alternative exposure scenarios (see Appendix F). The limits across studies—indicated by symbols of the same type—for each POD type (NOAEL or LOAEL) for each endpoint cover the full range of alternative PODs in Tables 4-8 to 4-10,

[61] (Michalek and Pavuk, 2008; Pavuk et al., 2003; Michalek et al., 2001a; Michalek et al., 2001b; Michalek et al., 2001c; Longnecker and Michalek, 2000)
[62] (Eskenazi et al., 2007; Baccarelli et al., 2005; Baccarelli et al., 2004; Eskenazi et al., 2003; Landi et al., 2003; Baccarelli et al., 2002; Eskenazi et al., 2002a)

without distinction of the relative plausibility of each one. That is, all the PODs are treated equally without considering the relative confidence held in each one, individually. The low end of most of the ranges is the critical-window average exposure, which does not take into account the influence of the much higher peak exposure. Conversely, the upper end of the range is generally the peak exposure, which does not account for the potential effect of longer-term continuous exposure. On the ~~"u~~ncertainty analysis only" side of Figure 4-9, most of the NOAELs and many of the LOAELs are somewhat speculative and would not be considered as candidates for the RfD POD. The range limits are themselves uncertain. The same DLC modeling issues presented in Section 4.5.1 apply to all the TEQ results here, so the TEQ results are approximations and are unlikely to be very accurate. Also, the lowest POD estimates are more affected by background DLC exposure than are the PODs closer to the RfD POD; generally, TCDD is a minor component of the total TEQ for the lower PODs, subjecting the lowest alternative PODs to the greatest uncertainty. The RfD LOAEL POD (0.02 ng/kg-day) and its RfD NOAEL Equivalent estimate (0.002 ng/kg-day, with the 10-fold UF), along with the RfD (7×10^{-4} ng/kg-day), are shown on the figure for comparison to the alternative POD ranges.

The LOAEL ranges for the two principal studies (Baccarelli et al., 2008; Mocarelli et al., 2008) span the RfD LOAEL POD, whether based on TCDD alone or total TEQ. The TCDD-only NOAEL estimate for Baccarelli et al. (2008) is only slightly below the RfD NOAEL Equivalent POD. The NOAEL and the lowest alternative LOAELs for Baccarelli et al. (2008) are not strong POD candidates because they are based on the raw observations and do not take into account the covariates that affect the exposure-response relationship, as does the regression model on which the RfD LOAEL POD is based. The ranges for the total TEQ LOAEL PODS for the coprincipal studies straddle the RfD LOAEL POD benchmark, in the range of twofold below to threefold above.[63] The POD ranges for the other candidate RfD endpoints are well above their respective comparison NOAEL/LOAEL benchmarks (i.e., RfD NOAEL Equivalent and RfD LOAEL). The NOAEL for Eskenazi et al. (2002b) is somewhat arbitrary, based simply on a continuous average exposure over a 13-year window corresponding to a normal 28-day menstrual cycle, without considering the possible range of normal durations.

Of the endpoints that were not selected as RfD POD candidates, there are three whose LOAEL ranges are wholly or mostly below the RfD LOAEL POD. The sperm effects in men

[63] See Sections 4.5.1.1.1 and 4.5.1.1.2 for more details

who were exposed in utero and by lactation reported by Mocarelli et al. (2011) are very similar to those in men exposed as boys in one of the principal studies (Mocarelli et al., 2008). The maternal exposures associated with the effects reported by Mocarelli et al. (2011) are very low with the TCDD-only LOAEL being 12-fold lower than the RfD LOAEL POD for the 30-year exposure scenario. For this study, a TCDD-only NOAEL can be established at 2.9×10^{-4} ng/kg-day (for the reference population), which is sevenfold below the equivalent RfD NOAEL POD. Both the TCDD-only NOAEL and LOAEL are much lower than the estimated DLC background exposure; however, assuming a simple TEQ additive model, and with the aforementioned uncertainties concerning DLC-TEQ estimation, a TEQ NOAEL and LOAEL of 2.9×10^{-3} and 4.11×10^{-3} ng/kg-day can be estimated (see Table 4-8 and Appendix F.3.7). Although the TEQ LOAEL is still well below that for the RfD POD, the TEQ NOAEL is in the range of the RfD NOAEL Equivalent POD. Given the large amount of uncertainty in the modeled NOAEL and LOAEL for this endpoint, EPA elected not to consider either as a POD.

The second endpoint with lower LOAELs than the RfD POD is age at menopause reported by Eskenazi et al. (2005). The figure for this endpoint includes two separate LOAEL candidates because of uncertainty in determining adversity at the lower exposure level in question (3rd quintile). For that reason, the daily intakes associated with the critical-window average and peak exposures are labeled (~~"~~W" and ~~"~~P," respectively). The intakes associated with the peak are in the range of the RfD LOAEL benchmark, while the window-average TCDD intakes are closer to the NOAEL benchmark. Considering background DLC intake, the window-average TEQ intakes are considerably higher, the DLC exposures being larger than the TCDD intakes, themselves, but still below the LOAEL benchmark. The range of the TEQ P/W average of 0.01−0.031 ng/kg-day (see Table 4-10), however, straddles the RfD LOAEL benchmark of 0.02 ng/kg-day. Uncertainty in the NOAEL is similar to that for the LOAEL, depending on whether the 1st or 2nd quintile can be called a NOAEL. Although the response in the 2nd quintile is not significant compared to the 1st quintile, the NOAEL determination is complicated by the lack of an absolute measure of ~~"~~normal."

The NOAELs and LOAELs for altered sex ratio reported by Mocarelli et al. (2000) span their respective RfD POD benchmarks and are above the benchmarks when considering the peak/window exposure averages or background DLC exposures. The uncertainties for lack of an identifiable critical exposure window also apply to this endpoint. The other two endpoints, age

at menarche (Warner et al., 2004) and ovarian function (Warner et al., 2007), are unbounded NOAELs at the highest exposures. The ovarian function endpoint also is uncertain for lack of an identifiable critical exposure window.

Additional uncertainties not covered explicitly in this analysis include exposure to other AhR agonists, either naturally occurring in food-stuffs (Connor et al., 2008) or by-products of combustion or manufacturing processes (e.g., poly-aromatic hydrocarbons), and choice of uncertainty factor. As a final note on background DLC exposure, the background DLC intake estimates for the standard scenario (Needham) used in this assessment are somewhat crude, in that they are simple multiples of modeled TCDD intake based on an approximation of the proportion of TCDD to total TEQ. TCDD exposures are modeled over durations of up to 35 years (1941−1976) using a single fixed background intake term (a model limitation). However, background TCDD/TEQ exposures are thought to have varied widely over that time period, increasing gradually in the United States from the early 20th century to a peak in 1965, then decreasing rapidly to near current levels in the early 1980s (Lorber, 2002). Based on a digitization of Figure 6 in Lorber (2002), depicting the estimated TEQ intake over the course of the 20th century, a time-weighted average total TEQ intake for the period 1941−1976 of 4.6×10^{-3} ng/kg-day can be estimated. Adjusting the TEF_{98}-based Lorber (2002) TEQ intakes to TEF_{05}-based values, assuming a 10% TCDD fraction and adjusting the TEFs from 1998 to 2005 (see Appendix F, Section F.1.2.1), yields a DLC-TEQ intake estimate of 3.4×10^{-3} ng/kg-day for that time period, which is similar to the estimated DLC background intake of 3.33×10^{-3} ng/kg-day for the standard scenario using the simple scaling model.

However, the DLC intake estimate based on Lorber (2002) is somewhat of an underestimate because it does not include dioxin-like PCBs. Pinsky and Lorber (1998) estimated a TCDD intake of 4×10^{-4} ng/kg-day for the U.S. population in the 1970s, which is almost the same as the modeled TCDD background intake for the Seveso population. However, there is no information on comparative environmental exposures for the United States and Italy during this period, and TCDD exposures before 1970 for these populations were not necessarily the same, on average. Higher TCDD background exposures have been estimated by others. Pinsky and Lorber (1998) estimated an average TCDD-only intake of 1.4×10^{-3} to 1.9×10^{-3} ng/kg-day for the U.S. population in the late 1960s and early 1970s using a 1st-order kinetics model with a variable intake term and a TCDD half-life of 7.1 years. Aylward and Hays (2002) estimated a

TCDD intake of at least 1.3×10^{-3} ng/kg-day for the United States, Canada, Germany, and France prior to 1972 using a 1st-order kinetics model assuming a TCDD half-life of 7.5 years. These estimates are 3.5−5 times higher than the background TCDD intake estimated by EPA using the Emond PBPK model for this assessment. Total TEQ background would increase proportionally. However, none of these estimates, including EPA's, is based on actual intake measurements and are all dependent on modeling assumptions. Raising the background DLC exposure would obviously increase the effective PODs. However, increasing the background TCDD intake for modeling purposes would decrease the contribution of the actual TCDD exposures experienced by the Seveso population in 1976, resulting in a lower TCDD POD, as can be seen in the Eskenazi background scenario for Mocarelli et al. (2008) (see Figure 4-6).

This analysis highlights several important research needs. While the disposition of TCDD following high exposures is reasonably understood and simulated in current models, the current scientific understanding of disposition following TCDD exposures that are closer to current background dietary intakes, likely the primary source of TCDD exposure for most of the U.S. population, is not understood as well at present. This uncertainty affects the estimation of TCDD intake rates corresponding to the lower blood TCDD levels associated with LOAELs and NOAELs. The disposition of DLCs following exposures at background levels is similarly not well understood. Furthermore, there is uncertainty in the relationship of DLC tissue concentrations to oral intakes in the current TEF approach. Finally, there is toxicological uncertainty regarding several of the endpoints. Additional studies corroborating these outcomes and their toxicological significance would further increase their utility in refining the TCDD RfD.

Overall, EPA believes that the results of this analysis of alternative endpoints and PODs increase the confidence in the TCDD RfD, both qualitatively and quantitatively. EPA's analyses of some studies show POD estimates higher than the RfD PODs—primarily those analyses that consider background DLCs. Other analyses show POD estimates lower than the RfD POD, such as the use of alternative age-adjusted background TCDD/DLC intake rates and some evaluations of more uncertain endpoints (e.g., age at menopause endpoint in Eskenazi et al. (2005)). The more extreme values on the lower end are also the most uncertain, particularly with respect to the contribution of TCDD relative to total TEQ. In addition, except for the male reproductive effects in Mocarelli et al. (2011), determination of adversity for the lower LOAELs is problematic,

leading to lower confidence in the PODs. The TCDD and TEQ LOAELs for semen quality in males exposed in utero and by lactation (Mocarelli et al., 2011) are much lower than the corresponding LOAELs for males exposed between ages 1 and 10 years (Mocarelli et al., 2008). However, the NOAEL established for in utero and lactational exposure is fairly strong in the qualitative sense; that is, there is fairly clear indication that semen quality is unaffected at the corresponding dioxin exposure level. Quantitatively, there is more uncertainty, but considering background DLC exposure, the NOAEL is close to the RfD NOAEL benchmark.

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