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

October 2011

NOTICE

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Office of Research and Development
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ABSTRACT

This document comprises the first of two EPA reports (*U.S. EPA'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 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

2,4,5-T	2,4,5-trichlorophenoxyacetic acid
2,4-D	2,4-dichlorophenoxyacetic acid
AA	ascorbic acid
ACOH	acetanilide-4-hydroxylase
AHH	aryl hydrocarbon hydroxylase
AhR	aryl hydrocarbon receptor
AhR-/-	AhR-deficient
AIC	Akaike Information Criterion
ANL	Argonne National Laboratory
ANOVA	analysis of variance
APE	airborne particulate extract
ASAT	aspartate aminotransferase
AUC	area under the curve
bHLH-PAS	basic helix-loop-helix, Per-Arnt-Sim
B_{max}	equilibrium maximum binding capacity
BMD	benchmark dose
BMDL	benchmark dose lower confidence bound
BMDS	Benchmark dose software
BMI	body mass index
BMR	benchmark response
BPS	balanopreputial separation
BROD	benzyloxy resoufin-O-deethylase
b-TSH	blood thyroid-stimulating hormone
BW	body weight
C	cerebellum
CADM	concentration- and age-dependent elimination model
Cc	cerebral cortex
CI	confidence interval
CSAF	chemical-specific adjustment factor
CSLC	cumulative serum lipid concentration
Cx	connexin
CYP	cytochrome P450
D_a:HED	ratio of administered dose to HED
DEN	diethylnitrosamine
df	degrees of freedom
DLC	dioxin-like compound
DRE/XRE	dioxin/xenobiotic response elements
DRL	differential reinforcement of low rate
DSA	delayed spatial alteration
E_2	17β-estradiol
ED_x	effective dose eliciting x percent response
EGFR	epidermal growth factor receptor

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

EPA	Environmental Protection Agency
ER	estrogen receptor
EROD	7-ethoxyresorufin-O-deethylase
ERα	estrogen receptor alpha
EU	European Union
FFA	free fatty acid
FR	fixed ratio
FSH	follicle stimulating hormone
FT4	free thyroxine
GD	gestation day
GSH	glutathione stimulating hormone
GSH-Px	glutathione stimulating hormone peroxidase
GST	glutathione-*S*-transferase
H	hippocampus
HCH	hexachlorocyclohexane
HED	human equivalent dose
HQ	hazard quotient
HR	hazard ratio
Hsp90	heat shock protein 90
IARC	International Agency for Research on Cancer
IGF	insulin-like growth factor
IL	interleukin
ILSI	International Life Sciences Institute
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
KABS	oral absorption parameters
LASC	lipid-adjusted serum concentration
LD_{50}	lethal dose eliciting x percent response
LED	lower confidence effective dose
LED_x	lower bound of the 95% confidence interval on the dose that yields an x% effect
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{HED}$	HED estimate based on LOAELs
LOEL	lowest-observed-adverse level
MCH	mean corpuscular hemoglobin
MCMC	Markov Chain Monte Carlo
MCV	mean corpuscular volume
MOA	mode of action
MOE	margin of exposure
MROD	7-methoxyresorufin-O-deethylase
MTD	maximum tolerated dose
NAS	National Academy of Sciences
NIOSH	National Institute for Occupational Safety and Health

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NRC	National Research Council
NTP	National Toxicology Program
OR	odds ratio
OSF	oral slope factor
PA	permeability x area
PAI2	plasminogen activator inhibitor 2
PBMC	peripheral blood mononuclear cells
PBPK	physiologically based pharmacokinetic
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDF	polychlorinated dibenzofuran
PEPCK	phosphoenolpyruvate carboxykinase
PF	adipose tissue:blood partition coefficient
PHAH	polyhalogenated aromatic hydrocarbons
PK	pharmacokinetic
PND	postnatal day
POD	point of departure
pp	phosphotyrosyl protein
PRA	probabilistic risk assessment
PRE	body:blood partition coefficient
PROD	7-pentoxyresorufin-O-deethylase
RAR	retinoic acid receptor
REP	relative potency
RfC	reference concentration
RfD	reference dose
RL	reversal learning
RL	risk level
RR	rate ratios
RR	relative risk
RT-PCR	reverse transcription polymerase chain reaction
RXR	retinoid X receptor
S	saline
SA	superoxide anion
SAhRM	SRM for AhRs
S-D	Sprague-Dawley
SD	standard deviation
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SOD	superoxide dismutase
SRBC	sheep red blood cell
SSB	single-strand break

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

SWHS	Seveso Women's Health Study
T4	thyroxine
TBARS	thiobarbituric acid-reactive substances
TCB	3,3',4,4'-tetrachlorobiphenyl
TCDD	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
TCP	2,4,5-trichlorophenol
TEF	toxicity equivalence factor
TEQ	toxicity equivalence
TGFα	transforming growth factor α
TK	toxicokinetic
TNF-α	tumor necrosis factor alpha
TOTTEQ	total toxicity equivalence
TSH	thyroid stimulating hormone
TT4	total thyroxine
TWA	time-weighted average
U.S. NRC	U.S. Nuclear Regulatory Commission
UDP	uridine diphosphate
UDPGT	UDP-glucoronosyl transferase
UED	upper confidence bound for the effective dose
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
UGT	UDP-glucuronosyltransferase
UGT1	uridine diphosphate glucuronosyltransferase I
Vd	volume of distribution
WHO	World Health Organization

PREFACE

This draft 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 entitled, *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds* ("2003 Reassessment"), and, in 2004, EPA sent the 2003 draft dioxin reassessment to the NAS for their review. In 2006, NAS released the report of their review entitled, *Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment*. 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. 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 entitled *EPA'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 epidemiological 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, SAB held public meetings in June, July, and October 2010, and in March and June 2011.

SAB released their final 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 cocritical 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, 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 assessment into two portions, the noncancer assessment (Volume 1) and the cancer assessment and quantitative uncertainty analysis (Volume 2). This document, Volume 1, comprises the noncancer portion of *EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments*. After completing the noncancer portion, EPA will complete Volume 2.

This document, Volume 1, responds to the recommendations and comments on noncancer TCDD dose-response assessment included in the 2006 NAS report, focusing on the NAS comments regarding TCDD dose-response assessment. Volume 1 systematically evaluates the epidemiologic studies and rodent bioassays relevant to TCDD dose response. It uses a TCDD PBPK model to simulate TCDD blood concentrations, the dose metric used in all dose-response analyses for TCDD. 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 Reanalysis Volume 2.

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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. Additionally, 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).

The EPA is committed to the development of health assessment information of the highest scientific integrity for use in protecting human health and the environment. 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 its comprehensive human health assessment external

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

review draft entitled, *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds* ("2003 Reassessment") (U.S. EPA, 2003).

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 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 states that EPA will release a draft report that responds to the recommendations and comments included in the NAS review of EPA's 2003 Reassessment, and that, in this draft report, EPA's National Center for Environmental Assessment, Office of Research and Development, will provide a limited response to key comments and recommendations in the NAS report.

As required in the Science Plan, in 2009, EPA developed a draft report titled *EPA'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 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).

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

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 cocritical studies whose developmental toxicity data were used to derive the RfD for TCDD;
- They agreed with EPA's evaluation of TCDD carcinogenicity (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

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.

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

In August 2011, EPA announced a plan for moving forward to complete the draft Reanalysis.[4] This plan included the completion and posting to the IRIS database of the noncancer portion of the draft Reanalysis separately followed soon thereafter by the completion and posting to the IRIS database of the cancer portion of the draft Reanalysis. As such, this current document is the first of two EPA reports (*U.S. EPA'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 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 these analyses 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. 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 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.

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

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 NAS (U.S. EPA, 2009b) (see Appendices B and J);
- 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 entitled *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, 2006).

PRELIMINARY ACTIVITIES UNDERTAKEN BY EPA TO ENSURE THAT THE REANLAYSIS VOLUMES 1 AND 2 REFLECTS 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 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. No data sets collected since October 2009 were 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, 2009b) (Appendix B). Some of the key outcomes from the workshop include the following recommendations:

- to further develop study selection criteria for evaluating the suitability of developing dose-response models based on animal bioassays and human epidemiologic studies;
- to 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;

- to consider newer human or animal bioassay (NTP, 2006a) publications when evaluating quantitative dose-response models for cancer;
- to 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 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 an RfD and a cancer OSF 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 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, information

concerning the latency period between TCDD exposure and the onset of the effect 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 included in EPA's TCDD dose‑response analysis.

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 were provided. Only studies meeting all of the criteria were included in 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. Final results of the study selection process for the epidemiologic studies are shown in Tables 2-1 and 2-2 (key cancer and noncancer studies, respectively) and for the animal bioassays are shown in Tables 2-3 and 2-4 (cancer and noncancer studies, respectively). Through this study selection process, EPA was able to identify a group of studies for TCDD dose-response evaluation that spanned across

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.

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 epidemiologice 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 transpartent 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.

USE OF KINETIC MODELING TO ESTIMATE TCDD DOSES

NAS recommended that EPA utilize state-of-the-science approaches to finalize the 2003 Reassessment. Although NAS concurred with EPA's use of first-order body burden models in the 2003 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 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 at the end of the model simulations by multiplying whole-blood concentrations by a 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 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, provided the duration of the experiment is at least 1 month, due to limitations in the Aylward et al. (2005a) model. 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.

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[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).

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. Additionally, 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, and models the blood compartment directly in the rat, mouse, and human; there are also gestational and life-time nongestational forms of the Emond et al. (2006) model. In this document, EPA chose the Emond rodent physiologically-based pharmacokinetic (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 yield observed oral bioavailability of TCDD (Poiger and 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 epidemiological 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 (*h* in Eq. 3-20, Section 3.3.4.3.2.2). Other sensitive 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 sensitive. 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 sensitive 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 exists 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.

For each noncancer epidemiologic study that EPA selected as key, 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 EPA then eliminated 13 studies from further analysis with both a human equivalent dose (HED) $LOAEL_{HED}$ >1 ng/kg-day and a $NOAEL_{HED}/BMDL_{HED}$ >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[6] for each study, to which appropriate uncertainty factors (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[7] 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

[6] In the standard order of consideration: BMDL, NOAEL, and LOAEL.
[7] However, studies with a lowest dose tested greater than 30 ng/kg-day were not included in the expanded evaluation.

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, given that the underlying epidemiologic and animal bioassay data are of comparable quality. This preference for epidemiologic study data also is consistent with recommendations of panelists at the Dioxin Workshop (U.S. EPA, 2009b) (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 co-principal 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 for TCDD. In addition, by using PODs derived from human data, the uncertainty of interspecies extrapolation is eliminated. The study subjects included infants (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[8] 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 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) mean TCDD LASC of 68 ppt, corresponding to decreased sperm concentrations (20%) and decreased motile sperm counts (11%) 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. 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 cut off for normal fertility and that sperm concentrations between 15 million sperm/mL and

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

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 co-principal 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 ($10^{.5}$) 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 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 principal 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.[9]

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 (e.g., those PODs that consider background DLCs), 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 these outcomes and their toxicological significance would further increase their utility in refining the TCDD RfD.

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

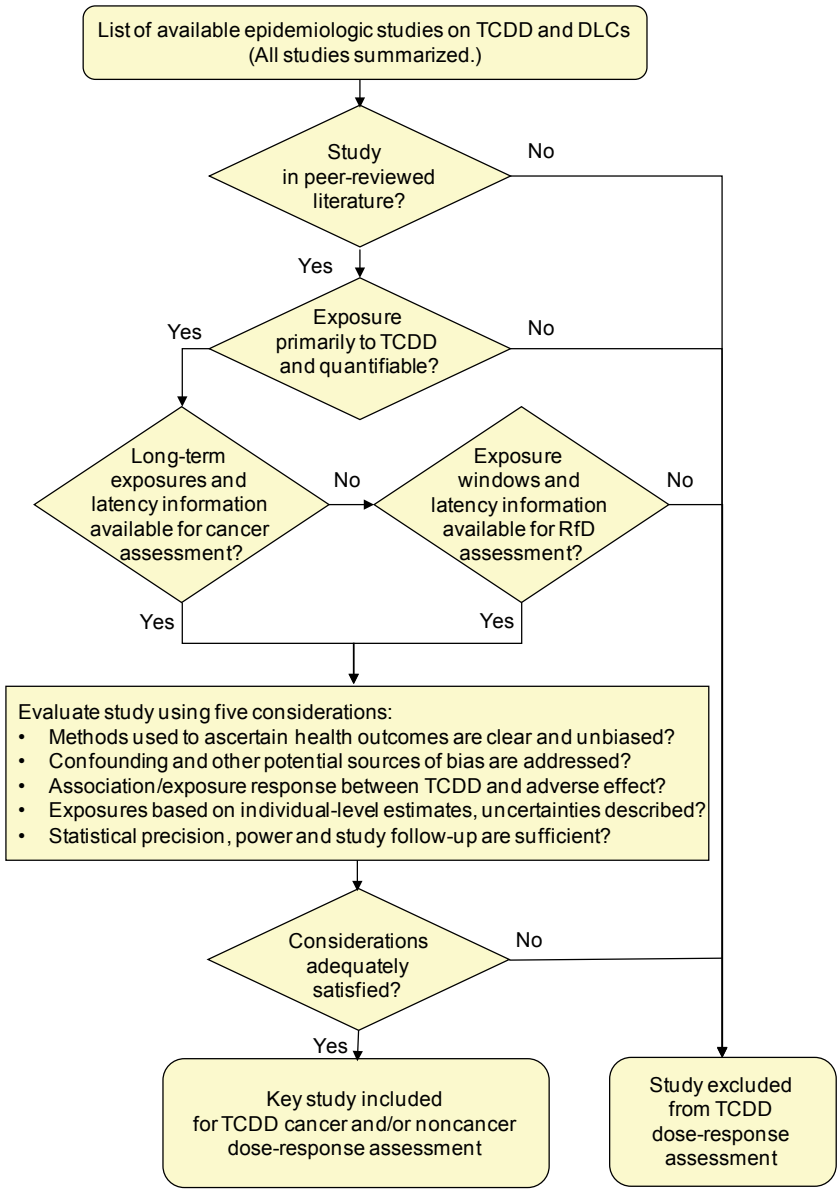


Figure ES-1. EPA's selection process to evaluate available epidemiologic studies using study inclusion criteria and other epidemiologic considerations for use in the dose-response analysis of TCDD.

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 included in EPA's TCDD dose-response analysis.

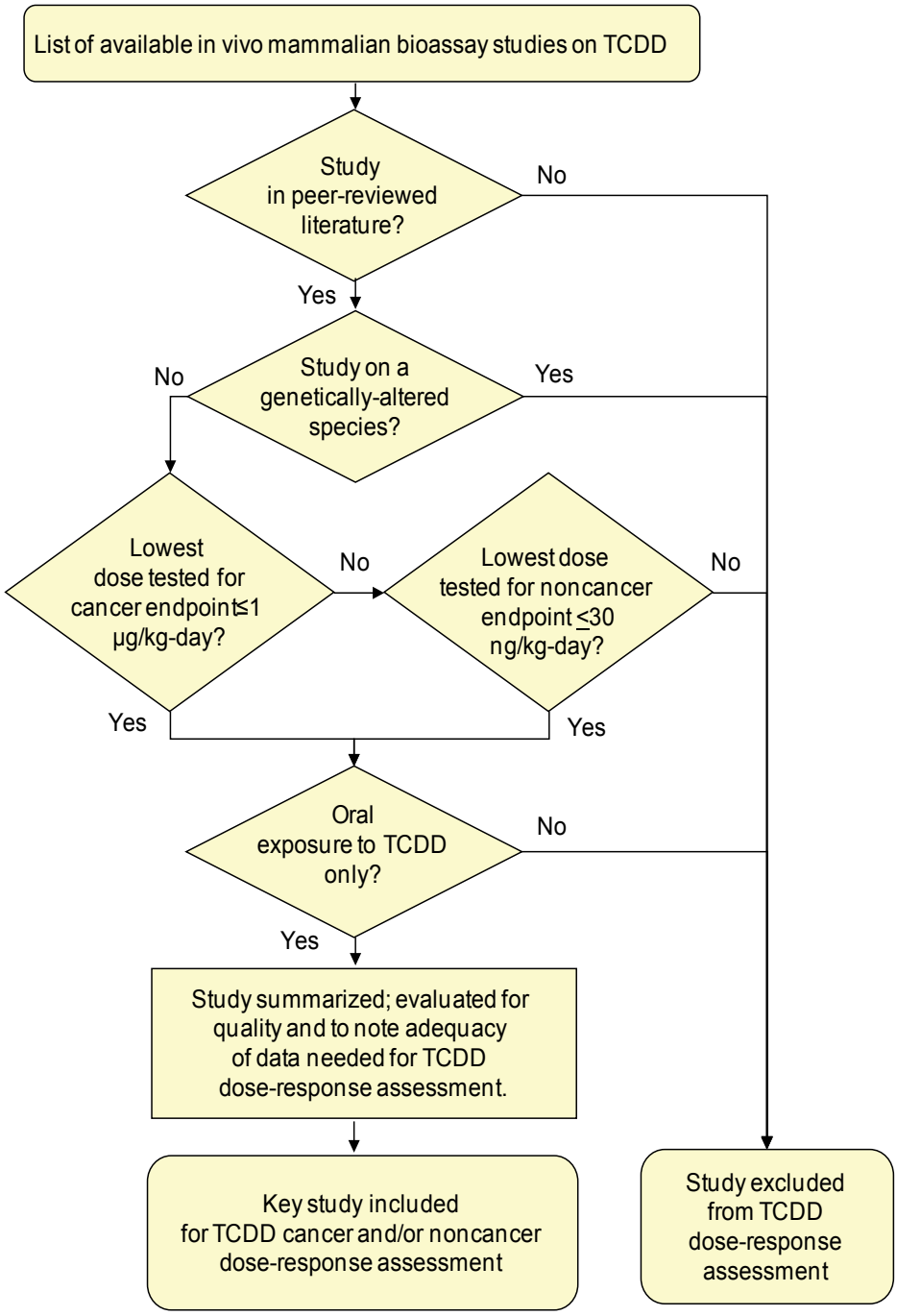
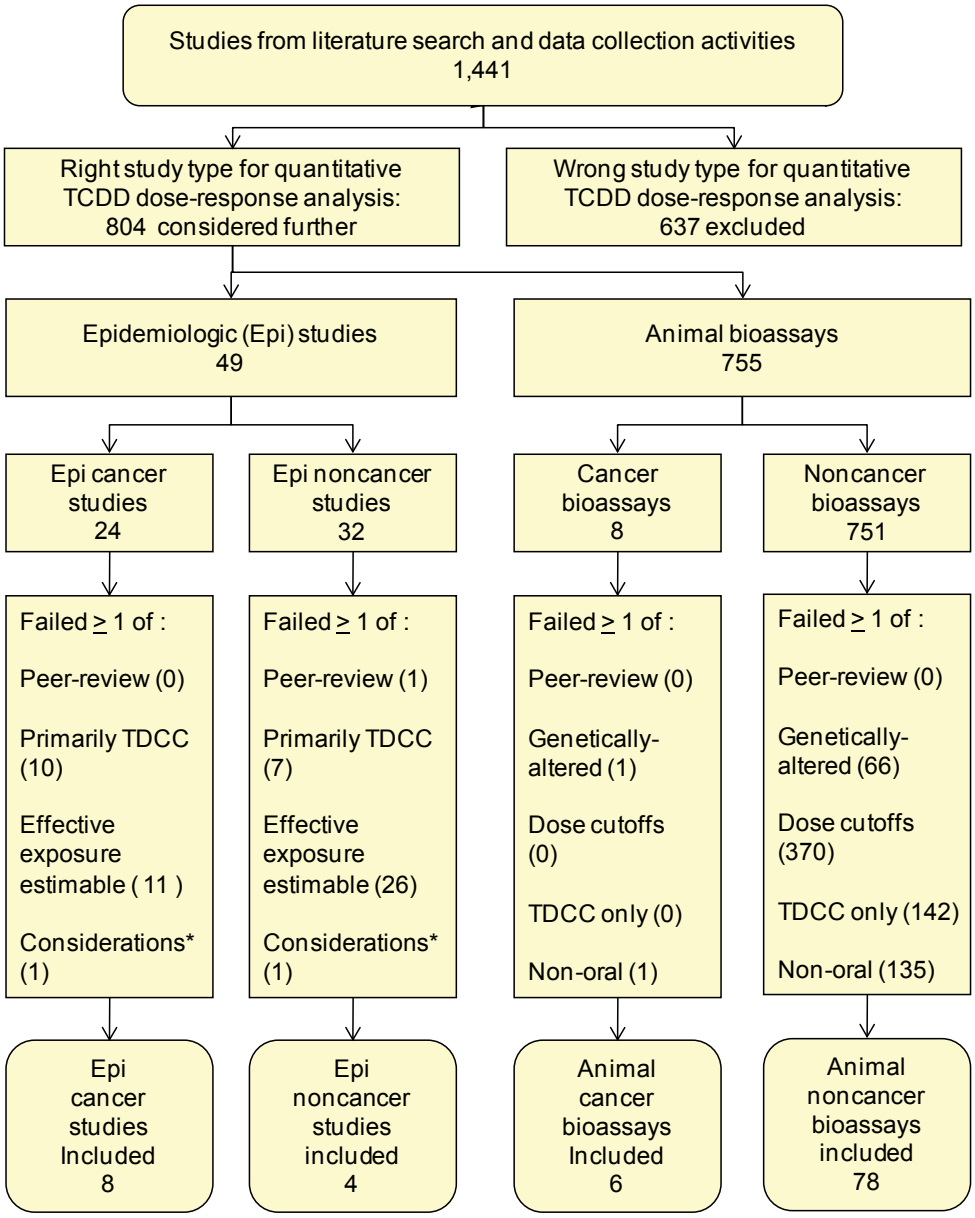


Figure ES-2. EPA's process to evaluate available animal bioassay studies using study inclusion criteria for use in the dose-response analysis of TCDD.

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 included in EPA's TCDD dose-response analysis.



*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.

Criteria not met are not mutually exclusive. 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.

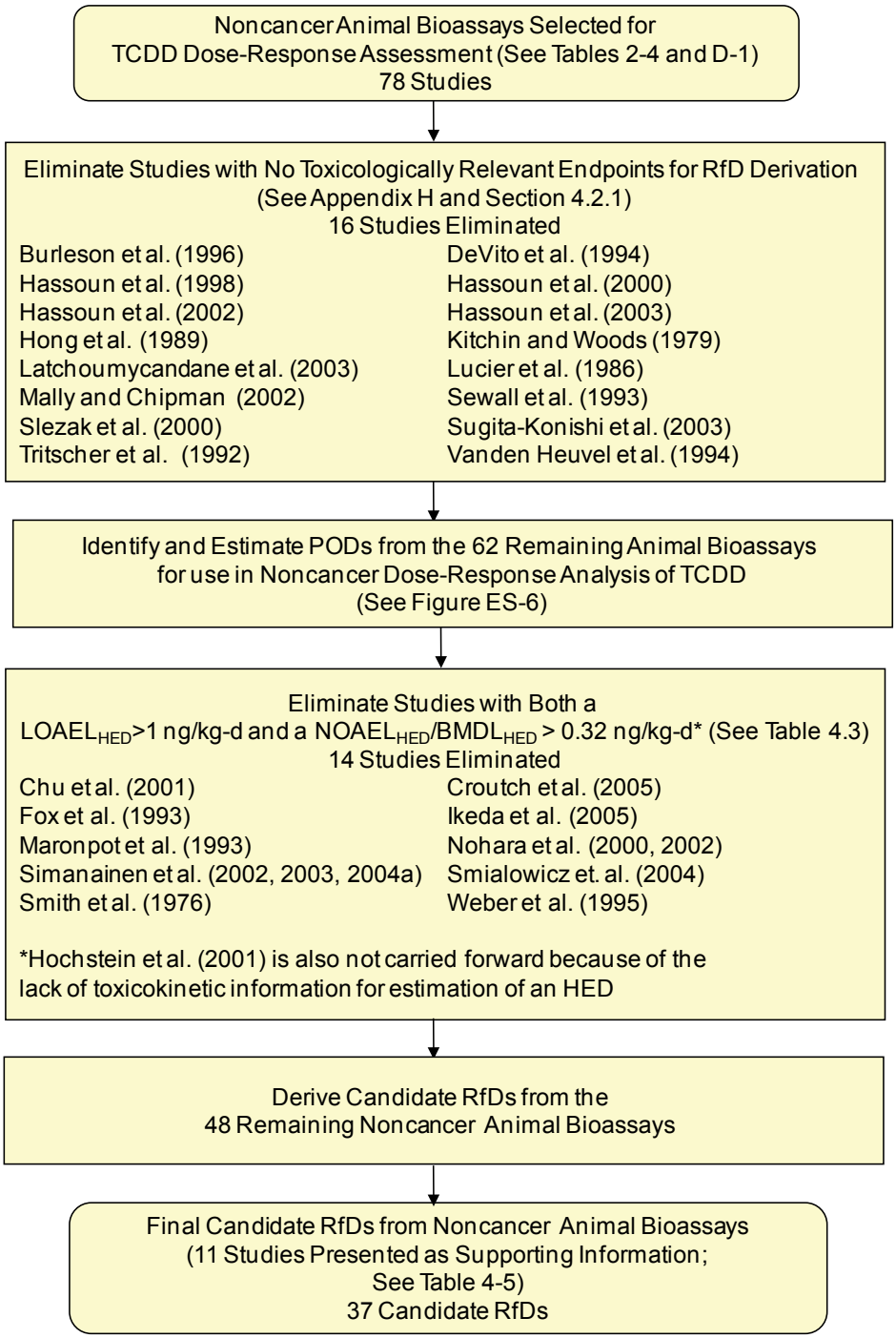


Figure ES-4. Disposition of animal noncancer bioassays selected for TCDD dose-response analysis.

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.

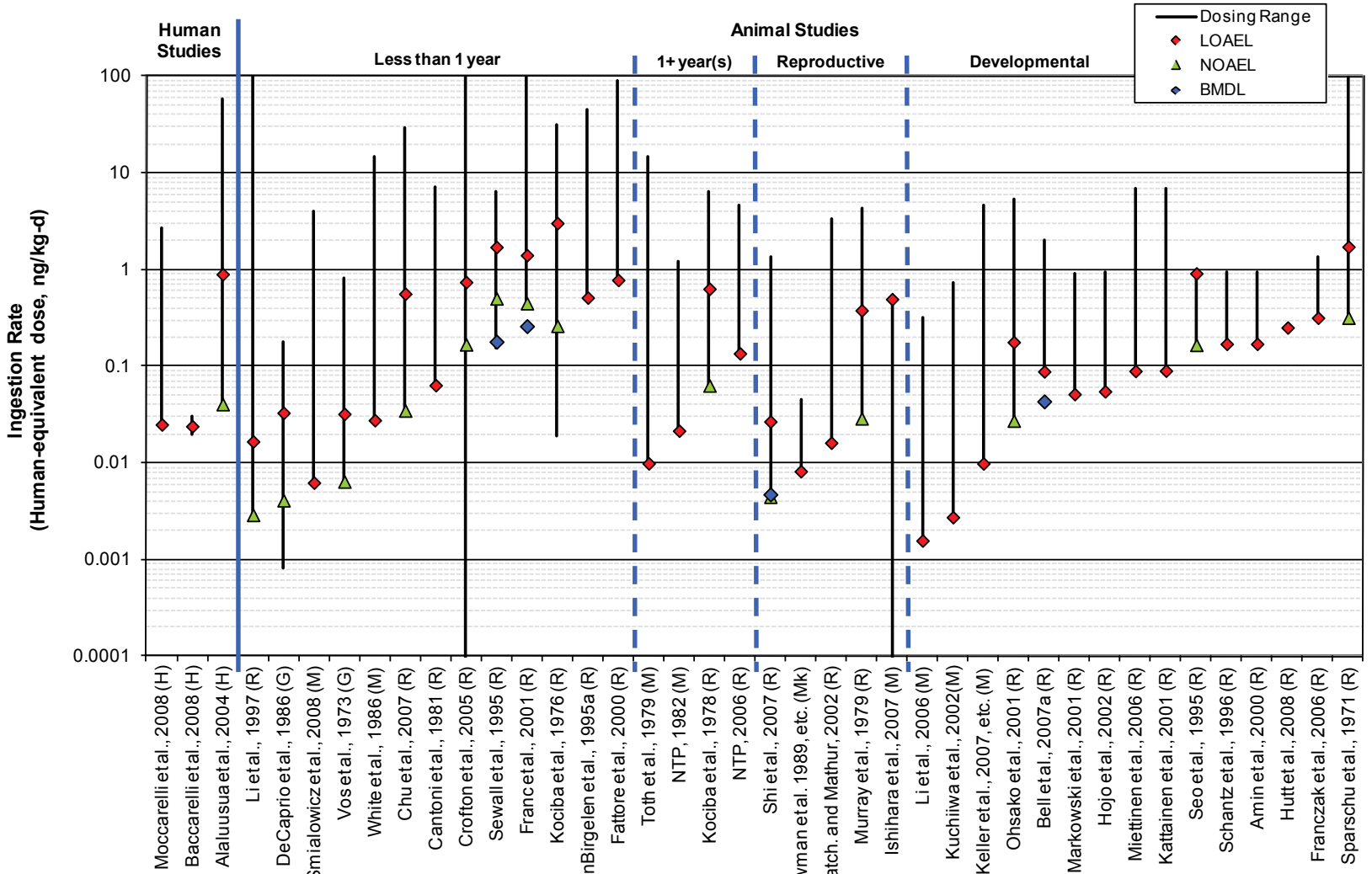


Figure ES-5. Exposure-response array for ingestion exposures to TCDD.

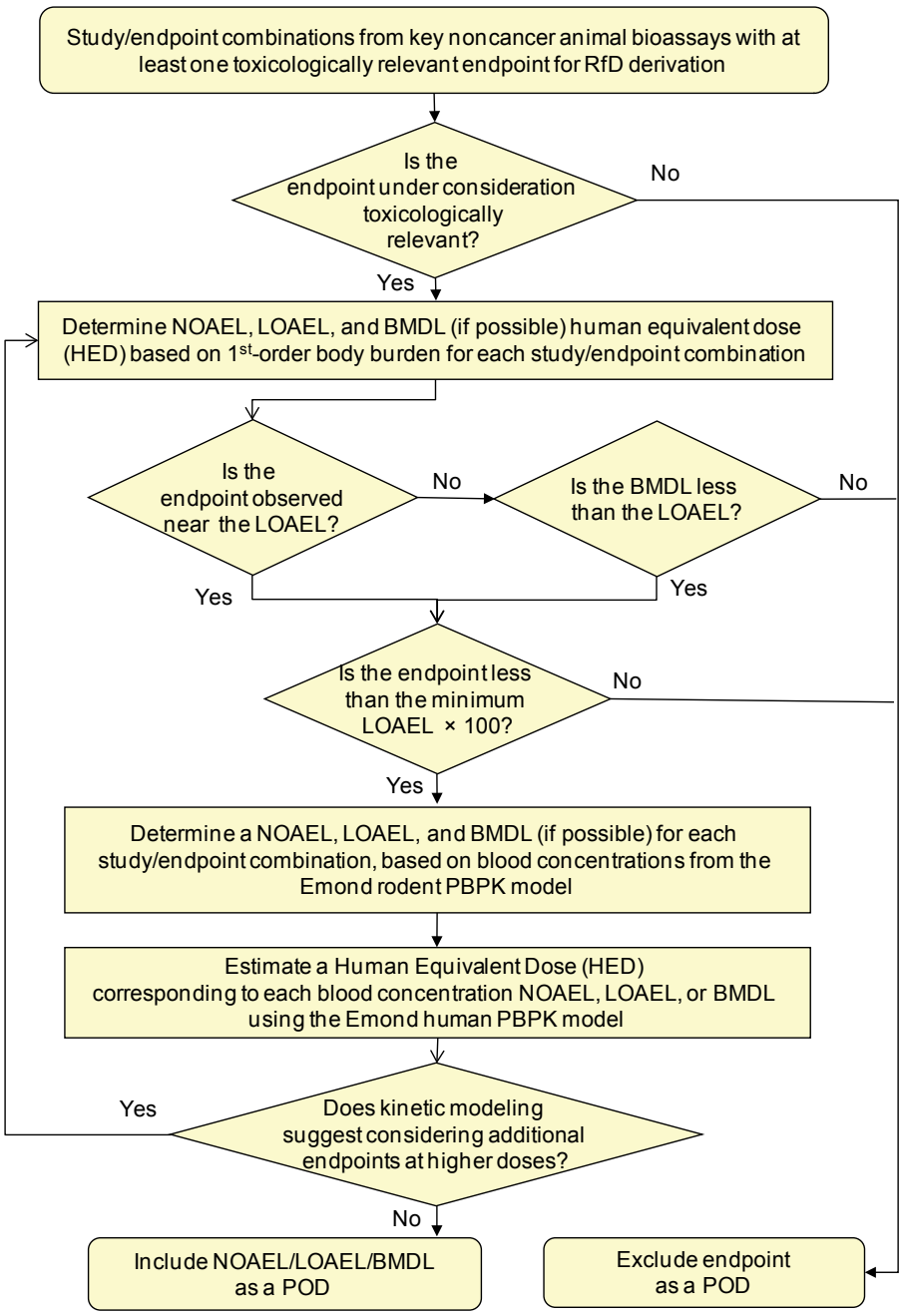


Figure ES-6. EPA's process to identify and estimate PODs from key animal bioassays for use in noncancer dose-response analysis of TCDD.
For the studies with at least one toxicologically relevant endpoint, EPA first determined if each endpoint was toxicologically relevant. If so, EPA determined the NOAEL, 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.

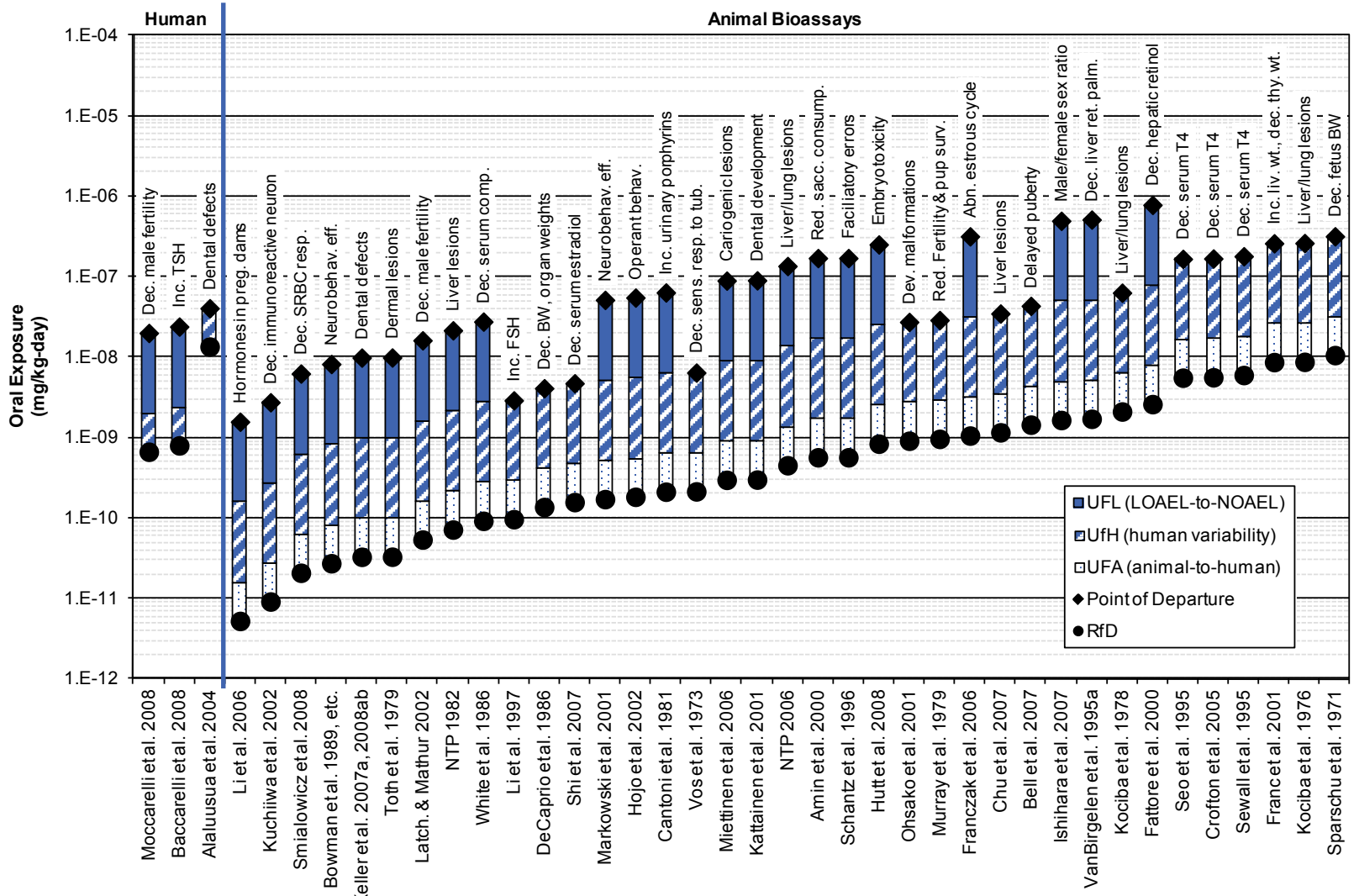


Figure ES-7. Exposure-response array for ingestion exposures to TCDD.