



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

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ABSTRACT

This draft report details EPA's technical response to the key comments and recommendations included in the 2006 NAS report, "Health Risks from Dioxin and Related Compounds: Evaluation of the EPA Reassessment," focusing on the NAS comments regarding TCDD dose-response assessment. After systematically evaluating the epidemiologic studies and rodent bioassays on TCDD, this draft report utilized a TCDD physiologically-based pharmacokinetic model to simulate TCDD blood concentrations, the dose metric used in the dose-response analyses. The draft report develops an oral reference dose (RfD) of 7×10^{-10} mg/kg-day based on two epidemiologic studies that associated TCDD exposures with decreased sperm concentration and sperm motility in men who were exposed during childhood (Mocarelli et al., 2008, [199595](#)) and increased thyroid-stimulating hormone levels in newborn infants (Baccarelli et al., 2008, [197059](#)). EPA also classifies TCDD as carcinogenic to humans, based on numerous lines of evidence, including primarily: multiple occupationally- and accidentally-exposed epidemiologic cohorts showing an association between TCDD exposure and certain cancers or increased mortality from all cancers and extensive evidence of carcinogenicity at multiple tumor sites in both sexes of multiple species of experimental animals. Based on a cancer mortality analysis of an occupational cohort (Cheng et al., 2006, [523122](#)), EPA also develops an oral cancer slope factor of 1×10^6 per (mg/kg-day) when the target risk range is 10^{-5} to 10^{-7} . While this draft report provides limited sensitivity analyses of several steps in the cancer and noncancer dose-response assessment, it concludes that a comprehensive uncertainty analysis is infeasible at this time.

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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

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LIST OF ABBREVIATIONS AND ACRONYMS (continued)

EPA	Environmental Protection Agency
ER	estrogen receptor
EROD	7-ethoxyresorufin-O-deethylase
ER α	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

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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- <i>p</i> -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-pentoxoresorufin-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

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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- <i>p</i> -dioxin
TCP	2,4,5-trichlorophenol
TEF	toxicity equivalence factor
TEQ	toxicity equivalence
TGF α	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-glucuronosyl 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
V _d	volume of distribution
WHO	World Health Organization
ZS@Z	zero slope at zero dose

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). Sections of the report, including Section 6 and the updated literature search, were developed through a collaborative effort between NCEA and the Department of Energy's Argonne National Laboratory (ANL).

In 2003, EPA, along with other federal agencies, asked the National Academy of Sciences (NAS) to review aspects of the science in EPA's draft dioxin reassessment entitled, "Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) and Related Compounds," and, in 2004, EPA sent the 2003 draft dioxin reassessment to the NAS for their review. In 2006, the NAS released the report of their review entitled, "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 substantial improvement to support a more scientifically robust risk characterization. These three areas were: (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. This draft report details EPA's response to the key comments and recommendations included in the 2006 NAS report.

In 2008, prior to developing this draft report, EPA, in collaboration with 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.

This draft report provides a technical response to the 2006 NAS report. It utilizes a TCDD physiologically-based pharmacokinetic model in its development of dose-response analyses of TCDD toxicological and epidemiological literature. This draft report presents new analyses of both the potential cancer and noncancer human health effects that may result from exposures to TCDD. The draft report develops an oral reference dose (RfD) for TCDD. It also presents a new cancer oral slope factor. Federal agencies and White House offices have been provided an opportunity for review and comment on this draft report prior to its public release.

This draft dioxin report is being released for public comment and will also be provided to EPA's Science Advisory Board (SAB) for independent external peer review. The SAB will convene an expert panel composed of scientists knowledgeable about technical issues related to dioxins and risk assessment. The SAB is expected to hold their first public meeting on July 13–15, 2010.

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EXECUTIVE SUMMARY

OVERVIEW

The U.S. Environmental Protection Agency (EPA) is committed to the development of risk 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 risk assessment external review draft entitled, *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and Related Compounds* (U.S. EPA, 2003, [537122](#); "2003 Reassessment"). This current document, *EPA's Reanalysis of Key Issues Related to Dioxin Toxicity and Response to NAS Comments*, directly and technically responds to key comments and recommendations pertaining to TCDD dose-response assessment published by the NAS in their review (NAS, 2006, [198441](#)). This document only addresses issues pertaining to TCDD dose-response assessment.

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 as quickly as possible.¹ 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 (draft response). This draft response is to focus on dose-response issues raised by the NAS and include analyses of relevant new key studies. The draft response is to be provided for public review and comment and for independent external peer review by EPA's Science Advisory Board. Following completion of this report, EPA is to review the impacts of the response to comments report on its 2003 Reassessment.

¹Available at <http://www.epa.gov/dioxin/scienceplan>.

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1 This draft document comprises EPA’s report that responds both directly and technically
2 to the recommendations and comments on TCDD dose-response assessment included in the NAS
3 review of EPA’s 2003 Reassessment. Because new data are analyzed in this report and toxicity
4 values are derived, this document will follow the IRIS process for review, clearance and
5 completion; however, it is not a traditional IRIS document. Information developed in this
6 document is intended to not only respond to the NAS review, but also to expand EPA’s
7 knowledge of TCDD cancer and noncancer dose-response based on the most current literature,
8 existing methods, and adherence to EPA risk assessment guidance documents.

9 In addition to this document, three separate EPA activities address additional NAS
10 comments pertaining to toxicity equivalence factors (TEFs) and background exposure levels.
11 Information on the application of the dioxin TEFs is published elsewhere by EPA for both
12 ecological (U.S. EPA, 2008, [543774](#)) and human health (U.S. EPA, 2009, [192196](#)) risk
13 assessment. EPA does not directly address TEFs herein, but makes use of the concept of toxicity
14 equivalence (TEQ)² as applicable to the analysis of exposure dose in epidemiologic studies and
15 to discussions on the effect of background TEQ on TCDD dose response. Furthermore,
16 information on updated background levels of dioxin in the U.S. population has been recently
17 reported by EPA (Lorber et al., 2009, [543766](#)), addressing the NAS recommendations pertaining
18 to the assessment of human exposures to TCDD and other dioxins.

19 The NAS identified three key recommendations requiring substantial improvement to
20 support a scientifically robust characterization of human responses to exposures to TCDD.
21 These three key areas are (1) improved transparency and clarity in the selection of key data sets
22 for dose-response analysis, (2) further justification of approaches to dose-response modeling for
23 cancer and noncancer endpoints, and (3) improved transparency, thoroughness, and clarity in
24 quantitative uncertainty analysis. The NAS also encouraged EPA to calculate a Reference Dose
25 (RfD), and provided numerous specific comments on various aspects of EPA’s 2003
26 Reassessment. The three key recommendations specifically pertain to dose-response assessment
27 and uncertainty analysis. Therefore, EPA’s response to the NAS in this document is focused on

²Toxicity equivalence (TEQ) is the product of the concentration of an individual dioxin like compound in an environmental mixture and the corresponding TCDD TEF for that compound. These products are summed to yield the TEQ of the mixture.

1 these issues. EPA thoroughly considered the recommendations of the NAS and responds with
2 scientific and technical evaluation of TCDD dose–response data via:

- 3
- 4 • an updated literature search that identified new TCDD dose-response studies (see
5 Section 2);
- 6 • a kickoff workshop that included the participation of external experts in TCDD health
7 effects, toxicokinetics, dose-response assessment and quantitative uncertainty analysis;
8 these experts discussed potential approaches to TCDD dose-response assessment and
9 considerations for EPA’s response to NAS; a Workshop Report was developed
10 (U.S. EPA, 2009, [543757](#), see Appendix A);
- 11 • detailed TCDD-specific study inclusion criteria and processes for the selection of key
12 studies (see Section 2.3) and epidemiologic and animal bioassay data for TCDD
13 dose-response assessment (see Section 2.4.1, Appendix B, and Section 2.4.2,
14 respectively);
- 15 • kinetic modeling to quantify appropriate dose metrics for use in TCDD dose-response
16 assessment (see Section 3 and Appendices C and D);
- 17 • dose-response modeling for all appropriate noncancer and cancer data sets (see
18 Section 4.2/Appendix E and Section 5.2.3/Appendix F, respectively);
- 19 • thorough and transparent evaluation of the selected TCDD data for use in the derivation
20 of an RfD and an oral slope factor (OSF) (see Sections 4.2 and 5.2.3, respectively);
- 21 • the development of an RfD (see Section 4.3);
- 22 • the development of a revised OSF (see Section 5.3) with an updated cancer weight of
23 evidence determination for TCDD based on EPA’s 2005 *Cancer Guidelines* (U.S. EPA,
24 2005, [086237](#)) (see Section 5.1.2);
- 25 • consideration of nonlinear dose-response approaches for cancer, including illustrative
26 RfDs for cancer precursor events and tumors (see Section 5.2.3.4) ; and
- 27 • discussion of the feasibility and utility of quantitative uncertainty analysis for TCDD
28 dose-response assessment (see Section 6).

29

30 Each of the activities listed above is briefly described in this Executive Summary, and is
31 described in detail in the related sections of this document.

32

33 **PRELIMINARY ACTIVITIES UNDERTAKEN BY EPA TO ENSURE THAT THIS**
34 **TECHNICAL RESPONSE REFLECTS THE CURRENT STATE-OF-THE-SCIENCE**

35 As part of the development of this document, EPA undertook two activities that included
36 public involvement: an updated literature search and a scientific expert workshop. The adverse

1 health effects associated with TCDD exposures are documented extensively in epidemiologic
2 and toxicologic studies. As such, the database of relevant information pertaining to the
3 dose-response assessment of TCDD is vast and constantly expanding. Responding directly to the
4 NAS recommendation to use the most current and up-to-date scientific information related to
5 TCDD, EPA, in collaboration with Argonne National Laboratory (ANL), developed an updated
6 literature database of peer-reviewed studies on TCDD toxicity, including in vivo mammalian
7 dose-response studies and epidemiologic studies. An initial literature search for studies
8 published since the 2003 Reassessment was conducted to identify studies published between the
9 year 2000 and October 31, 2008. EPA published the initial literature search results in the Federal
10 Register in November 2008 and invited the public to review the list and submit additional
11 peer-reviewed relevant studies. Additional studies identified by the public and through
12 continued work on this response have been incorporated into the final set of studies for TCDD
13 dose-response assessment (updated through October 2009). EPA believes that the
14 implementation of this rigorous search strategy ensures that the most current and relevant studies
15 were considered for the technical response to NAS and TCDD dose-response assessment
16 included herein.

17 To assist in responding to the NAS, EPA, in collaboration with ANL, convened a
18 scientific expert workshop (“Dioxin Workshop”) in February 2009 that was open to the public.
19 The primary goals of the Dioxin Workshop were to identify and address issues related to the
20 dose-response assessment of TCDD and to ensure that EPA’s response to the NAS focused on
21 the key issues, while reflecting the most meaningful science. EPA and ANL assembled expert
22 scientists and asked them to identify and discuss the technical challenges involved in addressing
23 the NAS comments, discuss approaches for addressing these key recommendations, and to assist
24 in the identification of important published and peer-reviewed literature on TCDD. The
25 workshop was structured into seven scientific topic sessions as follows: (1) quantitative
26 dose-response modeling issues, (2) immunotoxicity, (3) neurotoxicity and nonreproductive
27 endocrine effects, (4) cardiovascular toxicity and hepatotoxicity, (5) cancer, (6) reproductive and
28 developmental toxicity, and (7) quantitative uncertainty analysis of dose-response. External
29 co-chairs (i.e., scientists who were not members of EPA or ANL) were asked to facilitate the
30 sessions and then prepare summaries of discussions occurring in each session. The session
31 summaries formed the basis of a final workshop report (U.S. EPA, 2009, [543757](#), Appendix A of

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1 this document). Some of the key outcomes from the workshop include the following
2 recommendations:

- 3
- 4 • to further develop study selection criteria for evaluating the suitability of developing
5 dose-response models based on animal bioassays and human epidemiologic studies;
 - 6 • to use kinetic modeling to identify relevant dose metrics and dose conversions between
7 test animal species and humans, and between human internal dose measures and human
8 intakes;
 - 9 • to consider newer human or animal (e.g., NTP, 2006, [197605](#)) publications when
10 evaluating quantitative dose-response models for cancer;
 - 11 • to consider both linear and nonlinear modeling in the cancer dose-response analysis.
- 12

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

15

16 **EPA’S APPROACH TO CONSIDERING TRANSPARENCY AND CLARITY IN THE**
17 **SELECTION OF KEY STUDIES AND DATA SETS FOR DOSE-RESPONSE**
18 **MODELING**

19 One of the key NAS recommendations to EPA was to utilize a clear and transparent
20 process for the selection of key studies and data sets for dose-response assessment. EPA agrees
21 with the NAS and believes that clear delineation of the study selection process and decisions
22 regarding key studies and data sets will facilitate communication of critical decisions made in the
23 TCDD dose-response assessment. EPA developed detailed processes and TCDD-specific
24 criteria for the selection of key dose-response studies. These criteria are based on common
25 practices and current guidance for point of departure (POD) identification and RfD and OSF
26 derivation and also consider issues specifically related to TCDD. Following the selection of key
27 studies, EPA employed additional processes to further select and identify cancer and noncancer
28 datasets from these key studies for use in dose-response analysis of TCDD.

29 For the study evaluation and key data set selection, EPA has undertaken different
30 approaches for the epidemiologic and in vivo animal bioassay studies. The significant
31 differences between animal and human health effects data and their use in EPA risk assessment
32 support development of separate criteria for study inclusion and different approaches to study
33 evaluation. For the vast majority of compounds on EPA’s Integrated Risk Information System

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1 (IRIS, U.S. EPA, 2009, [192196](#)), cancer and noncancer toxicity values have been derived using
2 animal bioassay data; thus, some of the TCDD-specific study inclusion criteria for animal
3 bioassay data are based on EPA's common practices and guidance for POD selection and RfD
4 and OSF derivation. Far fewer IRIS toxicity values have been derived from human data,
5 although some examples do exist.³ The modeling and interpretation of such human data have
6 been conducted on a case-by-case basis because each cohort is uniquely defined and has its own
7 set of exposure conditions, significant confounders, and biases that may need to be considered in
8 dose-response modeling.

9 Figure ES-1 presents EPA's study evaluation process for the epidemiologic studies
10 considered for this TCDD dose-response assessment, including specific study inclusion criteria
11 (see Section 2.3.1). EPA applied TCDD-specific epidemiologic study inclusion criteria to all
12 epidemiologic studies published on TCDD and dioxin-like compounds (DLCs) that had been
13 identified in the TCDD literature database (see Section 2.4.1, Appendix B). The studies were
14 initially evaluated using five considerations (see Figure ES-1) that provide the most relevant
15 kinds of information needed to consider the feasibility of quantitative human health risk
16 analyses. Then EPA required that the studies meet three study inclusion criteria: 1) the study is
17 published in the peer-reviewed scientific literature and includes an appropriate discussion of
18 strengths and limitations; 2) the exposure is primarily to TCDD, rather than dioxin-like
19 compounds (DLCs), and is properly quantified so that dose-response relationships can be
20 assessed; and 3) the effective dose and oral exposure must be reasonably estimable. To meet the
21 third criterion, information is required on long-term exposures for cancer, and, for noncancer,
22 information is required regarding the appropriate time window of exposure that is relevant for a
23 specific, nonfatal health endpoint. Therefore, the study should include an appropriate latency
24 period between TCDD exposure and the onset of the effect. Only studies meeting these
25 three criteria were included in EPA's TCDD dose-response analyses (see Section 2.4.3).

26 Figure ES-2 presents EPA's study evaluation process for mammalian bioassays
27 considered for TCDD dose-response assessment, including the specific study inclusion criteria
28 (see Section 2.3.2). EPA applied TCDD-specific in vivo mammalian bioassay study inclusion

³ Examples of toxicity values on IRIS from human data include benzene, beryllium and compounds, chromium IV, and 1,3-butadiene that have RfDs, Reference Concentrations, Inhalation Unit Risks and/or OSFs all based on occupational cohort data and the methyl mercury RfD that is based on high fish consuming cohorts (U.S. EPA, 2009, [192196](#)).

1 criteria to all of the bioassay studies of TCDD that had been identified in the TCDD literature
2 database (see Section 2.4.2). After ascertaining that a study had been published in the
3 peer-reviewed literature, EPA applied dose requirements to the lowest tested average daily doses
4 in each study, with specific requirements for cancer ($\leq 1 \mu\text{g}/\text{kg}\text{-day}$) and noncancer
5 ($\leq 30 \text{ ng}/\text{kg}\text{-day}$) studies to ensure that only low-dose TCDD bioassays would be considered for
6 quantitative assessment. These dose requirements were used to eliminate those studies that
7 would not be selected for development of an RfD or an OSF because the lowest doses tested
8 were too high relative to other TCDD bioassays. EPA also required that the bioassays exposed
9 animals via the oral route to TCDD only and that the purity of TCDD was specified. Finally, the
10 studies were evaluated using four considerations (see Figure ES-2) regarded as providing the
11 most relevant information for development of quantitative human health risk analyses from
12 animal bioassay data. Only the bioassay studies meeting these criteria and considerations were
13 included in EPA's TCDD dose-response analyses (see Section 2.4.3).

14 Applying the study inclusion criteria for both epidemiologic and mammalian bioassay
15 datasets resulted in a list of key noncancer and cancer studies that were considered for
16 quantitative dose-response analyses of TCDD. Endpoints from these studies that were not
17 considered to be toxicologically relevant were eliminated from consideration (see Section 4.2.1,
18 Appendix G). The study/endpoint dataset combinations from the remaining studies were then
19 subjected to dose-response assessment, and PODs for use in developing RfDs or OSFs were
20 identified. PODs included no-observed-adverse-effect levels (NOAELs), lowest-observed-
21 adverse-effect levels (LOAELs) or lower bound benchmark dose levels (BMDLs). The most
22 sensitive PODs were selected as candidates for derivation of the RfD and OSF.

23

24 **USE OF KINETIC MODELING TO ESTIMATE TCDD DOSES**

25 NAS recommended that EPA utilize state-of-the-science approaches to finalize the
26 2003 Reassessment. Although NAS concurred with EPA's use of first-order body burden
27 models in the 2003 Reassessment, analyses of recent TCDD literature and comments by experts
28 at the Dioxin Workshop suggested that the understanding of TCDD kinetics had increased
29 significantly since the release of EPA's 2003 Reassessment. These advances led to the
30 development of several pharmacokinetic models for TCDD (Aylward et al., 2005, [197114](#); e.g.,

1 Emond et al., 2004, [197315](#); Emond et al., 2005, [197317](#); Emond et al., 2006, [197316](#)) and
2 resulted in EPA's incorporation of TCDD kinetics in the dose-response assessment of TCDD.

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

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

24 EPA considered the following possible dose metrics for TCDD: administered dose,
25 first-order body burden, lipid-adjusted serum concentration (LASC), whole blood concentration,
26 tissue concentration, and functional-related metrics of relevance to the mode of action (MOA)
27 (e.g., receptor occupancy) (see Section 3.3.4.1). After careful evaluation of these dose metrics,
28 EPA chose to use TCDD concentration in whole blood as the dose metric for assessing TCDD
29 dose response in this document. Although LASC is generally considered to be the most relevant
30 metric, whole blood concentration was chosen because of the structure of the PBPK models, in
31 which the target tissue compartments are connected to the whole blood compartment rather than

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1 to the serum compartment; LASC is related to whole blood by a scalar, so use of either is
2 equivalent in the model. Whole blood concentrations also reflect TCDD dose to target tissues
3 and, are biologically-relevant measures of internal dose. EPA used the time-weighted average
4 whole-blood concentration over the relevant exposure periods for all continuous dosing
5 protocols, dividing the area under the time-course concentration curve (AUC) by the exposure
6 duration.⁴

7 Several biologically-based kinetic models for TCDD exist in the literature. The more
8 recent pharmacokinetic models explicitly characterize the concentration-dependent elimination
9 of TCDD (Carrier et al., 1995, [197618](#); Carrier et al., 1995, [543780](#); Emond et al., 2004, [197315](#);
10 Emond et al., 2005, [197317](#); Emond et al., 2006, [197316](#); Aylward et al., 2005, [197114](#)). The
11 biologically-based pharmacokinetic models describing the concentration-dependent elimination
12 (i.e., the pharmacokinetic models of Aylward et al. (2005, [197114](#)) and Emond et al. (2005,
13 [197317](#); 2006, [197316](#)) are relevant for application to simulate the TCDD dose metrics in
14 humans and animals exposed via the oral route. The rationale for considering the application of
15 the Aylward et al. (2005, [197114](#)) and Emond et al. (2004, [197315](#); 2005, [197317](#); 2006,
16 [197316](#)) models was largely based on the fact that both models reflect research results from
17 recent peer-reviewed publications, and both models are formulated with dose-dependent hepatic
18 elimination consistent with the physiological understanding of TCDD kinetics. Dose-response
19 modeling based on body burden of TCDD in adult animals and humans can be conducted with
20 either of the models, provided the duration of the experiment is at least one month, due to
21 limitations in the Aylward et al. (2005, [197114](#)) model. The predicted slope and body burden
22 over a large dose range are quite comparable between the two models (generally within a factor
23 of two).

24 Results of simulations of serum lipid concentrations or liver concentrations vary for the
25 two models to a larger extent (up to a factor of 7), particularly for simulations of short duration.
26 These differences reflect two characteristics of the Emond et al. (2006, [197316](#)) model: first,
27 quasi-steady-state is not assumed in the Emond et al. (2006, [197316](#)) model; second, the serum
28 lipid composition used in the model is not the same as the adipose tissue lipids. The Aylward

⁴For the Seveso cohort, which had a high single exposure followed by low-level background exposures leading to a gradual decline in the internal TCDD concentrations, EPA estimated dose as the mean of the peak exposure and the average exposure over a defined critical exposure window (see Section 4.2.2).

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1 et al. (2005, [197114](#)) model does not account for differential solubility of TCDD in serum lipids
2 and adipose tissue lipids, nor does it account for the diffusion-limited uptake by adipose tissue.
3 Based on this evaluation, EPA determined that the Emond et al. (2006, [197316](#)) model
4 performed better than the Aylward et al. (2005, [197114](#)) model with respect to the ability to
5 simulate serum lipid and tissue concentrations during exposures that do not lead to the onset of
6 steady-state condition in the exposed organism. Additionally, of the two selected models, the
7 pharmacokinetic model developed by Emond et al. (2006, [197316](#)) is more
8 physiologically-based, as compared to the Aylward et al. (2005, [197114](#)) model, and models the
9 blood compartment directly in the rat, mouse, and human; there are also gestational and life-time
10 nongestational forms of the Emond et al. (2006, [197316](#)) model. In this document, EPA chose
11 the Emond rodent physiologically-based pharmacokinetic (PBPK) model to estimate blood
12 TCDD concentrations based on administered doses (see Section 3.3.4, Appendix C).

13 To enhance the biological basis of the PBPK model of Emond et al. (2006, [197316](#)),
14 three minor modifications, were made before its use in the computation of dose metrics for
15 TCDD: 1) recalculation of the volume of the “rest of the body compartment” after accounting for
16 volume of the liver and fat compartments; 2) calculation of the rate of TCDD excreted via urine
17 by multiplying the urinary clearance parameter by blood concentration in the equation instead of
18 by the concentration in the rest of the body compartment; and 3) recalibration for the human
19 gastric nonabsorption constant to yield observed oral bioavailability of TCDD (Poiger and
20 Schlatter, 1986) (see Section 3.3.4.4 for details). The modified PBPK model was evaluated
21 against all published data used in the original model. EPA assumed that the same blood TCDD
22 levels that led to effects in animals would also lead to effects in humans; therefore, the Emond
23 human PBPK model was used to estimate the lifetime average daily oral doses (consistent with
24 the chronic RfD and OSF) that would correspond to the blood TCDD concentrations estimated to
25 have occurred during the animal bioassays. EPA used the same Emond human PBPK model to
26 estimate the lifetime average daily doses that would correspond to the TCDD blood or tissue
27 concentrations reported in the epidemiological studies (Appendix D). These estimates are the
28 Human Equivalent Doses (HEDs) that are used to develop candidate RfDs and OSFs for TCDD.

29 Because TCDD elimination is inducible in the Emond model, ratios of daily averaged
30 intake to long-term blood concentrations are not linear. Because of the nonlinearity of blood
31 concentration and ingested dose in the Emond Human PBPK model, the cancer risk is only

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1 approximately linear with the TCDD blood concentration and low TCDD oral ingestion doses,
2 but is not linear with ingested TCDD at higher doses. Thus, to use these estimates in human
3 health risk assessment, risk-specific TCDD oral intake levels corresponding to the target risk
4 levels should be calculated (see Section 5.2.3.1.2.1).

6 **DERIVATION OF AN RfD FOR TCDD**

7 The NAS specifically recommended that EPA derive an RfD for TCDD. Through a
8 transparent study selection process, EPA identified key studies from both human epidemiologic
9 studies and animal bioassays. To select candidate PODs for its RfD methodology, EPA applied
10 additional processes to the key human epidemiologic studies and animal bioassays. Figure ES-3
11 (exposure-response array) shows the entire candidate PODs graphically in terms of
12 human-equivalent intake (ng/kg-day). The human study endpoints are shown at the far left of the
13 figure and, to the right, the rodent endpoints are arranged by the following study categories: less
14 than 1 year, greater than 1 year, reproductive, and developmental.

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

23 Through this process, EPA identified health effects from the following
24 four epidemiologic studies to be considered as the basis for the RfD: Eskenazi et al. (2002,
25 [197168](#))(reproductive—increased length of menstrual cycle), Alaluusua et al. (2004, [197142](#))
26 (developmental—tooth development), Mocarelli et al. (2008, [199595](#)) (reproductive—decreased
27 sperm concentrations and motility), and Baccarelli et al. (2008, [197059](#))
28 (developmental—increased thyroid-stimulating hormone levels in neonates). All four studies are
29 from the Seveso cohort, whose members were exposed environmentally to high peak
30 concentrations of TCDD as a consequence of an industrial accident. This complicated the
31 estimation of average daily doses associated with these specific endpoints, however EPA was

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1 able to calculate candidate PODs for derivation of an RfD from each of these human studies (see
2 Section 4.2.3). The Alaluusua et al. (2004, [197142](#)) and Eskenazi et al. (2002, [197168](#)) studies
3 had PODs well above the Mocarelli et al. (2008, [199595](#)) and Baccarelli et al. (2008, [197059](#));
4 because the LOAEL in Eskenazi et al. (2002, [197168](#)) is almost 2 orders of magnitude higher
5 than the LOAELs for Baccarelli et al. (2008, [197059](#)) and Mocarelli et al. (2008, [199595](#)), it was
6 not considered further as a candidate POD for derivation of the RfD.

7 Figure ES-4 summarizes the strategy employed for identifying and selecting candidate
8 PODs from the key animal bioassays EPA identified for use in noncancer dose-response analysis
9 of TCDD (see Section 4.2.4). For each noncancer endpoint, EPA first evaluated the
10 toxicological relevance of each endpoint, rejecting those judged not to be relevant for RfD
11 derivation (Section 4.2.1, Appendix G). Next, initial PODs (NOAELs, LOAELs, and BMDLs)
12 based on the first-order body burden metric, and expressed as continuous human-equivalent oral
13 daily doses (HEDs), were determined for all relevant endpoints.

14 Because there were very few NOAELs, and BMDL modeling was largely unsuccessful
15 due to data limitations, the next stage of evaluation was carried out using LOAELs only.
16 Endpoints not observed at the LOAEL (i.e., reported at higher doses) with BMDLs greater than
17 the LOAEL were eliminated from further analysis, as they would not be considered as candidates
18 for the final POD on either a BMDL or NOAEL/LOAEL basis (i.e. the POD would be higher
19 than the PODs of other relevant endpoints). In addition, all endpoints with HEDs for LOAELs
20 ($LOAEL_{HEDS}$) beyond a 100-fold range of the lowest identified $LOAEL_{HED}$ were eliminated
21 from further consideration, as they would not be potential POD candidates either (i.e. the POD
22 would be higher than the PODs of other relevant endpoints). For the remaining endpoints, EPA
23 then determined final potential PODs (NOAELs, LOAELs, and BMDLs) based on TCDD blood
24 concentrations obtained from the Emond rodent PBPK models. HEDs were then estimated for
25 each of these PODs using the Emond human PBPK model. From these HEDs, a POD_{HED} was
26 selected for each study as the basis for the candidate RfD, to which appropriate uncertainty
27 factors were applied following EPA guidelines. The resulting candidate RfDs were then
28 considered in the final selection process for the RfD. Other endpoints occurring at slightly
29 higher doses representing additional effects associated with TCDD exposure (beyond the
30 100-fold LOAEL range) were evaluated, modeled, and included in the final candidate RfD array

1 to examine endpoints not evaluated by studies with lower PODs. In addition, BMD modeling
2 based on administered dose was performed on all endpoints for comparison purposes.

3 For BMD modeling, EPA has used a 10% BMR for dichotomous data for all endpoints;
4 no developmental studies were identified with designs that incorporate litter effects, for which a
5 5% BMR would be used (U.S. EPA, 2000, [052150](#)). For continuous endpoints in this document,
6 EPA has used a BMR of 1 standard deviation from the control mean whenever a specific
7 toxicologically-relevant BMR could not be defined. Importantly, the 2003 Reassessment defined
8 the ED₀₁ as 1% of the maximal response for a given endpoint, not as a 1% change from control.
9 Because RfD derivation is one goal of this document, the noncancer modeling effort undertaken
10 here differs substantially from the modeling in the 2003 Reassessment. Evaluation of BMD
11 modeling performance, goodness-of-fit, dose-response data, and resulting BMD and BMDL
12 estimates included statistical criteria as well as expert judgment of their statistical and
13 toxicological properties. EPA has reported and evaluated the BMD results using the standard
14 suite of goodness-of-fit measures from the benchmark dose modeling software (BMDS 2.1).
15 These include chi-square *p*-values, Akaike's Information Criterion (AIC), scaled residuals at
16 each dose level and plots of the fitted models. In some cases, when restricted parameters hit a
17 bound, EPA used likelihood ratio tests to evaluate whether the improvement in fit afforded by
18 estimating additional parameters could be justified. Goodness-of-fit measures are reported for
19 all key data sets in Appendix E. (See Section 4.2.4.2 for a more complete description of the
20 benchmark dose modeling criteria for model evaluation.)

21 For selection of the POD to serve as the basis of the RfD, EPA gave the epidemiologic
22 studies the highest consideration because human data are preferred in the derivation of an RfD,
23 given that the underlying epidemiologic and animal bioassay data are of comparable quality.
24 This preference for epidemiologic study data also is consistent with recommendations of panelists
25 at the Dioxin Workshop (see U.S. EPA, 2009, [543757](#), Appendix A). Figure ES-5 arrays the
26 candidate RfDs from both the human and animal bioassays. The human studies included in
27 Figure ES-5 (Alaluusua et al., 2004, [197142](#); Baccarelli et al., 2008, [197059](#); Mocarelli et al.,
28 2008, [199595](#)) each evaluate a segment of the Seveso civilian population (i.e., not an
29 occupational cohort) exposed directly to TCDD released from an industrial accident. In this
30 document, EPA uses the Baccarelli et al. (2008, [197059](#)) and Mocarelli et al. (2008, [199595](#))

1 studies as co-critical studies in deriving the RfD (Section 4.3).⁵ In the Seveso cohort exposures
2 were primarily to TCDD, the chemical of concern, with apparently minimal DLC exposures
3 beyond those associated with background intake,⁶ making these studies highly appropriate for
4 use in RfD derivation for TCDD. In addition, health effects associated with TCDD exposures
5 were observed in humans, the species of concern whose health protection is represented by the
6 RfD, eliminating the uncertainty associated with interspecies extrapolation. The cohort members
7 who were evaluated included infants (exposed in utero) and adults who were exposed when they
8 were less than 10 years of age. The inclusion of these studies among the RfDs derived also may
9 characterize noncancer health effects associated with TCDD exposures in potentially vulnerable
10 populations, thus accounting for some part of the intraspecies uncertainty in the RfD. Finally,
11 the two virtually identical RfDs from different endpoints in the Baccarelli et al. (2008, [197059](#))
12 and Mocarelli et al. (2008, [199595](#)) studies provide an additional level of confidence in the use
13 of these data for derivation of the RfD for TCDD.

14 Although the human data are preferred, Figure ES-5 presents a number of animal studies
15 with RfDs that are lower than the human RfDs. To a large extent, this is expected because a
16 10-fold interspecies uncertainty factor is generally used to extrapolate from test-animal species to
17 humans, intended to provide a conservative estimate of an RfD that would be derived directly
18 from human data. Two of the rat bioassays among this group of studies—Bell et al. (2007,
19 [197041](#)) and NTP (2006, [197605](#))—are of particular note. Both studies were recently conducted
20 and very well designed and conducted, using 30 or more animals per dose group; both also are
21 consistent with and, in part, have helped to define the current state of practice in the field.
22 Bell et al. (2007, [197041](#)) evaluated several reproductive and developmental endpoints, initiating
23 TCDD exposures well before mating and continuing through gestation. NTP (2006, [197605](#)) is
24 the most comprehensive evaluation of TCDD chronic toxicity in rodents to date, evaluating
25 dozens of endpoints at several time points in all major tissues. Thus, proximity of the RfDs
26 derived from these two high quality, recent studies, provide additional support for the use of the
27 human data for RfD derivation.

⁵ The candidate RfD for Alaluusua et al. (2004, [197142](#)) was approximately 2 orders of magnitude higher than the RfDs for Mocarelli et al. (2008, [199595](#)) and Baccarelli et al. (2008, [197059](#)), thus, it was not included as a co-critical study for the RfD.

⁶As an example, note the lack of statistically significant effects reported by Baccarelli et al. (2008, [197059](#); Figure 2 C and D) in regression models based on either maternal plasma levels of non-coplaner PCBs or total TEQ on neonatal TSH levels.

1 There are several animal bioassay candidate RfDs at the lower end of the RfD range in
2 Figure ES-5 that are more than 10-fold below the human-based RfDs. Two of these studies
3 report effects that are analogous to the endpoints reported in the three human studies and support
4 the RfDs based on human data. Specifically, decreased sperm production in Latchoumydandane
5 and Mathur (2002, [197498](#)) is consistent with the decreased sperm counts and other sperm
6 effects in Baccarelli et al. (2008, [197059](#)), and missing molars in Keller et al. (2007, [198526](#);
7 2008, [198531](#); 2008, [198033](#)) are similar to the dental defects seen in Alaluusua et al. (2004,
8 [197142](#)). Thus, because these endpoints have been associated with TCDD exposures in humans,
9 these animal studies would not be selected for RfD derivation in preference to human data
10 showing similar effects.

11 Another characteristic of the remaining studies in the lower end of the candidate RfD
12 distribution is that they are dominated by mouse studies (comprising 6 of the 8 lowest
13 rodent-based RfDs). EPA considers the candidate RfD estimates based on mouse data to be
14 much more uncertain than either the rat or human candidate RfD estimates. The EPA considers
15 the Emond mouse PBPK model to be the most uncertain of toxicokinetic models used to estimate
16 the PODs because of the lack of key mouse-specific data, particularly for the gestational
17 component (see Section 3.3.4.3.2.5). The LOAEL_{HEDS} identified in mouse bioassays are low
18 primarily because of the large toxicokinetic interspecies extrapolation factors used for mice, for
19 which there is more potential for error. The ratio of administered dose to HED (D_a :HED) ranges
20 from 65 to 1,227 depending on the duration of exposure. The D_a :HED for mice is, on average,
21 about four times larger than that used for rats. In addition, each one of the mouse studies has
22 other qualitative limitations and uncertainties that make them less desirable candidates as the
23 basis for the RfD than the human studies.

24 The most relevant human PODs are based on the Baccarelli et al. (2008, [197059](#)) and
25 Mocarrelli et al. (2008, [199595](#)) studies, which exhibited similar LOAELs of 0.024 and
26 0.020 ng/kg-day, respectively. For Baccarelli et al. (2008, [197059](#)), EPA defined a LOAEL as
27 the group mean of 39 ppt TCDD in neonatal plasma which corresponds to thyroid-stimulating
28 hormone (TSH) values above 5 μ U/mL. The World Health Organization (WHO, 1994)
29 established the 5 μ U/mL standard as an indicator of potential iodine deficiency and potential
30 thyroid problems in neonates. Increased TSH levels are indicative of decreased thyroid hormone
31 (T4 and/or T3) levels. For TCDD, the toxicological concern is not likely to be iodine uptake

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1 inhibition, but rather increased metabolism and clearance of T4, as evidenced in a number of
2 animal studies (e.g., Seo et al., 1995, [197869](#)). Clinically, a TSH level of >4 $\mu\text{U}/\text{mL}$ in a
3 pregnant woman is followed up by an assessment of free T4, and treatment with L-thyroxine is
4 prescribed if T4 levels are low (Glinioer and Delange, 2000). This is to ensure a sufficient supply
5 of T4 for the fetus, which relies on maternal T4 exclusively during the 1st half of pregnancy
6 (Chan et al., 2005; Morreale de Escobar et al., 2000; Calvo et al., 2002). Adequate levels of
7 thyroid hormone also are essential in the newborn and young infant as this is a period of active
8 brain development (Glinioer and Delange, 2000; Zoeller and Rovet, 2004). Thyroid hormone
9 disruption during pregnancy and in the neonatal period can lead to neurological deficiencies.

10 Baccarelli et al. (2008, [197059](#)) showed, in graphical form, how the TSH distribution in
11 each of three categorical exposure groups (reference, zone A, and zone B—representing
12 increasing TCDD exposure) shifted to higher TSH values with increasing exposure. The
13 individuals comprising the above 5 $\mu\text{U}/\text{mL}$ group were from all three categorical exposure
14 groups, not just from the highest exposure group. Therefore, EPA was able to designate a
15 LOAEL independently of the nominal categorical exposure groups for TSH values above
16 5 $\mu\text{U}/\text{mL}$. Baccarelli et al. (2008, [197059](#)) did not estimate the equivalent oral intake associated
17 with TCDD serum concentrations, rather they provided neonatal serum TCDD concentrations for
18 the groups above and below 5 $\mu\text{U}/\text{mL}$. EPA estimated the maternal intake at the LOAEL from a
19 maternal serum-TCDD/TSH regression model presented in Baccarelli et al. (2008, [197059](#)) by
20 estimating the maternal TCDD lipid adjusted serum concentration (LASC) at which neonatal
21 TSH exceeded 5 $\mu\text{U}/\text{mL}$. EPA then used the Emond PBPK model to estimate the continuous
22 daily TCDD intake that would result in this TCDD LASC. The resulting predicted maternal
23 daily intake rate established the LOAEL (0.024 ng/kg-day). EPA did not defined a NOAEL
24 because it is not clear what maternal intake should be assigned to the group below 5 $\mu\text{U}/\text{mL}$.

25 For Mocarelli et al. (2008, [199595](#)), EPA defined a LOAEL as the lowest exposed group
26 mean of 68 ppt (1st-quartile) corresponding to decreased sperm concentrations (20%) and
27 decreased motile sperm counts (11%) in men who were 1–9 years old at the time of the Seveso
28 accident (initial TCDD exposure event). Although a decrease in sperm concentration of
29 20% likely would not have clinical significance for an individual, EPA’s concern is that such
30 decreases associated with TCDD exposures could lead to shifts in the distributions of these
31 measures in the general population. Such shifts could result in decreased fertility in men at the

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1 low end of these population distributions. In the group exposed due to the Seveso accident,
2 individuals one standard deviation below the mean are just above the cut-off used by clinicians
3 (20 million/ml) to indicate follow-up for potential reproductive impact in affected individuals,
4 indicating that a number of individuals in the exposed group likely had sperm concentrations less
5 than 20 million/ml; EPA could not obtain the individual data to determine the exact number of
6 men in this category. EPA judged that the impact on sperm concentration and quality reported
7 by Mocarelli et al. (2008, [199595](#)) is biologically significant given the potential for functional
8 impairment as a consequence of potential shifts in the distribution of these male fertility
9 measures in an exposed population.

10 For Mocarelli et al. (2008, [199595](#)), TCDD LASC levels were measured within
11 approximately one year of the initial exposure event. Because effects were only observed in men
12 who were under 10 years of age at the time of exposure, EPA assumed a maximum 10-year
13 critical exposure window for elicitation of these effects. EPA has estimated a continuous daily
14 oral intake of 0.020 ng/kg-day associated with the designated LOAEL from the lowest exposure
15 group (68 ppt), (see Section 4.2.3.2). The reference group is not designated as a NOAEL
16 because there is no clear zero-exposure measurement for any of these endpoints, particularly
17 considering the contribution of background exposure to DLCs, which further complicates the
18 interpretation of the reference group response as a true “control” response (see discussion in
19 Section 4.4). However, males less than 10 years old can be designated as a sensitive population
20 by comparison to older males who were not affected.

21 The two human studies, Baccarelli et al. (2008, [197059](#)) and Mocarelli et al. (2008,
22 [199595](#)), have similar LOAELs of 0.024 and 0.020 ng/kg-day, respectively. Together, these
23 two studies constitute the best foundation for establishing a POD for the RfD, and are designated
24 as co-principal studies. Therefore, increased TSH in neonates (Baccarelli et al., 2008, [197059](#))
25 and male reproductive effects (decreased sperm count and motility) are designated as cocritical
26 effects. Although the exposure estimate used in determination of the LOAEL for Mocarelli et al.
27 (2008, [199595](#)) is more uncertain than the Baccarelli et al. (2008, [197059](#)) exposure estimate, the
28 slightly lower LOAEL of 0.020 ng/kg-day from Mocarelli et al. (2008, [199595](#)) is designated as
29 the POD.

30 EPA used a composite UF of 30 for both studies. EPA applied a factor of 10 for UF_L to
31 account for lack of a NOAEL. EPA also applied a factor of 3 (10^{0.5}) for UF_H to account for

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1 human interindividual variability because the effects were elicited in sensitive populations. A
2 further reduction to 1 was not made because the sample sizes in these two epidemiologic studies
3 were relatively small, which, combined with uncertainty in exposure estimation, may not fully
4 capture the range of interindividual variability. The resulting RfD for TCDD in standard units is
5 7×10^{-10} mg/kg-day.

7 **WEIGHT-OF-EVIDENCE STATEMENT FOR CARCINOGENICITY**

8 The NAS recommended that EPA update its cancer classification for TCDD and the
9 weight-of-evidence (WOE) statement to reflect the current state of the science and incorporate
10 the latest EPA Cancer Guidelines (U.S. EPA, 2005, [086237](#)). Several notable new studies
11 addressing TCDD's carcinogenic potential have been published since the release of EPA's
12 2003 Reassessment, including several new studies of the Seveso epidemiologic cohort and an
13 NTP 2-year cancer bioassay in female rats (NTP, 2006, [197605](#)).

14 Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005, [086237](#))
15 TCDD is characterized as *carcinogenic to humans*, based on the available data as of 2009 (see
16 Section 5.1.2). When evaluating the carcinogenic potential of a compound, EPA employs a
17 WOE approach in which all available information is evaluated and considered. In the case of
18 TCDD, EPA based the classification on numerous lines of evidence, including: multiple
19 occupationally- and accidentally-exposed epidemiologic cohorts showing an association between
20 TCDD exposure and certain cancers or increased mortality from all cancers; extensive evidence
21 of carcinogenicity at multiple tumor sites in both sexes of multiple species of experimental
22 animals; consensus that the mode of TCDD's carcinogenic action in animals involves aryl
23 hydrocarbon receptor (AhR)-dependent key precursor events and proceeds through modification
24 of one or more of a number of cellular processes; the human AhR and rodent AhR are similar in
25 structure and function, and human and rodent tissue and organ cultures respond to TCDD in a
26 similar manner and at similar concentrations; and general scientific consensus that AhR
27 activation is anticipated to occur in humans and may progress to tumors.

28 Most evidence suggests that the majority of toxic effects of TCDD are mediated by
29 interaction with the AhR. EPA considers interaction with the AhR to be a necessary, but not
30 sufficient, event in TCDD carcinogenesis. Although AhR binding and activation by TCDD is
31 considered to be a key event in TCDD carcinogenesis, the sequence of key events following AhR

1 activation that ultimately leads to the development of cancer is unknown (See Section 5.1.2.3).
2 Therefore, EPA has determined that TCDD's mode of action, as defined by the 2005 Cancer
3 Guidelines, is unknown. Since the mode of action for TCDD carcinogenesis is not known, EPA
4 has used a low dose linear extrapolation approach in the development of a cancer oral slope
5 factor.

7 **DERIVATION OF CANDIDATE OSFs FROM EPIDEMIOLOGIC STUDIES AND** 8 **ANIMAL BIOASSAYS**

9 In response to the NAS concerns that EPA evaluate data published since the
10 2003 Reassessment and better justify its approach to cancer dose-response modeling, EPA has
11 developed candidate OSFs using epidemiologic studies and animal bioassays for TCDD,
12 including both new evaluations of data from the 2003 Reassessment and also the assessment of
13 new studies. The BMR level that has been used for the POD in deriving the cancer OSF is
14 one percent extra risk, which is close to the observable response data for most data sets and,
15 therefore, best represents low dose cancer risks (see Section 5.2.3.2.6.11). EPA has chosen a
16 single BMR for consistency across studies.

17 There are several well-studied occupationally-exposed epidemiologic cohorts showing an
18 association between TCDD and increased all-cancer mortality, and several epidemiologic
19 cohorts exposed to TCDD as a consequence of industrial accidents showing an association
20 between TCDD and cancer or cancer mortality (see Section 5.2.3.1). The 2003 Reassessment
21 included cancer dose-response analyses based on the following three occupational cohorts: the
22 NIOSH cohort, an occupational cohort subject to chronic TCDD exposures (Steenland et al.,
23 2001, [197433](#)); the Hamburg cohort, an occupational cohort also subject to chronic TCDD
24 exposures (Becher et al., 1998, [197173](#)); and the BASF cohort, an occupational cohort subject to
25 peak TCDD exposures through clean-up following an industrial accident (Ott and Zober, 1996,
26 [198101](#)). In this document, EPA determined that each of these studies met the epidemiologic
27 study inclusion criteria. Thus, after further evaluating the OSFs presented in the 2003
28 Reassessment for these three studies, EPA accepted those OSF estimates and retained them as
29 candidate OSFs in this document. These OSF estimates are arrayed in Figure ES-6, along with
30 the other OSFs calculated by EPA in this document. EPA also determined that three additional
31 studies met the epidemiologic study inclusion criteria: Cheng et al. (2006, [523122](#)) and Collins

1 et al. (2009, [197627](#)) (NIOSH cohort) and Warner et al. (2002, [197489](#)) (Seveso cohort). EPA
2 determined that the data presented in Collins et al. (2009, [197627](#)) were not sufficient to derive
3 an OSF, and EPA was unable to derive a credible OSF from the data presented by Warner et al.
4 (2002, [197489](#)) (see discussions in Section 5.2.3.1).

5 EPA did derive an OSF from Cheng et al. (2006, [523122](#)), as detailed in Text Box ES-1.
6 In Table ES-1, EPA presents estimates of OSFs for specific TCDD intake rates based on target
7 risk levels of 1×10^{-2} , through 1×10^{-7} based on Cheng et al. (2006, [523122](#)). Note that there
8 are two nonlinear steps in the estimation of risk-specific doses from the Cheng et al. model.
9 First, fat-AUC (AUC_{RL}) and the incremental cancer mortality risk (R_D) do not have a linear
10 relationship (Equation 5-4); however, the relationship becomes virtually linear below an
11 incremental risk of 10^{-3} (see Table ES-1). Second, TCDD fat concentration is not linear with
12 oral intake in the Emond human PBPK model (see Section 3); this relationship also is close to
13 linear below the 10^{-5} risk level. The resulting predicted cancer-mortality risk is approximately
14 linear with daily oral intake at low doses.

15 EPA also identified candidate OSFs for TCDD from key animal bioassays (see
16 Section 5.2.3.2). Based on the inclusion criteria, EPA selected five key rodent cancer bioassays
17 suitable for quantitative dose-response assessment. These included Della Porta et al. (1987,
18 [197405](#)), Kociba et al. (1978, [001818](#)), NTP (1982, [543764](#)), and Toth et al. (1979, [197109](#)) that
19 were evaluated in the 2003 Reassessment, and the new NTP (2006, [197605](#)) rat chronic bioassay.
20 EPA conducted dose-response modeling for each tumor type separately (individual tumor
21 models) as well as for composite tumor incidence (multiple tumor models). The tumor types that
22 EPA analyzed are shown in Table ES-2.

23 For each in vivo animal cancer study that qualified for TCDD dose-response assessment,
24 EPA selected the species/sex/tumor dataset combinations characterized as having statistically
25 significant increases in tumor incidences, then used the Emond rodent PBPK model to estimate
26 blood concentrations corresponding to each study's average daily administered dose for use in
27 dose-response modeling. BMDL_{01S} were then estimated for the blood concentration by
28 two different methodologies: (1) using the multistage cancer model for each species/sex/tumor
29 combination within each study, and (2) using a Bayesian Markov Chain Monte Carlo framework
30 that assumes independence of tumors, modeling all tumors together for each species/sex

Text Box ES-1. OSF Calculations Using Cheng et al. (2006, 523122) Information.

To develop cancer risks for TCDD, EPA used the modeling results of the Cheng analysis, with conversion to oral intake using the Emond human PBPK model as follows. The slope (β) from the Cheng analysis is the slope of the linear relationship between the natural logarithm of the rate ratio (RR) and the cumulative fat TCDD concentration (fat-AUC). Conceptually, the slope (β) is similar to an OSF, except that it is expressed in terms of fat-AUC rather than intake. Also, the slope represents the incremental increase in cancer mortality (expressed as an RR) above the background TCDD exposure experienced by the NIOSH cohort rather than above zero. Using the upper 95% bound on β and assuming that the slope is the same below the NIOSH cohort background exposure level (approximately 5 ppt/yr TCDD fat concentration), EPA calculated risk-specific doses (as daily oral intakes) for TCDD for risk levels of concern to EPA. The risk-specific doses were estimated from the Emond human PBPK model for the lifetime-average TCDD fat concentrations corresponding to the fat-AUC predicted by the Cheng et al. model for each of the risk levels of concern. The steps in this computation are as follows:

- Background cancer mortality risk estimate (R_0). EPA used an R_0 of 0.112 as reported by Cheng et al. (2006, 523122)
- Total cancer mortality risk in the exposed group associated with a specified (extra) risk level (RL) of fatal cancer (TR_{RL}). A TR_{RL} associated with any given extra risk level (e.g., 0.01, 1×10^{-6}) can be calculated using the following relationship for extra risk:

$$ER = \frac{TR_{RL} - R_0}{1 - R_0} \quad (\text{Eq. ES-1})$$

- Incremental cancer mortality risk in the exposed population based on a given extra risk (R_D). R_D is calculated as the difference between the total risk and background risk and expressed in terms of RL and R_0 by combining Equations 5-2 and 5-1.

$$R_D = TR_{RL} - R_0 \quad (\text{Eq. ES -2})$$

$$R_D = RL \times (1 - R_0) \quad (\text{Eq. ES -3})$$

- Cumulative TCDD concentration in the fat compartment for a given extra risk (AUC_{RL}). AUC_{RL} is then calculated by taking the natural logarithm of Equation 3 from Cheng et al. (2006, 523122), rearranging and substituting for RR^1 ($RR = [R_D + R_0]/R_0$):

$$AUC_{RL} = \ln((R_D + R_0)/R_0)/\beta^* \quad (\text{Eq. ES -4})$$

where β^* is the central-tendency regression slope or the 95% upper bound (β_{95}) determined by summing the regression coefficient (β) and the product of 1.96 and the standard error of the regression coefficient, yielding an estimate of 6.0×10^{-6} per ppt-year lipid adjusted serum TCDD, as follows:

$$\beta_{95} = \beta + 1.96 * SE \quad (\text{Eq. ES -5})$$

- Continuous daily TCDD intake associated with a given extra risk [D_{RL}]. Because the fat concentrations generated by CADM are not linear with oral exposure at higher doses, a single oral slope factor to be used for all risk levels cannot be obtained; the response is approximately linear with fat concentrations and oral intake at lower doses. Instead, a risk-specific D_{RL} must be estimated by converting the respective AUC_{RL} to the corresponding lifetime daily intake, using an appropriate human toxicokinetic model. EPA has chosen to use the Emond human PBPK model for this purpose because the CADM configuration does not facilitate this process and so that the dose conversions are consistent with those used in the derivation of the RfD. A D_{RL} is obtained from the Emond model by finding the average lifetime daily intake corresponding to the AUC_{RL} in the fat compartment.

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1 combination within each study. The final selected models were subjected to goodness-of-fit tests
2 and visual inspection of fit to the raw data. Thus, for each sex/species combination within each
3 study, EPA generated a BMDL₀₁ for each single tumor type and another BMDL₀₁ for the
4 combined tumors. Using the Emond human PBPK model, BMDL_{HEDS} were then calculated for
5 each of the BMDL₀₁s, and using a linear extrapolation, OSFs were calculated by
6 $OSF = 0.01/BMDL_{HED}$. The highest OSF for a species/sex combination for either a single tumor
7 type or all combined tumors was selected as a candidate OSF. The OSF candidates from the key
8 animal bioassays are shown in Table ES-2.

10 **DERIVATION OF TCDD ORAL SLOPE FACTOR AND RISK ESTIMATES**

11 EPA was able to derive OSFs for tumor incidence data from five animal cancer
12 bioassays, as well as for cancer mortality data from four epidemiological cohort studies that were
13 selected for TCDD dose-response modeling using the study inclusion criteria (see Section 5.3).
14 These OSFs are arrayed in Figure ES-6. For the animal data, OSFs based on individual tumors
15 were developed for 28 study/sex/endpoint combinations, and the results ranged from 1.8×10^4 to
16 5.8×10^6 (per mg/kg-day). The OSFs based on combined tumors were developed for
17 seven study/sex combinations, and the results ranged from 3.2×10^5 to 9.4×10^6 (per
18 mg/kg-day). EPA also developed OSFs based on four epidemiologic studies from three cohorts,
19 ranging from 3.75×10^5 to 2.5×10^6 (per mg/kg-day).

20 EPA has chosen to use the human data over the animal data as recommended by expert
21 panelists at EPA's 2009 Dioxin Workshop (U.S. EPA, 2009, [522927](#)) and in the 2005 Cancer
22 Guidelines (U.S. EPA, 2005, [086237](#)). OSFs derived from the human data are consistent with
23 the animal bioassay results; human OSFs fall within the same range as the animal bioassay
24 OSFs.

25 Among the human studies, the occupational TCDD exposures in the NIOSH and
26 Hamburg cohorts are assumed to be reasonably constant over the duration of occupational
27 exposure. In contrast, the TCDD exposure pattern for the Seveso and BASF accidents is acute,
28 high dose, followed by low-level background exposure. Such exposure patterns similar to those
29 experienced by the BASF and Seveso cohorts have been shown to yield higher estimates of risk
30 when compared to constant exposure scenarios with similar total exposure magnitudes (Kim
31 et al., 2003, [199146](#); Murdoch and Krewski, 1988, [548718](#); Murdoch et al., 1992, [548719](#)).

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1 Thus, EPA has judged that the NIOSH and Hamburg cohort response data are more relevant than
2 the BASF and Seveso data for assessing cancer risks from continuous ambient TCDD exposure
3 in the general population.

4 The NIOSH (Steenland et al., 2001, [197433](#); Cheng et al., 2006, [523122](#)) and Hamburg
5 (Becher et al., 1998, [197173](#)) cohort studies report cumulative TCDD levels in the serum for
6 cohort members. The most significant difference among the Cheng et al. (2006, [523122](#))
7 analysis and those of Steenland et al. (2001, [197433](#)) and Becher et al. (1998, [197173](#)) is the
8 method used to back-extrapolate exposure concentrations based on serum TCDD measurements.
9 Steenland et al. (2001, [197433](#)) and Becher et al. (1998, [197173](#)) back-extrapolated exposures
10 and body burdens using a first-order model with a constant half-life. In contrast, Cheng et al.
11 (2006, [523122](#)) back-extrapolated body burdens using a kinetic modeling approach that
12 incorporated concentration- and age-dependent elimination kinetics.

13 Although all three of these are high-quality studies, the kinetic modeling used by Cheng
14 et al. (2006, [523122](#)) is judged to better reflect TCDD pharmacokinetics, as currently
15 understood, than the first-order models used by Steenland et al. (2001, [197433](#)) and Becher et al.
16 (1998, [197173](#)). EPA believes that the representation of physiological processes provided by
17 Cheng et al (2006, [523122](#)) is more realistic than the assumption of simple first-order kinetics
18 and this outweighs the attendant modeling uncertainties. Furthermore, the use of kinetic
19 modeling is consistent with recommendations both by the NAS and the Dioxin Workshop panel.

20 EPA, therefore, has decided to use the results of the Cheng et al. (2006, [523122](#)) study for
21 derivation of the TCDD OSF based on total cancer mortality as calculated by EPA using data
22 and models from the Cheng et al. (2006, [523122](#)) study, as described in Section 5.2.3.1.2.

23 Although the OSF is only strictly defined for exposures above the background exposure
24 experienced by the NIOSH cohort, which was assumed to be 0.5 pg/kg-day TCDD, or
25 5 pg/kg-day total TEQ, EPA assumes that the slope (risk vs. blood concentration) is the same
26 below those background exposure levels as it is above. Table ES-1 shows the oral slope factors
27 at specific target risk levels (OSF_{RLS}) which range from 1.1×10^5 to 1.3×10^6 per (mg/kg-day).
28 EPA recommends the use of an OSF of 1×10^6 per (mg/kg-day) when the target risk range is 10^{-5}
29 to 10^{-7} .

1 **CONSIDERATION OF NONLINEAR DOSE-RESPONSE APPROACHES FOR**
2 **CANCER**

3 The NAS focused much of its review on EPA’s derivation of a cancer slope factor,
4 commenting extensively on the extrapolation of dose-response modeling below the POD. The
5 NAS questioned EPA’s choice of a linear, nonthreshold model for extrapolating risk associated
6 with exposure levels below the POD, concluding that the current scientific evidence was
7 sufficient to justify the use of nonlinear methods when extrapolating below the POD for dioxin
8 carcinogenicity.

9 While, based on the 2005 Cancer Guidelines, EPA deemed linear extrapolation to be
10 most appropriate for TCDD, EPA carefully considered the NAS recommendation to provide risk
11 estimates using both linear and nonlinear methods. In this document, EPA has evaluated the
12 information available for identifying a threshold and for estimating the shape of the
13 dose-response curve below the POD (see Section 5.2.3.4). EPA presents a hypothetical sublinear
14 dose-response modeling example of rodent carcinogenicity. EPA also presents two illustrative
15 examples of RfD development (i.e., nonlinear method) for carcinogenic effects of TCDD, using
16 data derived from animal bioassays. EPA derives illustrative RfDs for cancer based on
17 combined tumor response and also on hypothesized key events in TCDD’s MOA for female rat
18 liver and lung tumors. EPA identifies a number of limitations that prevent making strong
19 conclusions based on the nonlinear dose-response modeling exercises.

20

21 **FEASIBILITY OF QUANTITATIVE UNCERTAINTY ANALYSIS**

22 EPA also addresses the third key recommendation of the NAS, specifically, improving
23 transparency, thoroughness, and clarity in *quantitative uncertainty analysis* (see Section 6). In
24 summary, NAS suggested that EPA should

25

- 26 • describe and define (quantitatively to the extent possible) the variability and
27 uncertainty for key assumptions used for each key endpoint-specific risk
28 assessment (choices of data set, POD, model, and dose metric),
- 29 • incorporate probabilistic models to the extent possible to represent the range of
30 plausible values,
- 31 • clearly state it when quantitation is not possible and explain what would be
32 required to achieve quantitation (NAS, 2006, [198441](#), p. 9).

33

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1 Although the NAS summarized the shortfalls in the 2003 Reassessment categorically, the
2 elaborations within their report often contain the qualification “if possible” and do not take a
3 position with regard to the feasibility of many suggestions. With appreciation for the extent of
4 information available for dioxin, EPA’s goal herein was to examine the feasibility of a
5 data-driven quantitative uncertainty analysis for TCDD dose-response assessment.

6 In examining feasibility of quantitative uncertainty analysis, EPA recognized that
7 different kinds of uncertainty require different statistical treatment. *Cognitive uncertainty*
8 concerns uncertainty that can be expressed as probabilities and may be operationalized using
9 either frequentist or Bayesian approaches. For example, classical statistical methods yield
10 distributions on model parameters which reflect sample fluctuations, assuming that the model is
11 true. This type of uncertainty can be taken into account in the BMDL estimation. Also, for
12 TCDD epidemiologic data, the dose reconstruction often involves assumptions that may be
13 amenable to data-driven uncertainty analysis if sufficient data can be retrieved; back-
14 extrapolated TCDD levels, biological half-life, body fat, and background levels are example
15 variables that could be included in such an analysis. In addition, a Monte Carlo analysis has
16 been examined to develop quantitative uncertainty distributions for the RfD (e.g., Swartout et al.,
17 1998, [093460](#)). Given a set of animal bioassay data, quantifying dose-response uncertainty may
18 be approached in different ways. The differences reflect different types of uncertainty that are
19 captured. A recent evaluation enumerates the following possible methodologies (Bussard et al.,
20 2009, [543770](#)):

21
22 **Benchmark Dose Modeling (BMD):** Choose the ‘best’ model, and
23 assess uncertainty assuming this model is true. Supplemental results can compare
24 estimates obtained with different models, and sensitivity analyses can investigate
25 other modeling issues.

26 **Probabilistic Inversion with Isotonic Regression (PI-IR):** Define
27 model-independent ‘observational’ uncertainty, and look for a model that captures
28 this uncertainty by assuming the selected model is true and providing for a
29 distribution over its parameters.

30 **Non-Parametric Bayes (NPB):** Choose a prior mean response (potency)
31 curve (potentially a “non-informative prior”) and a precision parameter to express
32 prior uncertainty over all increasing dose-response relations, and update this prior
33 distribution with the bioassay data.

1 **Bayesian Model Averaging (BMA)** (as considered here): Choose an
2 initial set of models, and then estimate the parameters of each model with
3 maximum likelihood. Use classical methods to estimate parameter uncertainty,
4 given the truth of the model. Determine a probability weight for each model
5 using the Bayes Information Criterion (BIC), and use these weights to average the
6 model results.
7

8 The first of the above methods involves standard classical statistical methods and captures
9 sampling uncertainty conditional on the truth of the model used. The other methods are “exotic”
10 in the sense that they attempt to capture uncertainty that is not conditional on the truth of a given
11 model. In this response document, EPA has not applied such methods, but recognizes that
12 quantitative uncertainty analysis is possible in these cases.

13 In contrast to cognitive uncertainty, *Volitional uncertainty* concerns uncertainty regarding
14 choices on the best course of action to take; volitional uncertainty cannot be analyzed by
15 sampling from a probability distribution and, thus, is not amenable to a complete quantitative
16 uncertainty analysis. Some of the choices made in TCDD dose-response assessment that are
17 volitional include: choice of occupational cohort data set or bioassay data set; choice of PODs
18 (e.g., ED₀₁, ED₀₅, and ED₁₀); choice of species, strain, or sex within an animal bioassay; and
19 choice of dose metric (e.g., administered doses, blood concentrations, lipid-adjusted serum
20 concentrations). These volitional uncertainties cannot be quantified by sampling an input
21 distribution.

22 Although EPA has determined that a comprehensive quantitative uncertainty analysis is
23 not feasible because of the limitations discussed above, EPA believes the NAS was requesting
24 that dose-response modeling results be shown for specific choices of interest to TCDD
25 assessment. In response to the NAS concerns, this document provides some limited quantitative
26 comparisons. BMDs, BMDLs, and OSFs from the animal cancer bioassay benchmark dose
27 modeling assuming 1, 5, and 10% extra risk are compared in units of blood concentrations and
28 human equivalent doses (see Tables 5-18 and 5-19, respectively). In addition, central tendency
29 slope estimates and upper bound slope factor estimates based on Cheng et al. (2006, [523122](#)) are
30 presented (see Tables 5-3 and 5-4). For the noncancer effects, key animal study PODs
31 (ng/kg-day) are shown based on different dose metrics: administered dose, first-order body
32 burden HED, and blood concentration (Tables 4-3 and 4-4). EPA has undertaken some limited
33 quantitative uncertainty analyses for the kinetic modeling, presenting a sensitivity analysis and

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1 uncertainty analysis in dose metrics derived for the risk assessment of TCDD and a detailed
2 discussion on the uncertainty in choice of PBPK model-driven dose metrics. (see Sections 3.3.3
3 and 3.3.5). TCDD kinetic doses from the Emond et al. (2005, [197317](#); 2006, [197316](#)) PBPK
4 model that is primarily used in the technical analysis in this document are compared with those
5 predicted by the Aylward et al. (2005, [197114](#)) model.

6 Uncertainty quantification is an emerging area in science. There are many examples of
7 highly vetted and peer-reviewed uncertainty analyses based on structured expert judgment.
8 Under this process, experts in effect synthesize a wide diversity of information in generating
9 their subjective probability distributions. Where considerable data exist for an environmental
10 pollutant, such as for the well-studied TCDD, it is natural to ask whether these extensive data can
11 be leveraged more directly in uncertainty quantification. This is an area where research could be
12 focused. Additional research topics relevant to dioxin that could further inform health
13 assessments include population variability of biokinetic constants and threshold mechanisms for
14 the mass action model. Further data and improved methodologies in these areas, combined with
15 developments illustrated elsewhere in this report, will help reduce or better quantify uncertainties
16 and strengthen EPA’s understanding of potential health implications of environmental TCDD
17 exposures.

Table ES-1. Comparison of fat concentrations, risk specific dose estimates and equivalent oral slope factors based on upper 95th percentile estimate of regression coefficient^a of all fatal cancers reported by Cheng et al. (2006, [523122](#)) for selected risk levels

Risk level (RL)	AUC _{RL} (ppt-yr)	FAT _{RL} (ng/kg)	Risk specific dose ^b (D _{RL}) (ng/kg-day)	Equivalent oral slope factors (OSF _{RL}) per (mg/kg-day)
1×10^{-2}	1.262×10^4	1.803×10^2	8.79×10^{-2}	1.1×10^5
5×10^{-3}	6.432×10^3	9.189×10^1	3.14×10^{-2}	1.6×10^5
1×10^{-3}	1.307×10^3	1.867×10^1	2.88×10^{-3}	3.5×10^5
5×10^{-4}	6.546×10^2	9.352×10^0	9.56×10^{-4}	5.2×10^5
1×10^{-4}	1.311×10^2	1.873×10^0	1.29×10^{-4}	7.8×10^5
5×10^{-5}	6.558×10^1	9.368×10^{-1}	5.52×10^{-5}	9.1×10^5
1×10^{-5}	1.312×10^1	1.874×10^{-1}	8.94×10^{-6}	1.1×10^6
5×10^{-6}	6.559×10^0	9.370×10^{-2}	4.25×10^{-6}	1.2×10^6
1×10^{-6}	1.312×10^0	1.874×10^{-2}	8.08×10^{-7}	1.2×10^6
5×10^{-7}	6.559×10^{-1}	9.370×10^{-3}	4.00×10^{-7}	1.3×10^6
1×10^{-7}	1.312×10^{-1}	1.874×10^{-3}	7.92×10^{-8}	1.3×10^6

^aBased on regression coefficient of Cheng et al. (2006, [523122](#), Table III), excluding observations in the upper 5% range of the exposures; where reported $\beta = 3.3 \times 10^{-6}$ ppt-years and standard error = 1.4×10^{-6} . Upper 95th percentile estimate of regression coefficient (β_{95}) calculated to be $6.04 \times 10^{-6} = (3.3 \times 10^{-6}) + 1.96 \times (1.4 \times 10^{-6})$; background cancer mortality risk is assumed to be 0.112 as reported by Cheng et al. (2006, [523122](#)).

^bTo calculate the extra cancer risk (ER) and OSF for any TCDD daily oral intake (D):

1. For D in ng/kg-d, look up the corresponding fat concentration (ng/kg = ppt) from the conversion chart (nongestational lifetime dose metrics) in Appendix C.4.1.
2. Calculate the AUC in ppt-yrs by multiplying the fat concentration by 70 years.
3. Calculate Extra Risk (ER) using the following equation:
ER = $[\exp(\text{AUC} \times 6.04\text{E-}6) \times 0.112 - 0.112] \div 0.888$.
4. Calculate the OSF $(\text{mg/kg-d})^{-1} = 1\text{E}6 \times (\text{ER} \div \text{D})$.

Example for risk at the RfD: D = 7×10^{-4} ng/kg-d; fat concentration = 6.93 ng/kg;

AUC = 70 years \times 6.93 ppt = 485 ppt-year;

ER = $\exp(485 \text{ ppt-year} \times 6.04\text{E-}6 \text{ (ppt-yr)}^{-1}) \times 0.112 - 0.112 \div 0.888 = 3.7 \times 10^{-4}$

OSF = $1\text{E}6 \text{ ng/mg} \times (3.7 \times 10^{-4} \div 7 \times 10^{-4} \text{ ng/kg-d}) = 5.3 \times 10^5 \text{ (mg/kg-d)}^{-1}$.

Table ES-2. Tumor points of departure and oral slope factors using blood concentrations

Study	Sex/species: tumor sites	BMDL_{01HED}^a (ng/kg-day)	OSF (per mg/kg-day)
NTP, (1982, 543764)	Male mice: liver adenoma and carcinoma, lung	1.1E-03	9.4E+6
Toth et al., (1979, 197109)	Male mice: liver tumors	1.9E-03	5.2E+6
NTP, (1982, 543764)	Female mice: liver adenoma and carcinoma, thyroid adenoma, subcutaneous fibrosarcoma, all lymphomas	5.3E-03	1.9E+6
NTP, (1982, 543764)	Female rats: liver neoplastic nodules, liver adenoma and carcinoma, adrenal cortex adenoma or carcinoma, thyroid follicular cell adenoma	5.7E-03	1.8E+6
Kociba et al., (1978, 001818)	Female rats: liver adenoma carcinoma, oral cavity, lung	7.3E-03	1.4E+6
NTP, (1982, 543764)	Male rats: thyroid follicular cell adenoma, adrenal cortex adenoma	9.6E-03	1.0E+6
Della Porta et al., (1987, 197405)	Male mice: Hepatocellular carcinoma	3.1E-02	3.2E+5
NTP, (2006, 197605)	Female rats: liver cholangiocarcinoma, hepatocellular adenoma, oral mucosa squamous cell carcinoma, lung cystic keratinizing epithelioma, pancreas adenoma, carcinoma	2.3E-02	4.4E+5
Kociba et al., (1978, 001818)	Male rats: adrenal cortex adenoma, tongue carcinoma, nasal/palate carcinoma	3.1E-02	3.2E+5

^aBMDL_{HEDS} are from the multiple tumor analyses, with the exception of Toth et al. (1979, [197109](#)) and Della Porta et al. (1987, [197405](#)) which are the result of modeling single tumor sites.

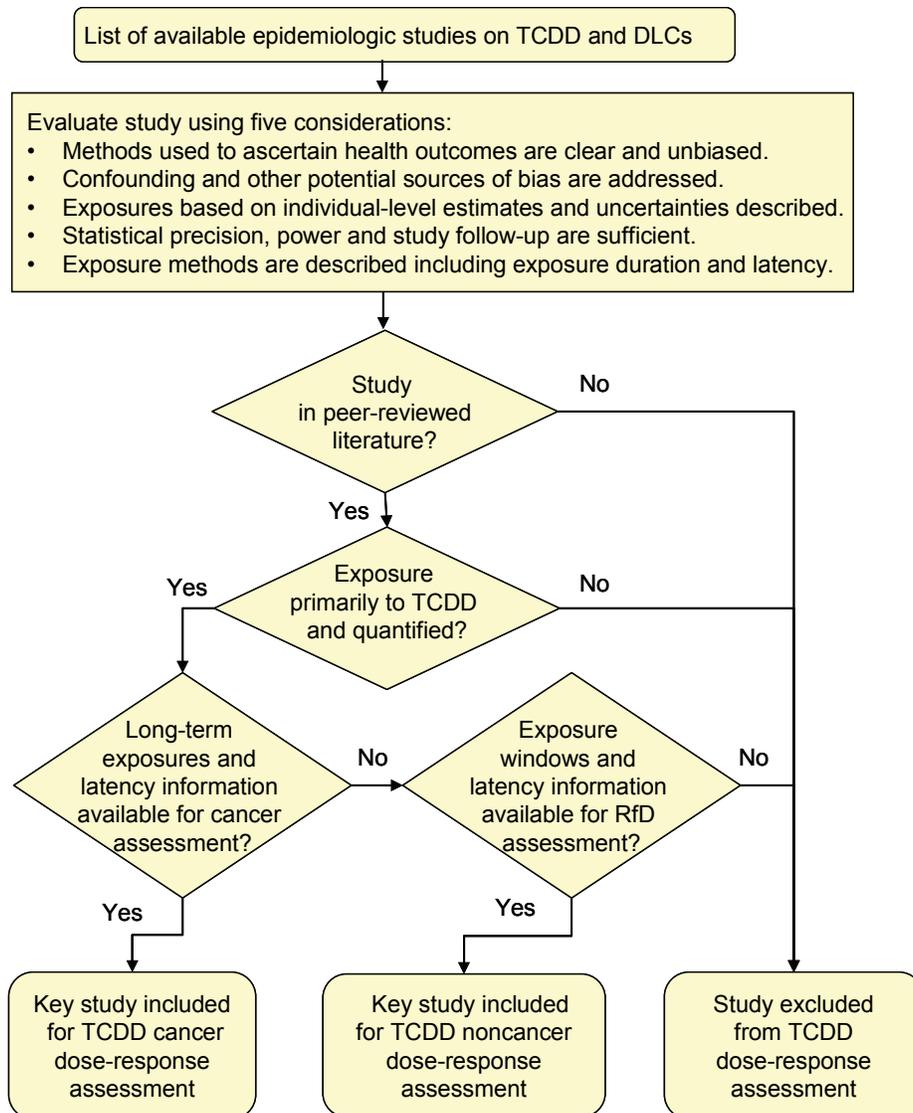


Figure ES-1. EPA’s process to evaluate available epidemiologic studies using study inclusion criteria for use in the dose-response analysis of TCDD. EPA applied its TCDD-specific epidemiologic study inclusion criteria to all studies published on TCDD and DLCs. The studies were initially evaluated using five considerations regarded as providing the most relevant kind of information needed for quantitative human health risk analyses. For each study that was published in the peer-reviewed literature, EPA then examined whether the exposures were primarily to TCDD and if the TCDD exposures could be quantified so that dose-response analyses could be conducted. Finally, 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 effect is needed. Only studies meeting these criteria were included in EPA’s TCDD dose-response analysis.

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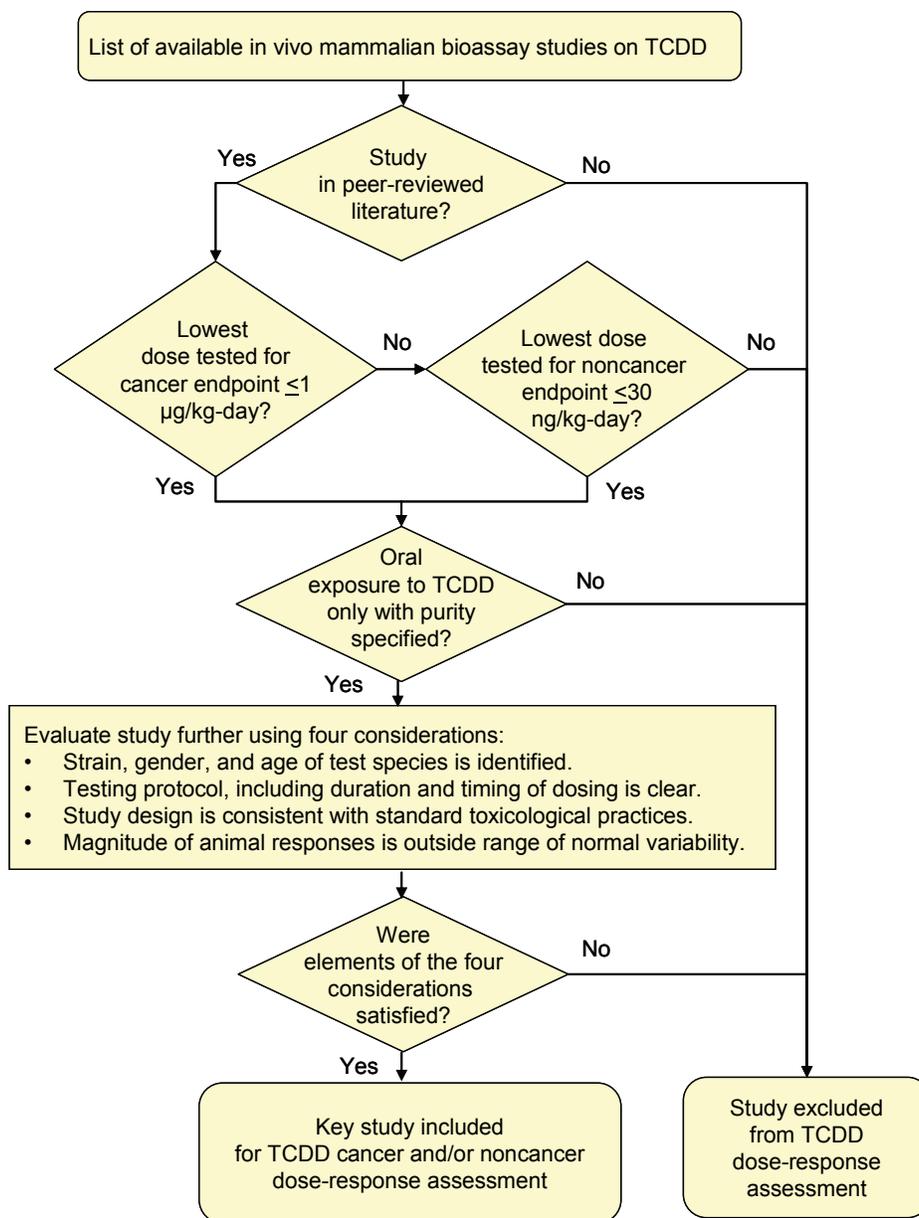


Figure ES-2. EPA’s process to evaluate available animal bioassay studies using study inclusion criteria for use in the dose-response analysis of TCDD. EPA evaluated all available in vivo mammalian bioassay studies on TCDD. Studies had to be published in the peer-reviewed literature. Next, to ensure working in the low-dose range for TCDD dose-response analysis, EPA applied dose requirements to the lowest tested average daily doses in each study, with specific requirements for cancer ($\leq 1 \mu\text{g}/\text{kg}\text{-day}$), and noncancer ($\leq 30 \text{ ng}/\text{kg}\text{-day}$) studies. Third, EPA required that the animals were exposed via the oral route to only TCDD and that the purity of the TCDD was specified. Finally, the studies were evaluated using four considerations regarded as providing the most relevant kind of information needed for quantitative human health risk analyses from animal bioassay data. Only studies meeting all of these criteria and considerations were included in EPA’s TCDD dose-response analysis.

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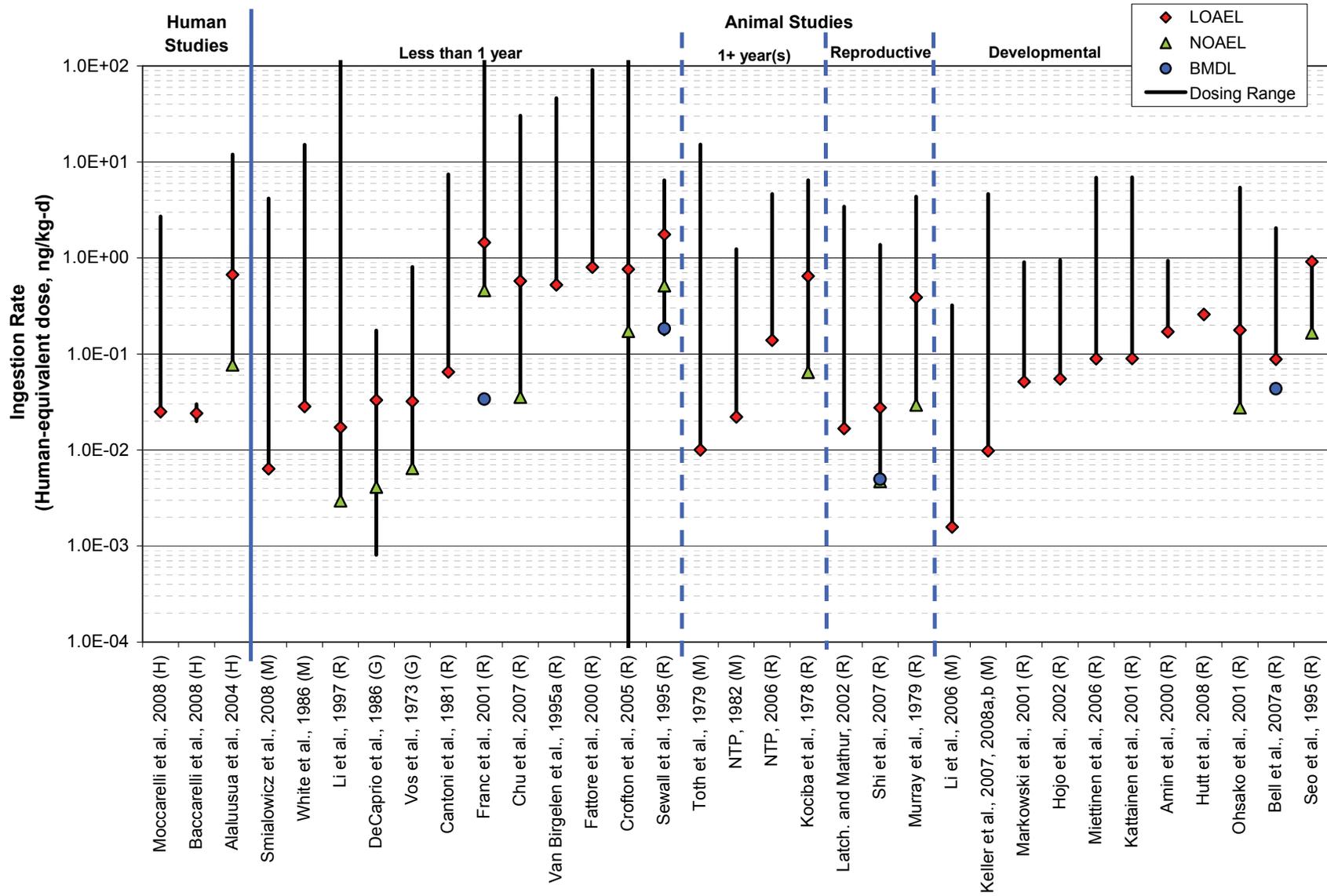


Figure ES-3. Exposure-response array for ingestion exposures to TCDD.

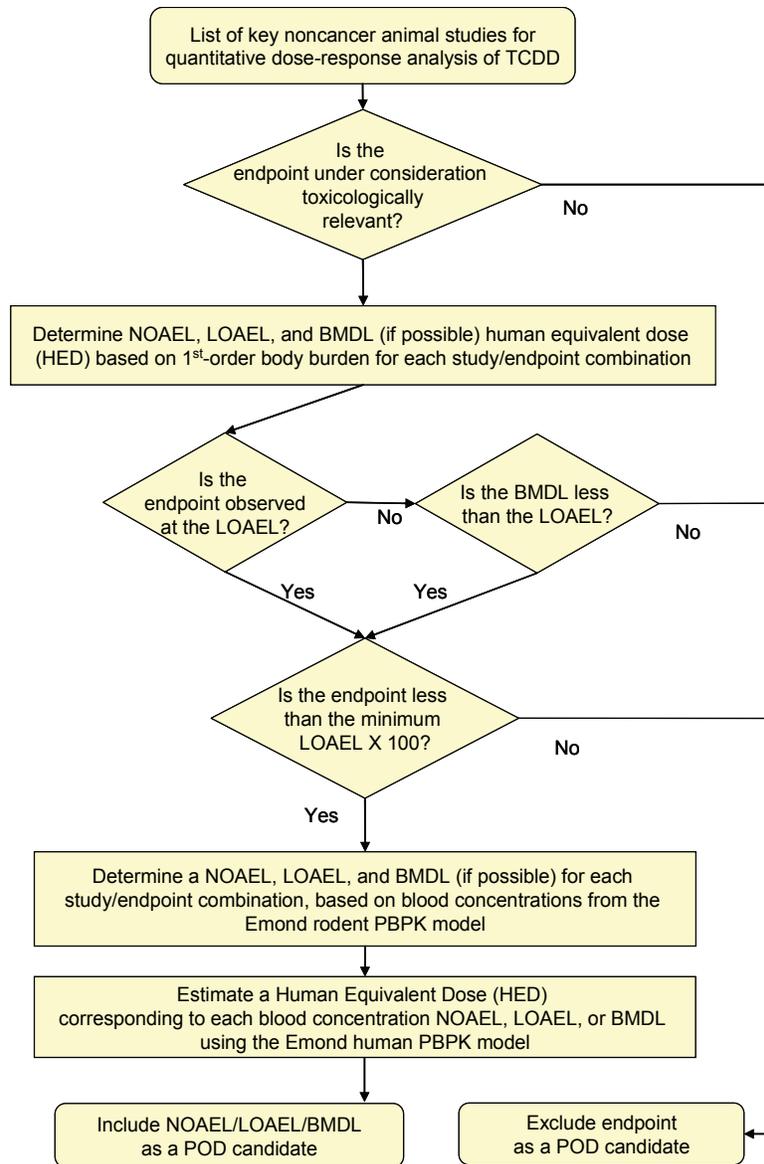


Figure ES-4. EPA’s process to select and identify candidate PODs from key animal bioassays for use in noncancer dose-response analysis of TCDD. For each noncancer endpoint found in the studies that qualified for TCDD dose-response assessment using the study inclusion criteria, EPA first determined if the 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. These potential PODs were examined for statistical relevance and included when the endpoint was observed at the LOAEL. If the BMDL was less than the LOAEL, and if the endpoint was less than the minimum LOAEL × 100, EPA then calculated NOAELs, LOAELs, or BMDLs based on blood concentrations from the Emond rodent PBPK model. Then, for all of the candidate PODs, HEDs were estimated using the Emond human PBPK model. Finally, the lowest group of the toxicologically relevant candidate PODs was selected for final use in derivation of an RfD.

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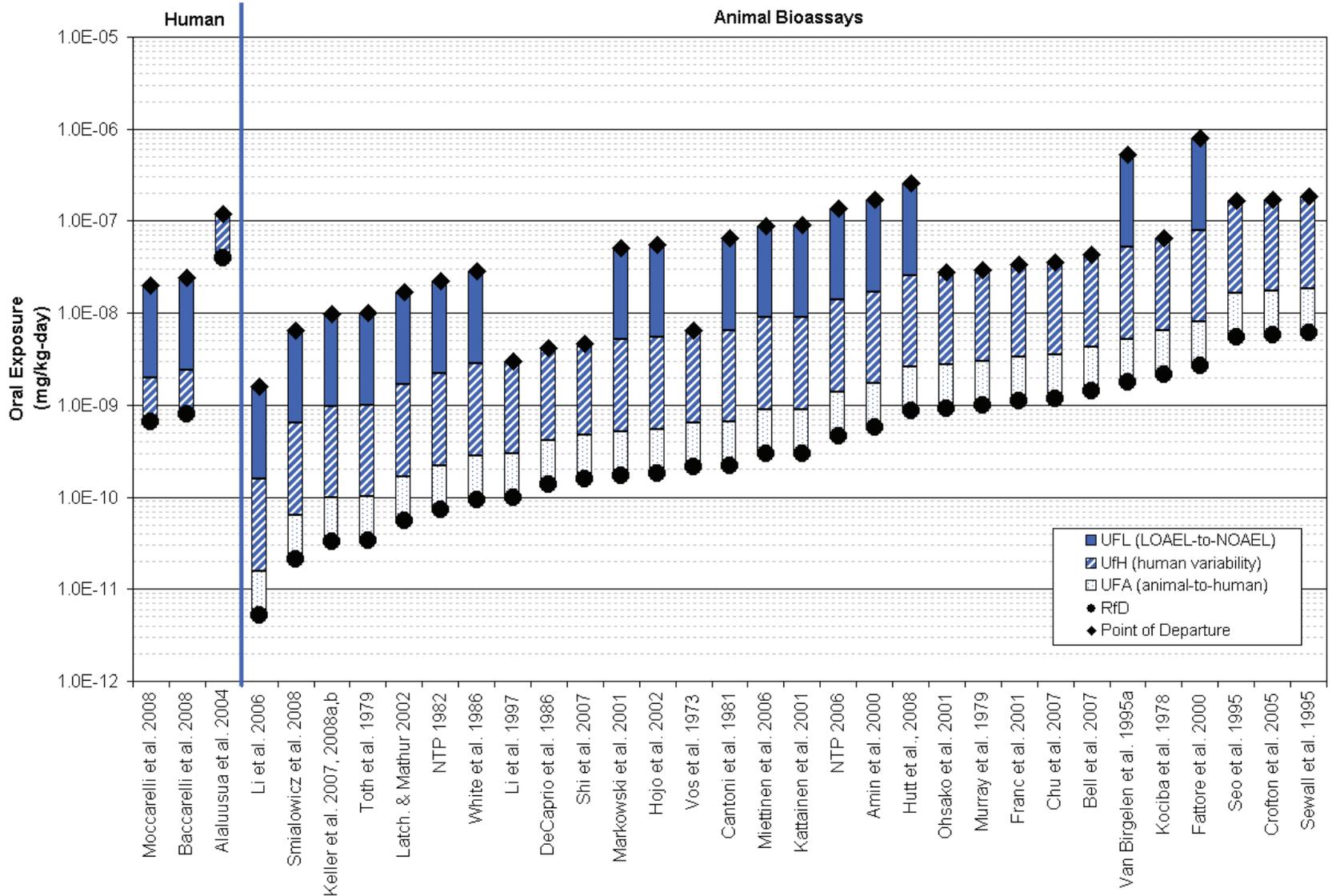


Figure ES-5. Candidate RfD array.

Cancer Slope Factors for 2,3,7,8-TCDD

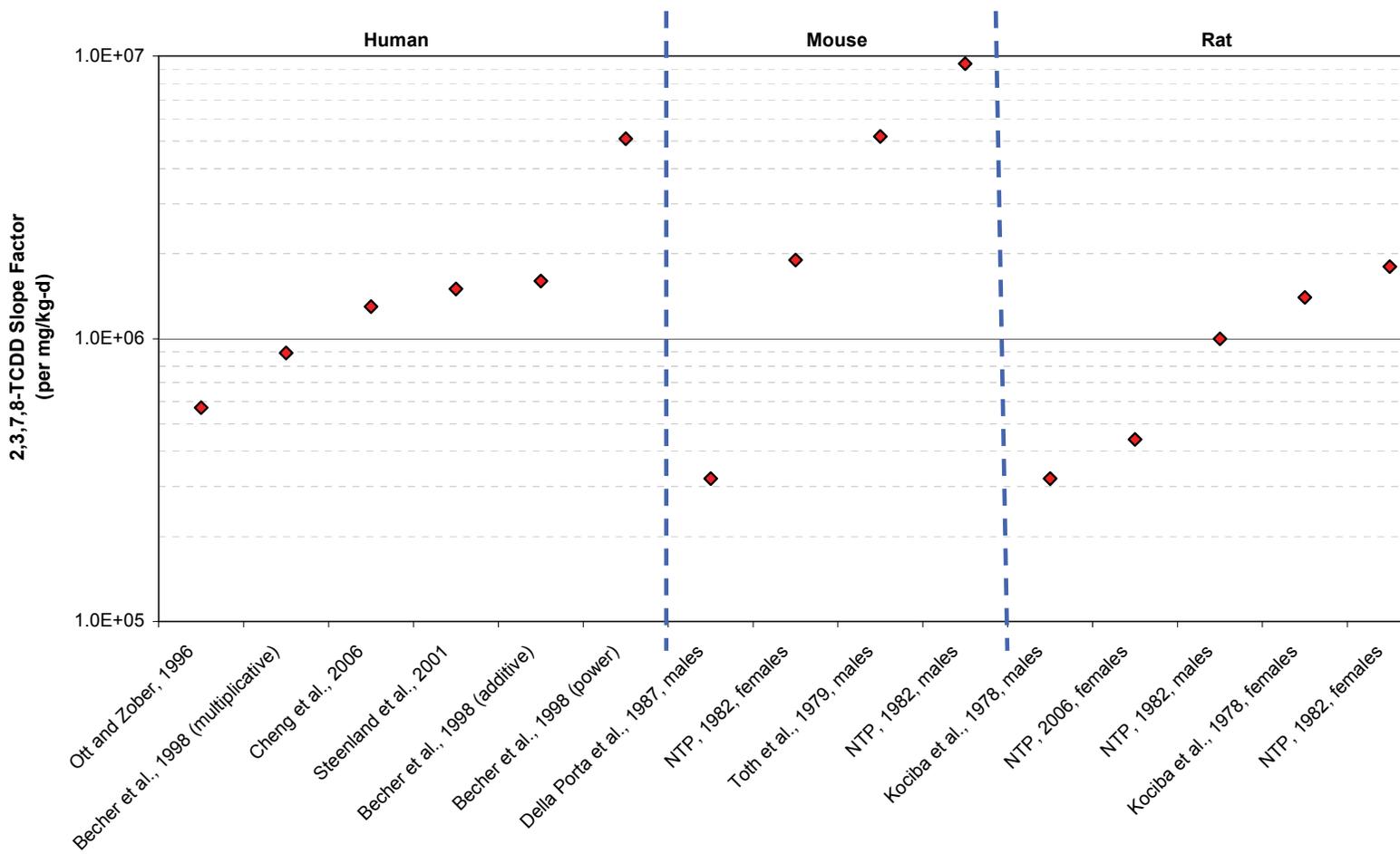


Figure ES-6. Candidate oral slope factor array.

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.⁷ 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, [543766](#)), 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 toxicologic 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 toxicologic 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” to serve as the basis for standardization of the toxicity of components in a mixture of dioxins and DLCs. The dose-response information for TCDD is used to evaluate risks from exposure to mixtures of DLCs (Van et al., 1998, [198345](#); Van den Berg et al., 2006, [543769](#); also see the World Health Organization’s Web site for the dioxin toxicity equivalence factors [TEFs]),⁸ therefore, it is imperative to correctly assess the dose response of TCDD and understand the uncertainties and limitations therein.

In 2003, the U.S. Environmental Protection Agency (EPA) produced an external review draft of the multiyear comprehensive reassessment of dioxin exposure and human health effects entitled, *Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) and Related Compounds* (U.S. EPA, 2003, [537122](#)). This draft report, herein called the

⁷For further information on the chemical structures of these compounds, see U.S. EPA (2003, [537122](#); 2008, [543774](#)).

⁸Available at http://www.who.int/ipcs/assessment/tef_update/en/.

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1 “2003 Reassessment,” consisted of (1) a scientific review of information relating to sources of
2 and exposures to TCDD, other dioxins, and DLCs in the environment; (2) detailed reviews of
3 scientific information on the health effects of TCDD, other dioxins, and DLCs; and (3) an
4 integrated risk characterization for TCDD and related compounds.

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

7

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, [198441](#), p. 43, Box 1-1).

8

9 In 2006, the NAS published its review of EPA’s 2003 Reassessment entitled *Health Risks from*
10 *Dioxin and Related Compounds: Evaluation of the EPA Reassessment* (NAS, 2006, [198441](#)).

11

12 **1.1. SUMMARY OF KEY NAS (2006, [198441](#)) COMMENTS ON DOSE-RESPONSE**
13 **MODELING IN THE 2003 REASSESSMENT**

14 While recognizing the effort that EPA expended to prepare the 2003 Reassessment, the
15 NAS committee identified three key areas that they believe require substantial improvement to
16 support a scientifically robust risk assessment. These three key areas are

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- 1 • transparency and clarity in selection of key data sets for analysis;
- 2 • justification of approaches to dose-response modeling for cancer and noncancer
- 3 endpoints; and
- 4 • transparency, thoroughness, and clarity in quantitative uncertainty analysis.

5

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

8

- 9 • EPA should compare cancer risks by using nonlinear models consistent with a receptor
10 mediated mechanism of action and by using epidemiological data and the new National
11 Toxicology Program (NTP) animal bioassay data (NTP, 2006, [197605](#)). The comparison
12 should include upper and lower bounds, as well as central estimates of risk. EPA should
13 clearly communicate this information as part of its risk characterization (NAS, 2006,
14 [198441](#), p. 9).
- 15 • EPA should identify the most important data sets to be used for quantitative risk
16 assessment for each of the four key end points (cancer, immunotoxicity, reproductive
17 effects, and developmental effects). EPA should specify inclusion criteria for the studies
18 (animal and human) used for derivation of the benchmark dose (BMD) for different
19 noncancer effects and potentially for the development of RfD (reference dose) values and
20 discuss the strengths and limitations of those key studies; describe and define
21 (quantitatively to the extent possible) the variability and uncertainty for key assumptions
22 used for each key end-point-specific risk assessment (choices of data set, POD [point of
23 departure], model, and dose metric); incorporate probabilistic models to the extent
24 possible to represent the range of plausible values; and assess goodness-of-fit of
25 dose-response models for data sets and provide both upper and lower bounds on central
26 estimates for all statistical estimates. When quantitation is not possible, EPA should
27 clearly state it and explain what would be required to achieve quantitation (NAS, 2006,
28 [198441](#), p. 9).
- 29 • When selecting a BMD as a POD, EPA should provide justification for selecting a
30 response level (e.g., at the 10%, 5%, or 1% level). In either case, the effects of this
31 choice on the final risk assessment values should be illustrated by comparing point
32 estimates and lower bounds derived from selected PODs (NAS, 2006, [198441](#), p. 9).
- 33 • EPA should continue to use body burden as the preferred dose metric but should also
34 consider physiologically based pharmacokinetic modeling as a means to adjust for
35 differences in body fat composition and for other differences between rodents and
36 humans (NAS, 2006, [198441](#), p. 9).
- 37 • Although EPA addressed many sources of variability and uncertainty qualitatively, the
38 committee noted that the 2003 Reassessment would be substantially improved if its risk
39 characterization included more quantitative approaches. Failure to characterize

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1 variability and uncertainty thoroughly can convey a false sense of precision in the
2 conclusions of the risk assessment (NAS, 2006, [198441](#), p. 5).

3
4 Importantly, the NAS encouraged EPA to calculate an RfD as the 2003 Reassessment
5 does not contain an RfD derivation. The committee suggested that

6
7 ...estimating an RfD would provide useful guidance to risk managers to help
8 them (1) assess potential health risks in that portion of the population with intakes
9 above the RfD, (2) assess risks to population subgroups, such as those with
10 occupational exposures, and (3) estimate the contributions to risk from the major
11 food sources and other environmental sources of TCDD, other dioxins, and DLCs
12 for those individuals with high intakes (NAS, 2006, [198441](#), p. 6).

13
14 The NAS made many thoughtful and specific recommendations throughout their review;
15 additional NAS recommendations and comments pertaining to the dose-response assessment of
16 TCDD will be presented and addressed in various sections throughout this document.

17 18 **1.2. EPA'S SCIENCE PLAN**

19 In May 2009, EPA Administrator Lisa P. Jackson announced the "*Science Plan for*
20 *Activities Related to Dioxins in the Environment*" ("Science Plan") that addressed the need to
21 finish EPA's dioxin reassessment and provide a completed health assessment on this high profile
22 chemical to the American public as quickly as possible.⁹ The Science Plan states that EPA will
23 release a draft report that responds to the recommendations and comments included in the NAS
24 review of EPA's 2003 Reassessment, and that, in this draft report, EPA's National Center for
25 Environmental Assessment, Office of Research and Development, will provide a limited
26 response to key comments and recommendations in the NAS report (draft response to comments
27 report). This draft response is to focus on dose-response issues raised by the NAS and include
28 analyses of relevant new key studies. The draft response is to be provided for public review and
29 comment and for independent external peer review by EPA's Science Advisory Board.
30 Following completion of this report, EPA is to review the impacts of the response to comments
31 report on its 2003 Reassessment.

⁹Available at <http://www.epa.gov/dioxin/scienceplan>.

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1 This draft document comprises EPA’s report that responds both directly and technically
2 to the recommendations and comments on TCDD dose-response assessment included in the NAS
3 review of EPA’s 2003 Reassessment. This document focuses on TCDD only. Because new data
4 are analyzed in this report and toxicity values are derived, this document will follow the IRIS
5 process for review, clearance and completion; however, it is not a traditional IRIS document.
6 Information developed in this document is intended to not only respond to the NAS review, but
7 also to expand EPA’s knowledge of TCDD cancer and noncancer dose-response based on the
8 most current literature, existing methods, and adherence to EPA risk assessment guidance
9 documents. Following completion of this document, EPA will consider its contents as it reviews
10 the TCDD risk assessment information presented in the 2003 Reassessment and moves forward
11 towards completion of the dioxin reassessment.

12

13 **1.3. OVERVIEW OF EPA’S RESPONSE TO NAS (2006, [198441](#)) “HEALTH RISKS**
14 **FROM DIOXIN AND RELATED COMPOUNDS: EVALUATION OF EPA’S 2003**
15 **REASSESSMENT”**

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

23 EPA thoroughly considered the recommendations of the NAS and responds with
24 scientific and technical evaluation of TCDD dose–response data via:

25

- 26 • an updated literature search that identified new TCDD dose-response studies (see
27 Section 2);
- 28 • a kickoff workshop that included the participation of external experts in TCDD health
29 effects, toxicokinetics, dose-response assessment and quantitative uncertainty analysis;
30 these experts discussed potential approaches to TCDD dose-response assessment and
31 considerations for EPA’s response to NAS (U.S. EPA, 2009, [543757](#), Appendix A);
- 32 • detailed study inclusion criteria and processes for the selection of key studies (see
33 Section 2.3) and epidemiologic and animal bioassay data for TCDD dose-response
34 assessment (see Section 2.4.1/Appendix B and Section 2.4.2, respectively);

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- 1 • kinetic modeling to quantify appropriate dose metrics for use in TCDD dose-response
2 assessment (see Section 3 and Appendices C and D);
- 3 • dose-response modeling for all appropriate noncancer and cancer data sets (see
4 Section 4.2/Appendix E and Section 5.2.3/Appendix F, respectively);
- 5 • thorough and transparent evaluation of the selected TCDD data for use in the derivation
6 of an RfD and an oral slope factor (OSF) (see Sections 4.2 and 5.2.3, respectively);
- 7 • the development of an RfD (see Section 4.3);
- 8 • the development of a revised OSF (see Section 5.3) with an updated cancer weight of
9 evidence determination for TCDD based on EPA's 2005 *Cancer Guidelines* (2005,
10 [086237](#)) (see Section 5.1.2);
- 11 • consideration of nonlinear dose-response approaches for cancer, including illustrative
12 RfDs for cancer precursor events and tumors (see Section 5.2.3.4); and
- 13 • discussion of the feasibility and utility of quantitative uncertainty analysis for TCDD
14 dose-response assessment (see Section 6).

15

16 Each of these activities is described in detail in subsequent sections of this document.

17 In addition to this document, it should be noted that three separate EPA activities address
18 other TCDD issues, specifically related to the application of dioxin TEFs and to TCDD and DLC
19 background exposure levels. Information on the application of the dioxin TEFs is published
20 elsewhere by EPA for both ecological (U.S. EPA, 2008, [543774](#)) and human health risk
21 assessment (U.S. EPA, 2009, [192196](#)). As a consequence, EPA does not directly address TEFs
22 herein, but makes use of the concept of toxicity equivalence¹⁰ as applicable to the analysis of
23 exposure dose in epidemiologic studies. Furthermore, this document does not address the NAS
24 recommendations pertaining to the assessment of human exposures to TCDD and other dioxins.
25 Information on updated background levels of dioxin in the U.S. population has been recently
26 reported (Lorber et al., 2009, [543766](#)).

27

28 **1.3.1. TCDD Literature Update**

29 EPA has developed a literature database of peer-reviewed studies on TCDD toxicity,
30 including in vivo mammalian dose-response studies and epidemiologic studies. An initial
31 literature search for studies published since the 2003 Reassessment was conducted by the U.S.
32 Department of Energy's Argonne National Laboratory (ANL) through an Interagency Agreement

¹⁰Toxicity equivalence (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.
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1 with EPA. ANL used the online National Library of Medicine database (PubMed) and identified
2 studies published between the year 2000 and October 31, 2008. Supporting references published
3 since the release of the 2003 Reassessment were also identified. Supporting studies were
4 classified as studies pertaining to TCDD kinetics, TCDD mode-of-action, in vitro TCDD studies,
5 and TCDD risk assessment approaches. The literature search strategy explicitly excluded studies
6 addressing (1) analytical/detection data and cellular screening assays; (2) environmental fate,
7 transport and concentration data; (3) dioxin-like compounds and toxic equivalents;
8 (4) nonmammalian dose-response data; (5) human exposure analyses only, including body
9 burden data; and (6) combustor or incinerator or other facility-related assessments absent
10 primary dose-response data. EPA published the initial literature search results in the Federal
11 Register on November 24, 2008 (73 FR 70999; November 24, 2008) and invited the public to
12 review the list and submit additional peer-reviewed in vivo mammalian dose-response studies for
13 TCDD, including epidemiologic studies that were absent from the list (U.S. EPA, 2008, [519261](#)).
14 Submissions were accepted by the EPA through an electronic docket, email and hand delivery,
15 and were evaluated for use in TCDD dose-response assessment. The literature search results and
16 subsequent submissions were used during a 2009 scientific workshop, which was open to the
17 public and featured a panel of experts on TCDD toxicity and dose-response modeling (discussed
18 below). Additional studies identified during the workshop and those collected by EPA scientists
19 during the development of this report through October 2009 have been incorporated into the final
20 set of studies for TCDD dose-response assessment.

21

22 **1.3.2. EPA’s 2009 Workshop on TCDD Dose Response**

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

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- 1
- 2
- 3 • Identify and discuss the technical challenges involved in addressing the NAS comments
4 related to the dose-response issues within each specific session topic and the TCDD
5 quantitative dose-response assessment.
 - 6 • Discuss approaches for addressing the key NAS recommendations.
 - 7 • Identify important published, independently peer-reviewed literature—particularly
8 studies describing epidemiologic studies and in vivo mammalian bioassays expected to
9 be most useful for informing EPA’s response.

10 The sessions were followed by open comment periods during which members of the
11 audience were invited to address the expert panels. The session’s Panel Co-chairs were asked to
12 summarize and present the results of the panel discussions—including the open comment
13 periods. The summaries incorporated points of agreement as well as minority opinions. Final
14 session summaries were prepared by the session Panel Co-chairs with input from the panelists,
15 and they formed the basis of a final workshop report (U.S. EPA, 2009, [543757](#), Appendix A of
16 this report). Because the sessions were not designed to achieve consensus among the panelists,
17 the summaries do not necessarily represent consensus opinions; rather reflect the core of the
18 panel discussions. Some of the key discussion points from the workshop that influenced EPA’s
19 development of this document are listed below (see Appendix A for detail):

- 20
- 21 • In the development of study selection criteria, more relevant exposure-level (i.e., dose)
22 decision points using tissue concentrations could be defined.
 - 23 • A linear approach to body-burden estimation, which was utilized in the 2003
24 Reassessment (U.S. EPA, 2003, [537122](#)), does not fully consider key toxicokinetic issues
25 related to TCDD—e.g., sequestration in the liver and fat, age-dependent elimination, and
26 changing elimination rates over time. Thus, kinetic/mechanistic modeling could be used
27 to quantify tissue-based metrics. In considering human data, lipid-adjusted serum levels
28 may be preferable over body burden, although the assumptions used in the back
29 calculation of the body burden in epidemiologic cohorts are of concern. In considering
30 rat bioassay data, lipid-adjusted body-burden estimates may be preferable.
 - 31 • New epidemiologic studies on noncancer endpoints have been published since the
32 2003 Reassessment that may need to be considered (e.g., thyroid dysfunction literature
33 from Wang et al. (2005, [198734](#)) and Baccarelli et al. (2008, [197059](#))).
 - 34 • The 1% of maximal response (ED₀₁) that was utilized in the 2003 Reassessment has not
35 typically been used in dose-response assessment. Some alternative ideas were as follows:
36 (1) the POD should depend on the specific endpoint; (2) for continuous measures, the
37 benchmark response (BMR) could be based on the difference from control and consider

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1 the adversity level; and (3) for incidence data, the BMR should be set to a fixed-risk
2 level.

- 3 • The quantitative dose-response modeling for cancer could be based on human or animal
4 data. There are new publications in the literature for four epidemiological cohort studies
5 (Dutch cohort, NIOSH cohort, BASF accident cohort, and Hamburg cohort). The
6 increase in total cancers could be considered for modeling human cancer data. However,
7 non-Hodgkin's lymphoma and lung tumors are the main TCDD-related cancer types seen
8 from human exposure. In reviewing the rat data, the NTP (2006, [197605](#)) data sets are
9 new and can be modeled. Although the liver and lungs are the main target organs,
10 modeling all cancers, as well as using tumor incidence in lieu of individual rats as a
11 measure, should be considered.
- 12 • Both linear and nonlinear model functions should be considered in the cancer
13 dose-response analysis because there are data and rationales to support use of either
14 below the POD.
- 15 • For quantitative uncertainty analysis, consider the impacts of choices among plausible
16 alternative data sets, dose metrics, models, and other more qualitative choices. Issues to
17 consider include how much difference these choices make and, also, how much relative
18 credence should be put toward each alternative as a means to gauge and describe the
19 landscape of imperfect knowledge with respect to possibilities for the true dose response.
20 This may be difficult to do quantitatively because the factors are not readily expressed as
21 statistical distributions. However, the rationale for accepting or questioning each
22 alternative in terms of the available supporting evidence, contrary evidence, and needed
23 assumptions, can be delineated.

24 25 **1.3.3. Overall Organization of EPA's Response to NAS Recommendations**

26 The remainder of this document is divided into five sections that address the
27 three primary areas of concern resulting from the NAS (2006, [198441](#)) review. Section 2
28 describes EPA's approach to the recommendation for transparency and clarity during selection of
29 key data sets—including criteria for the selection of key dose-response studies, evaluations of the
30 important epidemiologic studies and animal bioassays, and a summary of the key studies used
31 for subsequent dose-response modeling. Sections 3, 4, and 5 present EPA's response to the NAS
32 recommendation to better justify the approaches used in dose-response modeling of TCDD.
33 Section 3 discusses the toxicokinetic modeling EPA conducted to support the dose-response
34 analyses. Section 4 presents EPA's approach to noncancer data set selection, dose-response
35 modeling, and derivation of an RfD for TCDD, and contains a qualitative discussion of the
36 uncertainties associated with the RfD. Section 5 presents an updated cancer weight-of-evidence
37 summary, EPA's approach to cancer data set selection, dose-response modeling, derivation of an

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- 1 OSF for TCDD, and a qualitative discussion of the uncertainties associated with the OSF,
- 2 including an evaluation of illustrative nonlinear approaches to cancer assessment of TCDD.
- 3 Finally, Section 6 discusses the feasibility of conducting a quantitative uncertainty analysis of
- 4 TCDD dose response.

1 ...in its [EPA's] evaluation of the epidemiological literature of carcinogenicity, it
2 did not outline eligibility requirements or otherwise provide the criteria used to
3 assess the methodological quality of other included studies (NAS, 2006, [198441](#),
4 p. 56).

5 With regard to EPA's review of the animal bioassay data, the committee
6 recommends that EPA establish clear criteria for the inclusion of different data
7 sets (NAS, 2006, [198441](#), p. 191).

8 ...the committee expects that EPA could substantially improve its assessment
9 process if it more rigorously evaluated the quality of each study in the database
10 (NAS, 2006, [198441](#), p. 56).

11 EPA could also substantially improve the clarity and presentation of the risk
12 assessment process for TCDD...by using a summary table or a simple summary
13 graphical representation of the key data sets and assumptions...(NAS, 2006,
14 [198441](#), p. 56).

15

16 **2.2. EPA'S RESPONSE TO NAS COMMENTS ON TRANSPARENCY AND CLARITY** 17 **IN THE SELECTION OF KEY DATA SETS FOR DOSE-RESPONSE ANALYSIS**

18 EPA agrees with the NAS committee regarding the need for a transparent and clear
19 process for selecting studies and key data sets for TCDD dose-response analyses. The
20 delineation of the study selection process and decisions regarding key data sets will facilitate
21 communication regarding critical decisions made in the TCDD dose-response assessment. In
22 keeping with the NAS committee's recommendation to use a transparent process and improve
23 clarity and presentation of the risk assessment process for TCDD, Figure 2-1 overviews the
24 approach that EPA has used in this document to develop a final list of key cancer and noncancer
25 studies for quantitative dose-response analysis of TCDD. The steps in Figure 2-1 are further
26 explained below.

27

28 **Literature search for in vivo mammalian and epidemiologic TCDD studies**
29 **(2000–2008):** EPA conducted a literature search to identify peer-reviewed, dose-response
30 studies for TCDD that have been published since the 2003 Reassessment. This search
31 included in vivo mammalian and epidemiological studies of TCDD from 2000 to 2008.
32 Additional details describing the conduct of this literature search are presented in
33 Section 1.3.1 of this document.

34 **Federal Register Notice—Web publication of literature search for public comment:**
35 In November 2008, EPA published a list of ~500 citations from results of this literature
36 search (U.S. EPA, 2008, [519261](#)) and invited the public to review this preliminary list of
37 dose-response citations for use in TCDD dose-response assessment. EPA requested that
38 interested parties identify and submit peer-reviewed studies for TCDD that were absent

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1 from this list. Two parties identified additional references that were not included in the
2 2008 Federal Register notice and submitted additional references for EPA to consider.
3 These references were included in the final TCDD literature database considered by EPA
4 for TCDD dose-response analysis.

5 **Initial study inclusion criteria development for TCDD in vivo mammalian**

6 **bioassays:** EPA developed an initial set of draft criteria for evaluating the extensive
7 TCDD database of in vivo mammalian bioassays. These initial inclusion criteria had
8 three purposes. First, they provided a transparent and rigorous evaluation of the scientific
9 quality of each study in EPA’s database, a deficiency in the 2003 Reassessment identified
10 by the NAS committee. Second, given the vast TCDD mammalian bioassay database,
11 they provided a transparent method for initially screening studies to be considered for
12 TCDD dose-response analyses. Third, they served as a starting point for discussions of
13 study inclusion criteria by expert panelists who were convened by EPA for its scientific
14 workshop on TCDD dose-response analysis (the Dioxin Workshop), described next (also
15 see the workshop report in Appendix A, U.S. EPA [2009b]).

16 **Dioxin Workshop and expert refinement of TCDD in vivo mammalian bioassay**

17 **inclusion criteria:** In February 2009, EPA convened “A Scientific Workshop to Inform
18 EPA’s Response to NAS Comments on the Health Effects of Dioxin in EPA’s 2003
19 Dioxin Reassessment.” The goals of this 3-day public and scientific workshop were to
20 identify and address issues related to the dose-response assessment of TCDD. Sessions at
21 the workshop examined toxicities associated with TCDD, issues related to developing
22 dose-response estimates based on these data and associated uncertainties. At the
23 workshop, EPA presented the draft set of study inclusion criteria for evaluating the
24 extensive TCDD in vivo mammalian bioassay literature and asked workshop panelists to
25 discuss these criteria and make recommendations for their revision. Further details on
26 this workshop are presented in Section 1.3.2 of this document, and the complete report
27 from this workshop is available in Appendix A (U.S. EPA, 2009b), including detailed
28 summaries of the panels’ comments on the inclusion criteria in relation to the various
29 toxic endpoints that were discussed.

30 **Final development of inclusion criteria for TCDD in vivo mammalian studies:** Based
31 on discussions at the Dioxin Workshop, the initial draft inclusion criteria for evaluating
32 the TCDD mammalian bioassay literature were revised and are presented in Section 2.3.2
33 (see Figure 2-3). An initial criterion is that studies for consideration must be publically
34 available and published in a peer-reviewed scientific journal. Because the methodology
35 EPA uses to develop reference doses (RfDs) and cancer oral slope factors (OSFs) relies
36 on identification of studies reporting potential adverse effects at low doses (relative to the
37 overall database), another important criterion shown in Section 2.3.2 identifies a
38 maximum value for the lowest TCDD dose tested in a bioassay. This maximum value
39 was used to eliminate those studies that could not be selected for development of an RfD
40 or an oral slope factor because tested doses were too high relative to other TCDD
41 bioassays.

42 **Development of inclusion criteria for epidemiologic studies:** Following the Dioxin
43 Workshop, EPA determined that an evaluation process was also needed for inclusion of
44 epidemiologic studies for TCDD dose-response assessment. These criteria were

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1 developed and are detailed in Section 2.3.1 (see Figure 2-2). Analogous to animal
2 bioassay data, epidemiologic studies for consideration must also be publically available
3 and published in a peer-reviewed scientific journal. In addition to assessing the
4 methodological considerations relative to epidemiologic cohorts and studies (e.g.,
5 statistical power and precision of estimates, consideration of latency periods), key criteria
6 for use of a study in TCDD dose-response modeling were that the exposure be primarily
7 to TCDD and that the effective dose and oral exposure are reasonably estimable.

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

11 **Studies screened using inclusion criteria:** The two sets of TCDD-specific study
12 inclusion criteria presented in Section 2.3 were used to evaluate all studies included in the
13 2003 Reassessment, studies identified in the 2000–2008 literature search, studies
14 identified through public comment and submission, and studies collected in 2009 as
15 identified by EPA during the development of this document. Section 2.4 presents results
16 of EPA’s evaluation of epidemiologic and mammalian bioassay literature for both cancer
17 and noncancer endpoints.

18 **Final list of key cancer and noncancer studies for quantitative dose-response**
19 **analysis of TCDD:** Application of the study inclusion criteria concludes in Section 2.4
20 with development of a list of key noncancer and cancer studies that were considered for
21 quantitative dose-response analyses of TCDD in Sections 4 and 5, respectively. In those
22 sections, points of departure (PODs) are developed and evaluated for all biologically
23 relevant study/endpoint combinations from these final key study lists, and key data sets
24 and PODs for the development of TCDD toxicity values are identified.

26 **2.3. STUDY INCLUSION CRITERIA FOR TCDD DOSE-RESPONSE ANALYSIS**

27 One of the three major recommendations made by the NAS (2006, [198441](#)) committee
28 was that EPA should provide greater clarity and transparency on the selection of studies that
29 were used in the quantitative dose-response modeling of TCDD in the 2003 Reassessment. In
30 this section, EPA describes TCDD-specific study inclusion criteria that have been developed to
31 evaluate epidemiologic studies and animal bioassays for TCDD dose-response assessment.
32 These criteria reflect EPA’s goal of developing an RfD and a cancer OSF for TCDD through a
33 transparent study selection process; they are intended to be used by EPA for TCDD
34 dose-response assessment only. These criteria were applied to each of the ~500 studies listed in
35 *Preliminary Literature Search Results and Request for Additional Studies on*
36 *2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) Dose-Response Studies* (U.S. EPA, 2008,
37 [519261](#)); studies identified and submitted by the public and by participants in the Dioxin

1 Workshop (U.S. EPA, 2009, [522927](#)); studies included in the 2003 Reassessment, and other
2 relevant published studies collected by EPA scientists through October 2009.

3 EPA has undertaken different approaches for epidemiologic versus in vivo animal
4 bioassay study evaluation and key data set selection. The significant differences between animal
5 and human health effects data and their use in EPA risk assessment support development of
6 separate criteria for study inclusion and different approaches to study evaluation. For the vast
7 majority of compounds on EPA's Integrated Risk Information System (IRIS), cancer and
8 noncancer toxicity values have been derived using animal bioassay data; therefore, approaches to
9 dose-response modeling and POD selection from in vivo mammalian bioassays have been
10 standardized and codified (U.S. EPA, 2000, [052150](#)). The study criteria shown below and in
11 Figure 2-3 for animal bioassay data reflect EPA's preferences for TCDD-specific study
12 inclusion, some of which are based on common practices and guidance for POD selection and
13 RfD and OSF derivation. Far fewer IRIS toxicity values have been derived from human data,
14 although some examples do exist. For example, benzene, beryllium and compounds, chromium
15 IV, and 1,3-butadiene have RfDs, Reference Concentrations, Inhalation Unit Risks and/or OSFs
16 based on occupational cohort data and the methyl mercury RfD is based on high fish consuming
17 cohorts (U.S. EPA, 2009, [543757](#)). The modeling and interpretation of such human data have
18 been conducted on a case-by-case basis because each cohort is uniquely defined and has its own
19 set of exposure conditions, significant confounders, and biases that may need to be considered in
20 dose-response modeling. For TCDD, not all data are from occupational cohorts, but include
21 cohorts exposed for relatively short time periods to high concentrations as a consequence of
22 industrial accidents, a scenario that has not commonly been used to establish EPA toxicity
23 values.

24 Because of these differences in data characteristics, divergent selection approaches are
25 used in this document to present and evaluate the epidemiologic studies (see Section 2.3.1) and
26 the in vivo animal bioassays (see Section 2.3.2). In Section 2.4.1, all of the available
27 epidemiologic studies on TCDD are summarized and evaluated for suitability for dose-response
28 modeling using the TCDD-specific study inclusion criteria below and shown in Figure 2-2; only
29 studies meeting the inclusion criteria are presented as key studies in Section 2.4.3 (see Tables 2-4
30 and 2-5 for the cancer and noncancer endpoints, respectively). In Section 2.4.2, because
31 summarizing and showing the evaluation of the thousands of available animal bioassays on

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1 TCDD was prohibitive, only studies first meeting the in vivo animal bioassays study inclusion
2 criteria below (and shown in Figure 2-3) are summarized. These studies are also presented as
3 key studies in Section 2.4.3 (see Tables 2-6 and 2-7 for cancer and noncancer endpoints,
4 respectively).

6 **2.3.1. Study Inclusion Criteria for TCDD Epidemiologic Studies**

7 This section identifies the process EPA used to select epidemiologic studies for defining
8 candidate PODs for TCDD dose-response modeling. These criteria are based on EPA's
9 approaches for deriving OSFs and RfDs. A discussion of the considerations used in selecting
10 epidemiologic data for quantitative dose-response modeling is valuable, particularly given EPA's
11 preference to use high-quality human studies over animal studies because such human studies are
12 regarded as providing the most relevant information needed for quantitative human health risk
13 analyses (U.S. EPA, 2005, [086237](#)). As described by Hertz-Picciotto (1995, [065678](#)), key
14 components needed for the use of an epidemiologic study as a basis for quantitative risk
15 assessment include issues regarding exposure assessment (a well-quantified exposure assessment
16 with exposures linked to individuals) and study quality ("strong biases," for example with
17 respect to inclusion criteria for membership in the cohort and follow-up procedures "ruled out or
18 unlikely" and "confounding controlled or likely to be limited"). The strength of the association,
19 either within the full study or within a high exposure subgroup, can also be considered in the
20 evaluation of suitability for dose-response modeling (Hertz-Picciotto, 1995, [065678](#)). Stayner
21 et al. (1999, [198654](#)), however, note that even weak associations could be useful in terms of
22 providing an estimate of a potential upper bound for a quantitative risk estimate.

23 EPA's method for applying the TCDD study inclusion criteria to epidemiologic data is
24 detailed below and in Figure 2-2. Based on the framework discussed above, EPA evaluated the
25 available epidemiologic cohorts and studies based on the five following considerations:

- 26 1. The methods used to ascertain health outcomes are clearly identified and unbiased, with
27 high sensitivity and specificity.
- 28 2. The risk estimates generated from the study are not susceptible to important biases
29 arising from an inability to control for potential confounding exposures or other sources
30 of bias arising from either study design or statistical analysis.
31

- 1 3. The study demonstrates an association between TCDD and an adverse health effect
2 (assuming minimal misclassification of exposure and absence of important biases) with
3 some suggestion of an exposure-response relationship.
- 4 4. The exposure assessment methodology is clearly described and can be expected to
5 provide adequate characterization of exposure, with assignment of individual-level
6 exposures within a study (e.g., based on biomarker data, or based on a
7 job-exposure-matrix approach). Limitations and uncertainties in the exposure assessment
8 are considered.
- 9 5. The size and follow-up period of a cohort study are large enough and long enough,
10 respectively, to yield sufficiently precise estimates for use in development of quantitative
11 risk estimates and to ensure adequate statistical power to limit the possibility of not
12 detecting an association that might be present (i.e., to avoid Type II Errors due to failing
13 to reject the null hypothesis when the null hypothesis is true). Similar considerations
14 regarding sample size and statistical precision and power apply to case-control studies.

15
16 Three specific study inclusion criteria were used to select studies for further evaluation
17 and potential TCDD quantitative dose-response assessment

- 18
19 1. The study is published in the peer-reviewed scientific literature and includes an
20 appropriate discussion of strengths and limitations.
- 21 2. The exposure is primarily to TCDD, rather than dioxin-like compounds (DLCs), and is
22 properly quantified so that dose-response relationships can be assessed. All
23 epidemiologic cohorts will have background exposures to DLCs through the food chain
24 and these exposures are not included in this criterion.
- 25 3. The effective dose and oral exposure must be reasonably estimable. The measures of
26 exposure must be consistent with the current biological understanding of dose. For
27 TCDD dose-response assessment, it is critical that reported dose is consistent with a dose
28 that is likely to be toxicologically relevant. The timing of the measurement of effects
29 (i.e., the response) also must be consistent with current biological understanding of the
30 effect and its progression.

31 For cancer endpoints, EPA assumes that cumulative TCDD dose estimates are
32 toxicologically relevant measures. Thus, cancer studies must provide information
33 about long-term TCDD exposure levels. Further, EPA reasons that measures of
34 cancer occurrence or death need to allow for examination of issues of latency
35 between the end of effective exposure and cancer detection or death.

36 For noncancer endpoints, exposure estimates and analysis must allow for examination
37 of issues of latency and other issues regarding the appropriate time window of
38 exposure relevant for specific endpoints. Also, to be consistent with the RfD
39 methodology, the response must be to a nonfatal endpoint.

40
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1 Those studies that met these three inclusion criteria (see Sections 2.4.1, 2.4.3, and Appendix B)
2 were then subjected to further consideration for quantitative dose-response analyses.

4 **2.3.2. Study Inclusion Criteria for TCDD In Vivo Mammalian Bioassays**

5 This section identifies the criteria EPA applied to select nonhuman in vivo mammalian
6 studies for defining candidate PODs for use in TCDD dose-response modeling. These inclusion
7 criteria are based on EPA’s approaches for deriving OSFs and RfDs from bioassay data
8 (U.S. EPA, 2005, [086237](#)). EPA agrees with the NAS committee regarding the utility of an oral
9 RfD and the need for reevaluation of the OSF for TCDD, specifically in light of data that have
10 been published since the 2003 Reassessment was released. RfDs and OSFs are generally derived
11 using data sets that demonstrate the occurrence of adverse effects, or their precursors, in
12 low-dose range for that chemical. RfDs and OSFs are derived from a health protective
13 perspective for chronic exposures. Thus, when a group of studies is available on a chemical for
14 which a number of effects are observed at various doses across those studies, the studies using
15 the lowest exposures that show effects will typically drive the RfD and OSF derivations, all other
16 considerations being equal. Studies conducted at higher exposures relative to other available
17 studies are used as supporting evidence for the final RfD or OSF since they were conducted at
18 doses too high to impact the numeric derivations of toxicity values. EPA expresses RfDs and
19 OSFs in terms of average daily doses, usually as mg/kg-day and per mg/kg-day, respectively.
20 Thus, the study inclusion criteria for the animal bioassay data presented in this section include
21 requirements that average daily exposures in the studies are within a low dose range where,
22 relative to other studies, they could be considered for development of a toxicity value. These
23 low-dose requirements do not imply that TCDD studies conducted at higher doses are of poor
24 quality, simply that they are not quantitatively useful in the development of toxicity values
25 because other studies with lower exposures will drive the RfD and OSF derivations under current
26 EPA practice. Because EPA has identified ~2,000 studies on TCDD that may be considered for
27 this purpose, the development and application of these study inclusion criteria has been critical to
28 moving the risk assessment process forward.

29 EPA’s method for applying study inclusion criteria for mammalian bioassays is detailed
30 below and in Figure 2-3. The first study inclusion criterion is that the study is published in the
31 peer-reviewed scientific literature. Then, two specific study inclusion criteria were used to select

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1 studies for further evaluation and potential TCDD quantitative dose-response analyses and
2 identification of candidate PODs:

- 3
- 4 1. The lowest dose level tested is ≤ 1 $\mu\text{g}/\text{kg}\text{-day}$ for cancer studies and ≤ 30 $\text{ng}/\text{kg}\text{-day}$ for
5 noncancer studies.
 - 6 2. The study design consists of orally administered TCDD-only doses, and specifies the
7 purity and matrix used to administer the doses.

8

9 Then, EPA evaluated the remaining in vivo animal studies based on the following
10 four considerations.

- 11
- 12 1. The study tests mammalian species, identifying the strain, gender, and age of the tested
13 animals.
 - 14 2. The study clearly documents testing protocol, including dosing frequency, duration, and
15 timing of dose administration relative to age of the animals.
 - 16 3. The overall study design is consistent with standard toxicological principles and
17 practices. The control group or groups are appropriate, given the testing protocol, and are
18 well characterized. Clinical and pathological examinations conducted during the study
19 are endpoint-appropriate, particularly for negative findings.
 - 20 4. The magnitude of animal responses is outside the range of normal variability exhibited by
21 control animals (e.g., greater than or less than one standard deviation).

22

23 Those studies that met the aforementioned considerations and inclusion criteria (see
24 Sections 2.4.2 and 2.4.3) were then subjected to dose-response analysis.

25 The criteria for dose requirements, although somewhat arbitrary, are intended to be
26 reasonable cutoffs that restrict the number of studies that would need to be modeled while
27 ensuring that all study/data set combinations that could be candidates for the cancer slope factor
28 or RfD were modeled. Thus, the dose range under consideration allows for liberal ranges of
29 no-observed-adverse-effect levels (NOAELs), lowest-observed-adverse-effect levels (LOAELs),
30 and benchmark dose lower confidence bound (BMDLs) for assessment of both cancer and
31 noncancer effects.

32 For cancer studies, the dose requirements were selected based on an initial evaluation of
33 available average daily doses administered in TCDD animal bioassays in which adverse effects
34 were observed. For example, in cancer studies, a sample of the relatively low ranges of tested

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1 average daily doses include 1-1,000 ng/kg-day (Toth et al., 1979), 1–100 ng/kg-day (Kociba
2 et al., 1978), 1.43–286 ng/kg-day (NTP, 1982, [543764](#)) and 2.14–71.4 ng/kg-day (NTP, 2006,
3 [197605](#)) with statistically significant increases in tumor incidence via pair-wise or trend tests
4 found in all of these studies. The entire range of each these studies is ≤ 1 $\mu\text{g}/\text{kg}\text{-day}$. The
5 linearized multistage model used by EPA to estimate OSFs is most appropriately applied to
6 studies from which PODs can be estimated as closely as possible to the experimental data. Thus,
7 given the dose ranges in these studies that are available for modeling, the restriction to
8 ≤ 1 $\mu\text{g}/\text{kg}\text{-day}$ for cancer was considered to be a reasonable cutoff.

9 For noncancer studies, dose ranges are more complex and vary according to study
10 endpoint. Examples of the lowest administered doses that might be considered as NOAELs or
11 LOAELs in POD determinations for noncancer endpoints include 1 ng/kg-day (Toth et al., 1979,
12 [197109](#)), 1.43 ng/kg-day (Cantoni et al., 1981, [197092](#)), 1.07 ng/kg-day (Smialowicz et al., 2008,
13 [198341](#)) 1.43 ng/kg-day (NTP, 1982, [543764](#)) and 2.14 ng/kg-day (NTP, 2006, [197605](#)). Most
14 of the lowest tested doses in the TCDD studies have been designated as LOAELs (see
15 Section 4.1). Given the available database, it is likely that the same composite uncertainty factor
16 (e.g., of 300; 3 for UF_A [interspecies], 10 for UF_H [intraspecies], and 10 for UF_L [LOAEL to
17 NOAEL]) would be applied to any animal noncancer LOAEL used to derive an RfD for TCDD.
18 This implies that any study that has a LOAEL of 30 ng/kg-day or more would result in a
19 candidate RfD that is more than an order of magnitude higher than the example doses of
20 1–2 ng/kg-day shown here. BMDLs that might be derived from such data also would not be
21 expected to be lower than these example doses of 1–2 ng/kg-day. Thus, a tested dose
22 ≤ 30 ng/kg-day is considered to be a reasonable cutoff where the lowest tested dose would never
23 be used as a POD to derive an RfD given that much lower tested doses (associated with adverse
24 effects) are available from other studies of acceptable quality.

25 26 **2.4. EVALUATION OF KEY STUDIES FOR TCDD DOSE RESPONSE**

27 **2.4.1. Evaluation of Epidemiological Cohorts for Dose-Response Assessment**

28 This section summarizes and evaluates studies for potential use in TCDD dose-response
29 assessment using the study evaluation considerations and inclusion criteria for epidemiologic
30 data (see Section 2.3.1). Those studies that meet the study inclusion criteria are listed later in
31 this Section in Tables 2-4 and 2-5, for cancer and noncancer, respectively, and are considered in

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1 the dose-response modeling conducted later in this document (see Sections 4 and 5). The
2 following sections are organized by epidemiologic cohort. Following a brief summary of each
3 cohort, its associated studies are then summarized chronologically, assessed for methodological
4 considerations relative to epidemiologic cohorts and studies (e.g., statistical power and precision
5 of estimates, consideration of latency periods) and evaluated for suitability for TCDD dose-
6 response assessment.

7 8 **2.4.1.1. *Cancer***

9 In the 2003 Reassessment, EPA selected three cohort studies from which to conduct a
10 quantitative dose-response analysis: the National Institute for Occupational Safety and Health
11 (NIOSH) cohort (Steenland et al., 2001, [197433](#)), the BASF cohort (Ott and Zober, 1996,
12 [198408](#)), and the Hamburg cohort (Becher et al., 1998, [197173](#)). Although these studies were
13 deemed suitable for quantitative dose-response analysis, the criteria EPA used to reach this
14 conclusion were unclear. In this section, the study selection criteria and methodological
15 considerations presented in Section 2.3 are systematically applied to evaluate a number of studies
16 to determine their suitability for inclusion in dose-response modeling. In addition to the
17 three cohorts used in previous TCDD quantitative risk assessment, considerations are applied to
18 other relevant TCDD epidemiological data sets that were identified through a literature review
19 for epidemiological studies of TCDD and cancer. Study summaries and suitability for
20 quantitative dose-response analysis evaluations are discussed below.

21 22 **2.4.1.1.1. *Cancer cohorts.***

23 **2.4.1.1.1.1. *The NIOSH cohort.***

24 In 1978, the NIOSH undertook research that identified workers employed by U.S.
25 chemical companies that made products contaminated with TCDD between 1942 and 1982.
26 TCDD was generated in the production of 2,4,5-trichlorophenol and subsequent processes. This
27 chemical was used to make 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), which was a major
28 component of the widely-used defoliant, Agent Orange. The NIOSH cohort is the largest cohort
29 of occupational workers studied to date and has been the subject of a series of investigations
30 spanning more than two decades. It is important to note that this cohort consists mostly of male
31 workers that were exposed to TCDD via daily occupational exposure, as compared to an acute

1 accidental exposure scenario seen with other cohorts. The investigations have progressed from a
2 comparison of the mortality patterns of the cohort to the U.S. general population to
3 dose-response modeling using serum-derived estimates of TCDD that have been
4 back-extrapolated several decades. Analyses of cancer data from the NIOSH cohort that are
5 addressed in this section include Fingerhut et al. (1991, [197375](#)), Steenland et al. (1999, [197437](#);
6 2001, [197433](#)), Cheng et al. (2006, [523122](#)), and Collins et al. (2009, [197627](#)).

7
8 **2.4.1.1.1.1.1.** Fingerhut et al. (1991, [197375](#)).

9 **2.4.1.1.1.1.1.1.** *Study summary.*

10 The investigation of Fingerhut and her colleagues published nearly two decades ago
11 attracted widespread attention (Fingerhut et al., 1991, [197375](#)). This retrospective study
12 examined patterns of cancer mortality for 5,172 workers who comprised the NIOSH cohort,
13 which combined workers from the company-specific cohorts of Dow Chemical (Ott et al., 1987,
14 [064994](#))(Cook, 1981) and the Monsanto Company (Zack and Gaffey, 1983, [548783](#); Zack and
15 Suskind, 1980, [065005](#)). These workers were employed at 12 plants producing chemicals
16 contaminated with TCDD. Almost all workers in the cohort (97%) had production or
17 maintenance jobs with processes involving TCDD contamination. On average, workers were
18 employed for 2.7 years specifically in processes that involved TCDD contamination, and overall,
19 were employed for 12.6 years. The mortality follow-up began in 1940 and extended until the
20 end of 1987. Vital status was determined using records from the Social Security Administration,
21 the Internal Revenue Service, or the National Death Index. The ascertainment of vital status in
22 the cohort was nearly complete, with less than 1% of the cohort not followed up until death or
23 the end of the study period.

24 Comparisons of mortality were made relative to the U.S. male general population and
25 expressed using the standardized mortality ratio (SMR) metric and 95% confidence intervals
26 (CIs). Life-table methods were used to generate person-years of risk accrued by cohort members
27 at each plant. Person-years and corresponding deaths were tabulated across age, race, and year
28 of death strata, which permitted the SMRs to be examined for potential confounding from these
29 three characteristics. No unadjusted SMRs were presented in the paper. Cross-classification of
30 person-years and deaths was also done across several exposure-related groupings, including
31 duration of employment, years since first exposure, years since last exposure, and duration of

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1 exposure. Employment duration was categorized as <5, 5– <10, 10– <15, 15– <20, 20– <25,
2 25– <30, and ≥30 years. The variable “years since first exposure” (<10, 10– <20, and ≥20 years)
3 was used to evaluate associations in relation to different latency periods. The analysis was
4 jointly stratified by duration of employment and for varying latency intervals to evaluate whether
5 cohort members with higher cumulative TCDD levels had higher cancer mortality rates than
6 those cohort members with lower cumulative levels.

7 Overall, the cohort of workers had slightly elevated cancer mortality than the general
8 population (SMR = 1.15, 95% CI = 1.02–1.30). Comparisons to the general population,
9 however, yielded no statistically significant excess for any site-specific cancer. Cancer mortality
10 was examined for the subset of workers that worked for at least one year and had a latency
11 interval of at least 20 years ($n = 1,520$). The 1-year cut-point was selected based on analyses of
12 serum levels in a subset of 253 workers which revealed that every worker employed for at least
13 one year had a lipid-adjusted serum level that exceeded the mean (7 ppt). Relative to the
14 U.S. general population, statistically significant excesses in cancer mortality were observed for
15 all cancers (SMR = 1.46, 95% CI = 1.21–1.76), cancers of the respiratory system (SMR = 1.42,
16 95% CI = 1.03–1.92), and for soft tissue sarcoma (SMR = 9.22, 95% CI = 1.90–26.95) among
17 this subset of 1,520 male workers. The elevated SMR for soft tissue sarcoma, however, was
18 based on only three cases in this subset.

19 SMRs also were generated across joint categories of duration of exposure and period of
20 latency for deaths from all cancer sites (combined), and cancer of the trachea, bronchus, and
21 lung. Increased SMRs were observed in strata defined by longer exposure and latency, but no
22 statistically significant linear trends were found.

23

24 **2.4.1.1.1.1.2.** *Study evaluation.*

25 This cohort was the largest of four the International Agency for Research on Cancer
26 (IARC) considered in its 1997 classification of TCDD as a Group 1 human carcinogen (IARC,
27 1997, [537123](#)). Duration of employment in processes that involved TCDD contamination was
28 used as a surrogate measure of cumulative exposure. In using this exposure metric, Fingerhut
29 et al. (1991, [197375](#)) assumed that TCDD exposures were equivalent at all production plants.
30 Doses for individual cohort members were not reconstructed for these analyses, although they
31 were in subsequent analyses of this cohort.

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1 Workers in this cohort also were exposed to other chemicals, which could lead to bias
2 due to confounding if these exposures were associated with both TCDD exposure and the health
3 outcomes being examined. At one plant, workers were exposed to 4-aminobiphenyl. Previous
4 investigators also reported that workers at another plant were exposed to 2,4,5-T and
5 2,4-dichlorophenoxyacetic acid (2,4-D) (Bond et al., 1988, [197183](#); Bond et al., 1989, [064967](#);
6 Ott et al., 1987, [064994](#)). Although this study did not examine the impact of confounding by
7 other occupational coexposures, subsequent analyses of this cohort showed that associations
8 between cumulative TCDD and all cancer mortality persisted after excluding workers exposed to
9 pentachlorophenols from the analyses (Steenland et al., 1999, [197437](#)). Removal of workers
10 who died from bladder cancer also did not substantially change the dose-response association
11 between TCDD and cancer mortality from all other sites combined. This finding suggests that
12 exposures to 4-aminobiphenyl did not confound the association between cancer mortality and
13 TCDD exposure. Overall, there is little evidence of confounding by these co-exposures among
14 this cohort, however, exposure to other possible confounders, such as dioxin-like compounds,
15 was not examined.

16 The study collected no information on smoking behavior of the workers, and therefore,
17 the SMRs do not account for any differences in the prevalence of smoking that might have
18 existed between the workers and the general population. For several reasons, however, the
19 inability to take into account smoking is unlikely to have been an important source of bias. First,
20 mortality from other smoking-related causes of death such as nonmalignant respiratory disease
21 were not more common in the cohort than in the general population (SMR = 0.96,
22 95% CI = 0.54–1.58). Second, stratified analyses of workers with at least a 20-year latency
23 (assuming this subset shared similar smoking habits) revealed that excesses were apparent only
24 among those who were exposed for at least 1 year. Specifically, when compared to the general
25 population, the SMR among workers exposed for at least 1 year with a latency of 20 years was
26 1.46, (95% CI = 1.21–1.76) while those exposed for less than 1 year had an SMR of 1.02
27 (95% CI = 0.76–1.36). Third, for comparisons of cancer mortality between blue-collar workers
28 and the general population, smoking is unlikely to explain cancer excesses of greater than
29 10–20% (Siemiatycki et al., 1988, [198556](#)). Finally, the investigators found no substantial
30 changes in the results for lung cancer when risks were adjusted for smoking histories obtained in
31 1987 from 223 workers employed at two plants. These data were used to adjust for the expected

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1 number of lung cancer deaths expected in the entire cohort (Fingerhut et al., 1991, [197375](#)).
2 Following this adjustment, a small change was observed in the SMR for lung cancer in the
3 overall cohort from 1.11 (95% CI = 0.89–1.37) to 1.05 (95% CI = 0.85–1.30). Similarly, only a
4 slight change in the SMR for lung cancer in the higher exposure subcohort was noted from an
5 SMR of 1.39 (95% CI = 0.99–1.89) to 1.37 (95% CI = 0.98–1.87).

6 The use of death certificate information from the National Death Index is appropriate for
7 identifying cancer mortality outcomes. For site-specific cancers such as soft tissue sarcoma,
8 however, the coding of this underlying cause of death is more prone to misclassification (Percy
9 et al., 1981, [004891](#)). Indeed, a review of tissues from four men concluded to have died from
10 soft-tissue sarcoma determined that two deaths had been misclassified (Fingerhut et al., 1991,
11 [197375](#)). A review of hospital data revealed that two other individuals had soft tissue sarcomas
12 that were not identified by death certificate information. The use of death certificate information
13 to derive SMRs for cancer as a whole is likely not subject to significant bias; the same might not
14 hold true, however, for some site-specific cancers such as soft tissue sarcoma.

15 Using the SMR metric to compare an occupational cohort with the general population is
16 subject to what is commonly referred to as the “healthy worker effect” (Choi, 1992, [594250](#); Li
17 and Sung, 1999, [198427](#)). The healthy worker effect is a bias that arises because those healthy
18 enough to be employed have lower morbidity and mortality rates than the general population.
19 The healthy worker effect is likely to be larger for occupations that are more physically
20 demanding (Aittomaki et al., 2005, [197139](#); Checkoway et al., 1989, [027173](#)), and the healthy
21 worker effect is considered to be of little or no consequence in the interpretation of cancer
22 mortality (McMichael, 1976, [073484](#); Monson, 1986, [001410](#)). Few cancers are associated with
23 a prolonged period of poor health that would affect employability long before death. Also
24 recognized is that, as the employed population ages, the magnitude of the healthy worker effect
25 decreases as the absolute reduction in mortality becomes relatively smaller in older age groups
26 (McMichael, 1976, [073484](#)). The mortality follow-up of occupational cohorts generally spans
27 several decades, which should minimize the associated healthy worker effect in such studies.
28 Bias could also be introduced in that workers who are healthier might be more likely to stay
29 employed and therefore accrue higher levels of exposure. In the NIOSH cohort, however,
30 mortality was ascertained for those who could have left the workforce or retired by linking
31 subjects to the National Death Index. Although internal cohort comparisons can minimize the

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1 potential for the healthy worker effect for the reasons presented above, for cancer outcomes, the
2 SMR statistic is a valuable tool for characterizing whether occupational cohort are more likely to
3 die of cancer than the general population. Moreover, stratified analyses across categories of
4 duration of exposure, or latency periods within a cohort can yield important insights about which
5 workers are at greatest risk. Perhaps most important, subsequent analyses of the NIOSH cohort
6 that presented risk estimates derived from external comparisons using the SMR were remarkably
7 consistent with rate ratios derived using an internal referent (Steenland et al., 1999, [197437](#)).

8
9 **2.4.1.1.1.1.3.** *Suitability of data for TCDD dose-response modeling.*

10 This cohort meets most of the identified considerations for conducting a quantitative
11 dose-response analysis for mortality from all cancer sites combined. The NIOSH cohort is the
12 largest cohort of TCDD-exposed workers, exposure characterization at an individual level is
13 possible but not available in this particular study, and the follow-up period is long enough to
14 evaluate latent effects. Although there is no direct evidence of any important sources of bias,
15 confounding may be present due to a lack of consideration of dioxin-like compounds. For the
16 purpose of quantitative dose-response modeling, it is important to note that subsequent studies of
17 this cohort adopted methods that greatly improved the characterization of TCDD exposure in this
18 cohort and increased the follow-up interval (Cheng et al., 2006, [523122](#); Steenland et al., 2001,
19 [197433](#)). As such, for all practical purposes, due consideration for dose-response modeling
20 should focus on the more recently developed data sets.

21 For quantitative dose-response modeling for individual cancer sites, the data are much
22 more limited. A statistically significant positive association with TCDD was noted only for soft-
23 tissue sarcoma among those with more than 1 year of exposure and 20 years of latency
24 (SMR = 9.22, 95% CI = 1.90–26.95). However there were only three deaths from soft tissue
25 sarcoma among this exposed component of the cohort, and four deaths in total in the overall
26 cohort. Also, misclassification of outcome for soft-tissue sarcoma through death registries is
27 well recognized and supported with additional review of tissue from two of the men.
28 Specifically, tissues from the four men who died of soft-tissue sarcoma revealed that only two of
29 these cases were coded correctly.

30 Although subsequent analyses of the NIOSH cohort did not show evidence of
31 confounding by other occupational exposures, the design of this initial publication of the NIOSH

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1 cohort did not allow for examination of exposures to other possible confounders, such as dioxin-
2 like compounds. Duration of exposure was used as a surrogate for cumulative TCDD exposure;
3 therefore, effective doses could not be estimated. Therefore, dose-response modeling was not
4 conducted for this study.

5
6 **2.4.1.1.1.1.2.** Steenland et al. (1999, [197437](#)).

7 **2.4.1.1.1.1.2.1.** *Study summary.*

8 A subsequent analysis of the NIOSH cohort extended the follow-up interval of Fingerhut
9 et al. (1991, [197375](#)) by 6 years (i.e., from 1940–1993) and improved characterization of TCDD
10 exposure (Steenland et al., 1999, [197437](#)). A key distinction from the work of Fingerhut et al.
11 (1991, [197375](#)) was the exclusion of several workers that had been included in the previous
12 mortality analyses. The authors excluded 40 workers who were either female, had never worked
13 in TCDD-exposed departments, or had missing date of birth information. An additional
14 238 workers were excluded as occupational data for characterizing duration of exposure were
15 lacking, preventing their use in a subcohort dose-response analysis. This subcohort was further
16 reduced by excluding workers from four plants ($n = 591$) because the information on the degree
17 of TCDD contamination in work histories was limited, preventing the characterization of TCDD
18 levels by job type. Thirty-eight additional workers were excluded from the eight remaining
19 plants because TCDD contamination could not be estimated. Finally, 727 workers were
20 excluded because they had been exposed to pentachlorophenol. In total, exposures were
21 assigned to 3,538 (69%) members of the overall cohort, a cohort substantially reduced from the
22 5,172 on which Fingerhut et al. (1991, [197375](#)) reported. Steenland et al. (1999, [197437](#)) also
23 evaluated the mortality experience of a subcohort of 608 workers with chloracne who had no
24 exposure to pentachlorophenol.

25 For each worker, a quantitative exposure score for each day of work was calculated based
26 on the concentration of TCDD ($\mu\text{g/g}$) present in process materials, the fraction of the day
27 worked, and a qualitative contact level based on estimates of the amount of TCDD exposure via
28 dermal absorption or inhalation. The authors derived a cumulative measure of TCDD exposure
29 by summing the exposure scores across the working lifetime history for each worker. The
30 authors validated this cumulative exposure metric indirectly by comparing values obtained for
31 workers with and without chloracne. Such a validation is appropriate, given that chloracne is

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1 considered a clinical sign of exposure to high doses of dioxin (e.g., Ott et al., 1993, [594322](#)).
2 The median exposure score among those with chloracne was 11,546 compared with 77 among
3 those without (Steenland and Deddens, 2003, [198587](#)).

4 Cancer mortality was compared using two approaches. As in Fingerhut et al. (1991,
5 [197375](#)), external comparisons were made to the U.S. general population using the SMR
6 statistic. The authors adjusted the SMR statistics for race, age, and calendar time. They also
7 applied life-table methods to characterize risks across the subcohort of 3,538 workers with
8 exposure data by categorizing the workers into seven cumulative exposure groups. The
9 cut-points for these categories were selected so that the number of deaths in each category was
10 nearly equal to optimize study power. Life-table analyses were extended further to consider a
11 15-year lag interval, which in a practical sense means that person-years at risk would not begin
12 to accrue until 15 years after the first exposure occurred. The person-years and deaths that
13 occurred in the first 15 years were included in the lowest exposure grouping. The Cox
14 proportional hazards model was used to characterize risk within the cohort. Cox regression was
15 used to provide an estimate of the hazard ratios and the 95% CIs for ischemic heart disease, all
16 cancers combined, lung cancer, smoking related cancers, and all other cancers. The authors also
17 performed Cox regression analyses using the seven categories of exposure, adjusting the
18 regression coefficients for year of birth and age. The regression models were run for both
19 unlagged and lagged (15 years) cumulative exposure scores.

20 Overall, when compared with the U.S. general population, a slight excess of cancer
21 mortality (from all sites) was noted in the 5,132 cohort study population (SMR = 1.13,
22 95% CI = 1.02–1.25). This result did not substantially differ from the earlier finding that
23 Fingerhut et al. (1991) published (SMR = 1.15, 95% CI = 1.03–1.30). Site-specific analyses
24 revealed statistically significant excesses relative to the U.S. general population for bladder
25 cancer (SMR = 1.99, 95% CI = 1.13–3.23) and for cancer of the larynx (SMR = 2.22,
26 95% CI = 1.06–4.08). In the chloracne subcohort ($n = 608$), SMRs of 1.25
27 (95% CI = 0.98–1.57) and 1.45 (95% CI = 0.98–2.07) were found for all cancer sites and for
28 lung cancer, respectively, relative to the general population. The authors also found statistically
29 significant excesses for connective and soft tissue sarcomas (SMR = 11.32,
30 95% CI = 2.33–33.10) and for lymphatic and hematopoietic malignancies (SMR = 3.01,
31 95% CI = 1.43–8.52).

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1 External comparisons made by grouping workers into septiles of cumulative TCDD
2 exposure and generating an SMR for each septile using the U.S. population as the referent group
3 suggested a dose-response relationship. For all cancer sites combined, workers in the highest
4 exposure score category had an SMR of 1.60 (95% CI = 1.15–1.82); increases also were
5 observed in the sixth (SMR = 1.34) and fifth (SMR = 1.15) septiles. The two-sided p -value
6 associated with the test for trend for cumulative TCDD exposure was statistically significant
7 ($p = 0.02$). A similar approach for lung cancer revealed virtually the same pattern. The
8 incorporation of a 15-year latency for the analyses of all cancer deaths, in general, produced
9 slightly higher SMRs across the septiles, although a slight attenuation of effect was noted in the
10 highest septile (SMR_{unlagged} = 1.60 vs. SMR_{lagged} = 1.54). For a 15-year lag, the lung cancer
11 SMRs were mixed compared to the unlagged results with some septile exposure categories
12 increasing and others decreasing relative to the lowest exposure group.

13 For the internal cohort comparisons using Cox regression analyses higher hazard ratios
14 were found among workers in the higher exposure categories than in the lowest septile. The
15 linear test for trend, however, was not statistically significant ($p = 0.10$). The associations across
16 the septiles for the unlagged exposure for the internal cohort comparisons were not as strong as
17 for the external cohort comparisons. The opposite was true, however, for cumulative exposures
18 lagged 15 years.

19 Relative to the lowest septile, stratified analyses revealed increased hazard ratios in the
20 upper septiles of the internal cohort comparisons for both smoking- and nonsmoking-related
21 forms of cancer. The test for linear trend was statistically significant for all other cancers (after
22 smoking-related cancers were excluded). These analyses suggest that the overall cancer findings
23 were not limited to an interaction between TCDD and smoking. Additional sensitivity analyses
24 by the authors indicated the findings for smoking-related cancers were largely unaffected by the
25 exclusion of bladder cancer cases. This observation suggests that the exposure to
26 4-aminobiphenyl, which occurred at one plant and might have contributed to an increased
27 number of bladder cancers, did not substantially bias the dose-response relationship between
28 TCDD and all cancers combined.

29 The investigators also evaluated the dose-response relationship with a Cox regression
30 model separately for each plant using internal cohort comparisons and found some heterogeneity.
31 This finding is not unexpected particularly given the relatively small number of cancer deaths at

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1 each plant, and given that exposures were quite low for one plant at which no positive
2 association was found. The variability among plants was taken into account by modeling plant
3 as a random effect measure in the Cox model, which produced little change in the slope
4 coefficient ($\beta = 0.0422$ vs. $\beta = 0.0453$).

6 **2.4.1.1.1.1.2.2.** *Study evaluation.*

7 This study represents a valuable extension of that by Fingerhut et al. (1991, [197375](#)).
8 Internal comparisons were performed to help minimize potential biases associated with using an
9 external comparison group (e.g., healthy worker effect, and differences in other risk factors
10 between the cohort and the general population). That similar dose-response relationships were
11 found for internal and external comparison populations suggests that the bias due to the health
12 worker effect in the cohort might be minimal for cancer mortality. More importantly, the
13 construction of the cumulative exposure scores provides an improved opportunity to evaluate
14 dose-response relationships compared with the length of exposure and duration of employment
15 metrics that Fingerhut et al. (1991, [197375](#)) used.

16 A potential limitation of the NIOSH study was the inability to account for cigarette
17 smoking. If cigarette smoking did contribute to the increased cancer mortality rates in this and
18 other cohorts, increased cancer mortality from exposure to TCDD would be expected only for
19 smoking-attributable cancers. This study demonstrates associations with TCDD for both
20 smoking- and nonsmoking-related cancers, including a stronger association for
21 nonsmoking-related cancers. Therefore, the data provide evidence that associations between
22 TCDD and cancer mortality are not likely due to cigarette smoking.

23 The findings regarding latency should be interpreted cautiously as the statistical power in
24 the study to compare differences across latency intervals was limited. Caution also should be
25 heeded, given that latency intervals can vary on an individual basis as they are often
26 dose-dependent (Guess and Hoel, 1977, [197464](#)). The evaluation of whether TCDD acts as
27 either an initiating or promoting agent (or both) is severely constrained by the reliance on cancer
28 mortality data rather than incidence data. This constraint is due to the fact that survival time can
29 be quite lengthy and can vary substantially across individuals and by cancer subtype. For
30 example, the 5-year survival among U.S. males for all cancer sites combined ranged between 45
31 and 60% (Clegg et al., 2002, [594267](#)). When only mortality data are available, evaluating the

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1 time between when individuals are first exposed and when they are diagnosed with cancer is
2 nearly impossible.

3 Starr (2003, [594271](#)) suggested that Steenland et al. (1999, [197437](#)) focused too heavily
4 on the exposures that incorporated a 15-year period of latency and that those who experienced
5 high exposures would inappropriately contribute person-years to the lowest exposure group
6 “irrespective of how great the workers’ actual cumulative exposure scores may have been.”
7 Most cancer deaths would, however, typically occur many years postemployment. Given that
8 the follow-up interval of the cohort was long and the average exposure duration was 2.7 years, at
9 the time of death, person-years for those with high cumulative exposures would be captured
10 appropriately. The median 5-year survival for all cancers is approximately 50% (Clegg et al.,
11 2002, [594267](#)), so applying a minimum latency of 5 years when using cancer mortality rather
12 than cancer incidence data is needed to assure that the exposure metric is capturing exposures
13 that occur before diagnoses. Increasing this latency period, for example to 10 or 15 years, would
14 eliminate consideration of exposures that occur in the period between tumor occurrence and
15 tumor detection (diagnosis), and allows for an appropriate focus on exposures that act either
16 early or late in the pathogenic process. If the association of TCDD with cancer is causal, effects
17 might become apparent only at high exposures and with adequate latency. As such, IARC has
18 concluded that a latency interval of 15 years could be too short (IARC, 1997, [537123](#)). EPA
19 considers the Steenland et al. (1999, [197437](#)) presentation to be balanced in that they provided
20 the range in lifetime excess risk estimated across the various models used. The authors’ finding
21 that the models with a 15-year lag provided a statistically significant improvement in fit based on
22 the chi-square test statistic should not be readily dismissed.

23

24 **2.4.1.1.1.2.3.** *Suitability of data for TCDD dose-response modeling.*

25 This study meets most of the epidemiological considerations for conducting a
26 quantitative dose-response analysis for mortality from all cancer sites combined. This study
27 excludes a large number of workers who were exposed to pentachlorophenol, thus eliminating
28 the potential for bias from this exposure and used an improved methodology for assigning TCDD
29 exposures to the workers. However, given that exposures to other dioxin-like compounds were
30 not described, it is unclear if the exposures among this cohort were primarily to TCDD.

31 Therefore, dose-response modeling was not pursued for this study, but was for the subsequent

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1 NIOSH study by Steenland et al. (2001, [197433](#)), which did examine exposure to dioxin-like
2 compounds.

3

4 **2.4.1.1.1.3.** Steenland et al. (2001, [197433](#)).

5 **2.4.1.1.1.3.1.** *Study summary.*

6 In 2001, Steenland et al. published a risk analysis using the NIOSH cohort that for the
7 first time incorporated serum measures in the derivation of TCDD exposures for individual
8 workers. The authors applied the same exclusion criteria to the entire cohort of workers across
9 the 12 plants in the Steenland et al. (1999, [197437](#)) study, which left 3,538 workers for which
10 risk estimates could be calculated. Cumulative TCDD serum levels were estimated on an
11 individual basis for all 3,538 workers by developing predictive models that used a subset of
12 170 workers for which both serum measures and TCDD exposures scores were available
13 (Steenland et al., 2001, [197433](#)). Unlike previous analyses of the NIOSH cohort that considered
14 several different mortality outcomes, the analyses presented in Steenland et al. (2001, [197433](#))
15 focused exclusively on mortality from all cancers sites combined. The authors observed
16 256 cancer deaths in the cohort during the follow-up interval that extended from 1942 until the
17 end of 1993. All risks estimated in the Steenland et al. (2001, [197433](#)) study were based on
18 internal cohort comparisons.

19 Characterization of TCDD exposure levels among the workers was based on serum
20 measures obtained in 1988 from 199 workers who were employed in one of the eight plants. The
21 researchers restricted the development of the model to include only those workers whose
22 measured serum levels were deemed to be greater than the upper range of background levels
23 (10 ppt), which resulted in 170 workers.

24 The authors developed a regression model that could estimate the level of TCDD at the
25 time of last exposure for the 170 workers. The model was developed based on the estimated
26 half-life of TCDD, the known work history of each worker, a pharmacokinetic model for the
27 storage and excretion of TCDD, and exposure scores for each job held by each worker over time.
28 The resulting equation follows

29

30
$$y_{last\ exposure} = y_{1988} \exp(\lambda\Delta t) \tag{Eq. 2-1}$$

31

1 The first-order elimination rate constant (λ) was based on a half-life of 8.7 years
2 previously reported for the Ranch Hands cohort (Michalek et al., 1996, [198893](#)). The
3 background rate of TCDD exposure was assumed to be 6.1 parts per trillion (ppt), which was
4 based on the median level in a sample of 79 unexposed workers in the NIOSH cohort (Piacitelli
5 et al., 1992, [197275](#)). This value was subtracted when TCDD values were back-extrapolated,
6 and then added again after the back-extrapolation was completed. A background level of 5 ppt
7 also was used in some of the analyses with minimal demonstrable effects on the results.
8 Sensitivity analyses also were incorporated to consider a 7.1-year half-life estimate that had been
9 developed for the earlier Ranch Hands study (Pirkle et al., 1989, [197861](#)).

10 After back-extrapolating to obtain TCDD serums levels at the time of last exposure, the
11 investigators estimated cumulative (or “area under the curve”) TCDD serum levels for every
12 cohort member. This estimation procedure was the same method Flesch-Janys et al. (1998,
13 [197339](#)) applied to the Hamburg cohort to derive a coefficient for relating serum levels to
14 exposure scores. The “area under the curve” approach integrates time-specific serum levels over
15 the employment histories of the individual workers. The slope coefficient was estimated using a
16 no-intercept linear regression model. This model is based on the assumption that a cumulative
17 score of zero is associated with no serum levels above background.

18 Cox regression was also used to model the continuous measures of TCDD. A variety of
19 exposure metrics were considered that took into account different lags, nonlinear relationships
20 (e.g., log-transform and cubic spline), as well as threshold and nonthreshold exposure metrics.
21 Categorical analyses were used to evaluate risks across TCDD exposure groups, while different
22 shapes of dose-response curves were evaluated through the use of lagged and unlagged
23 continuous TCDD measures. Categorical analyses of TCDD exposure were conducted using the
24 Cox regression model to derive estimates of relative risk (RR) as described by hazard ratios and
25 95% CIs. The reference group in this analysis was those workers in the lowest septile
26 cumulative exposure grouping (<335 ppt-years). The septiles were chosen based on cumulative
27 serum levels that considered no lag and also a 15-year lag.

28 The investigators also conducted dose-response analyses using the toxicity equivalence
29 (TEQ) approach. The TEQ is calculated as the sum of all exposures to dioxins and furans
30 weighted by the potency of each specific compound. In this study, TCDD was assumed to be
31 account for all dioxin exposures in the workplace. For background TEQ levels, the investigators

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1 used a value of 50 ppt in the dose-response modeling. This is based on the assumption that
2 TCDD accounted for 10% of the toxicity of all dioxins and furans (WHO, 1988, [594278](#)), and is
3 equivalent to using a background level of 5 ppt/yr that was used in the derivation of cumulative
4 serum TCDD levels. A statistically significant dose-response pattern was observed for all cancer
5 mortality and TCDD exposure based on log of cumulative TEQs with a 15-year lag. A
6 comparison of the overall model chi-square values indicated that the fit of this model was not as
7 good as that for TCDD.

8 The hazard ratios among workers grouped by categories of cumulative TCDD exposure
9 (lagged 15 years) suggested a dose-response relationship. Steenland et al. (2001, [197433](#)) found
10 statistically significant excesses in the higher exposure categories compared to the lowest septile.
11 The RR was 1.82, 95% CI = 1.18–2.82 for the sixth septile (7,568–20,455 ppt-years) and 1.62,
12 95% CI = 1.03–2.56) for the seventh septile (>20,455 ppt-years). Cox regression indicated that
13 log TCDD serum concentrations (lagged 15 years) was positively associated with cancer
14 mortality ($\beta = 0.097$, standard error (β) = 0.032, $p < 0.003$). A statistically significant
15 improvement in fit was observed when a 15-year lag interval was incorporated into the model
16 compared to a model with no such lag [Model χ^2 with 4 degrees of freedom (df) = 7.5]. Results
17 were similar when using a half-life of 7.1 years rather than 8.7 years. The excess lifetime risk of
18 death from cancer at age 75 for TCDD intake (per 1.0-picogram per kilogram [pg/kg] of body
19 weight (BW) per day) was about 0.05–0.9% above a background lifetime risk of cancer death of
20 12.4%. The results from the best-fitting models provide lifetime risk estimates within the ranges
21 derived using data from the Hamburg cohort (Becher et al., 1998, [197173](#)).

22 In both categorical and continuous analyses of TCDD based on a linear exposure metric,
23 the dose-response pattern tailed off at high exposures suggesting nonlinear effects. This
24 phenomenon could be due to saturation effects (Stayner et al., 2003, [054922](#)) or, alternatively,
25 could have resulted from increased exposure misclassification of higher exposures (Steenland
26 et al., 2001, [197433](#)). As the authors highlighted, some of the highest exposures might have
27 been poorly estimated as they occurred in workers exposed to short-term high exposures during
28 the clean-up of a spill. The choice of a linear model to develop data from a single time point can
29 also result in exposure misclassification in those individuals that have differences in the length of
30 exposure (Emond et al., 2005, [197317](#)). Misclassification would be less likely at low
31 concentrations where dose-dependent elimination is minimal.

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1 **2.4.1.1.1.3.2. Study evaluation.**

2 An important consideration in the Steenland et al. (2001, [197433](#)) study was the use of a
3 small subset of workers ($n = 170$) to infer exposures for the remainder of the cohort. This subset
4 comprised surviving members of the cohort (in 1988), and therefore, their age distribution would
5 have differed from the rest of the cohort. Furthermore, these workers were employed at a single
6 plant, at which the work histories were less detailed than at other plants; thus, the development of
7 the exposure scores differed between this plant and that of the others. Also, many of the workers
8 at this plant had the same job title and were employed during the same calendar period. The use
9 of serum data from this subset adds a level of uncertainty that is not readily characterized.
10 Despite this limitation, the use of these sera data to derive cumulative measures for all cohort
11 workers has merit given the strong correlation observed between the exposure scores, and TCDD
12 serum levels estimates at the time of last exposure (Spearman $r = 0.90$).

13 The authors performed an extensive series of sensitivity analyses and considered several
14 alternative exposure metrics to the simple linear model. The lifetime excess risk above
15 background was nearly twice as high for the log cumulative serum measures with a 15-year lag
16 when compared to the piecewise linear models with no lag. An important observation was that
17 the exposure metric based on cumulative serum (lagged 15 years) did not fit the data as well as
18 the cumulative exposure score used in earlier analyses (Steenland et al., 1999, [197437](#)). A priori,
19 one would expect that a better fit would be obtained with serum-based measures because serum
20 levels are a better measure of relevant biological dose. As the authors noted, inaccuracies
21 introduced in estimating the external-based exposure scores could have contributed to a poorer
22 fit of the data. Alternatively, exposure misclassification error could be introduced if serum
23 samples based on the 170 workers were not representative of the entire cohort. Although the
24 serum-based measures did not fit the data as well as the exposures scores, the authors regarded
25 them as providing a reasonable fit based on an improvement in log likelihood of 3.99 (between
26 the log cumulative serum model and the log cumulative exposure score model). Moreover, the
27 serum-based measures enabled better characterization of risk in units (pg/kg-day) that can be
28 used in regulation exposures.

1 **2.4.1.1.1.3.3.** *Suitability of data for TCDD dose-response modeling.*

2 This study meets all of the epidemiological considerations for conducting a quantitative
3 dose-response analysis for mortality from all cancer sites combined. As mentioned previously,
4 the NIOSH cohort is the largest assembled to date for which TCDD-related risks of cancer
5 mortality can be estimated. The use of serum-based measures provides an objective measure of
6 TCDD exposure. Repeated measures in other study populations have provided reasonable
7 estimates of the half-life of TCDD, which permitted back-extrapolation of exposures.

8 The authors have made extensive efforts to evaluate a wide variety of nonlinear and
9 linear models with varying lengths of latency and log transformations. The model chi-square test
10 statistics were fairly similar for the log cumulative serum (15-year lag) (Model $\chi^2_{(4df)} = 11.3$)
11 model and the piecewise linear model (no lag) (Model $\chi^2_{(5df)} = 12.5$). These models, however,
12 produced results with twofold differences in lifetime excess risks. These differences underscore
13 the importance of characterizing uncertainty in modeling approaches when conducting
14 dose-response analysis.

15 The Steenland et al. (2001, [197433](#)) study characterizes risk in terms of pg/kg of body
16 weight per day. Given that tolerable daily intake dioxin levels are typically expressed in pg/kg
17 of body weight (WHO, 1988, [594278](#)), the presentation of risks in terms of these units is an
18 important advance from the earlier analyses that used exposure scores (Steenland et al., 1999,
19 [197437](#)). Many of the Steenland et al. (2001, [197433](#)) findings are consistent with earlier work
20 from this cohort, which is not surprising given that exposures scores were used to derive serum-
21 based levels for the cohort. The findings of excess lifetime risks obtained for the best- fitting
22 model are also consistent with those derived from the Hamburg cohort (Becher et al., 1998,
23 [197173](#)). This study meets the epidemiological considerations noted previously as there is no
24 evidence that the study is subject to bias from confounding due to cigarette smoking or other
25 occupational exposures. Given the considerable efforts to measure effective dose to TCDD
26 among the study participants, this study also meets the requisite dose-response modeling criteria
27 and will be used in quantitative dose-response analyses of cancer mortality.

1 **2.4.1.1.1.4.** Cheng et al. (2006, [523122](#)).

2 **2.4.1.1.1.4.1.** *Study summary.*

3 Cheng et al. (2006, [523122](#)) undertook a subsequent quantitative risk assessment of
4 3,538 workers in the NIOSH cohort using serum-derived estimates of TCDD. This
5 dose-response analysis was published after the 2003 Reassessment document was released. The
6 goal of this study was to examine the relationship between TCDD and cancer mortality (all sites
7 combined) using a new estimate of dose that estimated TCDD as a function of both exposure
8 intensity and age using a kinetic model. This physiologically based pharmacokinetic model has
9 been termed the “concentration- and age-dependent elimination model” (CADM) and was
10 developed by Aylward et al. (2005, [197014](#)). This model describes the kinetics of TCDD
11 following oral exposure to humans by accounting for key processes affecting kinetics by
12 simulating the total concentration of TCDD based on empirical consideration of hepatic
13 processes (see Section 3.3). An important feature of this kinetic model is that it incorporates
14 concentration- and age-dependent elimination of TCDD from the body; consequently, the
15 effective half-life of TCDD elimination varies based on exposure history, body burden, and age
16 of the exposed individuals. The study was motivated by the reasoning that back-calculations of
17 TCDD using a first-order elimination model and a constant half-life of 7–9 years underestimated
18 exposures to TCDD among workers. This underestimate, in turn, would result in overestimates
19 of the carcinogenic potency of TCDD.

20 As with the earlier Steenland et al. (2001, [197433](#)) analyses, the cohort follow-up period
21 was extended from 1942 until the end of 1993 and work histories were linked to a job exposure
22 matrix to obtain cumulative TCDD scores. Two cumulative serum lipid exposure metrics (in
23 ppt-years) were constructed using the data obtained from the sample of 170 workers. The first
24 replicated the metric used in a previous analysis of the cohort (Steenland et al., 2001, [197433](#))
25 and was based on a first-order elimination model with an 8.7-year half-life (Michalek et al.,
26 1996, [198893](#)). The second metric was based on CADM and had two first-order elimination
27 processes (Aylward et al., 2005, [197114](#)). This metric assumes that the elimination of TCDD in
28 humans occurs at a faster rate when body concentrations are high and at slower rates in older
29 individuals (Aylward et al., 2005, [197114](#); Aylward et al., 2005, [197014](#)). The model was
30 optimized using individuals for which serial measures of serum TCDD were available. These
31 measures were obtained from 39 adults with initial serum levels between 130 and 144,000 ppt

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1 (Aylward et al., 2005, [197014](#)). This group included 36 individuals who had been exposed in the
2 Seveso accident and 3 exposed in Vienna, Austria. In practice, for serum levels greater than
3 1,000 ppt, the effective half-life would be less than 3 years, and for serum TCDD levels less than
4 50 ppt, the effective half-life would be more than 10 years (Aylward et al., 2005, [197014](#)).
5 Results from the model indicate that men eliminate TCDD faster than women do as
6 demonstrated previously by Needham et al. (1994, [200030](#)). These age- and
7 concentration-dependent processes were assumed to operate independently on TCDD in hepatic
8 and adipose tissues, and TCDD levels in liver and adipose tissue were assumed to be a nonlinear
9 function of body concentration. Cheng et al. (2006, [523122](#)) calibrated CADM using a dose of
10 156 ng per unit of exposure score and assumed a background exposure rate of 0.01 ng/kg-month.
11 The average TCDD ppt-years derived from CADM with a 15-year lag was 4.5–5.2 times higher
12 than with the first-order elimination model. The two metrics, however, were highly correlated
13 based on a Pearson correlation coefficient of 0.98 ($p < 0.001$). Comparisons of fit between the
14 CADM and first-order elimination model were made using R^2 values and presented in Aylward
15 et al. (2005, [197014](#)).

16 Cheng et al. (2006, [523122](#)) compared the mortality experience of NIOSH workers to the
17 U.S. general population using the SMR statistic. SMR statistics also were generated separately
18 for each of the 8 plants and for all plants combined. Cox regression models were used to analyze
19 internal cohort dose-response. These models used age as the time variable, and penalized
20 smoothing spline functions of the CADM metric also were considered. The possible
21 confounding effects of other occupational exposures and other regional population differences
22 were assessed by repeating analyses after excluding one plant at a time. Lagged and unlagged
23 TCDD exposures were analyzed separately, and stratified analyses compared risk estimates for
24 smoking- and nonsmoking-related cancers. Cheng et al. (2006, [523122](#)) adjusted the slope
25 estimates derived from the Cox model for potential confounding effects of race and year of birth.

26 Overall, a statistically significant excess in all cancer mortality in the cohort occurred
27 relative to the general population (SMR = 1.17, 95% CI = 1.03–1.32). The plant-specific SMRs
28 ranged from 0.62–1.87, with a statistically significant excess evident only for plant 10
29 (SMR = 1.87, 95% CI = 1.35–2.52). For lung cancer mortality, the overall SMR was not
30 statistically significant (SMR = 1.11, 95% CI = 0.89–1.37). A statistically significant excess for
31 lung cancer also was found for plant 10 (SMR = 2.35, 95% CI = 1.44–3.64). The SMRs between

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1 smoking- (SMR = 1.22, 95% CI = 1.01–1.45) and nonsmoking-related cancers (SMR = 1.12,
2 95% CI = 0.94–1.33) were comparable.

3 For the internal cohort analyses of serum-derived measures, the authors were able to
4 replicate the one-compartmental model used previously (Steenland et al., 2001, [197433](#)). As had
5 been noted by Steenland et al. (2001, [197433](#)), an inverse-dose-response pattern was seen for
6 individuals with high exposures (above 95th percentile); this type of pattern is often seen in
7 occupational studies (Stayner et al., 2003, [054922](#)). Excluding these data produced a stronger
8 association between TCDD and all-cause mortality. In fact, only when the upper 2.5% or 5% of
9 observations was removed did a statistically significant positive association become evident with
10 the untransformed data. Similarly, when the model incorporated a lag of 15 years, a statistically
11 significant association was noted only for the untransformed TCDD ppt-years with the upper 5%
12 of observations removed. Stratified analyses revealed little difference between smoking- and
13 nonsmoking-related cancers, and the removal of one plant at a time from the analyses of TCDD
14 ppt-years changes did not substantially change the slope.

15

16 **2.4.1.1.1.4.2.** *Study evaluation.*

17 The authors reported that CADM provided an improved fit over the one-compartmental
18 model, but presented no evidence regarding any formal test of statistical significance. A
19 comparison of R² values presented in Aylward et al. (2005, [197014](#)), however, does reveal that
20 the R² value increased from 0.27 (first-order compartmental model with an 8.7-year half-life) to
21 0.40 for CADM. TCDD exposures estimated using CADM were approximately fivefold higher
22 than the one-compartmental model estimates among cohort members with higher levels of
23 exposure. Differences in exposure estimates between the two metrics were less striking among
24 individuals with lower TCDD exposures. The net effect was that CADM produced a 6- to
25 10-fold decrease in estimated risks compared to estimates previously reported (Steenland et al.,
26 2001, [197433](#)). Nonetheless, the estimates produced by CADM span more than two orders of
27 magnitude under various assumptions. Further uncertainties arise from between-worker
28 variability of TCDD elimination rates, possible residual confounding, and the variability
29 associated with the use of data obtained from other cohorts. Nevertheless, the use of the CADM
30 model to estimate TCDD exposure is considered a significant advantage over the previous first-
31 order body burden calculations.

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1 **2.4.1.1.1.4.3.** *Suitability of data for TCDD dose-response modeling.*

2 The value of including the NIOSH cohort data has already been established based on
3 investigations published by Steenland et al. (1999, [197437](#); 2001, [197433](#)). The decision to
4 include data from the quantitative dose-response analysis that Cheng et al. (2006, [523122](#))
5 conducted relates to the added value that the CADM exposure estimates would provide. The
6 earlier modeling work of Aylward et al. (2005, [197014](#)) provided some support for a modest
7 improvement of the fit of CADM over the first-order compartmental model, and they also
8 confirmed previous studies that found that TCDD elimination rates varied by age and sex.
9 Recent work by Kerger et al. (2006, [198651](#)) also demonstrates that the half-life for TCDD is
10 shorter among Seveso children than the corresponding half-life for adults, and that body burdens
11 influence the elimination of TCDD in humans. That estimates of half-lives among men have
12 been remarkably consistent, with mean estimates ranging between 6.9 and 8.7 years
13 (Flesch-Janys et al., 1996, [197351](#); Michalek et al., 2002, [199579](#); Needham et al., 2005,
14 [594295](#); Pirkle et al., 1989, [197861](#)), however, is noteworthy. Based on the underlying strengths
15 of the NIOSH cohort data and efforts by Cheng et al. (2006, [523122](#)) to improve estimates of
16 effective dose, these data support further dose-response modeling.

17
18 **2.4.1.1.1.5.** Collins et al. (2009, [197627](#)).

19 **2.4.1.1.1.5.1.** *Study summary.*

20 In a recent study, Collins et al. (2009, [197627](#)) investigated the relationship between
21 serum TCDD levels and mortality rates in a cohort of trichlorophenol workers exposed to
22 TCDD. These workers were part of the NIOSH cohort having accounted for approximately 45%
23 of the person-years in an earlier analysis (Bodner et al., 2003, [197135](#)). The investigators
24 completed an extensive dioxin serum evaluation of workers employed by the Dow Chemical
25 plant in Midland, Michigan, that made 2,4,5-trichlorophenol (TCP) from 1942 to 1979 and
26 2,4,5-T from 1948 to 1982. Collins et al. (2004, [197267](#)) developed historical TCDD exposure
27 estimates for all TCP and 2,4,5-T workers. This study represents the largest group of workers
28 from a single plant ever studied for the health effects of TCDD. Little information on how vital
29 status was ascertained, either in this paper or in the Bodner et al. (2003, [197135](#)) report of
30 mortality in this cohort. Although the authors indicate that death certificates were obtained from

1 the states in which the employees died, whether vital status was ascertained from company
2 records or through record linkage to the National Death Index is unclear.

3 The follow-up interval for these workers covered the period between 1942 and 2003.
4 Thus, the study included 10 more years of follow-up than earlier investigations of the entire
5 NIOSH cohort. Serum samples were obtained from 280 former workers collected during
6 2004–2005. A simple one-compartment first-order pharmacokinetic model and elimination rates
7 as estimated from the BASF cohort were used (Flesch-Janys et al., 1996, [197351](#)). The “area
8 under the curve” approach was used to characterize workers’ exposures over the course of their
9 working careers and provided a cumulative measure of exposure. Analyses were performed with
10 and without 165 of the 1,615 workers exposed to pentachlorophenol to evaluate the impact of
11 these exposures.

12 External comparisons of cancer mortality rates to the general U.S. population were made
13 using SMRs. Internal cohort comparisons of exposure-response relationships were made using
14 the Cox regression model. This model used age as the time variable, and was adjusted for year
15 of hire and birth year. Only those causes of death for which an excess was found based on the
16 external comparisons or for which previous studies had identified a positive association were
17 selected for dose-response analyses.

18 A total of 177 cancer deaths were observed in the cohort. For the external comparison
19 with the U.S. general population, overall, no statistically significant differences were observed in
20 all cancer mortality among all workers (SMR = 1.0, 95% CI = 0.8–1.1). Results obtained after
21 excluding workers exposed to pentachlorophenol were similar (SMR = 0.9, 95% CI = 0.8–1.1).
22 Excess mortality in the cohort were found for leukemia (SMR = 1.9, 95% CI = 1.0–3.2) and soft
23 tissue sarcoma (SMR = 4.1, 95% CI = 1.1–10.5). Although not statistically significant SMRs for
24 other lymphohemopoietic cancers included non-Hodgkin’s lymphoma SMR = 1.3; 95%CI = 0.6,
25 2.5) and Hodgkin’s disease (SMR = 2.2; 95% CI = 0.2, 6.4).

26 Internal cohort comparisons using the Cox regression model were performed for all
27 cancers combined, lung cancer, prostate cancer, leukemia, non-Hodgkin’s lymphoma, and
28 soft-tissue sarcoma. Whether the internal comparisons excluded those workers exposed to
29 pentachlorophenol is not entirely clear from the text or accompanying table, but presumably they
30 do not. The RR was 1.002 (95% CI = 0.991–1.013) for all cancer mortality per 1 ppb-year
31 increase in cumulative TCDD exposure was not statistically significant. Except for soft tissue

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1 sarcomas, no statistically significant exposure-response trends were observed for any cancer site.
2 For soft tissue sarcoma, analyses were based on only four deaths.

3
4 **2.4.1.1.1.5.2. Study evaluation.**

5 A key limitation of this study is that SMRs were not derived for different periods of
6 latency for the external comparison group analysis. The original publication on the NIOSH
7 cohort found that SMRs increased when a 20-year latency period was incorporated (Fingerhut
8 et al., 1991, [197375](#)), and similar patterns have been observed in other occupational cohorts
9 (Manz et al., 1991, [199061](#); Ott and Zober, 1996, [198101](#)) and among Seveso residents
10 (Consonni et al., 2008, [524825](#)). Additionally, dose-response analyses showed marked increases
11 in slopes with a 15-year latency period (Cheng et al., 2006, [523122](#); Steenland and Deddens,
12 2003, [198587](#)). In this context, the absence of an elevated SMR for cancer mortality is
13 consistent with previous findings of the NIOSH cohort. While the cohort did have sufficient
14 follow-up, no evaluation of possible latent effects was presented and this is a major limitation of
15 this study. Further, the evaluation of the exposure metrics should be expanded from what was
16 presented in Collins et al. (2009, [197627](#)) due to the previous analyses of the same workers
17 finding positive associations between cancer mortality and TCDD (Steenland et al., 2001,
18 [197433](#)).

19 Unfortunately, the Collins et al. (2009, [197627](#)) study did not include a categorical
20 analysis of TCDD exposure and cancer mortality. This categorical analysis would have enabled
21 an evaluation of whether a nonlinear association exists between TCDD exposure and cancer risk.
22 The analyses of both Cheng et al. (2006, [523122](#)) and Steenland et al. (2001, [197433](#)) suggest an
23 attenuation of effects at higher doses, and several investigations have considered log-transformed
24 associations as a means to address nonlinearity. Also, the earlier plant-specific dose-response
25 analyses of Steenland et al. (2001, [197433](#)) are not consistent with the findings for the Midland
26 plant that Collins et al. (2009, [197627](#)) presented. These differences could be due to differences
27 in the construction of exposure metrics, additional follow-up, or lagging of exposures.

28
29 **2.4.1.1.1.5.3. Suitability of data for dose-response modeling.**

30 The Collins et al. (2009, [197627](#)) study uses serum levels to derive TCDD exposure
31 estimates and does not appear to be subject to important biases. The reliance on data from one

1 plant offers some advantages over the multiplant analyses, as heterogeneity in exposure to other
2 occupational agents would be lower. The number of individuals who provided serum samples
3 ($n = 280$) is greater than the 170 individuals used to derive TCDD estimates for the NIOSH
4 cohort. The authors found a statistically significant dose-response trend for soft tissue sarcoma
5 mortality and TCDD exposures. Therefore, this study is considered for quantitative
6 dose-response analysis.

7 8 **2.4.1.1.1.2. The BASF cohort.**

9 In 1953, dioxin contamination occurred as a result of an autoclave accident during the
10 production of trichlorophenol at the BASF plant in Ludwigshafen, Germany. A second dioxin
11 incident occurred in 1988 that was attributed to the blending of thermoplastic polyesters with
12 brominated flame retardants. Of the two events, the one on November 13, 1953, was associated
13 with more severe acute health effects, including chloracne that resulted in immediate
14 hospitalizations for seven workers. These adverse events were not linked to TCDD until 1957
15 when TCDD was identified as a byproduct of the production of trichlorophenol and was shown
16 to induce chloracne (Zober et al., 1994, [197572](#)). Zober and colleagues (1998, [594300](#)) noted
17 that with the 1988 accident, affected individuals did not exhibit clinical symptoms or chloracne,
18 but rather were identified through “analytical measures.” In both instances, efforts were made to
19 limit the potential for exposure to employees.

20
21 **2.4.1.1.1.2.1.** Thiess and Frentzel-Beyme (1977, [594302](#)) and Thiess et al. (1982, [064999](#)).

22 **2.4.1.1.1.2.1.1. *Study summary.***

23 A study of the mortality of workers employed at the BASF plant was first presented in
24 1977 (Thiess and Frentzel-Beyme, 1977, [594302](#)) with subsequent updates in both 1982 (Thiess
25 et al., 1982, [064999](#)), and in 1990 (Zober et al., 1990, [197604](#)). In the first published paper
26 (Thiess et al., 1982, [064999](#)), 74 employees involved in the 1953 accident were traced and their
27 death certificate information extracted. Of these, 66 suffered chloracne or severe dermatitis.
28 Observed deaths were compared to the expected number using three external reference groups:
29 the town of Ludwigshafen ($n = 180,000$), the district of Rhinehessia-Palatinate ($n = 1.8$ million),
30 and the Federal Republic of Germany ($n = 60.5$ million). Another comparison group was
31 assembled by selecting age-matched employees taken from other cohorts under study. This

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1 additional comparison was aimed at avoiding potential biases associated with healthy worker
2 effect when using an external referent.

3 During a follow-up interval of up to 26 years (1953–1979), 21 individuals died. Of
4 these, seven deaths were from cancer. The expected number of cancer deaths derived for the
5 three external comparison groups ranged between 4.1 and 4.2, producing an SMR of 1.7
6 (p -values ranged between 0.12 and 0.14). Excess mortality was found for stomach cancer based
7 on the external comparisons ($p < 0.05$); however, this was based on only three cases. No other
8 statistically significant excesses were found with the external comparisons made to the other
9 cohorts of workers.

11 **2.4.1.1.1.2.1.2.** *Study evaluation.*

12 In the Thiess et al. (1982, [064999](#)) study, no TCDD exposures were derived for the
13 workers, thus no dose-reconstruction was performed. The findings from this study are limited by
14 the small size of the cohort. The 74 workers followed in this cohort represent the smallest
15 number of workers across the occupational cohorts (Becher et al., 1998, [197173](#); Fingerhut et al.,
16 1991, [197375](#); Hooiveld et al., 1998, [197829](#); McBride, 2009, [198490](#); McBride et al., 2009,
17 [197296](#); Michalek and Pavuk, 2008, [199573](#); Steenland et al., 2001, [197433](#)) that have
18 investigated TCDD exposures and cancer mortality. Mechanisms of follow-up were excellent as
19 all individuals were traced, and death certificates were obtained from all deceased workers.

20 Although the study does compare the mortality experience to other occupational cohorts,
21 the paper provides insufficient information to adequately interpret the associated findings. For
22 example, a description of these occupations is lacking making it impossible to determine whether
23 these cohorts were exposed to other occupational carcinogens that might have confounded the
24 associations between TCDD exposure and cancer mortality.

26 **2.4.1.1.1.2.1.3.** *Suitability of data for TCDD dose-response modeling.*

27 Subsequent data assembled for the BASF cohort provide more detailed exposure
28 characterization and also include information for 243 male workers employed at the plant. As
29 such, this study did not meet the considerations for further dose-response analysis.

1 2.4.1.1.1.2.2. Zober et al. (1990, [197604](#)).

2 2.4.1.1.1.2.2.1. *Study summary.*

3 Zober et al. (1990, [197604](#)) also examined the mortality patterns of 247 individuals
4 involved in the 1953 accident at the BASF plant. As detailed in their paper, the size of the
5 original cohort was expanded by efforts to locate all individuals who were exposed in the
6 accident or during the clean-up. Three approaches were followed in assembling the cohort.
7 Sixty-nine cohort members were identified from the company physician's list of employees
8 exposed as a result of the accident (Subcohort C1). Sixty-six of these workers were included in
9 the original study population of workers Thiess et al. (1982, [064999](#)) examined.
10 Eighty-four other workers who were potentially exposed to TCDD due to their involvement in
11 demolitions or operations were added to the cohort. This group included 43 firemen, 18 plant
12 workers, 7 bricklayers, 5 whitewashers, 4 mechanics, 2 roofers, and 5 individuals in other
13 occupations (Subcohort C2). The cohort was further augmented through the Dioxin
14 Investigation Program, which sought to locate those who were involved in the 1953 accident and
15 were still alive in 1986. Current and former workers enrolled in the study were asked to identify
16 other current or former coworkers (including deceased or retired) who might have been exposed
17 from the accident. This third component of 94 workers (Subcohort C3) included 27 plant
18 workers, 16 plumbers, 10 scaffolders, 10 professionals, 7 mechanics, 6 transportation workers,
19 5 bricklayers, 5 laboratory assistant, 3 insulators, and 5 individuals in other occupations. A
20 medical examination was performed for those identified through the Dioxin Investigation
21 Program, and blood measures were obtained for 28 of these workers.

22 External comparisons of the workers' mortality experience to the general population of
23 the Federal Republic of West Germany were made using SMRs. Person-years were tabulated
24 across strata defined by calendar period, sex, and age group. Sixty-nine deaths including
25 twenty-three from cancer were detected among the workers during the 34-year follow-up period
26 (November 17, 1953 through December 31, 1987). Cause-specific death rates for these same
27 strata were available for the Federal Republic of West Germany. Stratified analyses were
28 conducted to examine variations in the SMRs according to years since first exposure (0–9,
29 10–19, and ≥ 20 years) for each of the three subcohorts, as well as 114 workers with chloracne.

30 Although it was consistent in magnitude with findings from the NIOSH cohort, a
31 statistically significant SMR for all cancer mortality was not observed (SMR = 1.17,

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1 90% CI = 0.80–1.66). The SMRs for each of the three subcohorts varied substantially. For
2 Subcohorts C1, C2, and C3, the SMRs were 1.30 (90% CI = 0.68–2.26), 1.71
3 (90% CI = 0.96–2.83), and 0.48 (90% CI = 0.13–1.23), respectively. The SMRs increased
4 dramatically when analyses were restricted to those with 20 or more years since first exposure in
5 Subcohort C1 (SMR = 1.67, 90% CI = 0.78–3.13) and Subcohort C2 (SMR = 2.38,
6 90% CI = 1.18–4.29). Meanwhile, in a subgroup analysis of those with chloracne, for the period
7 of 20 or more years after first exposure, a statistically significant excess in cancer mortality was
8 noted (SMR = 2.01; 90% CI = 1.22–3.15).

9 10 **2.4.1.1.1.2.2.2.** *Study evaluation.*

11 An important limitation of the study is the manner in which the cohort was constructed.
12 Subcohort C3 was constructed by identifying individuals who were alive in 1986. This resulted
13 in 97 active and retired employees who participated in the program, with 94 included in the
14 analysis. Although these individuals did identify other workers who might have also retired or
15 died, inevitably, some individuals who had died were not included in the cohort. This would
16 serve to underestimate the SMRs that were generated with external comparisons to the German
17 population. Indeed, cancer mortality rates in this subcohort were about half of what would have
18 been expected based on general population rates (SMR = 0.48, 90% CI = 0.13–1.23).
19 Additionally, more than half of Subcohort C2 were firemen (43 of 84), who would likely have
20 been exposed to other carcinogens as a consequence of their employment. Quantitative analyses
21 of epidemiologic data for firefighters have demonstrated increased cancer risk for several
22 different forms of cancer (Youakim, 2006, [197295](#)). Therefore, potential confounding from
23 other occupational exposures of the firefighters could have contributed to the higher SMR in
24 Subcohort C2 cohort and is a concern. Data on cigarette smoking were not available either. No
25 excess for nonmalignant respiratory disease was found, however, suggesting this might not be an
26 important source of bias.

27 28 **2.4.1.1.1.2.2.3.** *Suitability of data for TCDD dose-response modeling.*

29 As with the Thiess et al. (1982, [064999](#)) publication, worker exposure was not estimated.
30 Lack of exposure estimates precludes a quantitative dose-response analysis using these data.
31 Also, the study design is not well suited to characterization of risk using the SMR statistic.

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1 Mortality is also likely under-ascertained in the large component of the cohort that was
2 constructed through the identification of surviving members of the cohort.

3
4 **2.4.1.1.1.2.3.** Ott and Zober (1996, [198101](#)).

5 **2.4.1.1.1.2.3.1.** *Study summary.*

6 Ott and Zober (1996, [198101](#)) extended the analyses of the BASF cohort to include
7 estimates of individual-level measures of TCDD. The researchers also investigated associations
8 with cancer mortality and identified incident cancer cases. The cohort follow-up period of
9 39 years extended until December 31, 1992, adding 5 years to a previous study (Zober et al.,
10 1990, [197604](#)). Ott and Zober (1996, [198101](#)) identified incident cases of cancer using
11 occupational medical records, death certificates, doctor's letters, necropsy reports, and
12 information from self-reported surveys sent to all surviving cohort members. Self-reported
13 cancer diagnoses were confirmed by contacting the attending physician.

14 This study characterized exposure by two methods: (1) determining chloracne status of
15 the cohort members and (2) estimating cumulative TCDD ($\mu\text{g}/\text{kg}$) levels. In 1989, serum
16 measures were sought for all surviving members of the 1953 accident, and serum TCDD levels
17 were quantified for 138 individuals. These serum levels were used to estimate cumulative
18 TCDD concentrations for all 254 members of the accident cohort. Ott et al. (1993, [594322](#))
19 published a description of the exposure estimation procedure, which was a regression model that
20 accounted for the circumstances and duration of individual exposure. The average internal
21 half-life of TCDD was estimated to be 5.8 years based on repeated serum sampling of
22 29 individuals. The regression model allowed for this half-life to vary according to the
23 percentage of body fat, and yielded half-lives of 5.1 and 8.9 years among those with 20% and
24 30% body fat, respectively. Previous analyses of this cohort had used a half-life of 7.0 years (Ott
25 et al., 1993, [594322](#)).

26 TCDD half-life has been reported to increase with percentage of body fat in both
27 laboratory mammals (Geyer et al., 1990, [197700](#)) and humans (Zober and Papke, 1993, [197602](#)).
28 Ott and Zober (1996, [198101](#)) contend that observed correlations with chloracne severity and
29 cumulative estimates of TCDD exposure indirectly validated this exposure metric. Specifically,
30 the mean TCDD concentration for those without chloracne was 38.4 ppt; for those with moderate
31 and severe forms of chloracne, the mean was 420.8 ppt and 1,008 ppt, respectively.

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1 Unlike for the NIOSH cohort, individual-level data were collected for other cancer risk
2 factors. These factors included body mass index at time of first exposure, history of
3 occupational exposure to β -naphthylamine and asbestos, and history of smoking. Smoking data
4 were available for 86% of the cohort. SMRs were based on the external referent population of
5 West Germany. For cancer incidence, Ott and Zober (1996, [198101](#)) generated standardized
6 incidence ratios (SIRs) using incidence rates for the state of Saarland (1970–1991) as the
7 external referent. They calculated SMRs (and SIRs) for three categories of cumulative TCDD
8 levels: $<0.1 \mu\text{g}/\text{kg}$, $0.1\text{--}0.99 \mu\text{g}/\text{kg}$ and $\geq 1 \mu\text{g}/\text{kg}$. The Cox regression model was used to
9 characterize risk within the cohort using a continuous measure of TCDD. These analyses
10 considered the potential confounding influence of age, smoking, and body mass index using a
11 stepwise regression modeling approach. The Cox modeling employed a stratified approach
12 using the date of first exposure to minimize possible confounding between calendar period and
13 exposure. The three first exposure groups were exposure within the first year of the accident,
14 exposure between 1 year after the accident and before 1960, and exposure after 1959. The Cox
15 regression estimates were presented in terms of conditional risk ratios (i.e., hazard ratios adjusted
16 for body mass index, smoking and age).

17 Although no statistically significant excesses relative to the general population were
18 detected for all cancer mortality, there was some suggestion of an exposure-response
19 relationship. In the $0.1\text{--}0.99 \mu\text{g}/\text{kg}$ and $\geq 1 \mu\text{g}/\text{kg}$ exposure groups, the all cancer SMRs were 1.2
20 (95% CI = 0.5–2.3) and 1.6 (95% CI = 0.9–2.6), respectively. Higher SMRs for cancer (all sites
21 combined) were also found with an increased interval since exposure first occurred.
22 Specifically, when observed versus expected counts of cancer were compared in the time interval
23 20 years after first exposure, the SMR in the highest exposure group ($\geq 1 \mu\text{g}/\text{kg}$) was 1.97
24 (95% CI = 1.05–5.36). An excess in lung cancer also was noted with the same lag in this
25 exposure group (SMR = 3.06, 95% CI = 1.12–6.66). For cancer incidence, a statistically
26 significant increased SIR for lung cancer was observed in the highest exposure category
27 (SIR = 2.2, 95% CI = 1.0–4.3), but no other statistically significant associations were detected
28 for any other cancer site. No cases of soft-tissue sarcoma were found among the cohort members
29 in this analysis.

30 Based on internal cohort comparisons, Cox regression models also were used to generate
31 hazard ratios as measures of relative risk for TCDD exposures following adjustment for

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1 smoking, age and body mass index. A statistically significant association between TCDD dose
2 (per $\mu\text{g}/\text{kg}$) and cancer mortality was detected (RR = 1.22, 95% CI = 1.00–1.50), but not for
3 cancer incidence (RR = 1.11, 95% CI = 0.91–1.35). Statistically significant findings were
4 observed for stomach cancer mortality (RR = 1.46, 95% CI = 1.13–1.89) and incidence
5 (RR = 1.39, 95% CI = 1.07–1.69).

6 The Ott and Zober (1996, [198101](#)) study also compared the relationship between TCDD
7 exposure categories and cancer mortality from all sites combined according to smoking status.
8 Associations were noted between increased exposure to TCDD and mortality from cancer among
9 smokers, but not among nonsmokers or former smokers.

11 **2.4.1.1.1.2.3.2.** *Study evaluation.*

12 The Ott and Zober (1996, [198101](#)) study characterizes exposure to TCDD at an
13 individual level. Therefore, unlike in past studies involving this cohort, these data can provide
14 an opportunity for conducting quantitative dose-response modeling. As with the more recent
15 studies involving the NIOSH cohort, serum samples were obtained from surviving cohort
16 members and then used to back-extrapolate TCDD values for all cohort members. In the BASF
17 cohort, however, serum data were available for a much higher percentage of cohort members
18 (54%) than in the NIOSH cohort (5%). An additional study strength was the collection of
19 questionnaire data, which allowed for the potential confounding from cigarette smoking and
20 body mass index to be examined.

21 The Ott and Zober (1996, [198101](#)) study also evaluates the relationship between TCDD
22 and cancer incidence. Most cohort studies of TCDD-exposed workers have relied solely on
23 mortality outcomes. The availability of incidence data better allows for period of latency to be
24 described, and moreover, to characterize risks associated with cancers that typically have long
25 survival periods. The authors provide few details on the expected completeness of ascertainment
26 for incident cancer cases, which makes determining any associated bias difficult. They do,
27 however, suggest that nonfatal cancers are more likely to have been missed in the earlier part of
28 the follow-up. The net result of differential case ascertainment over time makes evaluating
29 differences in risk estimates across different periods of latency impossible.

30 The small sample size of the cohort ($n = 243$ men) likely limited the statistical power to
31 detect small associations for some of the exposure measures. This also effectively limited the

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1 ability to analyze dose-response relationships quantitatively, particularly across strata such as
2 time since exposure. For site-specific analyses, the cancer site with the most cancer deaths was
3 the respiratory system ($n = 11$). Thus, quantitative dose-response analysis using these cohort
4 data would be limited to the evaluation of all cancer sites combined.

5 The most important limitation of this study is related to the construction of the
6 third component of the cohort. As mentioned earlier, this cohort was assembled by actively
7 seeking out surviving members of the cohort in the mid-1980s. The mortality experience of this
8 cohort is much lower than that of the general population over the entire follow-up, a result that is
9 expected given that the individuals were known to be alive as of 1986. The net result is likely an
10 underestimate of the SMR.

11 12 **2.4.1.1.1.2.3.3.** *Suitability of data for TCDD dose-response modeling.*

13 This study was included in the quantitative dose-response modeling for the
14 2003 Reassessment (U.S. EPA, 2003, [537122](#)). The characterization of exposure data and
15 availability of other risk factor data at an individual level are appropriate for use in quantitative
16 dose-response analyses.

17 18 **2.4.1.1.1.3.** **The Hamburg cohort.**

19 The Hamburg cohort has been the subject of several cancer risk assessments. As with the
20 NIOSH and BASF cohorts, analyses have progressed from basic comparisons of mortality
21 experience to general population rates to more sophisticated internal cohort analyses involving
22 the reconstruction of TCDD exposures using serum measures. This cohort consists of
23 approximately 1,600 workers who were employed in the production of herbicides at a plant in
24 Hamburg, Germany during 1950–1984 (Becher et al., 1998, [197173](#); Flesch-Janys et al., 1995,
25 [197261](#)). The herbicides produced included 2,4,5-T, β -hexachlorocyclohexane and lindane. The
26 production of TCP and 2,4,5-T was halted in 1954 following a chloracne outbreak. The plant
27 ceased operations in 1984. Approximately 20 different working areas were identified, which, in
28 turn, were grouped into five main areas based on putative TCDD exposure levels. One working
29 area was deemed to be extremely contaminated, having TCDD exposures at least 20-fold higher
30 than in other areas. In this section, the studies undertaken in this cohort that have examined
31 cancer mortality are summarized.

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1 **2.4.1.1.1.3.1.** Manz et al. (1991, [199061](#)).

2 **2.4.1.1.1.3.1.1.** *Study summary.*

3 Manz et al. (1991, [199061](#)) investigated patterns of mortality in the Hamburg cohort.
4 The study population consisted of 1,583 workers (1,184 men, 399 women) who were employed
5 for at least three months between 1952 and 1989. Casual workers were excluded as they lack
6 sufficient personal identifying information thereby not allowing for associations with mortality
7 outcomes to be examined. Vital status was determined using community-based registries of
8 inhabitants throughout West Germany. Cause of death until the end of 1989 was determined
9 from medical records for all cancer deaths and classified based on the ninth revision of the
10 International Classification of Diseases (WHO, 1978, [594329](#)). Although Manz et al. (1991,
11 [199061](#)) present some data on cancer incidence for the cohort, the data are incomplete as
12 information was available on only 12 cases; 93 cancer deaths were observed in the cohort.

13 In this study, the authors used information on production processes to group workers into
14 categories of low, medium, or high exposure to TCDD. This information was based on TCDD
15 concentrations in precursor materials, products, waste, and soil from the plant grounds, measured
16 after the plant closed in 1984. The distribution of workers into the low, medium, and high
17 exposure groups was 186, 901, and 496, respectively. The authors examined the validity of the
18 three exposure categories using a separate group of 48 workers who provided adipose tissue
19 samples. The median exposure of the 37 volunteers in the high group was 137 and 60 ng/kg in
20 the remaining 11. Information about chloracne in the cohort was incomplete, and, therefore, was
21 not used as a marker of TCDD exposure. Other surrogate measures of exposure were considered
22 in this study, including duration of exposure and year of first employment. For the latter
23 measure, employment that began after 1954 was assumed to result in much lower exposures
24 given that production of 2,4,5-T and TCP stopped in 1954.

25 External comparisons of cancer mortality were made by calculating SMRs using the
26 general population of West Germany as a referent. Comparisons of mortality in the cohort also
27 were made to a separate cohort of 3,417 gas supply workers to avoid bias from a healthy worker
28 effect. Vital status and cause of death in the gas supply workers were determined using the same
29 methods as used in the Hamburg cohort. SMRs were calculated relative to both referent
30 populations (West Germany and gas supply workers) across low, medium, and high TCDD
31 exposure groups. The comparison of mortality to the gas supply workers, however, extended

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1 only until the end of 1985, whereas, comparisons to the general population extended until 1989.
2 Stratified analyses were undertaken to calculate SMRs for each of the three exposure groups for
3 categories of duration of employment (<20 versus ≥20 years) and date of entry into the cohort
4 (≤1954 vs. >1954).

5 When compared to the general population, overall cancer mortality was elevated in male
6 cohort members (SMR = 1.24, 95% CI = 1.00–1.52) but not in females (SMR = 0.80,
7 95% CI = 0.60–1.05). A two-fold increase in female breast cancer mortality was noted although
8 it did not achieve statistical significance at the alpha level of 0.05 (SMR = 2.15,
9 95% CI = 0.98–4.09). The SMR among men was further increased when analyses were
10 restricted to workers who were employed for at least 20 years (SMR = 1.87,
11 95% CI = 1.11–2.95). Analyses restricted to those in the highest exposure group produced an
12 even higher SMR for those with at least 20 years of employment (SMR = 2.54,
13 95% CI = 1.10–5.00). Statistically significant excesses in risk were detected among those who
14 first worked before 1954, but not afterward. Furthermore, a dose-response trend was observed
15 across increasing exposure categories in the subset of workers employed before 1954. The
16 SMRs using the cohort of gas supply workers as the referent group for the low, medium, and
17 high groups in this subset were 1.41 (95% CI = 0.46–3.28), 1.61 (95% CI = 1.10–2.44), and 2.77
18 (95% CI = 1.59–4.53), respectively. This finding is consistent with what was known about
19 TCDD exposures levels at the plant, namely, that TCDD concentrations were much higher
20 between 1951 and 1954, with subsequent declining levels after 1954.

21 Generally speaking, patterns of excess mortality were similar when the cohort of gas
22 workers was used as a reference group. The overall SMR for men was 1.39
23 (95% CI = 1.10–1.75); and was 1.82 (95% CI = 0.97–3.11) when analyses were restricted to
24 workers with 20 or more years of employment. A dose-response trend also was observed across
25 exposure categories when analyses were restricted to those employed for at least 20 years. In
26 particular, with these analyses, no cancer deaths were observed among those in the lowest
27 exposure group, while the SMRs in the middle and high exposure groups were 1.36
28 (95% CI = 0.50–2.96) and 3.07 (95% CI = 1.24–6.33).

29 SMRs also were generated for several site-specific cancers relative to the West German
30 general population and the gas worker cohort. No statistically significant excesses were
31 observed using the general population reference. In contrast, statistically significant excesses

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1 were observed for lung cancer (SMR = 1.67, 95% CI = 1.09–2.44) and hematopoietic system
2 cancer (SMR = 2.65, 95% CI = 1.21–5.03) relative to the gas workers cohort.

3
4 **2.4.1.1.1.3.1.2. Study evaluation.**

5 The Manz et al. (1991, [199061](#)) findings indicate an excess of all cancer mortality among
6 the workers with the highest exposures, particularly those who worked for at least 20 years and
7 were employed before 1954. The findings across categories of exposure within the subsets of
8 workers employed for at least 20 years and before 1954, particularly using the cohort of gas
9 supply workers, are consistent with a dose-response relationship. These elevated cancer
10 mortality rates found among those employed before 1954 were likely due to higher TCDD
11 exposures. Other carcinogenic coexposures, such as benzene, asbestos, and dimethyl sulfate,
12 could have occurred among this population. Given that no substantial changes in the production
13 processes at the Hamburg plant occurred after 1954, comparable levels of these coexposures
14 would be expected before and after 1954. Exposures to these other chemicals varied across
15 different departments/groups; therefore, confounding was unlikely since a strong association
16 between concentrations of these chemicals and TCDD exposures was not evident. No
17 information, however, was presented on potential exposure to other dioxin-like compounds
18 which may confound the associations that were detected.

19 Detailed information on workers' smoking behaviors was not collected. Limited
20 evidence indicated, however, that smoking prevalence between the Hamburg cohort and the gas
21 supply workers cohort was quite similar. A nonrepresentative sample of 361 workers in the
22 Hamburg cohort and the sample of 2,860 workers in the gas supply cohort indicated that the
23 self-reported smoking prevalence was 73% and 76%, respectively. This suggests that the
24 two cohorts are comprised predominantly of smokers. The similarity in overall smoking
25 prevalence indicates that comparisons of cancer mortality between the two groups are not unduly
26 influenced by an inability to adjust for smoking.

27
28 **2.4.1.1.1.3.1.3. Suitability of data for TCDD dose-response modeling.**

29 The data compiled for the Manz et al. (1991, [199061](#)) study do satisfy many of the
30 considerations for conducting quantitative dose-response analysis; health outcomes appear to be
31 ascertained in an unbiased manner, and exposure was characterized on an individual-level basis.

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1 However, as demonstrated in later studies, there was a large dioxin-like compound component
2 that was not quantified or assessed in this study. Dose-response associations between TCDD and
3 cancer mortality were detected, with stronger associations observed with increased periods of
4 latency and for those who first worked when TCDD was at higher levels.

5 The size of the cohort, although not as large as the NIOSH cohort, does offer sufficient
6 statistical power to evaluate TCDD-related risk for cancers from all cancer sites. The data are
7 limited, however, for characterizing cancer risks among women; only 20 cancer deaths occurred
8 in the 399 women included in the cohort. It is unlikely that the findings are biased by
9 confounding due to cigarette smoking since dose-response patterns were strengthened when
10 comparisons were made to the cohort of gas supply workers rather the general population
11 referent where smoking rates were likely lower. The inability to account for other occupational
12 exposure when TCDD exposures were much higher (pre-1955) could result in confounding if
13 these other exposures were related to TCDD and the health outcomes under consideration. This
14 data set would be suitable for quantitative dose-response modeling if the exposure
15 characterization of the cohort could be improved using biological measures of dose.

16
17 **2.4.1.1.1.3.2.** Flesch-Janys et al. (1995, [197261](#)).

18 **2.4.1.1.1.3.2.1.** *Study summary.*

19 In 1995, Flesch-Janys et al. (1995, [197261](#)) published an analysis of the male employees
20 from the Hamburg cohort that extended the follow-up to 40 years (1952–1992). Inclusion of
21 these three additional years of follow-up resulted in a sample size of 1,189 male workers.

22 The authors estimated a quantitative exposure variable for concentrations of TCDD in
23 blood at the end of exposure (i.e., when employment in a department ended) and above German
24 median background TCDD levels. The TCDD exposure assessment defined 14 production
25 departments according to TCDD levels in various products in the plant, in waste products, and in
26 various buildings. The time (in years) each worker spent in each department then was
27 calculated. Concentrations of TCDD were determined in 190 male workers using serum
28 ($n = 142$) and adipose tissue samples ($n = 48$). The authors used a first-order kinetic model to
29 calculate TCDD levels at the end of exposure for the 190 workers with available polychlorinated
30 dibenzo-p-dioxin (PCDD) and -furan (PCDF) at various time points. Half-lives were calculated
31 from an elimination study of 48 workers from this cohort, and the median TCDD background

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1 level was estimated at 3.4 ng/kg blood fat from the German population (Flesch-Janys et al.,
2 1994, [197372](#); Pöpke et al., 1994, [198279](#)). Using the one-compartment, first-order kinetic
3 model, the half-life of TCDD was estimated to be 6.9 years (Flesch-Janys, 1997, [197305](#)).
4 Increased age and higher body fat percentage were associated with increased TCDD half-life,
5 while smoking was associated with a higher decay rate for most of the congeners examined
6 (Flesch-Janys et al., 1996, [197351](#)). Cumulative TCDD exposures were estimated by summing
7 exposures over the time spent in all production departments and were expressed in terms of
8 ng/kg of blood fat. The authors also applied a metric of total toxicity equivalence (TOTTEQ) as
9 the weighted sum of all congeners where weights were TEQs that denoted the toxicity of each
10 congener relative to TCDD.

11 Similar to previous analyses on this cohort, comparisons were made using an external
12 referent group of workers from a gas supply company (Manz et al., 1991, [199061](#)). In contrast to
13 previous analyses where SMR statistics were generated using this “external” reference, however,
14 Flesch-Janys et al. (1995, [197261](#)) used Cox regression. The Cox regression models treated the
15 gas worker cohort as the referent group, and six exposure groups were defined by serum-derived
16 cumulative TCDD estimates. The groups were determined by using the first four quintiles with
17 the upper two exposure categories corresponding to the ninth and tenth deciles of the cumulative
18 TCDD. Internal cohort comparisons used those workers in the lowest quintile as the referent
19 group, as opposed to the cohort of gas workers. A similar approach was used to model TEQs.
20 No known TCDD exposures occurred in the gas workers, so they were assigned exposures based
21 on the median background levels in the general population. RRs were calculated based on
22 exposure above background levels; in other words, background levels were assumed to be
23 equivalent across all workers and also for those employed by the gas supply company. The RRs
24 derived using the Cox model were adjusted for total duration of employment, age, and year when
25 employment began.

26 The Cox regression with the cohort of gas workers as the referent exposure group yielded
27 a linear dose-response relationship between cumulative TCDD exposure and cancer mortality for
28 all sites combined ($p < 0.01$). The RRs for all-cancer mortality were 1.59, 1.29, 1.66, 1.60, 1.70,
29 and 3.30. For four of the six categories (excluding the referent group), the RRs were statistically
30 significant ($p < 0.05$); in the highest TCDD exposure category (344.7–3,890.2 ng/kg) the RR
31 was 3.30 (95% CI = 2.05–5.31). Similar findings were evident with TOTTEQ. A dose-response

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1 pattern for all cancer mortality ($p < 0.01$) based on the internal cohort comparisons was also
2 detected.

3 The authors performed an additional analysis to evaluate the potential confounding role
4 of dimethylsulfate. Although no direct measures of dimethylsulfate were available, the
5 investigators repeated analyses by excluding 149 workers who were employed in the department
6 where dimethylsulfate was present. A dose-response pattern persisted for TCDD ($p < 0.01$), and
7 those in the highest exposure group (344.7–3,890.2 ng/kg of blood fat) had a RR of 2.28
8 (95% CI = 1.14–4.59).

9

10 **2.4.1.1.1.3.2.2.** *Study evaluation.*

11 The Flesch-Janys et al. (1995, [197261](#)) study used serum-based measures to determine
12 cumulative exposure to TCDD at the end of employment for all cohort members. They used the
13 standard one-compartment, first-order kinetic model and samples obtained from 190 male
14 workers. This quantitative measure of exposure permits an estimation of a dose-response
15 relationship.

16 Confounding for other occupational exposures is unlikely to have biased the results. A
17 dose-response relationship persisted after excluding workers exposed to dimethylsulfate. Other
18 potential exposures of interest included benzene and isomers of hexachlorocyclohexane.
19 Exposure to these agents, however, was highest in the hexachlorocyclohexane and lindane
20 department, where TCDD exposures were lower. Confounding was unlikely due to exposure to
21 these chemicals, since a strong association between concentrations of these chemicals and TCDD
22 exposures was not evident (due to considerable variability in concentrations across different
23 departments/groups). As outlined earlier, the study findings are unlikely to be biased for
24 cigarette smoking as cigarette smoking in the cohort was similar to that in the comparison
25 population. Moreover, more recent analyses of serum-based TCDD exposure measures found no
26 correlation with smoking status in this cohort (Flesch-Janys et al., 1995, [197261](#))—a necessary
27 condition for confounding.

28 The authors used an exposure metric that described cumulative TCDD exposure of
29 workers at the time they were last exposed. As a result, the authors were unable to characterize
30 risks associated with this metric for different periods of latency despite a sufficient follow-up

1 period. Subsequent analyses constructed time-dependent measures of cumulative TCDD and
2 accounted for excretion of TCDD during follow-up.

3 In contrast to most risk assessments of TCDD exposure, this study modeled the
4 relationship between other dioxin-like compounds and the risk of cancer mortality using the
5 TOTTEQ metric.

6 7 **2.4.1.1.1.3.2.3.** *Suitability of data for TCDD dose-response modeling.*

8 The data used in this study satisfy most of the considerations developed for performing a
9 quantitative dose-response analysis. However, latency period was not examined in this study.
10 Dose-response analyses were, therefore, limited to a subsequent study of this cohort (Becher
11 et al., 1998, [197173](#)), which did examine latency.

12 13 **2.4.1.1.1.3.3.** Flesch-Janys et al. (1998, [197339](#)).

14 **2.4.1.1.1.3.3.1.** *Study summary.*

15 Flesch-Janys et al. (1998, [197339](#)) undertook another analysis on this cohort that
16 incorporated additional sera data for 275 workers (39 females and 236 males). The follow-up
17 period was the same as that used in the 1995 analyses, with mortality follow-up extending until
18 December 31, 1992. Analyses were based on 1,189 males who were employed for at least
19 3 months from January 1, 1952 onward. The authors continued this dose-response analysis to
20 address limitations in their previous work. One limitation was that the previous method did not
21 account for the elimination of TCDD while exposures were being accrued during follow-up. A
22 second limitation was that the amount of time workers spent in different departments was not
23 considered. In the 1998 study, the “area under the curve” approach was used because it accounts
24 for variations in concentrations over time and reflects cumulative exposure to TCDD. The
25 authors used a first-order kinetic model to link blood levels and working histories to derive
26 department-specific dose rates for TCDD. The TCDD background level of 3.4 ng/kg blood fat
27 for the German population was used (Päpke et al., 1994, [198279](#)). The dose rates were applied
28 to estimate the concentration of TCDD at every point in time for all cohort members. A
29 cumulative measure expressed as ng/kg blood fat multiplied by years was calculated and used in
30 the SMR analysis. SMRs were calculated using general population mortality rates for the
31 German population between 1952 and 1992. No lag period was incorporated into the derivation

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1 of the SMRs. The SMRs were estimated for the entire cohort and for exposure groups based on
2 quartiles obtained from the area under the curve. Linear trend tests were also performed. The
3 overall SMR for cancer mortality in the cohort was 1.41 (95% CI = 1.17–1.68). This SMR value
4 was higher than the SMR of 1.21 reported for this same cohort with 3 fewer years of follow-up
5 (Manz et al., 1991, [199061](#)). In terms of site-specific cancer mortality, excesses were found for
6 respiratory cancer (SMR = 1.71, 95% CI = 1.24–2.29) and rectal cancer (SMR = 2.30,
7 95% CI = 1.05–2.47). Increased risk for lymphatic and hematopoietic cancer (SMR = 2.16,
8 95% CI = 1.11–3.17) were also noted largely attributable (SMR = 3.73, 95% CI = 1.20–8.71) to
9 lymphosarcoma (i.e., non-Hodgkin’s lymphoma). A dose-response relationship was observed
10 across quartiles of cumulative TCDD for all-cancer mortality ($p < 0.01$). The SMRs for these
11 quartiles were 1.24, 1.34, 1.34, and 1.73. Dose-response relationships were not observed for
12 lung cancer or hematopoietic cancers using this same metric. Dose-response relationships were
13 not observed with cumulative TEQ for any of the cancer sites examined (i.e., all cancers, lung
14 cancer, hematopoietic cancer).

15

16 **2.4.1.1.1.3.3.2.** *Study evaluation.*

17 The approach used in the Flesch-Janys et al. (1998, [197339](#)) study offers a distinct
18 advantage over earlier analyses involving the same cohort. Three more years of follow-up were
19 available, and the characterization of exposure using the “area under the curve” better captures
20 changes in cumulative exposure using a person-years approach rather than cumulative TCDD at
21 the time of last exposure. As noted previously, other occupational exposures or cigarette
22 smoking are unlikely to have biased the study findings. A sufficient length of follow-up had
23 accrued, and dose-response associations were evident. Dioxin-like compounds were evaluated in
24 this study. For TCDD, the mean concentration was 101.3 ng/kg at the time of measurement. For
25 other higher chlorinated congeners, the corresponding mean (without TCDD) was 89.3 ng/kg.

26

27 **2.4.1.1.1.3.3.3.** *Suitability of data for TCDD dose-response modeling.*

28 The data used in this study satisfy most of the considerations developed for performing a
29 quantitative dose-response analysis. However, latency was not examined in this study.
30 Dose-response analyses were, therefore, limited to a subsequent study of this cohort (Becher

1 et al., 1998, [197173](#)) which did examine latency and supersedes the Flesch-Janys et al. (1998,
2 [197339](#)) study.

3

4 **2.4.1.1.1.3.4.** Becher et al. (1998, [197173](#)).

5 **2.4.1.1.1.3.4.1.** *Study summary.*

6 The Becher et al. (1998, [197173](#)) quantitative cancer risk assessment for the Hamburg
7 cohort was highlighted in the 2003 Reassessment as being appropriate for conducting
8 dose-response analysis. The integrated TCDD concentration over time, as estimated in the
9 Flesch-Janys et al. (1998, [197339](#)) study, was used as the exposure variable. Estimates of the
10 half-life of TCDD based on the sample of 48 individuals with repeated measures were
11 incorporated into the model that back-calculated TCDD exposures to the end of the employment
12 (Flesch-Janys et al., 1996, [197351](#)). This method took into account the age and body fat
13 percentage of the workers. In Becher et al. (1998, [197173](#)), the analysis used the estimate of
14 cumulative dose (integrated dose or area under the curve) as a time-dependent variable.

15 Poisson and Cox regression models were used to characterize dose-response
16 relationships. Both models were applied to internal comparisons where a person-years offset
17 was used and to an external comparison where an offset of expected number of deaths was used.
18 The person-years offset was used to account for varying person-time accrued by workers across
19 exposure categories. The use of the expected number of deaths as an offset allows risks to be
20 described in relation to that expected in the general population. Within each classification cell of
21 deaths and person-years, a continuous value TCDD and TEQ levels based on the geometric mean
22 were entered into the Poisson model. For the Cox model, accumulated dose was estimated based
23 on area under the curve for TCDD, TEQ, TEQ without TCDD, and β -hexachlorocyclohexane.
24 These other coexposure metrics were adjusted for in the Cox regression analyses. Other
25 covariates considered included in the models were year of entry, year of birth, and age at entry
26 into the cohort. A background level of 3.4 ng/kg blood fat for the German population was used
27 (Päpke et al., 1994, [198279](#)). A variety of latencies was evaluated (0, 5, 10, 15, and 20 years),
28 and attributable risk and absolute risk were estimated. The unexposed cohort of gas workers was
29 used for most internal analyses.

30 Internal and external comparisons using the Poisson model found positive associations
31 with TCDD exposure and mortality from all cancers combined. The slope associated with the

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1 continuous measure of TCDD ($\mu\text{g}/\text{kg}$ blood fat \times years) for the internal comparison was 0.027
2 ($p < 0.001$), which decreased to 0.0156 ($p = 0.07$) after adjusting for age and calendar period.
3 The slope for the external comparison was 0.0163 ($p = 0.055$); this estimate was not adjusted for
4 other covariates. For TEQ, the slopes based on the internal comparisons were 0.0274 ($p < 0.001$)
5 in the univariate model and 0.0107 ($p = 0.175$) in the multivariate model after adjusting for age
6 and calendar period. The external estimate of slope for TEQ was 0.0109 ($p = 0.164$). Cox
7 regression of TCDD across six exposure categories, with a lag of 0 years, found a statistically
8 significant linear trend ($p = 0.03$) and those in the upper exposure group had a RR of 2.19
9 (95% CI = 0.76–6.29). These estimates were adjusted for year of entry, age at entry, and
10 duration of employment. A similar pattern was observed with the Cox regression analysis of
11 TEQ; the linear test for trend, however, was not statistically significant at the alpha level of 0.05
12 ($p = 0.06$).

13 Cox regression models that included both TCDD and TEQ (excluding TCDD) were
14 applied. In this model, the slope (β) for TCDD was 0.0089 ($p = 0.058$), while the coefficient for
15 TEQ (excluding TCDD) was -0.024 ($p = 0.70$). This suggests that confounding by other
16 dioxin-like compounds was unlikely and the increased risk of cancer was due to TCDD
17 exposure. For all TEQs combined, the slope was 0.0078 ($p = 0.066$).

18 The authors used multiple Cox models to evaluate the effect of latency. The slope
19 estimates for both TCDD and TEQ increased dramatically with increasing latency. The slope
20 estimates for TCDD increased from 0.0096 to 0.0160 ($p < 0.05$) when latency was increased
21 from 0 to 20 years. Similar changes in the TEQ slopes were noted (0.0093 to 0.0157).
22 Evaluations of dose-response curves found that the best-fitting curve was concave in shape,
23 thereby yielding higher risk at low exposure. Differences between the fit of the class of models
24 considered [i.e., $\text{RR}(x,\beta) = \exp(\beta \log(kx + 1))$], however, were small.

25 Attributable risks were generated only for TCDD, as the data suggested no effects with
26 other TEQs. The additional lifetime risk of cancer assuming a daily intake of 1 pg TCDD/kg
27 body weight/day was estimated to range between 0.001 and 0.01.

28

29 **2.4.1.1.1.3.4.2.** *Study evaluation.*

30 The Becher et al. (1998, [197173](#)) study represent perhaps the most detailed analyses
31 performed on any cohort to date. The findings were robust, as similar patterns were found with

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1 and without using the gas supply worker cohort as the referent group. Exposures to other
2 potential confounding coexposures, such as dioxin-like compounds, were taken into account, and
3 workers with exposure to other carcinogens (e.g., lindane) were excluded. Furthermore, latency
4 was examined in this study, unlike earlier studies of this cohort.

6 **2.4.1.1.1.3.4.3. *Suitability of data for TCDD dose-response modeling.***

7 This study was included in the quantitative dose-response modeling for the
8 2003 Reassessment (U.S. EPA, 2003, [537122](#)). The data in the Becher et al. (1998, [197173](#))
9 study are suitable for conducting quantitative dose-response modeling. The exposure data
10 capture cumulative exposure to TCDD as well as exposures to other dioxin-like compounds.
11 The length of the follow-up is sufficient, and the study appears to not be subject to confounding
12 or other types of biases. Therefore, this study is utilized in quantitative dose-response analysis.

14 **2.4.1.1.1.4. *The Seveso cohort.***

15 Several studies have evaluated the morbidity and mortality effects of residents exposed to
16 TCDD following a July 10, 1976, accidental release through an exhaust pipe at a chemical plant
17 in the town of Meda near Seveso, Italy. The released fluid mixture contained 2,4,5-T, sodium
18 trichlorophenate, ethylene glycol, and sodium hydroxide. Vegetation in the area showed
19 immediate signs of damage, and in the days following the accident, residents developed nausea,
20 headaches, eye irritation, and dermal lesions, particularly children.

21 This accident transported TCDD up to 6 km from the plant. Soil samples taken near the
22 plant revealed average levels of TCDD that ranged from 15.5 $\mu\text{g}/\text{m}^2$ to 580.4 $\mu\text{g}/\text{m}^2$ in the most
23 contaminated area near the plant (referred to as Zone A) (Bertazzi et al., 2001, [197005](#)). Zone A
24 covered 87 hectares and extended 2,200 m south from the plant. Another, more distant
25 contaminated zone (Zone B) covering 270 hectares also had contaminated soil levels, but the
26 TCDD concentration range was much lower (1.7–4.3 $\mu\text{g}/\text{m}^3$). A reference zone (Zone R), which
27 surrounded the two contaminated areas, had lower TCDD soil levels (range: 0.9–1.4 $\mu\text{g}/\text{m}^3$) and
28 included approximately 30,000 residents. Following the accident, most residents in Zone A left
29 the area. Although residents in Zone B remained, they were under strict regulations to avoid
30 consuming homegrown products. In total, 736, 4,737, and 31,800 individuals lived in Zones A,
31 B, and R, respectively. Within days of the accident, 3,300 animals (mostly poultry and rabbits)

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1 were found dead. Emergency slaughtering was undertaken to prevent TCDD from entering the
2 food chain, and within 2 years more than 80,000 animals had been slaughtered. Mechanisms
3 were put into place for long-term follow-up of these residents. Unlike the other studies based on
4 occupational cohorts, the follow-up of this population allows for risks to be characterized for
5 females.

6 The mortality studies from Seveso published to date have not incorporated serum TCDD
7 levels that were measured in individuals. Needham et al. (1997) describe the collection of serum
8 samples from a sample of the exposed population and control subjects in 1976. In 1988, human
9 exposure to TCDD was assessed by measuring small volumes of serum remaining from medical
10 examinations done in 1976. An examination of these data revealed some of the highest serum
11 TCDD levels ever reported, that the half-life of TCDD in this population was between 7 and
12 8 years, and that half-life varied between women and men. The half-life of TCDD in serum was
13 longer in women (~9 years) than in men (~7 years) (Needham et al., 1994, [200030](#)). In this
14 report, the findings of studies that characterized cancer risks in relation to exposure to TCDD
15 from the 1976 accident are highlighted. These studies include comparisons of cancer mortality
16 rates to the general population based on zone of residence at the time of accident (Bertazzi et al.,
17 2001, [197005](#); Consonni et al., 2008, [524825](#)). More recent work done by Warner et al. (2002,
18 [197489](#)) investigated the relationship between serum-based measures of TCDD and breast cancer
19 among participants in the Seveso Women’s Health Study (SWHS).

20

21 **2.4.1.1.1.4.1.** Bertazzi et al. (2001, [197005](#)).

22 **2.4.1.1.1.4.1.1.** *Study summary.*

23 Several studies have reported on the mortality experience of Seveso residents. The more
24 recent publications having a longer follow-up of the cohort are evaluated here. In 2001, the
25 findings from a 20-year mortality study of Seveso residents was published (Bertazzi et al., 2001,
26 [197005](#)). The Bertazzi et al. (2001, [197005](#)) study was an extension of the 10- and 15-year
27 follow-ups for mortality (Bertazzi et al., 1989, [197013](#); Bertazzi et al., 1997, [197097](#); Pesatori
28 et al., 1998, [523076](#)) and the 10-year follow-up for cancer incidence (Bertazzi et al., 1993,
29 [192445](#)).

30 In this cohort, TCDD exposures were assigned to the population using a three-level
31 categorical variable representative of the individual’s place of residence (Zones A, B, or R) at the

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1 time of the accident or when the person first became a resident of the zone, if that was after
2 1976. An external comparison to the province of Lombardy was made by generating rate ratios
3 (RR) using Poisson regression techniques. Person-years of follow-up were tabulated across
4 strata defined by age, zone of residence, duration of residence, gender, calendar time, and
5 number of years that had elapsed since the time of exposure. Mortality rates during the
6 preaccident period also were compared to evaluate potential changes in rates due to the accident
7 and to evaluate whether patterns were consistent before and after the accident.

8 No overall excess in mortality rates from all cancer sites combined was observed in
9 Zones A or B (combined) when compared to the reference population of Lombardy
10 ($n = 9$ million residents) (RR = 1.0, 95% CI = 0.9–1.2). Analyses of site-specific cancer
11 mortality revealed statistically significant excesses among residents in Zones A or B (combined)
12 for cancer of the rectum (RR = 1.8, 95% CI = 1.0–3.3) and lymphatic and hematopoietic
13 malignancies (RR = 1.7, 95% CI = 1.2–2.5). Lymphatic and hematopoietic malignancies were
14 elevated in women (RR = 1.8, 95% CI = 1.1–3.2) and in men (RR = 1.7, 95% CI = 1.0–2.8).

15 Analyses stratified by the number of years since first exposure (i.e., 1976) revealed
16 higher risk among men with an increased number of years elapsed. Similar to other studies, the
17 RR for all cancers (combined) was 1.3 (95% CI = 1.0–1.7) among men 15–20 years after first
18 exposure. No such increase after 15 years postexposure, however, was noted in women
19 (RR = 0.8, 95% CI = 0.6–1.2).

20 21 **2.4.1.1.1.4.1.2.** *Study evaluation.*

22 Ascertainment of mortality appears to be excellent. Vital status was established using
23 similar methods for both the exposed and reference populations. No individual data were
24 collected and, therefore, the possibility that confounding by individual characteristics such as
25 cigarette smoking cannot be entirely dismissed. Bertazzi et al. (2001, [197005](#)) do note that the
26 sociodemographic characteristics of residents in the three zones were similar based on
27 independently conducted surveys, and no differences in chronic respiratory disease were found
28 across the different zones. If excess mortality was attributable to cigarette smoking, such
29 excesses would be expected to be evident during the entire study period. Latency analyses
30 revealed elevated risks 15–20 years postaccident. Finally, no excesses were observed for other
31 smoking-related cancers of the larynx, esophagus, pancreas, and bladder. The observed excesses

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1 in all cancer mortality do not appear to be attributed to differential smoking rates between the
2 two populations.

3 To examine potential for bias due to noncomparability in the two study populations, a
4 comparison of cancer mortality rates between the Seveso regions and the reference population of
5 Lombardy was conducted. Elevated rates for brain cancer mortality were noted in Seveso
6 relative to Lombardy, but the higher rates of leukemia mortality were found in Lombardy
7 relative to Seveso. That no excess was reported for all cancer sites combined lends credence to
8 the hypothesis that the exposure to TCDD from the accident increased rates of cancer after a
9 sufficient period of latency.

10 Stratified analyses were performed across several categorical variables including gender
11 and time since exposure. The numbers of cancer site-specific deaths are quite small in many of
12 the 5-year increments since first exposure. The study, therefore, has limited statistical power to
13 detect differences in mortality rates among the comparison groups for many cancer sites.

14 Bertazzi et al. (2001, [197005](#)) assigned exposures based on zone of residence. Soil
15 sampling within each zone revealed considerable variability in TCDD soil levels within each
16 zone. Moreover, some individuals would have left the area shortly after the accident, and
17 determining the extent to which individuals in Zone B who were subject to the recommendations
18 near the time of the accident adhered to them is difficult. As a result, exposure misclassification
19 is possible, and the use of individual measures of TCDD level in serum is preferred over zone of
20 residence for determining exposure. As noted by the authors, the study is better suited to “hazard
21 identification” than to quantitative dose-response analysis.

22

23 **2.4.1.1.1.4.1.3.** *Suitability of data for TCDD dose-response modeling.*

24 Given the variability in soil TCDD levels within each zone and the lack of individual
25 level, no effective dose can be estimated for quantitative dose-response analyses. Uncertainty in
26 identifying the critical exposure window for the Seveso cohort is a key limitation. The
27 evaluation of this study indicates that this study is not suitable for quantitative dose-response
28 analysis.

29

1 2.4.1.1.1.4.2. Warner et al. (2002, [197489](#)).

2 2.4.1.1.1.4.2.1. *Study summary.*

3 To date, Warner et al. (2002, [197489](#)) is the only published investigation of the
4 relationship between serum-based measures of TCDD and cancer in Seveso. Eligible
5 participants from the Seveso Women's Health Study (SWHS; see Section 2.4.1.2.1.4 for details)
6 were women who, at the time of the accident in 1976, were 40 years of age or younger, had lived
7 in one of the most highly contaminated zones (A or B), and had adequate sera collected soon
8 after the explosion. Enrollment in SWHS was begun in March 1996 and lasted until July 1998.
9 Of the total 1,271 eligible women, 981 agreed to participate in the study. Cancer cases were
10 identified during interview and confirmed through review of medical records. Information on
11 other risk factors including reproductive history and cigarette smoking was obtained through
12 interview.

13 Serum volumes greater than 0.5 mL collected between 1976 and 1981 volume were
14 analyzed. Most sera were collected in 1976/77 ($n = 899$); samples were collected in 1978–1981
15 for 54 women, and in 1996/97 for 28 women. For most samples collected after 1977, serum
16 TCDD levels were back-extrapolated using a first-order kinetic model with a 9-year half-life
17 (Pirkle et al., 1989, [197861](#)). For 96 women with undetectable values, a serum level that was
18 equal to one-half the detection level was used.

19 Analyses were based only on women who provided serum samples; no extrapolation of
20 values to a larger population was done. Risks were therefore generated using data collected at an
21 individual level. Serum TCDD was analyzed as both a continuous variable and a categorical
22 variable. The distribution of serum TCDD levels of the 15 cases of breast cancer was examined
23 in relation to the distribution of all women in the SWHS. The median exposure was slightly
24 higher among with the 15 cases of breast cancer (71.8 ppt) compared to those without (55.1 ppt),
25 and the exposure distribution among breast cancer cases appeared to be shifted to the right (i.e.,
26 the exposures were higher but followed the same distribution); however, no formal test of
27 significance was conducted.

28 Warner et al. (2002, [197489](#)) used Cox proportional hazards modeling techniques to
29 evaluate risk of breast cancer in relation to TCDD serum levels while controlling for a variety of
30 potential risk factors. In all, 21 women had been diagnosed with cancer, and of these, 15 cases
31 were cancer of the breast. The analysis revealed that for every 10-fold increase in TCDD

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1 log-serum levels (e.g., from 10 to 100 ppt) the risk of breast cancer increased by 2.1
2 (95% CI = 1.0–4.6). Risk estimates also were generated across four categories (<20, 20.1–44,
3 44.1–100, >100 ppt), with the lowest category used as the reference. The RRs estimated in the
4 third and fourth highest exposure categories were 4.5 (95% CI = 0.6–36.8) and 3.3
5 (95% CI = 0.4–28.0). Although statistical significance was not achieved for either category,
6 likely because of the small number of cases, the greater than threefold risk evident in both
7 categories is worth noting. Given that the reference category had only one incident case
8 underscores the limited inferences that can be drawn from these analyses. The authors adjusted
9 for numerous potential confounders, but observed no differences between the crude and adjusted
10 results; the authors, therefore, presented unadjusted risks.

11

12 **2.4.1.1.1.4.2.2. Study evaluation.**

13 The findings from the Warner et al. (2002, [197489](#)) study differ from reports in earlier
14 studies in which mortality outcomes noted the absence of an SMR association. The design of
15 this study is much stronger than earlier ones, given the improved characterization of exposure,
16 the ability to compare incidence rates within the cohort, the ability to control for potential
17 confounding variables at an individual level, and the availability of incident outcomes. The use
18 of incident cases (versus mortality data) should also help minimize potential bias due to disease
19 survival. Another important advantage was the ability to measure TCDD near the time of the
20 accident, thereby reducing the potential for exposure measurement error.

21 A potentially important limitation of the Warner et al. (2002, [197489](#)) study was that
22 information was collected only from those who were alive as of March 1996. Therefore, TCDD
23 and other relevant risk factor data could not be collected for those who had previously died of
24 breast cancer. Thirty-three women could not participate because they were either too ill or had
25 died. Of these, three died of breast cancer. Given that there were only 15 breast cancer cases,
26 the exclusion of these 3 cases could have dramatically impacted the findings in either direction.

27 Another limitation was that, at the time of the follow-up, most women were still
28 premenopausal and therefore, most of the cohort (average age = 40.8 years) had not yet attained
29 the age of greater risk of breast cancer (average age at diagnosis among the cases in this cohort
30 was 45.2 years). Although comparable data from Italy were not found, the median age of
31 diagnosis for breast cancer among U.S. women from 2003–2007 was 61 years (Altekruse et al.,

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1 2010). An ongoing follow-up of the cohort should be completed by 2010, which should allow
2 for increased number of incident breast cancers to be identified. Given that the current analyses
3 were based only on 15 incident cases, this will substantially improve the statistical power of the
4 study. A secondary benefit is that the increased follow-up will allow for an investigation of
5 possible differential effects according to the age the women were at the time of exposure.

6
7 **2.4.1.1.1.4.2.3.** *Suitability of data for TCDD dose-response modeling.*

8 Several aspects of the Warner et al. (2002, [197489](#)) study are weaknesses in the
9 consideration of this study for further dose-response modeling. Only 15 cases of breast cancer
10 were available, and no increases in risk were found with serum TCDD exposures between 20.1
11 and 44 ppt ($n = 2$) when compared to those with <20 ppt ($n = 1$). The average age at the time of
12 enrollment was 40.8 years while the average age at diagnosis among the cases was 45.2 years.
13 As most women had not yet reached the age when breast cancer cases are typically diagnosed,
14 additional follow-up of the cohort would improve the quantitative dose-response analysis and
15 strengthen this study. A key strength of this study, however, is that Warner et al. (2002, [197489](#))
16 includes an investigation of the relationship between individual serum-based measures of TCDD
17 and cancer in Seveso. Despite the weaknesses, this study meets the evaluation considerations
18 and criteria for inclusion and will be analyzed for quantitative dose-response modeling.

19 **2.4.1.1.1.4.3.** Pesatori et al. (2003, [197001](#)).

20 **2.4.1.1.1.4.3.1.** *Study summary.*

21 Pesatori et al. (2003, [197001](#)) published a review of the short- and long-term studies of
22 morbidity and mortality outcomes in the Seveso cohort in 2003. This paper presented cancer
23 incidence data from 1977 to 1991 for Seveso males and females residing in Zones A, B and R
24 relative to an external population (i.e., uncontaminated areas). Mortality data are also presented
25 for a 20-year follow-up (1976–1996) relative to the reference population. As in the original
26 Bertazzi et al. (2001, [197005](#)) study, RRs were estimated using Poisson regression. No
27 associations were noted for zone of residence and all cancer mortality for either males or
28 females. Although no cases were reported in Zones A and B, soft tissues sarcoma was associated
29 with residence in males from Zone R (RR = 2.6, 95% CI = 1.1–6.3). Among males, residence in
30 Zones A and B was associated with lymphatic and hematopoietic cancer (RR = 1.9,

1 95% CI = 1.1–3.1). This increased risk was due primarily to non-Hodgkin’s lymphoma, which
2 accounted for 8 of the 15 incidence cases (RR = 2.6, 95% CI = 1.3–5.3). Among females,
3 increased incidence of multiple myeloma (RR = 4.9, 95% CI = 1.5–16.1), cancer of the vagina
4 (RR = 5.5, 95% CI = 1.3–23.8), and cancer of the biliary tract (RR = 3.0, 95% CI = 1.1–8.2) was
5 associated with residence in Zones A and B.

6 7 **2.4.1.1.1.4.3.2.** *Study evaluation.*

8 Study limitations of the Pesatori et al. (2003, [197001](#)) study included exposure
9 misclassification from the use of an ecological measure of exposure (region of residency at time
10 of accident) and low statistical power for some health endpoints. For e.g., all of the RRs
11 presented above for specific cancer mortality among females in the Pesatori et al. (2003, [197001](#))
12 study were based on fewer than five incident cases.

13 14 **2.4.1.1.1.4.3.3.** *Suitability of data for TCDD dose-response modeling.*

15 As with the studies of mortality among Seveso residents, the Pesatori et al. (2003,
16 [197001](#)) study does not capture TCDD exposure on an individual basis, and soil TCDD levels
17 considerably vary within each zone. Therefore, the quality of the exposure data is insufficient
18 for estimating the effective dose needed for quantitative dose-response analysis.

19 20 **2.4.1.1.1.4.4.** Baccarelli et al. (2006, [197036](#)).

21 **2.4.1.1.1.4.4.1.** *Study summary.*

22 Given previous findings from Seveso, Baccarelli et al. (2006, [197036](#)) examined t(14;18)
23 translocations in the DNA of circulating lymphocytes of healthy dioxin-exposed individuals.
24 These translocations are associated with the development of cancer, namely follicular
25 lymphomas. The study included 211 healthy subjects of the Seveso area, and 101 who had
26 developed chloracne. The investigators analyzed data from 72 high-TCDD plasma level
27 individuals (≥ 10 ppt) and 72 low-TCDD plasma levels (< 10 ppt). A three-level categorical
28 variable was used to evaluate dose-response. This variable was developed by dividing those
29 with exposures ≥ 10 ppt into two groups: 10– < 50 ppt, and 50–475.0 ppt. Trained interviewers
30 administered a questionnaire that collected data on demographic characteristics, diet, and
31 residential and occupational history.

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1 The prevalence of t(14;18) was estimated as those individuals having a t(14;18) positive
2 blood sample divided by the t(14;18) frequency (number of copies per million lymphocytes).
3 Baccarelli et al. (2006, [197036](#)) found that the frequency of t(14;18) was associated with plasma
4 TCDD levels, but no association between TCDD and the prevalence of t(14;18) was detected.

5
6 **2.4.1.1.1.4.4.2. Study evaluation.**

7 Whether the frequency of t(14;18) associated with plasma TCDD levels translates into an
8 increased risk of lymphoma is uncertain as prospective data of TCDD on those who developed
9 non-Hodgkin's lymphoma are lacking. Moreover, the t(14;18) translocation could be an
10 important event in the pre-B stage cell that contributes to tumorigenicity, however subsequent
11 exposure to carcinogenic agents might be necessary for t(14;18) cells to develop into a
12 malignancy (Höglund et al., 2004, [199130](#)).

13
14 **2.4.1.1.1.4.4.3. Suitability of data for TCDD dose-response modeling.**

15 Given that current TCDD plasma levels were measured for this study, it is unclear if the
16 effects of lymphocyte translocations may be due to initial high exposure or are a function of the
17 cumulative exposure for a longer exposure window. Additionally, whether the frequency of
18 t(14;18) associated with plasma TCDD levels translates into an increased risk of lymphoma is
19 unknown. Dose-response analysis for this outcome, therefore, was not conducted.

20
21 **2.4.1.1.1.4.5. Consonni et al. (2008, [524825](#)).**

22 **2.4.1.1.1.4.5.1. Study summary.**

23 Consonni et al. (2008, [524825](#)) analyzed cancer mortality in the Seveso cohort with the
24 addition of a 25-year follow up period. Similar analytic methods as Pesatori et al. (2003,
25 [197001](#)) were applied with 25 years of follow-up added to the analysis (Consonni et al., 2008,
26 [524825](#)). An important addition in this paper was the presentation of RRs for Zone R, which had
27 the lowest TCDD levels. Poisson regression models were used to calculate RRs of mortality
28 using Seregno as the reference population. Cancer deaths observed in Zones A and B were 42
29 and 244, respectively.

30 No statistically significant differences in all cancer mortality relative to the reference
31 population were noted in any of the zones (Zone A: RR = 1.03, 95% CI = 0.76–1.39; Zone B:

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1 RR = 0.92, 95% CI = 0.81–1.05; Zone R: RR = 0.97, 95% CI = 0.92–1.02). Statistically
2 significant excesses in mortality from non-Hodgkin’s lymphoma (RR = 3.35,
3 95% CI = 1.07–10.46) and multiple myeloma (RR = 4.34, 95% CI = 1.07–17.52) were observed
4 in the area with the highest TCDD levels (Zone A). No other statistically significant increases in
5 cancer mortality relative to the reference population were apparent. The absence of elevated
6 breast cancer mortality among women in this study was noteworthy, as this finding differs from
7 the results of a study of Seveso women for which TCDD exposures were estimated using serum
8 samples (Warner et al., 2002, [197489](#)).

9
10 **2.4.1.1.1.4.5.2. *Study evaluation.***

11 Although no individual-level data on smoking were available, the potential for
12 confounding is likely minimal. Independent smoking surveys found that the smoking prevalence
13 rates in Desio, one of cities affected by the accident, were similar to those in districts just outside
14 the study area (Cesana et al., 1995, [594366](#)). As mentioned earlier, one would expect elevated
15 RRs over the entire study period if smoking had biased the study results, and not just after
16 15–20 years since exposure to TCDD.

17
18 **2.4.1.1.1.4.5.3. *Suitability of data for TCDD dose-response modeling.***

19 The lack of individual-level exposure data precludes quantitative dose-response modeling
20 using these data.

21
22 **2.4.1.1.1.5. *Chapaevsk study.***

23 Industrial contamination of dioxin in the Chapaevsk region of Russia has been the focus
24 of research on the environmentally-induced cancer and other adverse health effects. The
25 Chapaevsk region is located in the Samara region of Russia and has a population of 83,000. The
26 region is home to a chemical plant that produced lindane and its derivatives between 1967 and
27 1987, which are believed to be responsible for local dioxin contamination. Soil sampling has
28 demonstrated a strong gradient of increased TCDD concentrations with decreased proximity to
29 the chemical plant (Revich et al., 2001, [199843](#)).

1 **2.4.1.1.1.5.1.** Revich et al. (2001, [199843](#)).

2 **2.4.1.1.1.5.1.1.** *Study summary.*

3 Revich et al. (2001, [199843](#)) used a cross-sectional study to compare mortality rates of
4 Chapaevsk residents to two external populations of Russia and the region of Samara. Mortality
5 rates for all cancers combined among males in Chapaevsk were found to be 1.2 times higher
6 when compared to the Samara region as a whole and 1.3 times higher than Russia. Similar to
7 other studies, statistically significant excess was noted in men (SMR = 1.8, 95% CI = 1.6–1.9)
8 but not in women (SMR = 0.9, 95% CI = 0.8–1.1). Among men, the excess was highest for the
9 smoking-related cancers of the lung (SMR = 3.1, 95% CI = 2.6–3.5) and larynx (SMR = 2.3,
10 95% CI = 1.2–3.8) and urinary organs (SMR = 2.6, 95% CI = 1.7–3.6). Among females, there
11 was no increased SMR for all cancer sites combined, but excesses for breast cancer (SMR = 2.1,
12 95% CI = 1.6–2.7) and cancer of the cervix (SMR = 1.5, 95% CI = 1.0–3.1) were statistically
13 significant.

14 Revich et al. (2001, [199843](#)) also compared age-standardized cancer incidence rates in
15 Chapaevsk to those in Samara. Although statistical tests examining these differences were not
16 reported, higher incidence rates were observed for all cancers combined, cancer of the lip, cancer
17 of the oral cavity, and lung and bladder cancer among males in Chapaevsk. Considerably lower
18 cancer incidence rates also were observed for prostate cancer, cancer of the esophagus, and
19 leukemia/lymphoma among males from Chapaevsk. Among females, incidence rates were
20 higher in 1998 for all cancers in Chapaevsk when compared to Russia and the Samara region, an
21 observation that appears somewhat counter to the presented SMR of 0.9 for all cancer mortality
22 from 1995–1998. Like mortality, rates of breast cancer incidence among women in Chapaevsk
23 were higher than in Russia, as were rates of cervical cancer. Leukemia/lymphoma rates were
24 higher among women in Chapaevsk than in those who lived in the reference populations of
25 Samara and Russia. This finding is contrary to the finding for males who had lower rates of
26 leukemia/lymphoma in Chapaevsk.

27

28 **2.4.1.1.1.5.1.2.** *Study evaluation.*

29 Although the Revich et al. (2001, [199843](#)) findings suggest TCDD exposures in
30 Chapaevsk are quite high relative to other parts of the world (Akhmedkhanov, 2002, [197140](#)),
31 evaluation of health outcomes to date have been based on ecological data only. This analysis did

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1 not adjust for the influence of other risk factors (e.g., smoking, reproductive characteristics) that
2 could contribute to increased cancer rates for lung cancer in men and breast cancer in women.
3 Given that both the SMRs and SIRs for cancer outcomes vary considerably between men and
4 women, this suggests the possibility that occupational exposures might be a contributing factor in
5 these adverse health outcomes.

6 Future research in Chapaevsk includes plans to conduct a breast cancer case-control
7 study. Women who were born from 1940 onward and who have been diagnosed with breast
8 cancer before the age of 55 were included in the study, although the plan to characterize TCDD
9 using serum is uncertain (Revich et al., 2005, [198777](#)).

10
11 **2.4.1.1.1.5.1.3. *Suitability of data for TCDD dose-response modeling.***

12 This study did not meet the considerations and criteria for inclusion in a quantitative
13 dose-response assessment. Given the lack of exposure data on an individual basis, no effective
14 dose can be estimated for this study population. As such, no dose-response modeling was
15 conducted.

16
17 **2.4.1.1.1.6. *The Air Force Health (“Ranch Hands” cohort) study.***

18 Between 1962 and 1971, the U.S. military sprayed herbicides over Vietnam to destroy
19 crops that opposition forces depended upon, to clear vegetation from the perimeter of U.S. bases,
20 and to reduce the ability of opposition forces to hide. These herbicides were predominantly a
21 mixture of 2,4-D, 2,4,5-T, picloram, and cacodylic acid (Institute of Medicine, 2006, [594374](#)). A
22 main chemical sprayed was Agent Orange, which was a 50% mixture of 2,4-D and 2,4,5-T.
23 TCDD was produced as a contaminant of 2,4,5-T and had levels ranging from 0.05 to 50 ppm
24 (Institute of Medicine, 1994, [594376](#)). A series of studies have investigated cancer outcomes
25 among Vietnam veterans. A review of military records to characterize exposure to
26 Agent Orange led Stellman and Stellman (1986, [594380](#)) to conclude that assignment of
27 herbicide levels should not be based solely on self-reports or a crude measure such as military
28 branch or area of service within Vietnam. Investigations have been performed on the Ranch
29 Hands cohort, which consisted of those who were involved in the aerial spraying of
30 Agent Orange between 1962 and 1971. More elaborate methods were used to characterize
31 exposures among these individuals, and these studies are summarized below.

1 **2.4.1.1.1.6.1.** Akhtar et al. (2004, [197141](#)).

2 **2.4.1.1.1.6.1.1.** *Study summary.*

3 Akhtar et al. (2004, [197141](#)) investigated the incidence of cancer in the Ranch Hand
4 cohort, which was published after the release of the 2003 Reassessment document (U.S. EPA,
5 2003, [537122](#)). The Ranch Hand Unit was responsible for aerial spraying of herbicides,
6 including Agent Orange, in Vietnam from 1962 to 1971. Cancer incidence in the Ranch Hand
7 cohort were compared to a cohort that included other Air Force personnel who served in
8 Southeast Asia during the same period but were not involved in the spraying of pesticides.
9 Health outcomes were identified during the postservice period that extended from the time each
10 veteran left Southeast Asia until December 31, 1999. In contrast to previous analyses of this
11 cohort, the Akhtar et al. (2004, [197141](#)) study took into account concerns that both the
12 comparison and spraying cohorts had increased risks of cancer, and addressed the possibility that
13 workers with service in Vietnam or Southeast Asia might have increased cancer risk. The
14 authors addressed the latter concern by adjusting risk estimates for the time spent in Southeast
15 Asia and for the proportion of time spent in Vietnam.

16 The Ranch Hand cohort comprised 1,196 individuals, and the comparison cohort had
17 1,785 individuals. The comparison cohort was selected by matching date of birth, race, and
18 occupation (i.e., officer pilot, officer navigator, nonflying officer, enlisted flyer, or enlisted
19 ground personnel). TCDD levels were determined using serum levels collected from veterans
20 who completed a medical examination in 1987. For those who did not have a serum measure
21 taken in 1987, but provided one in subsequent years, TCDD levels were back-extrapolated to
22 1987 using a first-order kinetic model that assumed a half-life of 7.6 years. Those with
23 nonquantifiable levels were assigned a value of the limit of detection divided by the square root
24 of 2. A total of 1,009 and 1,429 individuals in the Ranch Hand and comparison cohorts,
25 respectively, provided serum measures that were used in the risk assessment. Veterans also were
26 categorized according to the time their tours ended. This date corresponded to changes in
27 herbicide use. These categories were before 1962 or after 1972 (no herbicides were used),
28 1962–1965 (before Agent Orange was used), 1966–1970 (when Agent Orange use was greatest),
29 and 1971–1972 (after Agent Orange was used). Information on incident cases of cancer in the
30 cohort was determined from physical examinations and medical records. Some malignancies
31 were discovered at death and coded from the underlying causes of death as detailed on the death

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1 certificate. A total of 134 and 163 incident cases of cancer were identified in the Ranch Hand
2 and comparison cohort, respectively. Akhtar et al. (2004, [197141](#)) describe case ascertainment
3 verified by record review as being complete.

4 External comparisons were made based on the expected cancer experience derived from
5 U.S. national rates using SIRs and the corresponding 95% confidence interval. Person-years and
6 events were tabulated by 5-year calendar and age intervals.

7 When compared to the general population, no statistically significant excesses in all
8 cancer incidence were observed for either the Ranch Hand (SIR = 1.09, 95% CI = 0.91–1.28) or
9 the comparison cohort (SIR = 0.94, 95% CI = 0.81–1.10). Statistically significant differences
10 were found for three site-specific cancers in the Ranch Hands cohort relative to the general
11 population. Excesses were noted for malignant melanoma (SIR = 2.33, 95% CI = 1.40–3.65)
12 and prostate cancer (SIR = 1.46, 95% CI = 1.04–2.00). In contrast, a reduced SIR was found for
13 cancers of the digestive system (SIR = 0.61, 95% CI = 0.36–0.96). The excess in prostate cancer
14 was also noted in the comparison cohort (SIR = 1.62, 95% CI = 1.23–2.10) relative to the
15 general population. External comparisons were repeated by restricting the cohorts to the period
16 when Agent Orange was used (1966–1970). Again, no statistically significant excesses in all
17 cancer incidence were noted in the Ranch Hand (SIR = 1.14, 95% CI = 0.95–1.37) or
18 comparison cohort (SIR = 0.94, 95% CI = 0.80–1.11). Statistically significant excesses
19 continued to be observed for malignant melanoma (SIR = 2.57, 95% CI = 1.52–4.09) and
20 prostate cancer (SIR = 1.68, 95% CI = 1.19–2.33) in the Ranch Hand component of the cohort.
21 No other statistically significant differences were found among Ranch Hands personnel.

22 For internal cohort analyses, veterans were assigned to one of four exposure categories.
23 Those in the comparison cohort were assigned to the “comparison category.” Ranch Hand
24 veterans that had TCDD serum levels <10 ppt were assigned to the “background” category.
25 Those with a TCDD levels >10 ppt had their TCDD level estimated at the end of their Vietnam
26 service with a first-order kinetic model that used a half-life of 7.6 years. These
27 back-extrapolated values that were less than 118.5 ppt were assigned to a “low” exposure group,
28 while those with values above 118.5 ppt were classified as “high” exposure. Akhtar et al. (2004,
29 [197141](#)) used Cox regression models to describe risks across the exposure groups using the
30 comparison category as the reference. Risks were adjusted for age at tour, military occupation,
31 smoking history, skin reaction to sun exposure, and eye color. Internal cohort analyses were

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1 restricted to those who spent no more than 2 years in Southeast Asia and Ranch Hand workers
2 who served exclusively in Vietnam, and the comparison cohort who served exclusively outside
3 of Vietnam.

4 Statistically significant excesses of cancer incidence (all sites combined) were observed
5 in the highest two exposure groups. A statistically significant trend test ($p = 0.04$) was detected
6 based on the RRs for the background-, low-, and high- exposure groups: 1.44
7 (95% CI = 0.82–2.53); 2.23 (95% CI = 1.24–4.00), and 2.02 (95% CI = 1.03–3.95). For
8 malignant melanoma, the RRs across the three increasing exposure categories were 2.99, 7.42,
9 and 7.51. The corresponding risk estimates for prostate cancer were 1.50, 2.17, and 6.04.

11 **2.4.1.1.1.6.1.2.** *Study evaluation.*

12 An important strength of this study is the manner in which TCDD exposure was
13 estimated. Serum data were available for most veterans, and therefore, generalizing exposure
14 from a small sample of cohort members is not a concern as was the case with the NIOSH and
15 Hamburg cohorts. Back-extrapolating to derive past exposures was based on a methodology that
16 has been applied in many of the cohorts, thereby facilitating risk comparisons. An additional
17 strength of the study is the examination of incidence as a measure of disease occurrence rather
18 than mortality.

19 In contrast to the previous analysis (Ketchum et al., 1999, [198120](#)) the analysis by Akhtar
20 et al. (2004, [197141](#)) was restricted to individuals who spent no more than 2 years in Southeast
21 Asia. Previous research had demonstrated that increased time spent in Southeast Asia was
22 associated with an increased risk of cancer. Confounding might have been introduced given that
23 the comparison cohort spent much more time in Southeast Asia than the Ranch Hands. To
24 illustrate, the median number of days spent in Southeast Asia was 790 for comparison cohort
25 members, and the median days for the Ranch Hand cohort in the background, low, and high
26 exposure groups were 426, 457, and 397, respectively. After restricting to those who spent at
27 most 2 years, statistically significant associations were observed for all cancer sites combined,
28 prostate cancer, and malignant melanoma using the internal cohort comparisons.

29 An important issue in the study is the high correlation between 2,4,5-T and 2,4-D, given
30 that both were used in equal concentrations in Agent Orange. As a result, distinguishing the
31 effects of each is impossible. This point is relevant, given that 2,4-D has been associated with

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1 prostate cancer in several studies. As a result, the dose-response association with prostate cancer
2 might be due to 2,4-D exposure and not TCDD. This issue also has implications for the
3 interpretation of the dose-response pattern for all cancer sites combined, given that incident
4 prostate cancers accounted for 4 of the 12 incident cases in the high-exposure group.
5

6 **2.4.1.1.1.6.1.3.** *Suitability of data for TCDD dose-response modeling.*

7 The ascertainment of incident cases and characterization of exposure to TCDD based on
8 serum measures are strong features of the cohort. Confounding by 2,4-D is a major concern.
9 Since delineating the independent effects of other Agent Orange contaminants is not possible,
10 quantitative dose-response analysis was not conducted on this study.
11

12 **2.4.1.1.1.6.2.** Michalek and Pavuk (2008, [199573](#)).

13 **2.4.1.1.1.6.2.1.** *Study summary.*

14 Michalek and Pavuk (2008, [199573](#)) recently published an updated analysis of the
15 incidence of cancer and diabetes in the cohort of Ranch Hand veterans. As with the Akhtar et al.
16 (2004, [197141](#)) analysis, the study included a comparison cohort of other Air Force veterans who
17 served in Southeast Asia at the same time but were not involved with the spraying of herbicides.
18 This study extended previous analyses (Henriksen et al., 1997, [197645](#); Ketchum et al., 1999,
19 [198120](#)) by addressing the number of days of herbicide spraying, calendar period of service, and
20 the time spent in Southeast Asia. Veterans who attended at least one of five examinations were
21 eligible for inclusion. Incident cancer cases also were identified from medical records.

22 The methods used to determine TCDD exposures were as described above in the review
23 of the Akhtar et al. (2004, [197141](#)) study. Blood measures also were taken in 1992, 1997, and
24 2002 for subjects with no quantifiable TCDD levels in 1987, those who refused in 1987, and
25 those new to the study. TCDD dose at the end of service in Vietnam was assigned to Ranch
26 Hands that had TCDD levels above background using a a first-order kinetic model and constant
27 half-life of 7.6 years. Each veteran was then assigned to one of four dose categories: comparison
28 veteran, background (i.e., Ranch Hands with 1987 levels of TCDD ≤ 10 ppt), low (Ranch Hands
29 with 1987 levels of TCDD 10.1–91 ppt), and high (Ranch Hands with 1987 levels of TCDD
30 ≥ 118.5 ppt). Serum TCDD estimates are available for 1,597 veterans in the comparison cohort,

1 and 986 veterans in the Ranch Hand cohort. The comparison cohort was selected by matching
2 on date of birth, race, and occupation of the Ranch Hands.

3 Michalek and Pavuk (2008, [199573](#)) used Cox regression to characterize risks of cancer
4 incidence across the three upper exposure categories using the comparison category as the
5 referent group. Risk estimates were adjusted for year of birth, race, smoking, body mass index at
6 the qualifying tour, military occupation, and skin reaction to sun exposure. Tests for trend for
7 increased risk of cancer were conducted by testing the continuous covariate \log_{10} TCDD.

8 Overall, no association between the TCDD exposure categories and RR of all-site cancer
9 was observed. Those in the highest exposure group had an RR of 0.9 (95% CI = 0.6–1.4).
10 Stratified analyses by calendar period of service showed more pronounced risk for those who
11 served before 1986 (when higher amounts of Agent Orange were used). A statistically
12 significant dose-response trend ($p < 0.01$) was observed for cancer risk and \log_{10} TCDD
13 exposure. The RRs for the background, low, and high groups used in these comparisons were
14 0.7 (95% CI = 0.4–1.3), 1.7 (95% CI = 1.0–2.9), and 1.5 (95% CI = 0.9–2.6). A statistically
15 significant increase, however, was noted when analyses were restricted to those who had sprayed
16 for at least 30 days before 1967 and spent time in Southeast Asia (RR = 2.2, 95% CI = 1.1–4.4).
17

18 **2.4.1.1.1.6.2.2.** *Study evaluation.*

19 Michalek and Pavuk (2008, [199573](#)) used the same study population that Akhtar et al.
20 (2004, [197141](#)), and so it has the same strengths and limitations as noted above. The follow-up,
21 however, extends an additional 5 years (until the end of 2004). The findings for the
22 dose-response analyses were not as compelling as the earlier Akhtar et al. (2004, [197141](#))
23 findings.
24

25 **2.4.1.1.1.6.2.3.** *Suitability of data for TCDD dose-response modeling.*

26 The key limitation precluding dose-response analysis for the Michalek and Pavuk (2008,
27 [199573](#)) study is the possible confounding from the inability to control for 2,4-D and other
28 agents used in Agent Orange. As such, quantitative dose-response analysis was not conducted
29 on this study.
30

1 **2.4.1.1.1.7. Other studies of potential relevance to dose-response modeling.**

2 **2.4.1.1.1.7.1.** Hooiveld et al. (1998, [197829](#))—Netherlands workers.

3 **2.4.1.1.1.7.1.1. *Study summary.***

4 Hooiveld et al. (1998, [197829](#)) re-analyzed the mortality experience of a cohort of
5 workers employed in two chemical plants in the Netherlands using 6 additional years of
6 follow-up from an earlier study (Bueno et al., 1993, [196993](#)). The cohort consisted of those
7 employed between 1955 and June 30, 1985, and vital status was ascertained until
8 December 31, 1991 (i.e., 36 years of follow-up). These cohort members were involved in the
9 synthesis and formulation of phenoxy herbicides, of which the main product was
10 2,4,5-trichlorophenoxyacetic acid and monochloroacetic acid. This cohort, with a shorter
11 follow-up interval than the original study (t' Mannetje et al., 2005, [197593](#)), was included in the
12 IARC international cohort. The cohort consisted of 1,167 workers, of which 906 were known to
13 be alive at the end of the follow-up. The average length of follow-up was 22.3 years, and only
14 10 individuals were lost to follow-up.

15 The authors used detailed occupational histories to assign exposures. Workers were
16 classified as exposed to phenoxy herbicides or chlorophenols and contaminants if they worked in
17 selected departments (i.e., synthesis, finishing, formulation, packing, maintenance/repair,
18 laboratory, chemical effluent waste, cleaning, shipping-transport, or plant supervision); were
19 exposed to the accident in 1963; or were exposed by proximity (i.e., if they entered an exposed
20 department at least once a week). The 1963 accident was the result of an uncontrolled reaction
21 in the autoclave in which 2,4,5-trichlorophenol was synthesized; an explosion resulted, with
22 subsequent release of PCDDs that included TCDD. Based on these methods of exposure
23 assignment, 562 workers were deemed to be exposed to phenoxy herbicides or chlorophenols,
24 and 567 were unexposed. Due to limited information, 27 workers were classified as having
25 unknown exposure.

26 TCDD exposures also were assigned using serum measured on a sample of workers who
27 were employed for at least 1 year and first started working before 1975. Dioxin-like compounds
28 including PCDDs were also measured in the serum samples but were not analyzed for this study.
29 Of the 144 subjects who were invited to provide samples, 94 agreed. TCDD levels were
30 back-extrapolated to the time of maximum exposure using a one-compartment, first-order kinetic
31 model that used a half-life estimate of 7.1 years. The mathematical model used was

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1 $\ln(\text{TCDDmax}) = \ln(\text{TCDD}) + \text{lag} \times \ln(2)/7.1$. The lag was defined as the number of years since
2 last exposure for those exposed by virtue of their normal job duties. For those exposed as a
3 result of the accident in 1963, the lag was defined as the number of years since the accident
4 occurred.

5 The authors made external comparisons of cohort mortality to the Netherlands population
6 using the SMR statistics. Poisson regression was used to perform internal cohort comparisons
7 using unexposed workers as the referent. RRs (measured using rate ratios) generated from the
8 Poisson model also were used to compare mortality based on low, medium, and high TCDD
9 serum-derived categories. The Poisson model included the following covariates as adjustment
10 factors: age, calendar period at end of follow-up, and time since first exposure.

11 When compared to the general population, workers had an excess mortality from cancer
12 (SMR = 1.5, 95% CI = 1.1–1.9), based on 51 cancer deaths. Generally, no excesses were
13 observed for site-specific cancers. The exception included eight deaths from cancers of the
14 urinary organs (SMR = 3.9, 95% CI = 1.7–7.6). Although not statistically significant, SMRs
15 comparable in magnitude to other studies were detected for non-Hodgkin's lymphoma
16 (SMR = 3.8, 95% CI = 0.8–11.0) and Hodgkin's disease (SMR = 3.2, 95% CI = 0.1–17.6). A
17 statistically significant excess of cancer mortality ($n = 20$ deaths among occupational workers)
18 also was also observed relative to the general population when analyses were restricted to those
19 exposed as a result of the 1963 accident (SMR = 1.7, 95% CI = 1.1–2.7). Three deaths from
20 prostate cancer were also noted among these workers (SMR = 5.2, 95% CI = 1.1–15.3), but no
21 excess was observed with any other cancer site.

22 Internal cohort comparison also demonstrated an increased risk of all cancer mortality
23 among those exposed to phenoxy herbicides, chlorophenols, and contaminants relative to those
24 unexposed (RR = 4.1, 95% CI = 1.8–9.0). A statistically significant increased risk was also
25 noted for respiratory cancer mortality (RR = 7.5, 95% CI = 1.0–56.1). Analyses across
26 categories of TCDD exposure revealed excesses in cancer mortality for all cancer sites
27 combined; however, no dose-response trend was apparent.

28
29 **2.4.1.1.1.7.1.2.** *Study evaluation.*

30 Several other studies that have characterized cohorts by TCDD levels have used the area
31 under the curve approach and thus have derived an exposure metric that is time dependent.

1 Hooiveld et al. (1998, [197829](#)) instead created an exposure metric to capture the maximum
2 exposure attained during the worker's employment. Characterizing risks using this metric
3 assumes that other TCDD exposures accrued during a workers' lifetime are not relevant
4 predictors of cancer risk.

5
6 **2.4.1.1.1.7.1.3.** *Suitability of data for TCDD dose-response modeling.*

7 One study limitation is that although dioxin-like compounds were measured in the serum
8 samples, Hooiveld et al. (1998, [197829](#)) reported associations with mortality for TCDD only.
9 There is some utility to examining dose-response analyses using alternative exposure metrics as
10 those constructed in this cohort. However, the small number of identified cancer deaths,
11 limitations in terms of the exposure assignment (based on nonrepresentative sample, and
12 maximum exposure level) and concern over potential confounding by co-exposures preclude
13 using these data for a dose-response analysis.

14
15 **2.4.1.1.1.7.2.** t' Mannelje et al. (2005, [197593](#))—New Zealand herbicide sprayers.

16 **2.4.1.1.1.7.2.1.** *Study summary.*

17 t'Mannelje et al. (2005, [197593](#)) described the mortality experience of a cohort of New
18 Zealand workers who were employed in a plant located in New Plymouth. The plant produced
19 phenoxy herbicides and pentachlorophenol between 1950 and the mid-1980s. This study
20 population also was included in the international cohort of producers and sprayers of herbicides
21 that was analyzed by IARC (Kogevinas et al., 1997, [198598](#); Saracci et al., 1991, [199190](#)). In
22 this 2005 study, analyses were restricted to those who had worked at least 1 month; clerical,
23 kitchen, and field research staff were excluded. The authors followed up 1,025 herbicide
24 producers and 703 sprayers from 1969 and 1973, respectively, until the end of 2000.

25 The cohort consisted of two components: those involved with the production of
26 herbicides and those who were sprayers. For the herbicide producers, exposures were
27 determined by consulting occupational history records; no direct measures of exposure were
28 available. Each department of employment was assigned to one of 21 codes as in the IARC
29 international cohort (Saracci et al., 1991, [199190](#)). Industrial hygienists and factory personnel
30 with knowledge of potential exposures in this workforce classified each job according to
31 potential to be exposed to TCDD, other chlorinated dioxins, and phenoxy herbicides. Exposure

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1 was defined as a dichotomous variable (i.e., exposed and unexposed). Among producers, 813
2 were classified as exposed, with the remaining 212 considered unexposed.

3 The “sprayer” component of the cohort includes those who were registered in the national
4 registry of applicators at any time from January 1973 until the end of 1984. For the sprayers,
5 detailed occupational information was lacking. Exposure was, therefore, based on an exposure
6 history questionnaire completed in a previous study of congenital malformations (Smith et al.,
7 1982, [198586](#)). This questionnaire, administered to 548 applicators in 1980 and 232 applicators
8 in 1982, achieved a high response rate (89%). Participants were asked to provide information
9 about 2,4,5-T-containing product use on an annual basis from 1969 up to the year the survey was
10 completed. As the use of 2,4,5-T ceased in the mid-1980s, data on occupational exposure to
11 TCDD among these workers are fairly complete. Virtually all sprayers (699 of 703) were
12 exposed to TCDD, higher chlorinated dioxins, and phenoxy herbicides.

13 Deaths among workers were identified through record linkage to death registrations in the
14 New Zealand Health Information Service. Electoral rolls, drivers’ licenses, and social security
15 records also were consulted to confirm identified deaths. External comparisons of mortality
16 were made to the New Zealand population using the SMR statistic. The mortality follow-up for
17 the producers began on January 1, 1969 and extended until December 31, 2000. For the
18 sprayers, the follow-up period extended from January 1, 1973 until December 31, 2000. A total
19 of 43 cancer deaths occurred in the producer group and 35 cancer deaths occurred in the sprayer
20 group in the cohort. Where possible, stratified analyses by duration of employment and
21 department were conducted. The departments examined for producers included synthesis,
22 formulation and lab, maintenance and waste, packing and transport, other, and unexposed.
23 SMRs were generated using the New Zealand population as an external referent. A linear test
24 for trend was applied to evaluate dose-response trends according to categories of duration of
25 employment. Stratified analyses also were also done for sprayers who started working before
26 1973, as TCDD levels in 2,4,5-T produced at the New Zealand plant dropped dramatically after
27 1973. Although an SMR was presented for female producers, given that only one cancer death
28 was observed, this study can provide no insight on differential risks between the sexes.

29 Among TCDD-exposed producers, for all cancers combined, no statistically significant
30 excess mortality was found when compared to the general population (SMR = 1.24,
31 95% CI = 0.90–1.67). No dose-response trend in the SMRs for all cancers was observed with

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1 duration of employment ($p = 0.44$). No statistically significant elevated SMR was observed in
2 any of the duration of employment categories for any of the six specific departments examined.
3 A statistically significant positive linear trend, however, was noted among synthesis workers
4 ($p = 0.04$). There was some suggestion of reduced mortality in the upper exposure levels for
5 workers in the formulation and lab departments. For sprayers, the SMR for all cancer sites
6 combined was not elevated relative to the New Zealand general population (SMR = 0.82,
7 95% CI = 0.57–1.14), nor was a dose-response pattern observed with increasing duration of
8 employment ($p = 0.86$). Additionally, no statistically significant excess in cancer mortality for
9 all sites combined was evident in workers who were first employed either before 1973
10 (SMR = 0.75, 95% CI = 0.50–1.07) or from 1973 on (SMR = 1.81, 95% CI = 0.59–4.22). For
11 site-specific analyses of cancer mortality, an excess of multiple myeloma was observed among
12 production workers relative to the general population (SMR = 5.51, 95% CI = 1.14–16.1). This
13 SMR was based on three deaths. No statistically significant excess (or deficit) of mortality was
14 found for any other cancer site examined in either the sprayers or the producers.

15

16 **2.4.1.1.1.7.2.2.** *Study evaluation.*

17 The physical activity demands of spraying contribute to a healthy worker effect that
18 manifests itself in a lower SMR based on both external comparisons to the general population as
19 a referent, and the SMR generated for the producers in the cohort. The analyses conducted using
20 a simple dichotomy of exposure and duration of employment are limited, as nearly all of the
21 sprayers were unexposed.

22 The dose-response pattern with duration of employment coupled with the observation
23 that higher levels of exposure to TCDD occurred among workers in the synthesis department is
24 an important finding. These workers were also exposed to several other contaminants, however,
25 that include processing chemicals, technical products, intermediates, and byproducts (Kauppinen
26 et al., 1993, [594388](#)). These included phenoxy herbicides and dioxin-like compounds such as
27 chlorinated dioxins. Since the dichotomous exposure measure was based on exposure to TCDD,
28 chlorinated dioxins and phenoxy herbicides, the associated dose-response analyses presented in
29 this study should be interpreted cautiously in light of the inability to either characterize or control
30 for these potential confounders. As such, these co-exposures might have contributed to the
31 dose-response pattern observed with increased duration of employment in the synthesis workers.

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1 **2.4.1.1.1.7.2.3.** *Suitability of data for TCDD dose-response modeling.*

2 Although the study authors completed a subsequent analysis of this cohort using
3 serum-derived TCDD (McBride, 2009, [198490](#)), the lack of individual-level TCDD exposures
4 precludes dose-response modeling.

6 **2.4.1.1.1.7.3.** McBride et al. (2009, [198490](#))—New Zealand herbicide sprayers.

7 **2.4.1.1.1.7.3.1.** *Study summary.*

8 McBride et al. (2009, [198490](#)) recently published the mortality experience of the New
9 Zealand cohort in relation to serum estimates of TCDD levels. This study included
10 1,599 workers who were employed between 1969 and November 1, 1989, which was the date
11 that 2,4,5-T was last used. As in their study published earlier in the same year (McBride et al.,
12 2009, [197296](#)), the follow-up period extended from the first day of employment until
13 December 31, 2004. Vital status was ascertained through record linkage to the New Zealand
14 Health Information Service Mortality Collection and the Registrar General’s Index to Deaths for
15 deaths up to 1990.

16 All current and former workers who lived within 75 km of the plant were invited to
17 provide serum samples. A total of 346 of the eligible workers (68%) provided samples, which
18 represented 22% of the overall study population (346/1599). Based on the serum measures, 70%
19 (241/346) had been exposed to TCDD. This percentage is similar to the estimated 71% of
20 workers who were deemed to have been exposed based on a review of occupational records. The
21 mean serum TCDD value was 9.9 ppt. The highest exposures were observed for those employed
22 in the trichlorophenol operation (23.4 ppt). Values among unexposed workers averaged 4.9 ppt,
23 which is close to the background level of 3.9 ppt among individuals of similar age in the New
24 Zealand general population (Bates et al., 2004, [197113](#)). Details on smoking histories of
25 individuals were also collected for the 346 individuals who provided serum, allowing for an
26 examination of the potential confounding role that smoking might have on derived risk estimates
27 for TCDD.

28 Cumulative exposure to TCDD as a time-dependent metric was estimated for each
29 worker. A detailed description of the methods used to derive TCDD exposure was described in
30 Aylward et al. (2009, [197187](#)). The qualitative TCDD scores available for those with serum
31 measures were used to estimate the cumulative exposures based on a half-life of approximately

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1 7 years. A time-dependent estimate of TCDD exposure was derived and the area under the curve
2 was used to obtain cumulative workplace TCDD exposure above background levels. Model
3 performance appears modest as the model explained only 30% of the variance (adjusted R^2)
4 when these TCDD exposure estimates were compared with actual serum levels (Aylward et al.,
5 2009, [197187](#)).

6 As with previous analyses of the cohort (McBride et al., 2009, [197296](#); t' Mannetje et al.,
7 2005, [197593](#)), external comparisons to the New Zealand general population were made using
8 the SMR statistic. The SMR statistic also was used to compare mortality across four exposure
9 groups relative to the general population, as defined by the serum TCDD estimates: 0–68.3,
10 68.4–475.0, 475.1–2085.7, and ≥ 2085.8 ppt-month. The proportional hazards model also was
11 used to conduct internal cohort comparisons across these same four exposure groups. In these
12 analyses, age was used as the time variable, and the covariates of date of hire, sex, and birth year
13 were included in the proportional hazards model. The cut-points for these four exposure
14 categories were chosen so that approximately equal numbers of deaths were included in each
15 category.

16 Consistent with earlier SMR analyses of the same cohort, no increased cancer mortality
17 was observed among “ever” exposed workers in this cohort when compared to the general
18 population (SMR = 1.1, 95% CI = 0.9–1.4). No statistically significant excess was noted for any
19 of the site-specific cancers, although there was some suggestion of increased risk of soft tissue
20 sarcoma (SMR = 3.4, 95% CI = 0.1–19.5), multiple myeloma (SMR = 2.2, 95% CI = 0.2–8.1),
21 non-Hodgkin’s lymphoma (SMR = 1.6, 95% CI = 0.3–4.7), and cancer of the rectum
22 (SMR = 2.0, 95% CI = 0.7–4.4). No statistically significant increases in cancer mortality (all
23 sites combined) was found in any of the four exposure categories as measured by the SMR
24 statistic, nor was a dose-response trend noted with increasing exposure categories. No
25 dose-response trends (based on SMR analyses) were noted for five site-specific cancers
26 examined (i.e., digestive organs, bronchus, trachea and lung, soft tissue sarcomas, lymphatic and
27 hematopoietic tissue, and non-Hodgkin’s lymphoma), although SMRs for three of the
28 four exposure categories exceeded 2.0 for non-Hodgkin’s lymphoma.

29 In contrast to the external cohort comparisons, the RRs generated with the proportional
30 hazards model supported a dose-response trend, as rate ratios increased across increasing TCDD
31 exposure categories. The RRs and their 95% confidence intervals relative to the lowest of the

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1 four groups were 1.05 (95% CI = 0.48–2.26), 1.38 (95% CI = 0.64–2.97) and 1.58
2 (95% CI = 0.71–3.52). Neither the linear ($p = 0.29$) or quadratic ($p = 0.82$) test for trend,
3 however, was statistically significant. An increased risk of lung cancer mortality was observed
4 in the highest TCDD exposure category relative to the lowest (RR = 5.75,
5 95% CI = 0.76–42.24). The tests for trend for lung cancer, however, also were not statistically
6 significant.

7 A smoking survey was administered to a sample of surviving workers of this cohort, and
8 smoking prevalence was found to be slightly higher among those with higher cumulative
9 exposure (61%) compared to lower exposures (51–56%). These minor differences in smoking
10 prevalence unlikely was a strong enough confounder to explain the fivefold increase in risk of
11 lung cancer found in the highest exposure category. Although the smoking data assessment was
12 a strength of the study, it was limited to only sample of workers and was not available for those
13 who died of lung cancer.

14

15 **2.4.1.1.1.7.3.2.** *Study evaluation.*

16 Given high rates of emigration, loss to follow-up (22%) was a potential concern in this
17 study. If comparable emigration rates did occur among the general population then the SMRs
18 would be underestimated. It is unclear to what extent emigration occurred among the general
19 population and whether emigration in both the worker and general populations was dependent on
20 health status. If emigration rates were comparable among these two populations, the associated
21 bias from the under-ascertainment of mortality in the lost to follow-up group would likely
22 attenuate a positive association between TCDD and cancer mortality. Among the worker
23 population, there was not much evidence of differential loss to follow-up with respect to
24 exposure as average exposures were lower (3.2 ppt) among those loss to follow up compared to
25 those with complete follow-up (5.7 ppt). Previous studies among this population also found
26 slightly higher loss to follow-up rates among the unexposed (23%) compared to the exposed
27 (17%) workers (t' Mannetje et al., 2005, [197593](#)).

28 McBride et al. (2009, [198490](#)) did not present results using a continuous measure of
29 TCDD exposure (lagged or unlagged) as was done in most other occupational cohorts.
30 Additionally, the modeling did not consider the use of different periods of latency.

31

1 **2.4.1.1.1.7.3.3.** *Suitability of data for TCDD dose-response modeling.*

2 There is no evidence that the authors considered exposure metrics that are consistent with
3 environmental cancer-causing agents such as exposure modeling that takes latency into account.
4 Given that past occupational cohort studies of TCDD-exposed workers have consistently
5 demonstrated stronger association with lag interval of 15 years, such an approach should be
6 applied to this cohort. This precludes this study from consideration for quantitative
7 dose-response modeling.

8
9 **2.4.1.1.1.7.4.** McBride et al. (2009, [197296](#))—New Zealand herbicide sprayers.

10 **2.4.1.1.1.7.4.1.** *Study summary.*

11 McBride et al. (2009, [197296](#)) published an updated analysis of the mortality of the New
12 Zealand cohort. The follow-up period was from January 1, 1969 to December 31, 2004
13 extending the previous study by an additional 4 years. In contrast to the previous study where
14 the cohort comprised individuals employed for at least 1 month prior to 1982 (or 1984)
15 (t' Mannetje et al., 2005, [197593](#)), the cohort in this study consisted of all those who worked at
16 least one day between January 1, 1969 and October 1, 2003. This resulted in a cohort of
17 1,754 workers, of which 247 died in the follow-up interval. Seventeen percent of the cohort
18 members were lost to follow-up, which could be a source of selection bias if loss to follow-up
19 was related to both the exposure metrics and the health outcome of interest. Previous data from
20 this cohort (t' Mannetje et al., 2005, [197593](#)), however, showed fairly comparable loss to follow-
21 up rates among the unexposed (23%) and the exposed populations (17%).

22 Comparisons to the New Zealand general population were made using the SMR statistic.
23 Stratified analyses were conducted by duration of employment (<3 months, ≥3 months), sex,
24 latency (<15 years, ≥15 years), and period of hire (<1976, ≥1976). The authors defined latency
25 as the period between the day last worked and the earliest of date of death, date of emigration or
26 loss to follow-up, or December 31, 2004.

27 The overall SMR for mortality from all cancer sites combined relative to the New
28 Zealand population was 1.01 (95% CI = 0.85–1.10). Although not statistically significant there
29 was suggestion of an increased risk of rectal cancer (SMR = 2.03; 95%CI = 0.88–4.01) among
30 the employees. SMRs for lymphatic and hematopoietic cancers (overall SMR = 1.21,
31 95% CI = 0.52–2.39) included 3.12 (95% CI = 0.08–17.37) for Hodgkin's disease,

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1 1.59 (95% CI = 0.43–4.07) for non-Hodgkin’s lymphoma and 3.73, 95% CI = 1.20–8.71), and
2 1.66 (95% CI = 0.20–5.99) for multiple myeloma. No statistically significant excess of cancer
3 mortality was noted among workers employed for <3 months (SMR = 1.19,
4 95% CI = 0.65–2.00), or for ≥3 months (SMR = 0.98, 95% CI = 0.75–1.26). A statistically
5 significant excess of digestive cancers was found for those who worked fewer than 3 months
6 relative to the New Zealand population (SMR = 2.52, 95% CI = 1.15–4.78). No excesses were
7 observed for any site-specific cancers when analyses were restricted to those who worked for 3
8 or more months. No statistically significant elevated SMRs were found for all cancers
9 (combined) either for a latency period of fewer than 15 years (SMR = 1.14, 95% CI = 0.72–1.71)
10 or a latency period of ≥15 years (SMR = 0.96, 95% CI = 0.72–1.26). Similarly, no statistically
11 significant excess in cancer mortality was observed for all cancer sites combined, or any
12 site-specific cancer when analyses were stratified by date of hire (<1976, ≥1976) or by sex. The
13 SMR among women who were employed at the site was 0.68 (95% CI = 0.45–1.00).

14

15 **2.4.1.1.1.7.4.2. Study evaluation.**

16 High rates of emigration in New Zealand (9% among workers in the cohort) contributed
17 to a fairly high loss to follow-up (22% among workers) during the study period. The loss to
18 follow-up would reduce the overall mortality estimates among the workers, which could
19 underestimate the SMRs if loss to follow-up (and health status) was not comparable in the
20 general population. For example, it is unclear if workers and the general population who
21 emigrated were sicker than those remaining in the cohort. Previous data from the cohort workers
22 suggests that loss to follow-up rates were slightly higher among the low and unexposed
23 populations (McBride, 2009, [198490](#); t' Mannetje et al., 2005, [197593](#)) worker population, so
24 presumably the highly exposed workers were not lost to follow-up more so than other workers.

25

26 **2.4.1.1.1.7.4.3. Suitability of data for TCDD dose-response modeling.**

27 This study extended the mortality follow-up and included stratified analyses to
28 investigate effect modification by period of latency, sex, and date of hire. A key limitation was
29 the lack of direct measures of exposure for study participants which precluded estimating
30 effective dose needed for dose-response modeling. This study did not meet the considerations
31 and criteria for inclusion in quantitative dose-response analysis.

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1 **2.4.1.1.2. *Key characteristics of epidemiologic cancer studies***

2 See Table 2-1 at the end of the chapter for a comparison of the length of follow-up,
3 latency period used, the half-life for TCDD used, and the fraction of TEQs accounted for by
4 TCDD (when applicable) for each study.
5

6 **2.4.1.1.3. *Feasibility of TCDD cancer dose-response modeling—summary discussion by***
7 ***cohort.***

8 **2.4.1.1.3.1. *Using the NIOSH cohort in dose-response modeling.***

9 It is important to evaluate the NIOSH cohort in cancer dose-response modeling of TCDD.
10 This cohort is the largest assembled to date, direct measures of TCDD based on sampling are
11 available, and the lengthy follow-up interval allows for latent effects to be taken into account.
12 Further, although this cohort consists mostly of male workers, these workers were occupationally
13 exposed to TCDD daily, as compared to the acute accidental exposures of other occupational
14 cohorts. Although the most recent analyses of a subset of the NIOSH cohort showed no
15 association between serum TCDD levels and cancer mortality, the study authors did not examine
16 latency effects (Collins et al., 2009, [197627](#)). Incorporation of latency intervals is important in
17 light of the stronger dose-response relationships that consistently have been observed with a
18 15–20 year latency interval in previous investigations of the NIOSH and other cohorts
19 (Steenland et al., 2001, [197433](#)).

20 Most published studies of the NIOSH cohort did not evaluate exposures to dioxin-like
21 compounds. An exception is the analysis by Steenland et al. (2001, [197433](#)). Although
22 Steenland et al. (2001, [197433](#)) did not incorporate individual-level data on dioxin-like
23 compounds, based on their previous work (Piacitelli et al., 1992, [197275](#)) they assumed that TEQ
24 occupational exposures occurred as a result of TCDD alone in this population. TCDD exposures
25 provided a better fit to the data than the TEQ-based metric, and 15-year latencies improved the
26 fit for both metrics (relative to unlagged exposures). The lifetime risk estimates for an increase
27 in 10 TEQs (pg/kg of body weight/day/sex) ranged from 0.05–0.18%. The value added for this
28 measure is the incorporation of the contribution of other dioxin-like compounds to the
29 background rates.

30 Blue collar workers, such as those in the NIOSH cohort, typically have higher rates of
31 smoking than the general population (Bang and Kim, 2001, [197081](#); Lee et al., 2007, [594391](#)).

1 This potential source of confounding would be expected to produce a higher SMR for lung
2 cancer mortality, and could contribute to the excess noted in the cohort with longer lag intervals.
3 This bias, however, likely is not large as no statistically significant excess of nonmalignant
4 respiratory mortality was found in these workers. Any associated bias from smoking would be
5 expected to be smaller for comparisons conducted within the cohort, as fellow workers would be
6 expected to be more homogeneous with respect to their risk factor profile than with an external
7 general population referent group. Stratified analyses using both internal and external
8 comparison groups also did not identify important differences in associations with TCDD
9 exposure between smoking and nonsmoking cancers. Thus, fatal cancer risk estimates reported
10 for workers in the NIOSH cohort appear to provide a reasonable estimate of the carcinogenic
11 potency of TCDD.

12 Although the Steenland et al. (2001, [197433](#)) study did not directly account for the
13 possible confounding effects of other occupational exposure, the authors did address this source
14 of potential bias. No known occupational exposures to carcinogens occurred, with the exception
15 of 4-aminobiphenyl, which occurred at one plant. Two deaths from mesothelioma also occurred
16 in the cohort, so some exposure to asbestos might also have occurred in the cohort (Fingerhut
17 et al., 1991, [197375](#)). The statistical analyses suggested that the inability to control for other
18 occupational exposures would not have unduly affected risk estimates generated from internal
19 cohort comparisons. For instance, the removal of one plant at a time from the analysis did not
20 materially change dose-response estimates generated from the Cox model (Cheng et al., 2006,
21 [523122](#)). Moreover, adding a variable to represent plant in the Cox regression had little impact
22 on the risk estimates. Given that other occupational exposures varied by plant, a change in risk
23 estimates would be expected if such exposures were strong confounders.

24 The Cheng et al. (2006, [523122](#)) analysis provides important information about the
25 impact of applying kinetic models to the data. The CADM TCDD kinetic model resulted in
26 dramatic decreases in the TCDD cancer mortality risk estimates when compared to the one-stage
27 compartmental model that had been applied. Although Cheng et al. (2006, [523122](#)) suggested
28 that the CADM model provides a better fit to the data than the typically used simple
29 one-compartmental model, statistical comparisons of model fit were not reported. Therefore,
30 there is value in presenting the range in risk estimates across different models when
31 characterizing dose-response relationships.

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1 Finally, the half-life of TCDD is generally recognized to vary according to body fat
2 percentage, data that were not available for the NIOSH workers. The inability to account for
3 between-worker variability in body fat would introduce exposure measurement error. That body
4 fat percentage would not be expected to correlate with cumulative exposure to TCDD exposure,
5 however, would limit the potential for misclassification bias. The effect of any nondifferential
6 exposure measurement error likely would serve to attenuate the risk estimates of the study.

7
8 **2.4.1.1.3.2. Using the BASF cohort in dose-response modeling.**

9 The availability of blood lipid data for TCDD allows for characterization of cumulative
10 TCDD exposures in the BASF cohort. TCDD blood lipid data were collected for 90% of the
11 surviving members of the cohort (138 of 154) and these serum measures were used to generate
12 TCDD exposure estimates for all 254 cohort members. Therefore, the potential for
13 misclassification from extrapolating these exposures to the entire cohort may not be as likely as
14 for the NIOSH cohort where sera data were available for only a small fraction of workers. These
15 data were, however, collected long after the accident (36 years) and had to be back-extrapolated
16 to derive the initial exposures.

17 The data on this cohort included several risk factors such as cigarette smoking and body
18 mass index. One advantage is that cumulative TCDD levels by body mass index can be
19 estimates on an individual-level basis. As expected, the derived cumulative measures appear to
20 compare well with severity scores of chloracne. The finding that more pronounced risks are
21 found 15–20 years after first exposure are also consistent with findings from several other
22 cohorts (Bertazzi et al., 2001, [197005](#); Fingerhut et al., 1991, [197375](#); Manz et al., 1991,
23 [199061](#)).

24 One key limitation of the BASF cohort is its relatively small sample size ($n = 243$), which
25 limits the ability to evaluate dose-response relationships for site-specific cancers. Also, the
26 quality of the ascertainment of cancer incidence cannot be readily evaluated as the geographic
27 area of the cohort is not covered by a tumor registry. Ott and Zober (1996, [198101](#)) state that
28 nonfatal cancers could have been more likely to be missed in early years, which could partially
29 contribute to the larger standardized incidence ratio found for cancer with longer latencies.
30 Commenting on risk differences derived from incident and decedent cancer outcomes is difficult.
31 Among those comprising the cohort, the ascertainment of incident outcomes was recognized to

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1 be less complete in early years. Although the ascertainment of mortality outcomes was generally
2 regarded to be good among the 243 workers, some workers who died or moved likely were
3 missed when the cohort was constructed. These deaths would have been more likely to have
4 occurred several years before the second component of the cohort was assembled.

5 The use of the SMR statistic for this study population is associated with important
6 sources of uncertainties. Deaths were surely missed, particularly for the third component of the
7 cohort that accounts for approximately 38% (94/247) of the entire cohort; this factor would serve
8 to underestimate the overall SMR. As mentioned before, this component of the cohort was
9 assembled through the recruitment of workers known to be alive in 1986. Despite this limitation,
10 the characterization of exposure data and availability of other risk factor data at an individual
11 level allow the development of quantitative dose-response analyses.

12 13 **2.4.1.1.3.3. Using the Hamburg cohort in dose-response modeling.**

14 The Hamburg cohort lacked data on cigarette smoking, and, therefore, effect estimates
15 could not be adjusted for this covariate. Additional analyses that excluded lung cancers resulted
16 in an even stronger dose-response relationship between all cancer mortality and TCDD. Serum
17 levels of TCDD also were also not associated with smoking status in a subgroup of these workers
18 (Flesch-Janys et al., 1995, [197261](#)) suggesting that smoking is not likely a confounder of the
19 association between all cancer mortality and TCDD.

20 An important limitation of the cohort is the reliance on blood and tissue measurements of
21 190 workers that likely represent a highly selective component of the cohort. This subset of
22 workers was identified at the end of the observation period, and therefore, excludes workers who
23 died or could not be traced. There are uncertainties in deriving department- and period-specific
24 estimates for a period that extends over three decades using this number of workers.

25 Additionally, the criteria applied to the reference population could have introduced some bias.
26 Workers were included only in the reference group if they had been employed for at least
27 10 years in a gas supply industry. The criteria were much different for the workers who were
28 exposed to TCDD (only 3 months of employment). As a result, the reference group likely would
29 be more susceptible to the healthy worker effect. Internal cohort comparisons, which should be
30 void of such bias, however, generally produced results similar to those based on the external
31 comparison population. Therefore, the Becher et al. (1998, [197173](#)) study meets the criteria and

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1 additional epidemiological considerations which allowed for development of quantitative
2 dose-response analyses.

3

4 **2.4.1.1.3.4. *Using the Seveso cohort in dose-response modeling.***

5 Unlike many of the occupational cohorts that were examined, data from the Seveso
6 cohort are representative of a residential population whose primary exposure was from a single
7 TCDD release. A notable exception is the BASF cohort where workers were exposed primarily
8 through two accidents that occurred in the plant. The Seveso data, therefore, might permit
9 cancer dose-response investigations in women and children.

10 Uncertainty in identifying the critical exposure window for most of the outcomes related
11 to the Seveso cohort is a key limitation. An important feature of the Seveso cohort, however, is
12 that TCDD levels were much lower among those in the highest exposure zones in Seveso
13 (medians range from 56–136 ng/kg) (Eskenazi et al., 2004, [197160](#)) than those in the
14 occupational cohorts who had TCDD exposures that were sometimes more than 1,000 ng/kg.
15 Given these dramatic differences in exposures, the standardized mortality ratios (after
16 incorporating a 15–20 year latency period) for all cancer sites combined are remarkably similar
17 between the Seveso and the occupational cohort analyses. Perhaps more importantly, the data
18 from Seveso might be more relevant for extrapolating to lower levels, given that exposures to
19 TCDD are two orders of magnitude higher than background levels (Smith and Lopipero, 2001,
20 [198585](#)).

21 The Warner et al. (2002, [197489](#)) study found a positive association between serum
22 levels of TCDD and breast cancer. As noted previously, ascertainment of incident cases for all
23 cancers would allow for a dose-response relationship to be evaluated. Moreover, future breast
24 cancer analyses in this cohort should strengthen the quantitative dose response analyses of this
25 specific cancer site. The strengths of the Warner et al. (2002, [197489](#)) study outlined earlier
26 suggest that this study should be considered for cancer dose-response modeling.

27 Earlier Seveso studies likely are unsuitable for conducting quantitative risk assessment.
28 These previous studies used an indirect measure of TCDD exposure, namely, zone of residence.
29 Soil concentrations of TCDD varied widely in these three zones (Zone A: 15.5–580.4 ppt;
30 Zone B: 1.7–4.3 ppt; and Zone R: 0.9–1.4 ppt), which could have resulted in considerable
31 exposure misclassification. The Warner et al. (2002, [197489](#)) study greatly improved the

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1 characterization of TCDD exposure using serum measures, and also allowed for control of
2 salient risk factors that may have resulted in bias due to confounding.

3 At this time it is unclear whether any study has examined the relationship between cancer
4 and serum estimates of TCDD among Seveso males exposed from the 1976 accident.

5
6 **2.4.1.1.3.5. Using the Chapaevsk related data in dose-response modeling.**

7 Currently, individual-level exposure data are lacking for residents of this area and there is
8 no established cohort for which cancer outcomes can be ascertained. These limitations,
9 therefore, preclude the inclusion of Chapaevsk data in a quantitative dose-response analysis.

10
11 **2.4.1.1.3.6. Using the Ranch Hands cohort in dose-response modeling.**

12 An important limitation of the Ranch Hands cohort for TCDD and cancer dose-response
13 modeling is an inability to isolate TCDD effects from the effects of other agents found in the
14 associated herbicides. Exposure to other dioxin-like compounds was not estimated in this study
15 and could confound the previously reported associations. As such, dose-response analyses on
16 this population were not conducted.

17
18 **2.4.1.1.4. *Discussion of general issues related to dose-response modeling***

19 **2.4.1.1.4.1. Ascertainment of exposures.**

20 Several series of epidemiological data have used serum measures to estimate TCDD
21 levels. Serum data offer a distinct advantage in that they provide an objective means to
22 characterize TCDD exposure at the individual level. The serum measures in the occupational
23 cohorts, however, are limited in two important ways. First, these samples are generally collected
24 from small subsets of the larger cohorts; therefore, using these measures to extrapolate to the
25 remainder of the cohort could introduce bias due to exposure misclassification. The
26 second limitation is related to estimating the half-life of TCDD. As noted previously, exposures
27 to TCDD were back-extrapolated several decades from serum samples collected among
28 surviving members of several cohorts. This approach was used in the NIOSH, Ranch Hands,
29 BASF, New Zealand, and Hamburg cohorts. The reported half-life of TCDD among these
30 populations was reported between 7.1 to 9.0 years and shown to vary with several individual
31 characteristics including age, body fat composition, and smoking. The derivation of half-lives

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1 from a sample of workers, and application of these estimates to retrospectively characterize
2 exposure can introduce uncertainty into the lifetime exposure estimates. It is important to note,
3 however, that sensitivity analyses results in several studies have been fairly consistent when
4 evaluating the impact of half-life of TCDD (Flesch-Janys et al., 1995, [197261](#); Steenland et al.,
5 2001, [197433](#)).

6 A unique advantage of the Seveso study is that serum measures were taken shortly after
7 the accident, and therefore characterization of TCDD exposure in this population does not
8 depend on assumptions needed to back-extrapolate exposures several decades.

9 10 **2.4.1.1.4.2. Latency intervals.**

11 Many of the epidemiological studies indicate stronger associations between TCDD and
12 cancer outcomes once a latency period has been considered. Generally, risks are higher when a
13 lag period of 15–20 years is included. As noted previously, this observation is consistent with
14 many other environmental carcinogens such as radon, radiation, and cigarette smoking. That
15 recent exposures do not contribute to increased cancer risk provides some support that the
16 initiation and promotion phases might occur many years before death making recent exposures
17 irrelevant for these analyses. The ability to discriminate between models of varying latency,
18 however, was limited in many studies. The application of biologically based modeling could
19 provide additional important insights on which phase(s) of carcinogenesis TCDD exerts an
20 influence. Such modeling, however, would necessitate having data on an individual-level basis.
21 Ideally, this modeling would use cancer incident data rather than mortality outcomes, given that
22 for many cancers, the median survival time exceeds 5 years.

23 24 **2.4.1.1.4.3. Use of the SMR metric.**

25 The occupational cohorts and the studies in Seveso and Chapaevsk have made inferences
26 regarding the effects of TCDD on mortality using the SMR. When compared to the general
27 population, the healthy worker effect may result in a downward bias in the SMR. This often can
28 manifest as SMRs less than 1 for several causes of mortality. The effect of this bias is, however,
29 generally lower for cancer outcomes. Cancer outcomes, whether incidence or death, typically
30 occur later in life and do not generally affect an individual's ability to work at earlier ages.

1 There are several approaches that can be taken to minimize potential biases introduced by
2 the healthy worker effect, which would account for workers being healthier than the general
3 population. Comparisons of mortality (or cancer incidence) can be made to other cohorts of
4 similar workers. If done properly, this can allow for some control of characteristics such as
5 sociodemographic characteristics and smoking as the two populations can be matched by these
6 factors. However, it may be the case that other working populations are exposed to other
7 harmful exposures, thereby making it difficult to estimate risk associated with a specific agent
8 (such as TCDD) in the cohort of interest. A second and preferred approach to control for the
9 healthy worker effect, should it prove feasible, is to conduct comparisons of health outcomes in
10 relation to exposure within the cohort. These comparisons are less likely to be influenced by
11 other potential confounding variables such as smoking, socioeconomic status, and other
12 occupational exposures that are generally more homogeneous within the cohort relative to
13 external populations. Moreover, the mechanisms used to identify health outcomes and follow
14 individuals over time are generally applied in the same manner to all cohort members. Taken
15 together, where different comparisons have been made to generate risk estimates, those that have
16 been conducted using internal cohort comparisons are preferable.

17 In addition to potential bias from the health worker effect, the comparison of SMRs
18 between studies is not always straightforward and is not recommended by some (Myers and
19 Thompson, 1998, [594395](#); Rothman, 1986, [046091](#)). The SMR is the ratio of the observed
20 number of deaths to the expected number of deaths and is often referred to as the method of
21 indirect standardization. The expected number of deaths is estimated by multiplying the number
22 of person-years tabulated across individuals in the cohort, stratified by age, by rates from a
23 reference population that are available for the same strata. Therefore, each population cohort
24 will have an estimated number of cases derived using a different underlying age structure. As
25 outlined by Rothman (1986, [046091](#)), the mortality rates might not be directly comparable to
26 each other, although the impact of such bias will be much less if the age-distribution of the
27 cohorts is similar. While it might be reasoned that the TCDD exposed workers would have
28 similar age distributions this is in fact not the case (Becher et al., 1998, [197173](#); Ott et al., 1993,
29 [594322](#); Thiess et al., 1982, [064999](#)). This may be due to exposure occurring both chronically,
30 as well as from acute exposures due to accidental releases that happened at various times at
31 different plants. This is evident with the Hamburg and the BASF cohorts, as most individuals

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1 comprising the BASF cohort were employed at the time of the accident (1953/1954), while most
2 of the Hamburg cohort (852/1048) was employed after 1954; the follow-up of these cohorts
3 ended at approximately the same time.

4 The method of direct standardization allows for a more meaningful comparison of
5 mortality rates to be made between cohorts. With this approach, weights (usually based on age
6 and sex) are drawn from a standard population and are, in turn, applied to disease rates for the
7 same strata observed in the cohort of interest. A comparison of weighted rates between different
8 cohorts would then be based on the same population standard.

9 Despite these limitations in comparing SMRs between studies, Armstrong (1995,
10 [594397](#)) argues that the comparisons are valid if the underlying stratum specific rates in each
11 exposure grouping are in constant proportion to external rates. Comparisons of the SMRs
12 between studies will be biased only if there is an interaction between age and TCDD (i.e., the RR
13 of disease due to exposure differs by age). For cancer outcomes, the finding that associations
14 become stronger after a period of latency is incorporated into the analyses suggests that this
15 assumption does not hold true. That is, risk estimates would be lower among young workers.
16 Similarly, for noncancer outcomes, some of the data from the Seveso cohort suggests differential
17 effects according to the age at exposure.

18 The use of the SMR might also be biased in that workers exposed to TCDD could be
19 subject to more intensive follow-up than the general population, and as a result, differential
20 coding biases with cause of death might occur. Moreover, some cohorts (e.g., the BASF cohort)
21 have been assembled, in part, by actively seeking out survivors exposed to accidental releases of
22 dioxins. As such, they would not include persons who have died or who were lost to follow-up.
23 This would result in underascertainment of deaths and SMRs developed from these data. The
24 use of an internal cohort comparison offers distinct advantages to overcome potential sources of
25 selection bias. Given these uncertainty about comparability across the different studies,
26 conducting a meta-analysis of cancer outcomes for TCDD using the SMR statistic is not
27 warranted for this analysis.

28 29 **2.4.1.1.4.4. All cancers versus site-specific.**

30 An important consideration for quantitative dose-response modeling is the application of
31 models for all cancers combined, or for site-specific cancers. Consistency is often lacking for

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1 site-specific cancers, which might be due in large part to the relatively small number of cases
2 identified for site-specific cancers in the cohorts. Although the risk estimates produced for all
3 cancer sites have important limitations and uncertainties, the data are far more consistent in
4 terms of the magnitude of an association and latency intervals. The IARC evaluation has put
5 forth the possibility of a pleuripotential mode of action between TCDD and the occurrence of
6 cancer. Despite the criticism of this assertion by some (Cole et al., 2003, [197626](#)), the general
7 consistency of an increased risk for all-cancer mortality across the occupational cohorts when
8 latency intervals have been incorporated, provides adequate justification for dose-response
9 quantification of all cancer sites combined.

10
11 **2.4.1.1.4.5. Summary of epidemiologic cancer study evaluations for dose-response**
12 **modeling.**

13 All epidemiologic cancer studies summarized above were evaluated for suitability of
14 quantitative dose-response assessment using the TCDD-specific considerations and study
15 inclusion criteria. The results of this evaluation are summarized in a matrix style array (see
16 Table 2-2) at the end of this section, and descriptively in Appendix B. Table 2-4 summarizes the
17 key epidemiologic cancer studies suitable for further TCDD dose-response analyses.

18
19 **2.4.1.2. Noncancer**

20 In this section, the available epidemiological data that could be used in a dose-response
21 analysis for noncancer endpoints are evaluated. Because many of the key studies also evaluated
22 cancer outcomes, the noncancer studies are presented in the same order as presented in
23 Section 2.4.1.1. Generally, the strengths and limitations of the cancer studies also apply to the
24 noncancer outcomes. In this section, key features of these studies that have direct relevance to
25 modeling of noncancer outcomes in particular are highlighted. To reduce redundancy, a detailed
26 overview of many of these cohorts and studies are not provided here. Instead, the reader should
27 refer to Section 2.4.1.1.1.

1 **2.4.1.2.1. *Noncancer cohorts.***

2 **2.4.1.2.1.1. *The NIOSH cohort.***

3 **2.4.1.2.1.1.1. Steenland et al. (1999, [197437](#)).**

4 **2.4.1.2.1.1.1.1. *Study summary.***

5 The 1999 published report of NIOSH workers exposed to TCDD also conducted external
6 cohort comparisons to the U.S. general population using SMRs for mortality outcomes other than
7 cancer (Steenland et al., 1999, [197437](#)). Analyses are based on 3,538 workers employed at
8 8 plants from 1942 to 1984. SMRs were based on a mortality follow-up that was extended until
9 the end of 1993. Cox regression analyses were used to compare mortality risk in relation to
10 TCDD exposure within the cohort.

11

12 **2.4.1.2.1.1.1.2. *Study evaluation.***

13 Overall, no statistically significant differences in all-cause mortality (SMR = 1.03,
14 95% CI = 0.97–1.08) were observed. Mortality from ischemic heart disease (SMR = 1.09,
15 95% CI = 1.00–1.20) and accidents (SMR = 1.25, 95% CI = 1.03–1.50) was slightly elevated.
16 Based on the external comparison population, the dose-response relationship for ischemic heart
17 disease observed with the SMRs calculated across TCDD exposure septiles was not statistically
18 significant ($p = 0.14$). Overall, excess risk was not evident for diabetes, cerebrovascular disease,
19 or nonmalignant respiratory disease using the external population comparisons. Internal cohort
20 comparisons using the Cox regression model were performed using 0 and 15-year lag intervals.
21 A dose-response trend was observed for the derived ratios across the unlagged cumulative
22 TCDD exposure septiles for ischemic heart disease ($p = 0.05$) and diabetes ($p = 0.02$). For
23 ischemic heart disease mortality, those in the upper two septiles had rate ratios of 1.57
24 (95% CI = 0.96–2.56) and 1.75 (95% CI = 1.07–2.87), respectively, relative to those in the
25 lowest septile. In contrast, an inverse dose-response relationship was observed for diabetes
26 mortality. The inverse association found for diabetes is inconsistent with the positive association
27 reported in the Ranch Hands study (Michalek and Pavuk, 2008, [199573](#)). However, previous
28 reports have questioned the use of death certificates as the means to ascertain outcome as
29 diabetes may be under-reported especially among descendants with diabetes who die from cancer
30 (McEwen and TRIAD, 2006, [594400](#)).

31

1 **2.4.1.2.1.1.1.3.** *Suitability of data for TCDD dose-response modeling.*

2 The inverse association with diabetes precludes dose-response analysis for this outcome.
3 The dose-response relationship between TCDD exposure and ischemic heart disease mortality
4 was not statistically significant at the alpha level of 0.05 and was not observed in other cohorts.
5 Furthermore, fatal outcomes are not a suitable basis for development of an RfD. For these
6 reasons, dose-response analysis for this outcome is precluded.

7
8 **2.4.1.2.1.1.2.** Collins et al. (2009, [197627](#)).

9 **2.4.1.2.1.1.2.1.** *Study summary.*

10 Collins et al. (2009, [197627](#)) recently described the mortality experience of Dow
11 employees who worked in Midland, Michigan. This plant produced 2,4,5-trichlorophenol
12 between 1942 and 1979, and 2,4,5-T between 1948 and 1982. The cohort consisted of
13 1,615 workers exposed to TCDD from as early as 1942; the follow-up of the cohort extended
14 until 2003.

15 TCDD exposures were derived using serum samples obtained from 280 surviving
16 individuals. A simple one-compartment, first-order pharmacokinetic model was used to estimate
17 time-dependent TCDD measures. The area under the curve approach was then applied to
18 estimate cumulative TCDD exposure above background. A half-life of 7.2 years for TCDD
19 based on earlier work was incorporated into the exposure estimation (Flesch-Janys et al., 1996,
20 [197351](#)).

21 Collins et al. (2009, [197627](#)) made an external comparison of the mortality rates of the
22 cohort to the U.S. general population using the SMR statistic. Noncancer causes of death
23 included all causes, diabetes, cerebrovascular disease, nonmalignant respiratory disease, cirrhosis
24 of the liver, and accidents. Overall, no statistically significant difference in all-cause mortality of
25 these workers was detected when compared to the general population (SMR = 0.9,
26 95% CI = 0.9–1.0). Except for cirrhosis of the liver (SMR = 0.4, 95% CI = 0.1–0.8), no
27 differences were found for any of the noncancer causes of death relative to the general
28 population.

29 Internal cohort analyses based on cumulative measures of TCDD were conducted for
30 mortality from diabetes, ischemic heart disease, and nonmalignant respiratory disease using the
31 Cox regression model. These models adjusted for possible confounders such as year of hire and

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1 birth year. No statistically significant association was found between continuous measure of
2 TCDD and these causes of death.

4 **2.4.1.2.1.1.2.2.** *Study evaluation.*

5 Given that the external comparisons may result in bias from the healthy worker effect,
6 results from the internal cohort comparisons using the Cox regression model are preferred.
7 These analyses were performed for diabetes, ischemic heart disease, and nonmalignant
8 respiratory disease. TCDD levels for these workers were estimated using a simple
9 one-compartment pharmacokinetic model (Aylward et al., 2007, [197175](#)). The hazard ratios
10 generated from the Cox regression model were not statistically significant for any of the
11 three noncancer outcomes modeled.

13 **2.4.1.2.1.1.2.3.** *Suitability of data for TCDD dose-response modeling.*

14 No association of an increased risk for an adverse effect was observed with any of the
15 noncancer outcomes. In addition, since noncancer mortality was the endpoint being examined,
16 dose-response modeling based on this population was not conducted.

18 **2.4.1.2.1.2.** **The BASF cohort.**

19 **2.4.1.2.1.2.1.** Ott and Zober (1996, [198101](#)).

20 **2.4.1.2.1.2.1.1.** *Study summary.*

21 In 1996, Ott and Zober published a report on the mortality experience of the cohort of
22 243 BASF male workers who were accidentally exposed to 2,3,7,8-TCDD in 1954 or in the clean
23 up that followed. The mortality follow-up of this cohort extended until the end of 1992.
24 External comparisons of mortality were made to the German population using the SMR statistic.
25 Internal cohort comparisons were also made by estimating cumulative TCDD for the cohort
26 using serum measures that were obtained from 138 workers. Ott et al. (1993, [594322](#)) provided
27 a detailed account of the methodology to estimate TCDD. Briefly, a cumulative measure of
28 TCDD expressed in $\mu\text{g}/\text{kg}$ was derived, by first estimating the half-life of TCDD using
29 individuals who had repeated serum measures; the half-life was estimated to be 5.8 years.
30 Individual-level data on body fat were used to account for the influence of body fat on decay
31 rates. Half-life estimates of TCDD varied (range: 5.1–8.9 years) and were dependent on body fat

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1 composition (20% and 30%, respectively). This approach differed from previous analysis of this
2 cohort that used a constant 7-year half-life (Ott et al., 1993, [594322](#)). TCDD levels at the time of
3 serum sampling were then estimated as the product of TCDD concentration in blood lipid and
4 the total lipid weight for each worker. Nonlinear models then were applied to estimate the
5 contribution of duration of exposure to TCDD dose extrapolated to the time of exposure.

6 External comparisons to the German population using the SMR statistic also were
7 examined across dose categories. The noncancer causes of death examined by Ott and Zober
8 (1996, [198101](#)) included all-cause mortality, diseases of the circulatory system, ischemic heart
9 disease, diseases of the digestive system, external causes, suicide, and residual causes of death.
10 Overall, no statistically significant differences in the SMR with the general population for
11 all-causes of death (SMR = 0.9, 95% CI = 0.7–1.1) were found. No statistically significant
12 differences were noted for any of the other causes of death examined.

13 Ott and Zober (1996, [198101](#)) performed internal cohort comparisons using the Cox
14 regression model. These analyses found no dose-response patterns when cause-specific
15 mortality was examined across increasing cumulative TCDD exposure categories. Although an
16 inverse association for diseases of the respiratory system (SMR = 0.1, 95% CI = 0.0–0.8) was
17 detected, it was based only on 1 reported case. Many of these comparisons are limited by small
18 sample sizes as 92 deaths occurred in the cohort, and of these, 31 were from cancer. Also, the
19 third component of the cohort was identified primarily from former employees who were alive in
20 1986. As a result, the SMR based on the general population might be underestimated by the
21 exclusion of deceased workers.

22 23 **2.4.1.2.1.2.1.2. Study evaluation.**

24 As noted previously, caution should be exercised in the interpretation of SMR values of
25 noncancer outcomes as they could be influenced by the healthy worker effect. Although the
26 mechanism of identifying vital status appears to be excellent and unbiased, SMRs might be
27 underestimated for the cohort due to the manner in which they were constructed. Specifically, a
28 large component of the cohort was assembled by actively seeking out former workers who were
29 known to be alive in 1986.

1 **2.4.1.2.1.2.1.3.** *Suitability of data for TCDD dose-response modeling.*

2 No dose-response patterns were observed between TCDD and the noncancer outcomes in
3 the Ott and Zober (1996, [198101](#)) study. Therefore, dose-response modeling was not conducted.

4
5 **2.4.1.2.1.3.** ***The Hamburg cohort.***

6 **2.4.1.2.1.3.1.** Flesch-Janys et al. (1995, [197261](#)).

7 **2.4.1.2.1.3.1.1.** *Study summary.*

8 Flesch-Janys et al. (1995, [197261](#)) reported on the mortality experience of a cohort of
9 individuals employed by an herbicide-producing plant in Hamburg, Germany, covering the
10 period 1952 to 1992. As described in more detail in Section 2.4.1.1.1.3, the authors developed a
11 cumulative measure of TCDD using serum measures from 190 workers. This study also
12 examined the relationship between total TEQ and mortality. In the study population, the mean
13 TEQ without TCDD was 155 ng/kg, and for the mean TEQ including TCDD was 296.5 ng/kg.

14 Risks relative to the unexposed referent group of gas workers were estimated using Cox
15 regression across six exposed TCDD groups (i.e., the first four quintiles, and the ninth and
16 tenth deciles). A linear dose-response relationship was found with all causes of mortality and
17 cardiovascular mortality ($p < 0.01$). The RR for all cardiovascular deaths in the upper exposure
18 category was 1.96 (95% CI = 1.15–3.34), although there was no evidence of a linear
19 dose-response trend ($p = 0.27$). The dose-response relationship was most marked for ischemic
20 heart disease, with a RR of 2.48 (95% CI = 1.32–4.66) in the highest exposure group. A
21 dose-response relationship was also observed across TEQ groupings for all cause mortality,
22 cardiovascular disease mortality, and ischemic heart disease mortality. The authors did not
23 perform joint modeling of TEQ (without TCDD) and TCDD, so determining the extent that
24 dioxin-like compounds contributed to an increased risk of mortality is not possible.

25
26 **2.4.1.2.1.3.1.2.** *Study evaluation.*

27 The Flesch-Janys et al. (1995, [197261](#)) study lacks information on other potential risk
28 factors for cardiovascular disease, which could result in confounding if those risk factors are also
29 related to TCDD exposure. Dose-response patterns were strong, however, and persisted across
30 numerous TCDD (and TEQ) exposure categories based on the use of an external reference group
31 (i.e., gas workers) or based on the internal comparison. The findings based on the internal

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1 comparison are noteworthy in that these groups should be more homogenous with respect to
2 confounding factors. As noted previously, the poor correlation between TCDD and smoking
3 among workers and similar smoking prevalence between the workers and the external gas
4 company workers suggest that smoking was not likely a confounder of the TCDD and
5 cardiovascular disease relationship. No other evaluation of noncancer mortality outcomes has
6 been undertaken in this cohort since 1995.

7 A strength of the Flesch-Janys et al. (1995, [197261](#)) study was that it included the
8 collection of blood serum measures, which provided an objective measure of TCDD exposure.
9 Blood serum data, however, were obtained only for 16% of the cohort. The assumption of the
10 first-order kinetic elimination model is critical, given that measures were taken at the end of
11 follow-up. The model also assumed the half-life of TCDD was 6.9 years. If the kinetics are not
12 first order, or if the half-life estimate is inaccurate, estimates of TCDD levels during exposure
13 would be biased, particularly for workers having longer periods between exposure and PCDD
14 and PCDF assays. Sensitivity analyses completed by the authors suggest that such bias is not
15 likely to present because the results were unaffected when different model assumptions regarding
16 kinetic and half-lives were examined. The lack of an impact on RR estimates with varying
17 half-life estimates was similar to findings by Steenland et al. (2001, [197433](#)).

18 19 **2.4.1.2.1.3.1.3.** *Suitability of data for TCDD dose-response modeling.*

20 Despite the aforementioned study strengths, the study focused on fatal outcomes such as
21 all cause mortality, cardiovascular disease mortality, and ischemic heart disease mortality. As
22 such, dose-response analysis was not conducted since these outcomes are not suitable for
23 development of an RfD.

24 25 **2.4.1.2.1.4.** **The Seveso Women's Health Study (SWHS).**

26 Eskenazi et al. (2000, [197162](#)) presented an overview of the SWHS. The SWHS is the
27 first comprehensive epidemiologic study of the reproductive health of a female population
28 exposed to TCDD. The primary objective of the SWHS is to investigate the relationship of
29 TCDD and several reproductive endpoints, including endometriosis, menstrual cycle
30 characteristics, birth outcomes, infertility, and age at menopause. A second phase of follow-up

1 that focuses on osteoporosis, thyroid hormone, breast cancer, diabetes, and metabolic syndrome
2 is expected to be completed in 2010.

3 Women were eligible for participation in the SWHS if they resided in Zones A and B (the
4 most contaminated areas) at the time of the explosion, were 40 years of age or younger at the
5 time of the explosion in 1976, and samples of their blood were collected and stored between
6 1976 and 1980. The enrollment of women in the SWHS began in March 1996 and continued
7 until July 1998. Of the 1,271 eligible women, 17 could not be found, 21 had died, and 12 were
8 too ill to participate. Of the 96% of the remaining women, 80% ($n = 981$) participated in the
9 study. Participation in the SWHS included a blood draw and an interview by a trained nurse who
10 was blind to subjects' TCDD level and zones of residence at the time of the accident. The
11 interview included detailed information on potential confounders including occupational,
12 medical, and reproductive, and pregnancy history. Also, women who were premenopausal were
13 asked to undergo a vaginal ultrasound and pelvic exam and to complete a daily diary on
14 menstruation.

15 Depending on the health outcome under study, TCDD exposures were characterized for
16 the women at different times. For example, TCDD exposure levels were estimated at the time of
17 the accident for some studies and at the time of conception for others. The SWHS study
18 population has been used to investigate associations between maternal TCDD levels and the
19 following health outcomes: menstrual cycle characteristics (Eskenazi et al., 2002, [197168](#));
20 endometriosis (Eskenazi et al., 2002, [197164](#)); birth outcomes (Eskenazi et al., 2003, [197158](#));
21 age at menarche (Warner et al., 2004, [197490](#)); age at menopause (Eskenazi et al., 2005,
22 [197166](#)); uterine leiomyomas (Eskenazi et al., 2007, [197170](#)); and ovarian function (Warner
23 et al., 2007, [197486](#)). An evaluation of the studies in chronological order is presented in this
24 section.

25
26 **2.4.1.2.1.4.1.** Eskenazi et al. (2002, [197168](#))—Menstrual cycle characteristics.

27 **2.4.1.2.1.4.1.1.** *Study summary.*

28 Eskenazi et al. (2002, [197168](#)) evaluated serum TCDD exposures in relation to several
29 menstrual cycle characteristics in the SWHS. A total of 981 women who were 40 years of age or
30 younger at the time of the accident comprised the SWHS. The following exclusion criteria was
31 applied 44 years of age or older, women with surgical or natural menopause, those with Turner's

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1 syndrome, and those who in the past year had been pregnant, breastfed, or used an intrauterine
2 device or oral contraceptives.

3 A trained interviewer collected data on menstrual cycle characteristics using a
4 questionnaire. Women were asked to indicate how long their cycles were, whether the cycles
5 were regular (e.g., irregular cycle defined as length varied by more than 4 days), how many days
6 the menstrual flow lasted, and whether this flow was “scanty, moderate, or heavy.” Information
7 was also collected on obstetric and gynecological conditions. TCDD exposures were derived
8 from serum samples collected in 1976–1985. The authors selected the earliest available serum
9 sample, and back-extrapolated to 1976 values using either the Filser model (Kreuzer et al., 1997,
10 [198088](#)) for women aged 16 years or younger in 1976 ($n = 20$) or the first-order kinetic model
11 ($n = 6$) (Pirkle et al., 1989, [197861](#)).

12 Serum TCDD levels were transformed using the log₁₀ scale, and the relationships
13 between these levels and length of menstrual cycle and days of menstrual flow were examined
14 using linear regression. The authors applied logistic regression to characterize the risk between
15 log₁₀TCDD and heaviness of flow or regularity of cycle. In these analyses, moderate or heavy
16 flow and regular cycle were used as the reference categories. Stratified analysis was performed
17 by menarcheal status at the time of the accident.

18 Overall, the association with TCDD exposure (per 10-fold increase) and length of
19 menstrual cycle was not statistically significant for premenarcheal ($\beta = 0.93$, 95% CI = -0.01 ,
20 1.86) women or postmenarcheal women ($\beta = -0.03$, 95% CI = -0.61 , 0.54). The corresponding
21 estimates found for days of menstrual flow were $\beta = 0.18$ (95% CI = -0.15 , 0.51) and $\beta = 0.16$
22 (95% CI = -0.18 , 0.50), respectively. Reduced flow was not associated with TCDD when
23 compared to moderate or heavy flow (odds ratio [OR] = 0.84, 95% CI = 0.44, 1.61); effect
24 modification by menarcheal status, however, was evident ($p = 0.03$). Specifically, women
25 exposed to TCDD who were premenarcheal had lower odds of reduced flow, while those
26 exposed to TCDD who were postmenarcheal did not. These findings counter the hypothesis that
27 TCDD exposure is related to ovarian dysfunction. Finally, statistically significant ORs were
28 found between serum TCDD levels (per 10-fold increase) and having an irregular cycle
29 (OR = 0.46, 95% CI = 0.23, 0.95). This inverse association was evident in both premenarcheal
30 women (OR = 0.50, 95% CI = 0.18, 1.38) and postmenarcheal women (OR = 0.41,
31 95% CI = 0.15, 1.16).

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1 **2.4.1.2.1.4.1.2.** *Study evaluation.*

2 Overall, the findings from the Eskenazi et al. (2002, [197168](#)) study suggest that
3 exposures to TCDD can affect menstrual cycle characteristics among women who were exposed
4 before menarche. Exposures to TCDD were well characterized using serum samples available
5 on an individual-level basis, and the design allowed for the influence of other risk factor data to
6 be controlled for in regression analyses. Analysis of TCDD levels and the length of menstrual
7 cycle in premenarcheal women produced associations that were largely not statistically
8 significant at the alpha level of 0.05, but may have some biological significance. However, it is
9 unclear whether the endpoints that were measured constitute adverse health outcomes as they are
10 not definitive markers of ovarian dysfunction. Another source of uncertainty is measurement
11 error due to the subjective nature of menstrual flow reporting. Any resulting misclassification of
12 the outcome should be nondifferential, as the measurement error is unlikely to be dependent on
13 TCDD exposure.

14
15 **2.4.1.2.1.4.1.3.** *Suitability of data for TCDD dose-response modeling.*

16 The lack of a clear adverse health outcome related to TCDD exposure is a weakness of
17 this study. Although it is difficult to define the critical window of exposure for quantitative
18 exposure calculations, it can be estimated for the women that were premenarcheal at the time of
19 the accident as 13 years. Therefore, this study is suitable for further consideration for
20 quantitative dose-response modeling.

21
22 **2.4.1.2.1.4.2.** Eskenazi et al. (2002, [197164](#))—Endometriosis.

23 **2.4.1.2.1.4.2.1.** *Study summary.*

24 The SWHS provided the opportunity to investigate the association between serum TCDD
25 levels and endometriosis (Eskenazi et al., 2002, [197164](#)). The rationale the authors provided for
26 undertaking this study was the experimental animal studies that suggested an association, the
27 high prevalence of endometriosis among infertile women where breast milk concentrations of
28 dioxin are high, and the unknown etiology of endometriosis. The study consisted of 601 women
29 who were younger than 30 years at the time of the Seveso accident. Stored sera that had been
30 collected between 1976 and 1980 were also available for these women.

1 Given that laparoscopy could not be performed on women unless clinically indicated, no
2 “gold” standard was available for endometriosis diagnosis. Based on the results of a validation
3 study they conducted in a clinical population, the researchers classified women as having
4 endometriosis based on symptom report, gynecologic exam results, and vaginal ultrasound.

5 TCDD was measured in sera in 1976 for 93% of the women. Values for women whose
6 serum TCDD levels were collected after 1977 and had values exceeding 10 ppt were
7 back-extrapolated to 1976 using either the Filser model (<16 years of age) (Kreuzer et al., 1997,
8 [198088](#)) or a first-order kinetic model (≥ 16 years) (Pirkle et al., 1989, [197861](#)). These estimates
9 of TCDD were then modeled as both continuous (on a log scale) and categorical (≤ 20 , 20.1–100,
10 and >100 ppt) exposures.

11 Polytomous logistic regression was applied within the cohort used to generate RRs. In
12 relation to women in the lowest exposure category, the RR for endometriosis among women in
13 the middle and upper categories was 1.2 (90% CI = 0.3–4.5) and 2.1 (90% CI = 0.5–8.0),
14 respectively. The trend tests were not statistically significant for either the categorical ($p = 0.25$)
15 and continuous measures of TCDD ($p = 0.84$).

16 17 **2.4.1.2.1.4.2.2.** *Study evaluation.*

18 It is important to note that disease misclassification could have led to an underestimate of
19 the true risk of endometriosis if this misclassification was not differential with respect to TCDD
20 exposure. Also, younger women were likely to be under-represented as those who had never
21 been sexually active could not be examined due to cultural reasons. Other dioxin-like
22 compounds (PCDD, PCDFs, or polychlorinated biphenyls [PCBs]) were not considered because
23 of small serum volumes, but any potential TEQ exposures occurring in the population were
24 thought to be mostly attributable to TCDD in the exposed women.

25 26 **2.4.1.2.1.4.2.3.** *Suitability of data for TCDD dose-response modeling.*

27 Given that no statistically significant dose-response patterns were observed with either
28 log-transformed or across TCDD exposure categories, and that the elevated risks among those
29 with higher exposures had very wide confidence intervals (that included unity) quantitative
30 dose-response analyses were not recommended for this outcome.

1 **2.4.1.2.1.4.3.** Eskenazi et al. (2003, [197158](#))—Adverse birth outcomes.

2 **2.4.1.2.1.4.3.1.** *Study summary.*

3 Eskenazi et al. (2003, [197158](#)) examined the relationship between serum TCDD levels
4 and birth outcome measures. Analyses were based on 745 of the 981 women enrolled in the
5 SWHS who reported having been pregnant ($n = 1,822$). Most of these pregnancies
6 (888 pregnancies among 510 women) occurred after the accident. Analysis of spontaneous
7 abortions was restricted to 769 pregnancies among 476 women that did not end in abortion or in
8 ectopic or molar pregnancy. Congenital anomalies were evaluated for the 672 pregnancies that
9 did not end in spontaneous abortion. For the birth outcomes of fetal growth and gestational age,
10 analysis was performed using 608 singleton births from women without hypertensive pregnancy
11 disorders.

12 TCDD exposures were based on serum measures, most of which were taken shortly after
13 the accident. Serum was collected in 1976–1977 for 413 women, between 1978 and 1981 for
14 12 women, and in 1996 for 19 women. TCDD exposures based on serum samples collected from
15 1977 onward were back-extrapolated to 1976.

16 Statistical analyses were performed on pregnancies that ended between 1976 and the time
17 of interview. A continuous measure of \log_{10} TCDD (base 10 scale) was used to investigate
18 associations with adverse birth outcomes. Logistic regression was used to characterize the
19 relationship between TCDD exposure spontaneous abortions, small for gestational age, and
20 preterm birth (<37 weeks gestation). Linear regression was used to describe the relationship
21 between TCDD and birth weight (in grams) and gestational age (in weeks).

22 The risk estimates were adjusted for a series of characteristics that included sex of infant,
23 history of low birth weight child, maternal height, maternal body mass index, maternal
24 education, maternal smoking during pregnancy, and parity. No association was evident between
25 TCDD serum levels and spontaneous abortion for pregnancies between 1976 and 1998
26 ($OR = 0.8$, 95% $CI = 0.6$ – 1.2), or those between 1976 and 1984 ($OR = 1.0$, 95% $CI = 0.6$ – 1.6).
27 No statistically significant associations (ORs ranged from 1.2–1.8) were found between
28 \log_{10} TCDD levels and preterm delivery, small for gestational age. Although the mean change in
29 birth weight for pregnancies between 1976 and 1984 was fairly large ($\beta = -92$, 95% $CI = -204$
30 to 19), it also was not statistically significant at the alpha level of 0.05.

31

1 **2.4.1.2.1.4.3.2.** *Study evaluation.*

2 This study was well-designed with well characterized exposures. Statistically significant
3 associations were not evident, although the birth-weight findings should be pursued with further
4 follow-up of the cohort. As the authors point out, those who were most vulnerable at the time of
5 the accident (the youngest) had not yet completed their childbearing years. While the study
6 lacked exposure data for the fathers, the authors indicated that only a small proportion were
7 believed to have high exposures to TCDD. The key limitation of the study was a reliance on
8 self-reported measures of pregnancy history, which may lead to some misclassification of the
9 birth outcomes. The observation that a large proportion of Seveso women had a voluntary
10 abortion because of fears of possible birth defects due to exposures from the accident suggest an
11 awareness bias is possible as a result of differential reporting of birth outcomes according to
12 exposure status.

13
14 **2.4.1.2.1.4.3.3.** *Suitability of data for TCDD dose-response modeling.*

15 No statistically significant associations were found in the study; in addition, possible
16 awareness bias could have influenced the self-reported measures of birth outcomes. Therefore,
17 quantitative dose-response assessment was not considered for this study.

18
19 **2.4.1.2.1.4.4.** Warner et al. (2004, [197490](#))—Age at menarche.

20 **2.4.1.2.1.4.4.1.** *Study summary.*

21 Warner et al. (2004, [197490](#)) examined the relationship between TCDD and age at
22 menarche in the SWHS cohort. As described earlier in this report, the SWHS comprised
23 981 participants. This study was restricted only to those who were premenarcheal at the time of
24 the accident ($n = 282$). The proportional hazards model was used to model TCDD exposures and
25 age at menarche. Age at menarche was determined by questionnaire administered by a trained
26 interviewer. Covariates examined as potential confounders included height, weight, body mass
27 index, athletic training at the time of interview, smoking, and alcohol consumption.

28 TCDD exposures were determined using serum samples collected from 257 of these
29 women between 1976 and 1977. For the remaining women, TCDD levels were quantified from
30 measures collected between 1978 and 1981 ($n = 23$) and in 1996 ($n = 2$). TCDD levels were
31 back-extrapolated to the time of the explosion in 1976. TCDD was modeled as both a

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1 continuous variable (\log_{10} TCDD) and a categorical variable based on quartile values (≤ 55.9 ,
2 56–140.2, 140.3–300, >300 ppt). The lowest group was further subdivided into those with levels
3 ≤ 20 , and >20 ppt; this cut-point represented background levels found in a sample of women
4 living in an unexposed area.

5 No association was found between the continuous measure of TCDD and age at
6 menarche (hazard ratio [HR] = 0.95, 95% CI = 0.83–1.09). Analyses restricted to those who
7 were younger than 8 in 1976 produced similar results (HR = 1.08, 95% CI = 0.89–1.30).
8 Additionally, no dose-response trend was observed with categorical measures of TCDD among
9 all women, as well as those under the age of 8. Although not statistically significant at the alpha
10 level of 0.05, TCDD exposures were later reported to be associated with age of menarche
11 (HR = 1.20, 95% CI = 0.98–1.60) when analyses were restricted to 84 women under the age of 5
12 at the time of the accident (Warner and Eskenazi, 2005).

13 14 **2.4.1.2.1.4.4.2.** *Study evaluation.*

15 An important strength of the Warner et al. (2004, [197490](#)) study is the ability to
16 characterize TCDD exposures using serum samples that were collected shortly after the accident
17 occurred. The outcome of interest, age at menarche, was determined by asking women “At what
18 age did you get your first menstrual period?” Recent work suggests that self-reported measures
19 of age at menarche decades later have modest agreement with responses provided during
20 adolescence with recall varying by education and by history of an adverse birth outcome (Cooper
21 et al., 2005, [594401](#)). In the Seveso study, bias would be introduced if recall varied according to
22 exposure levels.

23 24 **2.4.1.2.1.4.4.3.** *Suitability of data for TCDD dose-response modeling.*

25 Although the TCDD exposure characterization of study subjects was based on serum
26 data, and no major biases were introduced from the study design, the analyses produced largely
27 null associations. Therefore, quantitative dose-response assessment was not considered for this
28 study.

1 **2.4.1.2.1.4.5.** Eskenazi et al. (2005, [197166](#))—Age at menopause.

2 **2.4.1.2.1.4.5.1.** *Study summary.*

3 Eskenazi et al. (2005, [197166](#)) evaluated the relationship between age at onset of
4 menopause and serum levels of TCDD among women in the SWHS. Of the 981 women who
5 agreed to participate in SWHS, this analysis was restricted to those who had not reached natural
6 menopause before the time of the accident and who were at least 35 years of age at the time of
7 the interview. The recruitment and interview of women occurred approximately 20 to 22 years
8 after the accident (March 1996–July 1998).

9 The population was divided into quintiles of serum TCDD levels for the categorical
10 analysis. For most women ($n = 564$), TCDD levels were estimated from samples provided in
11 1976–1977. For the remaining women included in these analyses, TCDD levels were estimated
12 from samples collected between 1978 and 1982 ($n = 28$) and between 1996 and 1997 ($n = 24$).
13 As noted previously, exposure levels for women with post-1977 detectable levels of TCDD were
14 back-extrapolated to 1976 using either the first-order kinetic model (Pirkle et al., 1989, [197861](#))
15 (>16 years at time of accident) or the Filser model (<16 years at time of accident) (Kreuzer et al.,
16 1997, [198088](#)). Women were classified as premenopausal if they were still menstruating or if
17 they had amenorrhea as a result of pregnancy or lactation (at the time of interview) with an
18 indication of subsequent menstruation based on maintained diaries or further examination.
19 Subjects for which amenorrhea had persisted for at least 1 year with no apparent medical
20 explanation were classified into a natural menopause category. The category, surgical
21 menopause, pertained to women with a medically confirmed hysterectomy or an oophorectomy.
22 Finally, impending menopause was defined for subjects in which menstruation had been absent
23 for 2 months, but who provided evidence of subsequent menstruation, or had a secretory
24 endometrial lining, or indicated less predictable cycles in the previous 2–5 years. If participants’
25 menopausal status could not be determined, they were grouped into the “other” category. This
26 category included those for whom status could not be determined due to current use of oral
27 contraceptives, hormone replacement therapy, or previous cancer chemotherapy.

28 Statistical analysis was based on both a continuous measure of log-transformed TCDD
29 exposures and categories based on quintiles (<20.4 ppt; 20.4–34.2 ppt; 34.3–54.1 ppt;
30 54.2–118.0 ppt; >118.0 ppt). The Cox model was used to generate hazard ratios as estimates of
31 relative risks and their 95% confidence intervals examining natural menopause as the outcome.

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1 Several covariates previously identified as associated with menopausal status in the literature
2 were considered as potential confounders. These covariates included body mass index, physical
3 activity, premenopausal smoking, education, marital status, history of heart disease and other
4 medical conditions, and other reproductive characteristics.

5 The RRs were found to increase across the second through fourth quintiles (RRs = 1.1,
6 1.4, and 1.6, respectively) of serum TCDD categories in relation to those in the lowest category,
7 but not in the upper quintile (RR = 1.0, 95% CI = 0.6–1.8). A statistically significant test of
8 trend was detected across the first four quartiles ($p = 0.04$) but not across all five quintiles
9 ($p = 0.44$). A statistically significant association with onset of menopause was not detected
10 (RR = 1.02, 95% CI = 0.8–1.3) based on the logTCDD continuous measure.

11 12 **2.4.1.2.1.4.5.2.** *Study evaluation.*

13 The categorical exposure results from this study support a nonmonotonic
14 dose-related-association for earlier menopause with increased serum TCDD levels up to
15 approximately 100-ppt TCDD serum, but not above. Eskenazi et al. (2005, [197166](#)) speculated
16 that the inverse “U” shape of the dose-response relationship is explained by the mimicking of
17 hormones at lower doses of a chemical, while at higher levels the toxic effect of a chemical does
18 not have the capacity to either inhibit or stimulate hormonal effects.

19 A study limitation is the potential for residual confounding due to adjustment based on
20 current smoking status and not at the time of onset of menopause. It is unclear to what extent
21 smoking status may differ between these two time periods and whether smoking is related to
22 TCDD exposures in this cohort. Exposures to other dioxin-like compounds were not considered
23 in this study because of small serum volumes, but any potential TEQ exposures occurring in the
24 exposed population were thought to be mostly attributable to TCDD in the exposed women.

25 26 **2.4.1.2.1.4.5.3.** *Suitability of data for TCDD dose-response modeling.*

27 To date, this study is the only one that has examined the relationship between TCDD
28 levels and onset of menopause. Although the findings suggest the possibility of a nonlinear
29 dose-response function, the \log_{10} TCDD exposure metric was not statistically significant, nor
30 were any category-specific hazard ratios statistically significant relative to the lowest category.
31 Therefore, a quantitative dose-response analysis was not undertaken.

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1 **2.4.1.2.1.4.6.** Warner et al. (2007, [197486](#))—Ovarian function.

2 **2.4.1.2.1.4.6.1.** *Study summary.*

3 Warner et al. (2007, [197486](#)) investigated the association between serum TCDD levels
4 and ovarian function in subjects in the SWHS who were younger than 40 in 1976 and for whom
5 sera collected after the accident had been stored. These women were recruited from March 1996
6 until July 1998. Ovarian function analysis was limited to 363 women between 20 and 40 years
7 of age and who were not using oral contraceptives. Of these, 310 underwent transvaginal
8 ultrasound and were included in the functional ovarian cyst analysis. Ninety-six women were in
9 the preovulatory stage of their menstrual cycles and were included in the follicle analysis. For
10 the hormone analysis, 126 women who were in the last 2 weeks of their cycle were included.

11 The authors used logistic regression to examine the relationship between TCDD and the
12 prevalence of ovarian follicles greater than 10 mm. Linear regression models examined the
13 continuous outcome variables: number of ovarian follicles >10 mm and diameter of dominant
14 ovarian follicle. Covariates considered for inclusion in the model were age at ultrasound, age at
15 accident, age at menarche, marital status, parity, gravidity, lactation history, current body mass
16 index, age at last birth, and smoking history. For the serum hormone analyses, estradiol and
17 progesterone were measured in blood at the time of interview. Ovulation status was defined as a
18 dichotomous variable (yes/no) based on a serum progesterone cut-point value of 3 ng/mL.

19 The adjusted ORs across categories of TCDD exhibited no dose-response trend for the
20 presence of follicles in relation to TCDD in the follicular phase; also, no statistically significant
21 differences were noted in any of the upper exposure categories relative to those in the lowest.
22 The adjusted OR for the continuous measure of \log_{10} TCDD was 0.99 (95% CI = 0.4–2.2). A
23 similar nonstatistically significant finding was found for \log_{10} TCDD in relation to ovulation in
24 both the luteal (OR = 0.99, 95% CI = 0.5–1.9) and mid-luteal phases (OR = 1.03,
25 95% CI = 0.4–2.7). Analyses of progesterone and estradiol also were not related to serum
26 TCDD levels for either the luteal or mid-luteal phases ($p = 0.51$ and $p = 0.47$).

27

28 **2.4.1.2.1.4.6.2.** *Study evaluation.*

29 The investigators found no relationship between serum TCDD levels and serum
30 progesterone and estradiol levels among women who were in the luteal phase at the time of
31 blood draw. No association with number of ovarian follicles detected from ultrasound.

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1 Although no association was found, the authors suggested that the lack of significant results
2 could be because the women in SWHS were all exposed postnatally and the relevant and critical
3 time period for an effect might be in utero (animal studies support relevance of in utero
4 exposures).

5
6 **2.4.1.2.1.4.6.3.** *Suitability of data for TCDD dose-response modeling.*

7 One limitation of the study was the lack of examination of confounding by dioxin-like
8 compounds. The absence of associations between TCDD and adverse health effects in this study
9 precludes conducting quantitative dose-response analyses.

10
11 **2.4.1.2.1.4.7.** Eskenazi et al. (2007, [197170](#))—Uterine leiomyoma.

12 **2.4.1.2.1.4.7.1.** *Study summary.*

13 Associations between TCDD exposures and uterine leiomyoma (i.e., fibroids) were also
14 examined among 956 women in the SWHS (Eskenazi et al., 2007, [197170](#)). The sample
15 population was based on the on the original 981 SWHS participants excluding 25 women
16 diagnosed with fibroids before the date of the accident (July 10, 1976). Women who previously
17 had fibroids were identified both through the administered questionnaire and the review of
18 medical records. Transvaginal ultrasounds were performed for 634 women to determine if they
19 had fibroids at the time of follow-up. Similar to other SWHS studies, exposure to TCDD was
20 estimated using serum collected from women shortly after the time of the accident, between
21 1978 and 1981 and in 1996. TCDD levels were back-extrapolated to 1976 levels.

22 The study authors performed statistical analyses using two definitions of fibroids as
23 outcome measures. The first was fibroids detected before the study, and the second was fibroids
24 detected via ultrasound. A proportional odds method Dunson and Baird (2001, [197248](#))
25 developed was used to model the cumulative odds of onset of fibroids. This method combines
26 historical and current information of diagnoses of fibroids. Continuous and categorical measures
27 of TCDD were modeled. Regression models were adjusted for known or suspected risk factors
28 of fibroids including parity, family history of fibroids, age at menarche, body mass index,
29 smoking, alcohol use, and education.

1 **2.4.1.2.1.4.7.2. Study evaluation.**

2 Categorical measures of TCDD suggested an inverse dose-response relationship with the
3 onset of fibroids. Relative to those with TCDD levels less than 20 ppt, those having TCDD
4 exposures between 20.1 and 75.0 ppt and greater than 75.0 ppt had RRs of 0.58
5 (95% CI = 0.41–0.81), and 0.62 (95% CI = 0.44–0.89), respectively. The continuous measure of
6 \log_{10} TCDD produced a hazard ratio of 0.83 (95% CI = 0.65–1.07).

7
8 **2.4.1.2.1.4.7.3. Suitability of data for TCDD dose-response modeling.**

9 The inverse association between TCDD and uterine fibroids supports the possibility of an
10 anti-estrogenic effect of TCDD. The observed direction of the reported associations precludes
11 quantitative dose-response modeling.

12
13 **2.4.1.2.1.5. Other Seveso noncancer studies.**

14 **2.4.1.2.1.5.1.** Bertazzi et al. (1989, [197013](#)); Consonni et al. (2008, [524825](#))—Mortality
15 outcomes.

16 **2.4.1.2.1.5.1.1. Study summary.**

17 Several studies have evaluated the mortality of Seveso residents exposed to TCDD
18 following the 1976 accident. The earlier section of this report described the designs of these
19 studies and discussed their findings as they relate to cancer mortality. In this section, some of
20 the findings for other causes of death are described. A key feature of these studies is that
21 patterns of mortality among Seveso residents were investigated according to their zone of
22 residence at the time of explosion relative to general population rates.

23 A 10-year mortality follow-up of residents of Seveso was published in 1989 (Bertazzi
24 et al., 1989, [197013](#)). Poisson regression was used to derive RRs for those who had lived in
25 Zone A at the time of explosion using a referent group consisting of inhabitants who had lived in
26 the uncontaminated study area. Between 1976 and 1986, no statistically significant difference
27 was observed in all-cause mortality relative to the general population among those who lived in
28 the most highly exposed area (Zone A) at the time of the accident. This finding was evident in
29 both males (RR = 0.86, 95% CI = 0.5–1.4) and females (RR = 1.14, 95% CI = 0.6–2.1). A
30 statistically significant excess in circulatory disease mortality was found among males relative to
31 those in the referent population (RR = 1.75, 95% CI = 1.0–3.2); this increased risk was more

1 pronounced when the follow-up period was restricted to the first 5 years after the accident
2 (1976–1981) (RR = 2.04, 95% CI = 1.04–4.2). Between 1982 and 1986, the RR decreased
3 substantially and was not statistically significant (RR = 1.19, 95% CI = 0.4–3.5). Among
4 females, a risk similar in magnitude was detected for circulatory disease mortality although it
5 was not statistically significant (RR = 1.89, 95% CI = 0.8–4.2). Contrary to the calendar
6 period-specific findings for males, the excess of circulatory mortality among females occurred
7 between 1982 and 1986 (RR = 2.91, 95% CI = 1.1–7.8) and not between 1976 and 1981
8 (RR = 1.12, 95% CI = 0.3–4.5). The number of deaths in this cohort with the 10 years of
9 follow-up was relatively small; in Zone A, 16 deaths were observed among males and 11 among
10 females.

11 The most recently published account of the mortality experience of Seveso residents
12 provides further information on follow-up of these residents until the end of 2001 (25 years after
13 the accident) (Consonni et al., 2008, [524825](#)). Three exposure groups were considered: Zone A
14 (very high contamination), Zone B (high contamination), and Zone R (low contamination). The
15 reference population consisted of those residents who lived in unaffected surrounding areas, as
16 well as residents of five nearby towns. The authors used Poisson regression to compare
17 mortality rates for each zone relative to the reference population.

18 For all causes of death, no excess was found in Zone A, B, or R relative to the reference
19 population. Statistically significant excesses were noted for those who lived in Zone A relative
20 to the reference population for chronic rheumatic heart disease (RR = 5.74,
21 95% CI = 1.83–17.99) and chronic obstructive pulmonary disease (RR = 2.53,
22 95% CI = 1.20–5.32). These risks, however, were based on only 3 and 7 deaths, respectively.
23 For those in Zone A, no statistically significant excesses in mortality were noted for diabetes,
24 accidents, digestive diseases, ischemic heart disease, or stroke. Among Zone A residents,
25 stratified analysis by time since accident showed increased rates of circulatory disease 5–9 years
26 since the accident (RR = 1.84, 95% CI = 1.09–3.12). Increased mortality from diabetes relative
27 to the reference population was noted among females who lived in Zone B (RR = 1.78,
28 95% CI = 1.14–2.77).

29

1 **2.4.1.2.1.5.1.2.** *Study evaluation.*

2 The ascertainment of mortality in this cohort is nearly complete. Misclassification of
3 some health outcomes, such as diabetes, may occur due to use of death certificate data.

4 The characterization of exposure is based on zone of residence. Soil sampling indicated
5 considerable variability in TCDD soil levels, and therefore, the generation of risks based on zone
6 of residence likely does not accurately reflect individual exposure. Exposure misclassification
7 might also occur because residency in the areas does not necessarily reflect whether the
8 individual would have been present in the area at the time the accident occurred. Any exposure
9 misclassification would likely be nondifferential which would tend to bias the risk estimates
10 towards the null.

11 Although some excess of circulatory disease mortality was found, the finding was not
12 consistent between men and women. Moreover, excess circulatory disease mortality was more
13 pronounced among men within the first 5 years of exposure, while, for women, the excess was
14 more pronounced in years 5–10. Numerous other risk factors for circulatory disease were not
15 controlled for in these analyses and may be confounders if related to TCDD exposure. Taken
16 together, the possibility that TCDD increased circulatory disease mortality based on these data is
17 tenuous at best.

18
19 **2.4.1.2.1.5.1.3.** *Suitability of data for TCDD dose-response modeling.*

20 There is considerable uncertainty in these data due to the potential for outcome and
21 exposure misclassification. The lack of the individual-level TCDD levels and the examination of
22 fatal outcomes reported in this study are not a suitable basis for development of an RfD. For
23 these reasons, dose-response analysis for this outcome is not conducted.

24
25 **2.4.1.2.1.5.2.** Mocarelli et al. (1996, [197637](#); 2000, [197448](#))—Sex ratio.

26 **2.4.1.2.1.5.2.1.** *Study summary.*

27 A letter to the editor was the first report of a possible change in the sex ratio from dioxin
28 among Seveso residents following the July 10, 1976 accident (Mocarelli et al., 1996, [197637](#)).
29 The authors reported that 65% ($n = 48$) of the 74 total births that had occurred from April 1977
30 to December 1984 were females. This male to female ratio of 26:48 (35%) is significantly
31 different from the worldwide birth ratio of 106 males to 100 females (51%) (James, 1995,

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1 [197722](#)). Between 1985 and 1994, the Seveso male to female ratio leveled out at 60:64 (48%).
2 The authors suggested that the finding supported the hypothesis that dioxin might alter the sex
3 ratio through several possible mechanistic pathways.

4 Mocarelli et al. (2000, [197448](#)) later reported on an investigation between serum-based
5 TCDD measures in parents and the sex ratio of offspring. In this study, serum samples were
6 collected from mothers and fathers who lived in the areas at the time of the explosion, were
7 between the ages of 3 and 45 at the time of the explosion, and produced offspring between
8 April 1, 1977 and December 31, 1996. The study population included 452 families and
9 674 offspring, and serum measures were available for 296 mothers and 239 fathers. An estimate
10 of TCDD at the time of conception was also examined in relation to male to female birth ratios.
11 TCDD exposure estimates between the years of 1976 and 1996 were estimated using Filser's
12 model (Kreuzer et al., 1997, [198088](#)).

13 Mocarelli et al. (2000, [197448](#)) used chi-square test statistics to compare observed sex
14 ratio to an expected value of 0.51 in this Seveso population. Concentrations of TCDD were
15 modeled as categorical variables in several ways. First, a dichotomous variable was used
16 whereby unexposed parents were defined as those who lived outside Zones A, B, and R or had a
17 serum TCDD concentration of less than 15 ppt; parents with exposures of 15 ppt or higher were
18 considered exposed. Second, a trichotomous exposure variable was created that consisted of
19 parents who (1) lived outside Zones A, B, and R or had serum concentrations of less than 15 ppt,
20 (2) had serum concentrations of 15–80 ppt, and (3) had serum concentrations that exceeded
21 80 ppt. These cut-points were chosen as they represented tertiles based on the distribution of
22 TCDD among parents. Analyses were conducted separately for paternal and maternal TCDD
23 levels.

24 The overall proportion of 0.49 male births (based on male to female ratio of 328:346) was
25 not significantly different from the expected proportion of 0.51 ($p > 0.05$). Statistically
26 significant differences were found, however, if both parents had TCDD levels >15 ppt (sex
27 ratio = 0.44) or just the father had serum TCDD levels >15 ppt (sex ratio = 0.44). No
28 statistically significant differences were found when the fathers had TCDD levels less than
29 15 ppt, irrespective of the maternal levels. A dose-response pattern in the sex ratio was found
30 across the paternal exposure categories. That is, the sex ratio decreased with increased paternal
31 TCDD levels (linear test for trend, $p = 0.008$). In the unexposed group, the sex ratio (male to

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1 female) was 0.56 (95% CI = 0.49–0.61), while in the highest exposure group
2 (281.0–26,400.0 ppt) the corresponding sex ratio was 0.38 (95% CI = 0.28–0.49).

3 Stratified analyses by age at paternal exposure revealed that the sex ratio was altered to a
4 greater degree among fathers who were younger than 19 at the time of the explosion. The male
5 to female ratio among the unexposed fathers was 0.56 (95% CI = 0.50–0.62), while it was 0.38
6 (95% CI = 0.30–0.47) for those younger than 19 when exposed and 0.47 (95% CI = 0.41–0.53)
7 for those exposed after 19. Regardless of the age at the time of exposure, however, fathers who
8 were exposed had a statistically significantly different birth ratio (they were more likely to father
9 girls) than those who were unexposed ($p < 0.05$).

10 Separate analysis of birth ratios based on paternal TCDD exposure estimated at the time
11 of conception did not show the same dose-response pattern but did show strong evidence of
12 consistently decreased male births relative to females. More specifically, the male to female
13 birth ratios among the four successive quartiles (first through fourth) were 0.41, 0.33, 0.33,
14 and 0.46.

15 16 **2.4.1.2.1.5.2.2. Study evaluation.**

17 Mocarelli et al. (2000, [197448](#)) based the characterization of TCDD exposure on serum
18 samples, which is an objective method for characterizing dose. Unlike for the occupational
19 cohorts, serum measures for this study were taken close to the time of the accident, and
20 therefore, back-extrapolation of TCDD exposures is unnecessary. Exposure received before the
21 age of 19 at the time of the explosion were more strongly associated with a reduced male to
22 female ratio than those received after the age of 19. The cut off age of 19 seems to be somewhat
23 arbitrary, resulting in a highly uncertain critical exposure window. TCDD levels at the time of
24 conception did not demonstrate a dose-response relationship, but paternal exposures resulted in
25 consistently reduced male to female birth ratios (range: 0.33–0.46).

26 The study findings are unlikely to be influenced by age at conception as these values
27 were found, on average, to be similar across calendar years. This suggests that age at conception
28 was not an important confounder and that the birth ratio findings may be related to paternal
29 exposures.

30 The methods used to identify births appear to be appropriate. Even if some
31 under-ascertainment of births occurred, there is no reason to believe that ascertainment would be

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1 related to TCDD exposure and the sex of the baby. Therefore, no bias is suspected due to
2 incomplete birth ascertainment.

3
4 **2.4.1.2.1.5.2.3.** *Suitability of data for TCDD dose-response modeling.*

5 TCDD exposures were well-characterized, and internal cohort analyses demonstrate
6 association between paternal TCDD levels at the time of the accident and birth ratio. However,
7 the change in sex ratio was only statistically significant when exposure occurred before 19 years
8 of age. It is impossible to identify the relevant time interval over which TCDD dose should be
9 considered for dose-response analysis; specifically, it is difficult to discern whether the different
10 sex ratio is a consequence of the initial peak exposure before 19 years of age or a function of the
11 average cumulative exposure over this entire exposure window. Assuming the initial high
12 exposure is the correct exposure window, using the initial exposures in a dose-response model
13 would yield LOAELs that are too high to be relevant to factor into the RfD calculation. The
14 differences between the two dose estimates are quite large. Dose-response analysis for this
15 outcome, therefore, was not conducted.

16
17 **2.4.1.2.1.5.3.** Baccarelli et al. (2002, [197062](#); 2004, [197045](#))—Immunologic effects.

18 **2.4.1.2.1.5.3.1.** *Study summary.*

19 The relationship between TCDD and immunological effects was evaluated in a sample of
20 Seveso residents (Baccarelli et al., 2002, [197062](#); Baccarelli et al., 2004, [197045](#)). Both studies
21 were based on findings from 62 individuals who were randomly selected from Zones A and B.
22 An additional 59 subjects were chosen from the surrounding noncontaminated areas. Residency
23 was based on where subjects lived at the time of the accident (July 10, 1976) (Landi, 1998,
24 [594409](#)). Frequency matching ensured that the two groups of subjects were similar with respect
25 to age, sex, and cigarette smoking status.

26 TCDD levels were determined by mass spectrometric analysis of plasma samples.
27 TCDD levels at the time of sampling were obtained, and estimates of levels at the time of the
28 accident also were estimated by assuming an 8.2-year half-life (Landi, 1998, [594409](#)). The
29 plasma was also used to characterize levels of the immunoglobulins (Ig) IgG and IgM and the
30 complement components C3 and C4. One subject was excluded due to lack of an immunological

1 evaluation. Analyses are, therefore, based on 58 subjects in the noncontaminated areas and
2 62 individuals from the contaminated areas.

3 Nonparametric tests were applied to test for differences between the two groups.
4 Multiple regression also was used to describe the relationship between the variables. Adjustment
5 was made for several potentially confounding variables that were collected via a questionnaire.

6 An inverse association was noted with increasing TCDD levels and plasma IgG levels;
7 this result remained statistically significant after adjusting for other potential confounding
8 variables in the regression models. Specifically, the slope coefficient and p -value for the
9 unadjusted model were -0.35 ($p = 0.0002$) and for the adjusted model the p -value was 0.0004.

10 The authors did not present the slope coefficient for the adjusted model in either paper but noted
11 minimal differences between the adjusted and unadjusted results. In the 2004 analysis, the
12 authors present IgG, IgM, IgA, C3, and C4 median and interquartile values across TCDD
13 exposure quintiles. Decreased levels of IgG were observed in the highest exposure groups.
14 Specifically, the median values across the five quintiles (for lowest to highest) were 1,526;
15 1,422; 1,363; 1,302; and 1,163. The Kruskal-Wallis test for differences across the TCDD
16 categories was statistically significant ($p = 0.002$), which is consistent with the findings for the
17 continuous measures of TCDD. This finding persisted after excluding those subjects with
18 inflammatory diseases and those who used antibiotics or nonsteroidal anti-inflammatory drugs.
19 For the other plasma measures, no dose-response relationship was apparent based on median
20 values for IgM, IgA, C3, or C4 across TCDD quintiles. The authors highlight the need for
21 additional research, particularly given the excess of lymphatic tumors noted in the area.

22 Exposure to other dioxin-like compounds for both the TCDD and nonexposed areas were
23 reported to be at background levels.

24 25 **2.4.1.2.1.5.3.2. Study evaluation.**

26 Both TCDD exposure and health outcome measures are well characterized. TCDD
27 exposures, in particular, are based on current serum measures and, therefore, are not dependent
28 on assumptions needed to back-extrapolate to earlier time periods of exposure.

29 A dose-response relationship between TCDD and IgG is well documented for the
30 unadjusted model, but no details are provided on the change in the slope coefficient when other
31 covariates were added to the model.

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1 Interpreting the inverse association between TCDD exposure and IgG in terms of clinical
2 significance is not possible. The IgG values reported are much higher than those subjects with
3 antibody immunodeficiency disorders.

4
5 **2.4.1.2.1.5.3.3.** *Suitability of data for TCDD dose-response modeling.*

6 Although the data support an inverse dose-response association between IgG and TCDD,
7 because the relationship cannot be described in terms of clinical relevance with respect to a
8 specific adverse health outcome, these data were not suitable for quantitative dose-response
9 modeling.

10
11 **2.4.1.2.1.5.4.** Landi et al. (2003, [198362](#))—Gene expression.

12 **2.4.1.2.1.5.4.1.** *Study summary*

13 The impact of TCDD on the aryl hydrocarbon receptor (AhR) was evaluated by Landi
14 et al. (2003, [198362](#)) in a population-based study of Seveso residents. AhR, a mechanistically
15 based biomarker of dioxin response, must be present for manifestation of most of the toxic
16 effects of TCDD, including tumor promotion and immunological and reproductive system effects
17 (Safe, 1986; Puga et al., 2000). AhR activates the transcription of several metabolizing enzymes
18 in addition to certain genes (Whitlock, 1999). The primary objective of the study was to
19 determine whether plasma levels of TCDD and TEQ are associated with the AhR-dependent
20 pathway in lymphocytes among Seveso residents. The genes involved in the pathway that were
21 examined included: AhR, aryl hydrocarbon receptor nuclear translocator, CYP1A1 and
22 CYP1B1 transcripts, and CYP1A1-associated 7-ethoxyresorufin O-deethylase (EROD).

23 Study recruitment occurred from December 1992 to March 1994. A total of 62 subjects
24 were randomly chosen from the highest exposed zones in Seveso (Zones A and B), while 59
25 were chosen from the noncontaminated area (non-ABR). Those chosen from the
26 noncontaminated zone were matched by age, sex, and smoking. Assignment of zones was based
27 on place of residence where subjects lived at the time of the accident in 1976. Subjects provided
28 data via questionnaire on a variety of sociodemographic and behavioral risk factors, including
29 cigarette smoking. Multivariate models were adjusted for a variety of confounders including;
30 adjustment for age, gender, date of assay, actin expression, postculture viability, experimental
31 group, and cell growth.

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1 TCDD levels were determined using high-resolution gas chromatography, and 21 other
2 dioxins, or dioxin-like compounds, were measured to examine TEQ. Eleven measurements
3 taken on the 121 subjects were deemed inadequate and excluded, but no further information was
4 provided on these exclusions. Nine subjects from Zone B and fourteen subjects from Zone ABR
5 had TCDD levels below that of detection, and were assigned a value equal to the lipid-adjusted
6 detection limit divided by the square root of 2. The toxic equivalent for the mixture of
7 dioxin-like compounds (i.e., TEQ) was calculated by summing the products of the concentration
8 of each congener by its specific toxic equivalency factor.

9 The subjects provided between 5 and 50 mL of whole blood, which was centrifuged to
10 separate mononuclear cells. The cells were frozen and later thawed. Cells were cultured,
11 removed from the culture medium, and resuspended in a stimulation medium, 14 mL of which
12 was used for RNA analysis. Reverse transcription-PCR was conducted and EROD was assayed.
13 Differences in gene expression and EROD activity observed for various cell culture conditions
14 were compared using paired t-tests. The unpaired Student's t-test was applied to test for
15 differences between groups, while a Bonferroni factor was used to account for multiple
16 comparisons. Data for continuous variables were log-transformed.

17 TCDD accounted for 26% of the TEQ among the study subjects, but varied by zone (35%
18 in zone A and 18% in zone non-ABR). After adjusting for potential confounding, AhR was
19 inversely related to plasma TCDD levels in uncultured cells ($p < 0.03$) and in mitogen-stimulated
20 cells ($p < 0.05$). EROD was lower in cells cultured from subjects with higher plasma TCDD and
21 TEQ levels, and the corresponding continuous measure of EROD was statistically significant
22 ($p < 0.05$). No statistically significant associations with TCDD or TEQ were found with ARNT
23 or CYP1B1 in uncultured cell medium, nor with CYP1A1 or CYP1B1 in mitogen-stimulated
24 cells. In general, females had lower AhR transcripts and higher levels of dioxin.

25 Collectively, the findings suggest that TCDD exposure might reduce AhR expression in
26 unstimulated cells. Therefore, TCDD could exert an influence on the AhR pathway regulation.

27 28 **2.4.1.2.1.5.4.2. Study evaluation.**

29 The study used biologically based measures of both TCDD exposures and biomarkers or
30 AhR. Subject recruitment was based on randomly sampling of the cohort study population;
31 some individuals with severe medical illnesses were excluded (Landi, 1998, [594409](#)). Although

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1 few details are provided on the number of subjects excluded for these reasons, given the
2 objective nature of the biomarker outcomes that were evaluated, such exclusions are unlikely to
3 be an important source of bias. The exclusion rates were also reported to be low and comparable
4 across the zones (five subjects from the noncontaminated zone non-ABR and four subjects from
5 zone B).

6 A strength of the study was the examination of other dioxin-like compounds via the TEQ
7 analysis. A limitation of the study included the relatively small number of subjects which
8 resulted in the grouping of several covariates, including TCDD exposures, into a small number
9 of categories. As such, slope coefficients derived from modeling continuous measures were
10 emphasized in the data presentation. Another key limitation of the study is the uncertainty of
11 how effects on AhR translate into subsequent development of cancer and other chronic health
12 effects.

13

14 **2.4.1.2.1.5.4.3.** *Suitability of data for TCDD dose-response modeling.*

15 It is unclear how associations between AhR biomarkers and TCDD levels translate into
16 an increased risk of cancer. Dose-response analysis for this outcome, therefore, was not
17 conducted.

18

19 **2.4.1.2.1.5.5.** Alaluusua et al. (2004, [197142](#))—Developmental dental effects.

20 **2.4.1.2.1.5.5.1.** *Study summary.*

21 Alaluusua et al. (2004, [197142](#)) examined the relationship between TCDD and dental
22 defects, dental caries, and periodontal disease among Seveso residents who were children at the
23 time of the accident. Subjects were randomly selected from those individuals who had
24 previously provided serum samples in 1976, which was shortly after the accident. A total of
25 65 subjects who were less than 9.5 years of age at the time of the accident, and who lived in
26 Zones A, B, or R were invited to participate. Recruitment was initiated 25 years after the time of
27 the Seveso accident. An additional 130 subjects from the surrounding area (outside Zones A, B,
28 or R or “non-ABR zone”) having the same age restriction were recruited. Subjects were
29 frequency matched for age, sex, and education. Questionnaires were administered to these
30 individuals to collect detailed information on dental and medical histories, education, and
31 smoking behaviors. Ten subjects who had completed at least high school were randomly

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1 excluded from the non-ABR zone to create groups with similar educational profiles.
2 Participation rates for the ABR and non-ABR zones were 74 and 58%, respectively.

3 One dentist who was blind to the patients' TCDD exposure levels assessed dental
4 aberrations. Dental caries was assessed using recommendations of the World Health
5 Organization. Periodontal status was described following a detailed evaluation of the surfaces of
6 the teeth. A radiographic examination was done to identify missing teeth, alveolar bone loss,
7 deformities in the roots, and jaw cysts.

8 Comparisons of the presence of dental enamel defects according to exposure status were
9 performed using logistic regression. Chi-square test statistics were applied to compare the
10 distributions in the prevalence of dental defects across several categorical covariates (i.e.,
11 education, age, and serum TCDD level). For those who were younger than 5 at the time of the
12 accident, dental defects were more prevalent among patients in zone ABR (42%) than those in
13 the non-ABR zone (26%) ($p = 0.14$). Zone ABR is characterized by higher levels of soil TCDD
14 levels relative to non-ABR. Serum levels permitted an improved characterization of risk as they
15 were available at an individual level, rather than using a zone of residence. Defect prevalence
16 was highest among those in the upper serum TCDD category (700–26,000 ng/kg) with 60% of
17 subjects having dental defects. The continuous measure of serum TCDD was associated with
18 developmental dental defects ($p = 0.007$) and hypodontia ($p = 0.05$).

19
20 **2.4.1.2.1.5.5.2. Study evaluation.**

21 Although the subjects with serum measures were selected randomly, no direct measures
22 of TCDD were made in subjects from the unexposed area (i.e., non-ABR zones). That those who
23 resided in the non-ABR areas had lower TCDD exposures would be a reasonable assumption.
24 Alaluusua et al. (2004, [197142](#)), however, provide few details about the sampling frame used to
25 identify these participants. Despite this fact, it is important to note that a dose-response pattern
26 was observed between TCDD exposure and presence of developmental defects in the ABR
27 population alone ($p = 0.016$). This finding is based on 27 subjects with developmental dental
28 defects. This positive association provides support for a quantitative dose-response modeling of
29 dental aberrations. The numbers of such subjects are small, however, with one, five, and
30 nine subjects having defects in the exposure groups of 31–226, 238–592, and
31 700–26,000 ng/kg TCDD, respectively.

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1 TCDD exposures were characterized using serum measures for those who resided in
2 zone ABR in 1976 (near the time of the accident). The authors could not account for additional
3 exposure to TCDD across subjects that might have occurred since the time of the accident, so
4 there is considerable uncertainty in delineating the critical exposure window for the reported
5 effects. In addition, the lack of exposure data for those in the non-ABR zone, however, makes
6 interpretation of the findings difficult. This difficulty is particularly evident, given that the
7 prevalence of dental defects was less among those in the low exposure category of zone ABR
8 (31–226 ng/kg TCDD) (10%) when compared to those in the non-ABR zone (26%).

9
10 **2.4.1.2.1.5.5.3.** *Suitability of data for TCDD dose-response modeling.*

11 Most of the considerations for conducting a dose-response analysis have been satisfied
12 with the study population, although, exposure assessment uncertainties are a limitation of this
13 study. For example, it is difficult to discern whether these health effects are a consequence of
14 the initial high exposure during childhood or a function of the cumulative exposure for this entire
15 exposure window beginning at the early age. If the latter is true, averaging exposure over the
16 critical window would add considerable uncertainty to effective dose estimates given the large
17 difference between initial TCDD body burden and body burden at the end of the critical
18 exposure window. Despite the uncertainty in defining the critical window of exposure,
19 dose-response analysis was conducted for this outcome.

20
21 **2.4.1.2.1.5.6.** Baccarelli et al. (2005, [197053](#))—Chloracne.

22 **2.4.1.2.1.5.6.1.** *Study summary.*

23 Baccarelli et al. (2005, [197053](#)) published findings from a case-control study of
24 110 chloracne cases and 211 controls. The authors collected information on pigment
25 characteristics and an extensive list of diseases. This study was performed to yield information
26 about the health status of chloracne cases, TCDD-chloracne exposure response, and factors that
27 could modify TCDD toxicity. TCDD was measured from plasma. Following adjustment for
28 confounding, TCDD was associated with chloracne (OR = 3.7, 95% CI = 1.5–8.8), and the risk
29 of chloracne was considerably higher in subjects younger than 8 at the time of the accidents
30 (OR = 7.4, 95% CI = 1.8–30.3). Among individuals with lighter hair, the association between
31 TCDD and chloracne was stronger than among those with darker hair.

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1 **2.4.1.2.1.5.6.2.** *Study evaluation.*

2 Although a dose-response association was observed, chloracne is a rare health outcome
3 likely only to occur among those highly exposed.

4
5 **2.4.1.2.1.5.6.3.** *Suitability of data for TCDD dose-response modeling.*

6 Given the very high TCDD levels needed to cause chloracne (e.g., Ott et al., 1993,
7 [594322](#)), quantitative dose-response modeling to characterize risks for the general population
8 with much lower TCDD exposures would be of little value. Therefore, quantitative
9 dose-response assessment for the Baccarelli et al. (2005, [197053](#)) study was not conducted.

10
11 **2.4.1.2.1.5.7.** Baccarelli et al. (2008, [197059](#))—Neonatal thyroid hormone levels.

12 **2.4.1.2.1.5.7.1.** *Study summary.*

13 Baccarelli et al. (2008, [197059](#)) investigated the relationship between thyroid function
14 and TCDD among offspring of women of reproductive age who were exposed in the
15 1976 accident. This health endpoint is relevant because thyroid function is important for energy
16 metabolism and nutrients and for stimulating growth and development of tissues. Neonatal
17 thyroid function at birth is evaluated through blood thyroid-stimulating hormone (b-TSH).

18 The study population was drawn from 1,772 women who were identified as having lived
19 in the highly contaminated areas (Zones A or B) at the time of the accident or between
20 July 10, 1976 and December 31, 1947; were of fertile age (born after 1947); and were alive as of
21 January 1, 1994. A random sample of 1,772 unexposed women who lived in the reference area
22 was selected using frequency matching by year of birth to the exposed women, and residency in
23 the reference area at the time of the accident. The reference area represents the noncontaminated
24 areas that surround the three zones of decreasing exposure (Zones A, B and R). In total,
25 55,576 women had lived in the reference area. Population registry offices ($n = 472$) were
26 contacted to detect children born to these women. Records could be traced for virtually all
27 subjects (1761/1772 exposed; 1762/1772 unexposed). Children born outside the Lombardy area
28 were excluded as b-TSH could not be obtained for them. This accounted for 156 of the
29 1,170 children identified. The analyses were based on the remaining 56, 425, and 533 singletons
30 born between January 1, 1994, and June 30, 2005 in Zone A, B, and from the reference area,
31 respectively.

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1 Thyroid function is tested in all newborns by b-TSH measures in the region of Lombardy
2 where Seveso is located. These measures are obtained from blood samples taken 72 hours after
3 birth using a standardized protocol. The b-TSH levels were log transformed to approximate a
4 normal distribution. Linear regression analysis was used to conduct test for trends in mean
5 b-TSH levels across different covariates. Logistic regression was used to assess associations
6 between elevated b-TSH levels defined by the cutpoint of 5 $\mu\text{U}/\text{mL}$ and residence in particular
7 zones of contamination. The 5 $\mu\text{U}/\text{mL}$ cutpoint for TSH measurements in neonates was
8 recommended by WHO (1994) for use in neonatal population surveillance programs. Although
9 WHO established the standard for increased neonatal TSH in the context of iodine deficiency
10 disease, the toxicological implications are the same for TCDD exposure and include increased
11 metabolism and clearance of T4. Generalized estimating equations were used to adjust the
12 standard errors of the ORs for correlation between siblings.

13 The mean levels of b-TSH were positively associated with average soil TCDD
14 concentrations in the three areas (Zone A: 1.66 $\mu\text{U}/\text{mL}$; Zone B: 1.35 $\mu\text{U}/\text{mL}$; and Zone R:
15 0.98 $\mu\text{U}/\text{mL}$) ($p < 0.001$). Plasma TCDD levels also were shown to be much higher in a group of
16 51 newborns that had b-TSH levels $>5 \mu\text{U}/\text{mL}$. Compared to the reference population, adjusted
17 ORs were elevated for Zone B (OR = 1.90, 95% CI = 0.94–3.86) and Zone A (OR = 6.63,
18 95% CI = 2.36–18.6). These ORs were adjusted for gender, birth weight, birth order, maternal
19 age at delivery, hospital, and type of delivery. The adjusted ORs however differed only slightly
20 from those that were unadjusted (Zone B, OR = 1.79, 95% CI = 0.92–3.50; Zone A OR = 6.60,
21 95% CI = 2.45–17.8). Of the risk factors considered, both gender and birth weights were
22 associated with neonatal b-TSH.

23 The paper also included an analysis of children born to 109 women who were part of the
24 Seveso Chloracne Study (Baccarelli et al., 2005, [197053](#)). A total of 51 children were born to
25 38 of these women, of these 12 lived in Zone A, 10 in Zone B, 20 in Zone R, and 9 from the
26 reference population. Several congeners including TCDD were measured in maternal plasma.
27 TCDD levels were extrapolated to the date of delivery using a first-order pharmacokinetic model
28 (Michalek et al., 1996, [198893](#)). The elimination rate used was 9.8 years based on the mean
29 half-life estimate from a previous study of women in the Seveso region (Michalek et al., 2002,
30 [199579](#)). TEQs were calculated for a mixture of dioxin-like compounds by multiplying the
31 concentration of each congener by its toxicity equivalence factor. The maternal average TEQ

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1 was 44.8 ppt (range: 11.6–330.4) among 51 mothers. The measurement of noncoplanar PCBs
2 occurred only later in the study (1996) and, therefore, total mean TEQs (i.e., including the sum
3 of PCDDs, PCDFs, coplanar PCBs, and noncoplanar PCBs) are available only on a subset
4 ($n = 37$) of the population. Dioxin-like congeners were examined in this study as several studies
5 suggest associations between the sum of PCBs, or individual congeners having decreased
6 thyroxine (T4; Longnecker et al., 2000, [201463](#); Sandau et al., 2002, [594406](#)), and increased
7 TSH (Alvarez-Pedrerol et al., 2008, [594407](#); Chevrier et al., 2007, [594408](#)). The following
8 confounders were examined by the authors in the plasma dioxin models: maternal body mass
9 index, smoking habits, alcohol consumption, and neonatal age in hours at b-TSH measurement.

10 The authors used a linear model to examine the association between maternal TCDD
11 levels and b-TSH. The standardized regression coefficient obtained from this model was 0.47
12 ($p < 0.001$). For the evaluation of TEQs, a similar association was noted for PCDDs, PCDFs,
13 and coplanar PCBs ($n = 51$, $\beta = 0.45$, $p = 0.005$) but not with noncoplanar PCBs ($n = 37$,
14 $\beta = 0.16$, $p = 0.45$). Multivariate regression models that were adjusted for several covariates
15 (i.e., gender, birth weight, birth order, maternal age at delivery, hospital, and type of delivery)
16 found statistically significant associations with plasma TCDD, PCDDs, PCDFs, and coplanar
17 PCBs, but not with noncoplanar PCBs. The sum of all total TEQs from the measured
18 compounds was not statistically significant ($n = 37$, $\beta = 0.31$, $p = 0.14$).

19 20 **2.4.1.2.1.5.7.2. Study evaluation.**

21 The Baccarelli et al. (2008, [197059](#)) study satisfies the epidemiological considerations
22 and criteria for determining whether dose-response modeling should be pursued. The outcome is
23 well defined, and a dose-response pattern was observed. The study also contained a substudy
24 that characterized TCDD and exposures to other dioxin-like congeners and used serum measures
25 for a sample of mothers. Results were consistent among the zone of residence analysis and the
26 substudy based on serum measures.

27 28 **2.4.1.2.1.5.7.3. Suitability of data for TCDD dose-response modeling.**

29 Given the potential for exposure misclassification due to variability in TCDD soil levels
30 within each zone, modeling should rely on individual-level TCDD exposures derived from the
31 serum sampling substudy. The study data provide an opportunity for quantitative dose-response

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1 analyses as the critical exposure window of 9 months can be used for exposure assessment
2 purposes.

3

4 **2.4.1.2.1.5.8.** Mocarelli et al. (2008, [199595](#))—Sperm effects.

5 **2.4.1.2.1.5.8.1.** *Study summary.*

6 Mocarelli et al. (2008, [199595](#)) examined the relationship between TCDD and endocrine
7 disruption and semen quality in a cohort of Seveso men. A total of 397 subjects of the eligible
8 417 males (<26 years old in 1976) from Zone A and nearby contaminated areas were invited to
9 participate. Frozen serum samples were used to derive TCCD exposures. Also, 372 healthy
10 blood donors not living in the TCCD-contaminated area were invited to participate. The
11 researchers collected a health questionnaire and semen samples from participants. Analyses
12 were based on 257 individuals in the exposed group and 372 in the comparison group.

13 Semen samples were collected postmasturbatory at home. Ejaculate volume, sperm
14 motility, and sperm concentration were measured on these samples. Fasting blood samples also
15 were collected from the subjects for reproductive hormone analyses, including 17 β -estradiol
16 (E₂), follicle stimulating hormone (FSH), inhibin B, luteinizing hormone (LH), and testosterone.

17 The researchers estimated serum concentrations of TCDD from samples provided in
18 1976–1977, and also in 1997–1998 for individuals whose earlier samples had TCDD values that
19 exceeded 15 ppt. Serum concentrations for the comparison group were assumed to be less than
20 15 ppt in 1976 and 1977 and <6 ppt in 1998/2002 on the basis of serum results for residents in
21 uncontaminated areas. The exposed and comparison groups were divided into three groups
22 based on their age in 1976: 1–9, 10–17, and 18–26 years. Mocarelli et al. (2008, [199595](#))
23 applied a general linear model to the sperm and hormone data and included exposure status, age,
24 smoking status, body mass index, and occupational exposures as covariates. The study authors
25 thoroughly addressed the potential for confounding.

26 Men exposed between the ages of 1 and 9 had reduced semen quality 22 years later.
27 Reduced sperm quality included decreases in sperm count ($p = 0.025$), progressive sperm
28 motility ($p = 0.001$), and total number of motile sperm ($p = 0.01$) relative to the comparison
29 group. The opposite pattern was observed for several indices of semen quality among those aged
30 10–17 at the time of the accident; this included a statistically significant increase in sperm count
31 ($p = 0.042$). The clinical significance of this increase is unknown. For the hormone analyses,

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1 those in the exposed group had lower serum E₂ levels, and higher follicle stimulating hormone
2 concentrations. Neither testosterone levels nor inhibin B concentrations were associated with
3 TCDD exposure.

4 5 **2.4.1.2.1.5.8.2. *Study evaluation.***

6 The findings of the Mocarelli et al. (2008, [199595](#)) study support the hypothesis that
7 exposure to TCDD in infancy/prepuberty reduces sperm quality. The changes in serum E₂ and
8 FSH concentrations are of unknown clinical significance, and cannot be considered adverse.
9 Although most semen analysis studies have low compliance rates in general population samples
10 (20–40%) (Jørgensen et al., 2001, [594402](#); Muller et al., 2004, [594403](#)), the compliance rate in
11 this study was much higher (60%). Given that the compliance rates were similar between the
12 exposed and comparison groups and the strong differences detected across the two age groups,
13 selection bias appears unlikely in this study.

14 15 **2.4.1.2.1.5.8.3. *Suitability of data for TCDD dose-response modeling.***

16 Health outcomes are well defined in the Mocarelli et al. (2008, [199595](#)) study, and
17 exposures are well characterized using serum data. Because the men exposed to elevated TCDD
18 levels between the ages of 1 and 9 had reduced semen quality 22 years later, it is difficult to
19 identify the relevant time interval over which TCDD dose should be considered. Specifically, it
20 is difficult to discern whether this effect is a consequence of the initial high exposure between
21 1 and 9 years of age or a function of the cumulative exposure for this entire exposure window
22 beginning at the early age. However, the differences between these two dose estimates (the
23 initial high exposure versus the cumulative exposure for the 9 year window) are minimal (i.e.,
24 within an order of magnitude). Despite the uncertainty in estimating the critical window of
25 exposure, dose-response analysis for this outcome was conducted.

26 27 **2.4.1.2.1.6. *The Chapaevsk study.***

28 **2.4.1.2.1.6.1.** Revich et al. (2001, [199843](#))—Mortality and reproductive health.

29 **2.4.1.2.1.6.1.1. *Study summary.***

30 Revich et al. (2001, [199843](#)) describe a series of investigations that have evaluated
31 adverse health outcomes among residents of Chapaevsk where ecological measures of TCDD

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1 have been noted to be higher than expected. In the earlier cancer section of this report, the
2 cross-sectional comparisons of mortality that the authors carried out between Chapaevsk
3 residents and a general population reference were described. Although the general focus of this
4 paper is on cancer, the authors examined other adverse health outcomes.

5 For all-cause mortality, rates were found to be higher in Chapaevsk relative to the Samara
6 region and other nearby towns. The magnitude of this increase, however, was not quantified in
7 the review by Revich. Cardiovascular mortality accounted for nearly two-thirds of women's
8 deaths and almost half of those among men. The rates of cardiovascular mortality among
9 Chapaevsk men have been reported to be 1.14 times higher than those in Russia.

10 Revich et al. (2001, [199843](#)) also reported on the occurrence of adverse reproductive
11 events. Although the authors indicated that official medical information was used to make
12 comparisons between regions, no details were provided about data quality, completeness, or
13 surveillance differences across areas. The presented rates for reproductive health outcomes
14 should be interpreted cautiously. A higher rate of spontaneous abortions (24.4 per
15 100 pregnancies finished by delivery) was found in Chapaevsk women relative to rates that
16 ranged between 10.6 and 15.2 found in five other areas. The frequency of preeclampsia also was
17 found to be higher in Chapaevsk women (44.1/100) relative to other towns, as was the proportion
18 of low birth-weight babies and preterm births. The percentage of newborns with low birth
19 weight was slightly larger in Chapaevsk (7.1%) when compared to other towns in Samara
20 (5.1–6.2%); observed differences, however, were not statistically significant. The authors also
21 reported on the sex ratio of newborns born between 1983 and 1997. These ratios (boys:girls)
22 were highly variable and ranged between 0.79 and 1.29. Given the annual variability of this ratio
23 on a year-to-year basis, it is unclear if this is largely due to natural fluctuations and to what
24 extent this may result from prior TCDD (or other contaminants) exposure TCDD and other
25 contaminants.

26 27 **2.4.1.2.1.6.1.2. Study evaluation.**

28 The review by Revich et al. (2001, [199843](#)) highlights analyses that have been
29 undertaken using largely cross-sectional data. Although soil sampling measures appear to
30 demonstrate decreasing levels of TCDD in the soil with increasing distance from the plant, at this
31 time, no individual-level TCDD exposure data are available. Increased rates of mortality relative

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1 to the Samara region in Russia were observed among Chapaevsk men for all cancer sites
2 combined; this excess risk however, was not observed among women. Although the authors
3 provide compelling evidence of increased adverse events among residents of Chapaevsk, the
4 study lacks a discussion about the validity of comparing health data across regions, and suffers
5 from inherent limitations from ecological studies such as exposure misclassification.

6
7 **2.4.1.2.1.6.1.3. *Suitability of data for TCDD dose-response modeling.***

8 As with the cancer outcomes presented in this study, the data for noncancer outcomes are
9 limited by the absence of TCDD levels on an individual-level basis and information on other
10 potential confounding variables that could have biased the comparisons. Additional studies are
11 being undertaken to evaluate the relationship between TCDD and the sexual and physical
12 development of boys. The cross-sectional nature of the data that were presented does not
13 provide the necessary level of detail needed to estimate effective dose given the lack of
14 individual-level exposure data. Therefore, a quantitative dose-response analysis was not
15 conducted.

16
17 **2.4.1.2.1.7. *The Air Force Health (“Ranch Hands” cohort) study.***

18 **2.4.1.2.1.7.1.** Michalek and Pavuk (2008, [199573](#))—Diabetes.

19 **2.4.1.2.1.7.1.1. *Study summary.***

20 Michalek and Pavuk (2008, [199573](#)) examined both the incidence of cancer and the
21 prevalence of diabetes in the cohort of Ranch Hand workers exposed to TCDD. As noted
22 previously, these veterans were responsible for aerial spraying of Agent Orange in Vietnam
23 between 1962 and 1971. Exposure to TCDD was estimated using serum collected from
24 participants in 1987 and assayed for TCDD. Exposure to TCDD was estimated using a
25 first-order pharmacokinetic model with a half-life of 7.6 years and provided an estimate of
26 TCDD at the end of the tour of duty in Vietnam. Veterans were grouped into four categories:
27 comparison, background, low, and high. Diabetes was identified from diagnoses during the
28 post-Vietnam era from medical records. Overall, no differences were shown in the RR of
29 diabetes between the Ranch Hand unit and the reference group (RR = 1.21, $p = 0.16$). Stratified
30 analyses by days of spraying (<90 days, ≥ 90 days), however, revealed a significant increase in
31 risk of diabetes (RR = 1.32, $p = 0.04$) among those who sprayed for at least 90 days. A dose-

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1 response relationship was also evident when log₁₀TCDD was modeled in the combined cohort.
2 Also, stratification by calendar period showed a dose-response relationship for those whose last
3 year of service was during or before 1969.

4 5 **2.4.1.2.1.7.1.2. Study evaluation.**

6 The Michalek and Pavuk (2008, [199573](#)) study provides an opportunity to characterize
7 risks of diabetes as the study is not subject to some of the potential bias of case ascertainment
8 based on death certificates (D'Amico et al., 1999, [197389](#)). The quality of the TCDD exposure
9 estimates is high, given that serum data were available at an individual-level basis for all Ranch
10 Hand and comparison veterans used in the cohort. Although disentangling the effects of 2,4-D
11 and TCDD is not possible because their concentrations in Agent Orange are equivalent, 2,4-D
12 has not been associated with diabetes.

13 14 **2.4.1.2.1.7.1.3. Suitability of data for TCDD dose-response modeling.**

15 The reported dose-response relationship between TCDD and diabetes is supported by
16 study strengths including the use of the individual-level level TCDD serum measures and the
17 identification of diabetes through medical records are important strengths of the Michalek and
18 Pavuk (2008, [199573](#)) study. Nonetheless, the possible confounding from the inability to control
19 for 2,4-D and other agents used in Agent Orange precludes a quantitative dose-response analysis.

20 21 **2.4.1.2.1.8. Other noncancer studies of TCDD.**

22 **2.4.1.2.1.8.1. Ryan et al. (2002, [198508](#))—Sex ratio.**

23 **2.4.1.2.1.8.1.1. Study summary.**

24 Ryan et al. (2002, [198508](#)) conducted an investigation on the sex ratio in offspring of
25 children of pesticide workers who were involved with the production of trichlorophenol and the
26 herbicide 2,4,5-T in Ufa, Bashkortostan, Russia. Ufa was the site of a state agrochemical plant
27 that has been in operation since the 1940s. Between 1961 and 1988, the plant employed more
28 than 600 workers, most in their early 20s. Females, however, accounted for about 15% of the
29 workforce that produced 2,4,5-T and 30% for 2,4,5-trichlorophenol.

30 Serum samples previously taken in 1992 among 60 men, women, and children from the
31 factory and city of Ufa showed TCDD exposures that were approximately 30 times higher than

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1 background levels (Ryan and Schechter, 2000, [594412](#)). Blood data were subsequently measured
2 on a sample of 20 workers between 1997 and 2000, and on 23 2,4,5-trichlorophenol workers
3 between 1997 and 2001. In all, 84 individuals who provided blood samples formed the basis of
4 the analysis in this study. Of these, 55 were exposed to 2,4,5-T and 29 were exposed to
5 2,4,5-trichlorophenol.

6 Ryan et al. (2002, [198508](#)) reviewed company records for these workers to determine the
7 number, sex, and date of birth of any children; birth data were available for 198 workers.
8 Awareness of the study led other workers who had not provided serum to provide information on
9 births that occurred 9 months after the time of first employment in the factory.

10 The authors calculated descriptive statistics for the 198 workers and compared them to
11 values for the city of Ufa between 1959 and 1996. Tests of statistical significance were made
12 using the z-test, and the chi-square test. The observed proportion of male births (0.40) among
13 the factory workers was much lower than that for the city of Ufa (0.51) ($p < 0.001$). Stratified
14 analyses revealed that this lower ratio was observed only among those paternally exposed to
15 TCDD. Specifically, the proportion of male births among exposed fathers was 0.38 and among
16 exposed mothers was 0.51. This pattern was observed in both the workers exposed to 2,4,5-T
17 (proportion of male births = 0.40) and 2,4,5-trichlorophenol (proportion of male births = 0.35).

18 19 **2.4.1.2.1.8.1.2.** *Study evaluation.*

20 The Ryan et al. (2002, [198508](#)) findings are consistent with earlier work completed for
21 Seveso residents (Mocarelli et al., 2000, [197448](#)). Although serum measures were available for
22 84 individuals, no dose-response of birth ratios was performed using exposure quantified at an
23 individual-level basis. This approach would have been preferred and consistent with that which
24 Mocarelli et al. (2000, [197448](#)) used. All comparisons were made using an external comparison
25 group, namely the sex ratio observed in Ufa between 1959 and 1996.

26 Although serum measures were used to describe TCDD exposure for a sample of the
27 workers, individual-level dose estimates were not calculated for the study population.
28 Specifically, exposures were characterized many years after exposure, and no attempt was made
29 to back-extrapolate to the time of conception. The two groups of workers in the study also
30 reportedly had high exposure levels of 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin. So, the group
31 level exposure classification (by plant) did not allow consideration of confounding due to other

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1 dioxin-like compounds. Another limitation of the study is that the study population is likely
2 nonrepresentative of all workers employed at the plant. Participants included only those willing
3 to provide serum samples and those who volunteered to participate in the study after learning
4 about it in a public forum. If participation was dependent on TCDD exposures and the
5 reproductive health of these subjects, then bias may have occurred.

6
7 **2.4.1.2.1.8.1.3.** *Suitability of data for TCDD dose-response modeling.*

8 The findings are notable in their consistency with those found in Seveso residents by
9 Mocarelli et al. (2000, [197448](#)). For the Ryan et al. (2002, [198508](#)) study, serum data were
10 quantified at an individual-level basis. Risk estimates, however, were not derived in relation to
11 these exposures but instead in two separate subgroups (2,4,5-T and 2,4,5-trichlorophenol
12 workers). This important limitation precludes the use of these data for quantitative
13 dose-response modeling.

14
15 **2.4.1.2.1.8.2.** Kang et al. (2006, [199133](#))—Long-term health effects.

16 **2.4.1.2.1.8.2.1.** *Study summary.*

17 Kang et al. (2006, [199133](#)) investigated the relationship between self-reported health
18 measures and serum-based measures of TCDD in a group of 1,499 Vietnam veterans and a
19 control group of 1,428 non-Vietnam veterans. The study subjects were identified from
20 (1) reports of Army Chemical Corps detachments in Vietnam between 1966 and 1971,
21 (2) personnel records of individuals involved in chemical operations who were on active duty
22 between 1971 and 1974, and (3) class rosters of personnel who were trained at Fort McClellan in
23 Alabama between 1965 and 1973. The comparison group was selected so that branch of service,
24 time period, and military occupation were similar to those of the subjects with the exception that
25 they did not serve in Vietnam. Although 2,872 Vietnam veterans and 2,732 non-Vietnam
26 veterans were identified as potential subjects, those who were deceased as of December 1998
27 and those who had previously participated in a pilot study were excluded. The study targeted
28 2,247 Vietnam and 2,242 non-Vietnam veterans.

29 Exposure to TCDD was characterized for subsets of the study population that provided
30 blood samples, specifically 795 of 1,085 (73%) Vietnam veterans and 102 of 157 (65%)
31 non-Vietnam veterans. Details on these individuals selected for participation in the serum dioxin

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1 study were not presented. The authors did state, however, that due to economic constraints, only
2 897 serum samples could be analyzed. Blood specimens were collected in 1999–2000 at
3 individuals' homes. TCDD concentrations were analyzed by laboratory staff blind to the group
4 status (i.e., Vietnam or non-Vietnam) of the study subjects.

5 Prevalent health outcomes were ascertained by self-reported information on selected
6 conditions diagnosed by a medical doctor. The following conditions were included: diabetes,
7 hepatitis (all types combined), heart disease, all cancer, nonmalignant chronic respiratory
8 diseases, and hypertension. Health-related quality of life was evaluated using the SF-36 survey
9 instrument (Ware et al., 1993, [004687](#)).

10 Eligible veterans whose current residences (4,119 total) could be identified were
11 contacted for study participation. Survey participation rates were 72.9% for Vietnam veterans,
12 yielding data for 1,499 individuals, and 69.2% for non-Vietnam veterans, yielding data for
13 1,428 non-Vietnam veterans. The survey data showed that, relative to non-Vietnam veterans,
14 Vietnam veterans were more likely to be regular smokers and to be obese. They also were more
15 likely to be enlisted personnel, and a much higher proportion was 51 years of age or older
16 (83.4% vs. 58.4%). After adjusting for age, race, smoking status, rank, and body mass index, the
17 prevalence of self-reported health conditions was found to be statistically significantly higher in
18 the Vietnam group. The adjusted odds ratios (OR) were as follows: diabetes, OR = 1.16
19 (95% CI = 0.91, 1.49); hepatitis, OR = 1.85 (95% CI = 1.30, 2.64); heart condition, OR = 1.09
20 (95% CI = 0.87, 1.38); all cancer, OR = 1.46 (95% CI = 1.02, 2.10); nonmalignant respiratory
21 condition, OR = 1.41 (95% CI = 1.13, 1.76); and hypertension, OR = 1.06 (95% CI = 0.89, 1.27).

22 For those with Vietnam service, the mean serum TCDD concentrations were higher
23 among those who reported spraying herbicides (4.3 parts per thousand [ppt]) than those who did
24 not (2.7 ppt) ($p < 0.001$). The investigators did not back-extrapolate serum levels to the time
25 when individuals last sprayed. The adjusted ORs (adjusted for age, cigarette smoking, body
26 mass index, rank, and race) for most chronic health conditions examined revealed increased
27 prevalence among Vietnam sprayers relative to non-Vietnam sprayers. These ORs were:
28 diabetes, OR = 1.49 (95% CI = 1.10, 2.02); hepatitis, OR = 1.40 (95% CI = 0.92, 2.12); heart
29 condition, OR = 1.41 (95% CI = 1.06, 1.89); all cancer, OR = 1.36 (95% CI = 0.91, 2.04);
30 nonmalignant respiratory condition, OR = 1.57 (95% CI = 1.20, 2.07); and hypertension,
31 OR = 1.26 (95% CI = 1.00, 1.58).

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1 The investigators also examine the possibility of over-reporting of chronic health
2 conditions by comparing the prevalence of self-reported conditions among 357 Vietnam sprayers
3 who mean serum TCDD levels of 2.5 ppt compared to those who had levels less than 2.5 ppt.
4 Prevalence of diabetes, heart condition, and hypertension, was higher among those with mean
5 serum TCDD levels of 2.5 ppt, although no levels of statistical significance were reported. Data
6 for cancer were not presented.

7
8 **2.4.1.2.1.8.2.2. Study evaluation.**

9 Because data were collected from only half of the individuals in the study target
10 population, there is some potential for selection bias in this study. First, the study excluded those
11 who had died before 1999, excluding potentially important TCDD-related adverse health effects
12 that could result in death more than two decades after veterans had been actively spraying.
13 Second, survey participation rates were modest: 72.9% for Vietnam veterans and 69.2% for
14 non-Vietnam veterans. If those in poorer health were less inclined to participate, the prevalence
15 of the selected chronic health conditions would be understated. Selection bias due to study
16 participation could also be possible if, for example, those in poorer health also had high (or
17 lower) exposures than those not participating in the study. The lack of direct evidence of
18 differential participation and reports of comparable prevalence rates of hypertension and diabetes
19 to other general populations suggests that selection bias may be minimal.

20 Because the data collected are cross-sectional, they are ill-suited for evaluating the
21 relationship between the timing of exposure and the onset of disease. Whether any of the data
22 could help identify when the chronic health conditions were diagnosed is unclear. Given the
23 long period covered by the study, many of the self-reported health conditions likely were
24 diagnosed some time ago, perhaps closer to the time of potential TCDD exposure. Such detail is
25 needed to characterize health risks associated with specific TCDD levels, particularly given that
26 TCDD levels have been demonstrated to decrease from time of last exposure.

27 An important strength of the study is the availability of blood sera for a subset of the
28 study population, which allows for an objective determination of TCDD exposure. That serum
29 TCDD levels were available for only 897 subjects, however, limits the ability to examine the
30 relationship between measures of TCDD and prevalence of health outcomes without restricting
31 the sample size or extrapolating exposure levels to the whole study population. For example,

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1 among sprayers with available TCDD exposure data only 60 cases of diabetes and 69 cases of
2 heart disease were examined relative to exposure. Also, the small number of cancers precluded a
3 cancer site-specific analysis. Moreover, whether these TCDD levels are representative of the
4 larger eligible population is difficult to gauge, given that deceased veterans and those whose
5 current residences could not be determined were excluded.

6 The study relied on self-reported measures of disease prevalence. The ascertainment of
7 chronic health conditions using self-reported data can be fraught with difficulties. For example,
8 the sensitivity of self-reported data when compared to medical diagnosis has been shown to be
9 poor for conditions such as diabetes and hypertension (Okura et al., 2004). As Kang et al. (2006,
10 [199133](#)) state, prevalence studies are not well suited to examine rare diseases with short
11 survival times such as cancer. In addition, self-reports of physician-diagnosed cancers by study
12 subjects often lacks the sensitivity needed in most epidemiological studies as they can be
13 influenced by a variety of factors including age and education (Navarro et al., 2006).

14 The potential for biases in the reporting of health outcomes between the sprayers and the
15 non-Vietnam veterans (i.e., differential by TCDD exposure status) also is plausible, given the
16 public attention that spraying of Agent Orange has received. Although the authors examined
17 whether over-reporting was related to outcome prevalence among herbicide sprayers (prior to
18 collection and determination of actual TCDD serum levels), the possibility exists that these
19 subjects reporting could be influenced by their perceived level of exposure from herbicide
20 spraying. The authors also examined the potential for misreported diabetes by conducting a
21 medical records review of 362 veterans. Seventy-nine percent of the self-reported diabetes cases
22 were confirmed with medical records. The documentation rate was also comparable between the
23 Vietnam veterans and the non-Vietnam veterans suggesting that differential reporting was not an
24 issue for this health outcome.

25 Because the Vietnam veterans group comprised professional sprayers, it is not
26 unreasonable to assume that they would have been exposed to other potentially harmful agents
27 either during their service in Vietnam, or from the end of their service to when they provided
28 data in 1999–2000. This study did not control for other, potentially relevant occupational
29 exposures.

30

1 **2.4.1.2.1.8.2.3.** *Suitability of data for TCDD dose-response modeling.*

2 Although the study demonstrates increased prevalence of several chronic health
3 conditions, these findings should be interpreted with caution due to potential for selection and
4 recall biases. The lack of demonstrated dose-response relationships with cancer or other
5 outcomes precluded the use of these data for characterizing the dose response from TCDD.

6
7 **2.4.1.2.1.8.3.** McBride et al. (2009, [198490](#); 2009, [197296](#))—Noncancer mortality.

8 **2.4.1.2.1.8.3.1.** *Study summary.*

9 The McBride et al. (2009, [198490](#)) mortality study of New Zealand workers employed as
10 producer or sprayers with potential exposure to TCDD was described earlier in this report.
11 These individuals were employed at a plant that manufactured 2,4,-dichlorophenoxyacetic acid,
12 and later 2,4,5-T and 4-chloro-2-methyphenoxyacetic acid. In 1987, the plant closed and 2,4,5-T
13 production ceased in 1988.

14 The cohort consisted of 1,754 individuals who were employed for at least one day at the
15 New Plymouth site between January 1, 1969, and October 1, 2003. Vital status was determined
16 until the end of 2004. Comparisons of mortality were made to the New Zealand general
17 population using the SMR statistic. Exposure was characterized by duration of employment.
18 Person-years of follow-up were tabulated across strata defined by age, calendar period, duration
19 of employment, sex, latency, and period of hire. Analyses were stratified to compare risks by
20 duration of employment (<3 or ≥3 months), latency (<15 or ≥15 years), and period of hire
21 (<1976, ≥1976).

22 Overall, no statistically significant differences in all-cause mortality relative to the
23 general population were found among those who worked for at least 3 months (SMR = 0.92,
24 95% CI = 0.80–1.06) or for less than 3 months (SMR = 1.23, 95% CI = 0.91–1.62). No
25 statistically significant excesses were found for mortality from diabetes, cerebrovascular disease,
26 heart diseases, or accidents. The incorporation of a latency period of 15 years revealed no
27 statistically significant excesses for these same causes of death. Similarly, no excesses for any
28 cause of death were noted among those who were hired either before or after 1976.

29 In subsequent analyses of the same cohort that used estimated TCDD levels from serum
30 samples, McBride et al. (2009, [197296](#)) found no excesses for all-cause mortality or mortality
31 from diabetes or heart disease.

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1 **2.4.1.2.1.8.3.2. Study evaluation.**

2 For the McBride et al. (2009, [198490](#)) study, the size of the cohort is large enough to
3 characterize mortality risks relative to the general population for most common causes of deaths.
4 An important limitation of this study is the loss to follow-up of a substantial percentage of
5 workers (22%). This would have impacted statistical power by reducing the number of deaths
6 among the workers. If this incomplete ascertainment of mortality outcomes did not occur in a
7 similar fashion with the general population then the SMR may also be biased.

8 For noncancer causes of death, the use of the SMR statistic is more likely to be
9 influenced by the healthy-worker effect. Therefore, the findings obtained for these outcomes
10 should be interpreted with caution. Subsequent analyses published by the same authors
11 (McBride et al., 2009, [197296](#)) provide improved characterization of TCDD exposure using
12 serum samples.

13
14 **2.4.1.2.1.8.3.3. Suitability of data for dose-response analysis.**

15 Overall, no associations were evident between surrogate measures of TCDD (duration of
16 employment, year of hire) and noncancer mortality outcomes. Further, the use of mortality
17 endpoints is inconsistent with EPA RfD methodology. As such, these data do not support further
18 use in a quantitative dose-response analysis.

19
20 **2.4.1.2.1.8.4. McBride et al. (2009, [197296](#))—Noncancer mortality.**

21 **2.4.1.2.1.8.4.1. Study summary.**

22 McBride et al. (2009, [197296](#)) further analyzed the cohort of New Zealand workers to
23 include estimates of TCDD exposure based on serum samples. Current and former employees
24 who were still alive and living within 75 km of the site were asked to provide serum samples.
25 Samples were collected from 346 workers representing 22% (346/1599) of the entire study
26 population. These serum measures were used to estimate cumulative TCDD levels for all
27 workers. The exposure assessment approach by Flesch-Janys et al. (1996, [197351](#)) was used to
28 estimate time-dependent exposures based on area under the curve models. This was based on a
29 one-compartment first-order kinetic model with a half-life of 7.2 years.

30 Comparisons of mortality were made to the general population using the SMR statistic.
31 The Cox proportional hazards model was used to conduct an internal cohort analysis across

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1 four categories of cumulative TCDD levels for diabetes and ischemic heart disease mortality.
2 The RRs generated from these models were adjusted for sex, hire year, and birth year. No
3 diabetes deaths were observed among women, and therefore, analysis of this outcome was
4 limited to men.

5 Relative to the general population, no difference in the all-cause mortality experience was
6 observed in exposed cohort members (SMR = 1.0, 95% CI = 0.9–1.2). Similarly, no excess in
7 these workers was observed for heart disease (SMR = 1.1, 95% CI = 0.9–1.5); cerebrovascular
8 disease (SMR = 1.1, 95% CI = 0.6–1.9); diabetes (SMR = 0.7, 95% CI = 0.2–2.2); or
9 nonmalignant respiratory disease (SMR = 0.8, 95% CI = 0.4–1.4). For the internal cohort
10 analysis, the RR associated with cumulative categorical TCDD measure was 1.0 for both
11 diabetes and ischemic heart disease.

12

13 **2.4.1.2.1.8.4.2.** *Study evaluation.*

14 The McBride et al. (2009, [197296](#)) study extends the earlier work the same authors
15 completed in two ways. First, serum measures were used to estimate cumulative TCDD with
16 methodology that has been applied to several other cohorts of workers exposed to TCDD.
17 Second, the authors used regression analyses that examined individual-level TCDD exposures in
18 relation to various outcomes as part of the internal cohort comparisons. For noncancer
19 outcomes, no dose-response associations with TCDD were observed with the internal
20 comparisons. Also, as found with earlier analyses of this same cohort, no excess noncancer
21 mortality relative to the New Zealand general population was observed.

22 Associations between TCDD and diabetes have been found previously in TCDD-exposed
23 populations, most notably in the Ranch Hands cohort (Michalek and Pavuk, 2008, [199573](#)). In
24 this cohort, only five deaths from diabetes were identified, and of these, only three occurred
25 among those who were exposed to TCDD. The study, therefore, has limited statistical power to
26 characterize associations between TCDD and mortality from diabetes. Further, the identification
27 of diabetes deaths is subject to misclassification errors due to under-reporting (McEwen and
28 TRIAD, 2006, [594400](#)).

29

1 **2.4.1.2.1.8.4.3.** *Suitability of data for TCDD dose-response modeling.*

2 McBride et al. (2009, [197296](#)) found no statistically significant associations in any of the
3 noncancer causes of death. Furthermore, the use of mortality endpoints is inconsistent with EPA
4 RfD methodology. Therefore, the data were not suitable for quantitative dose-response analysis
5 for these outcomes.

6
7 **2.4.1.2.2.** *Feasibility of dose-response modeling for noncancer.*

8 Relatively few study populations permit quantitative dose-response modeling to be
9 performed for noncancer outcomes. The serum collected among Seveso men and women
10 provide an opportunity to characterize risks for several health conditions in relation to TCDD
11 exposure. The collection of these serum samples, shortly after the accident does not require the
12 back-extrapolation of TCDD levels as in the occupational cohorts, which should reduce the
13 exposure assessment uncertainty and minimize the potential for exposure misclassification.

14 An added feature of the SWHS is the detailed collection of other risk factor data from
15 trained interviewers. These data allow for risk estimates to be adjusted for potential confounding
16 variables. For the evaluations of reproductive health outcomes, this adjustment is critical given
17 there are various documented risk factors for the different outcomes that were examined. For
18 some health outcomes, continued follow-up of the cohort is needed, given that several of the
19 Seveso studies suggest that those exposed at a very young age might be more susceptible to
20 subsequent adverse health effects.

21 The findings of positive associations and dose-response relationships with serum-based
22 measures of TCDD suggest several noncancer health outcomes could be associated with TCDD
23 exposure. These health outcomes include neonatal thyroid function, sex ratio, diabetes, and
24 semen quality. Although findings have suggested an association between TCDD and age at
25 menopause, they were not statistically significant and no dose-response trend was observed.
26 Weak or nonstatistically significant associations have been noted for endometriosis and
27 menstrual cycle characteristics and do not support quantitative dose-response analyses.

28 Associations between TCDD exposure and cardiovascular disease have been noted in
29 some, but not all, of the occupational cohorts, and also shortly after the accident among Seveso
30 residents. Findings from the cohort studies based on external comparisons using the SMR
31 statistic should be interpreted cautiously due to potential bias from the healthy worker effect.

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1 Because the magnitude of the healthy worker bias is recognized to be larger for cardiovascular
2 diseases than for cancer outcomes, risk estimates in some occupational cohorts might be
3 underestimated for cardiovascular outcomes. Information on cardiovascular risk factors
4 generally was not captured in these studies, and sensitivity analyses were generally designed to
5 examine risk estimates generated for cancer outcomes.

6 7 **2.4.1.2.3. Summary of epidemiologic noncancer study evaluations for dose-response** 8 **modeling.**

9 All epidemiologic noncancer studies summarized above were evaluated for suitability of
10 quantitative dose-response assessment using the TCDD-specific considerations and study
11 inclusion criteria. The results of this evaluation are summarized in a matrix style array (see
12 Table 2-3) at the end of the chapter, and descriptively in Appendix B. The key epidemiologic
13 noncancer studies suitable for further TCDD dose-response assessment are presented in
14 Table 2-5.

15 16 **2.4.2. Summary of Animal Bioassay Studies Included for TCDD Dose-Response Modeling**

17 This section summarizes studies that have already met the in vivo animal bioassay TCDD
18 study inclusion criteria (see Section 2.3.2). These studies are listed later in this section in
19 Tables 2-6 and 2-7, for cancer and noncancer, respectively, and are considered in the
20 dose-response modeling conducted later in this document (see Sections 4 and 5). The following
21 sections are organized by reproductive studies, developmental studies, and general toxicity
22 studies (subdivided by duration). They summarize the experimental protocol, the results, and the
23 NOAELs and LOAELs EPA has identified for each study.

24 To evaluate and discuss studies consistently, doses were converted to nanograms per
25 kilogram body weight per day (ng/kg-day) and were also adjusted for continuous exposure.
26 Some doses were adjusted based on daily dietary intake and body weight. For these studies,
27 EPA uses 10% of an animal's body weight as the daily feed rate. More commonly, doses were
28 adjusted from 5 days/week to a 7 days/week standard adjustment, in which case administered
29 doses were multiplied by 5 and divided by 7 to obtain continuous doses. To adjust for weekly
30 dosing, the weekly administered doses were multiplied by the administration frequency per week
31 (in days) and divided by 7 to give continuous doses.

1 Other exposure protocols used a single loading dose followed by weekly maintenance
2 doses. To adjust these doses, the loading dose was added to the maintenance doses multiplied by
3 the administration frequency, and this sum was divided by the exposure duration to give a
4 continuous dosing rate. The doses administered in single dose studies were not averaged over
5 the observation period.

6 7 **2.4.2.1. Reproductive Studies**

8 **2.4.2.1.1. Bowman et al. (1989, [543744](#); 1989, [543745](#)) (and related Schantz and Bowman** 9 **(1989, [198104](#)); Schantz et al. (1986, [088206](#))).**

10 Female rhesus monkeys (6 to 10 years old; 8 per treatment) were exposed to 0 or 5 ppt
11 (for 3.5 years), or 25 ppt (for 4 years) TCDD (purity not specified) (Bowman et al., 1989,
12 [543744](#); Bowman et al., 1989, [543745](#); Schantz and Bowman, 1989, [198104](#); Schantz et al.,
13 1986, [088206](#)). Female monkeys were mated to unexposed males after 7 months (Cohort I) and
14 27 months (Cohort II) of exposure, then again 10 months postexposure (Cohort III). The average
15 daily doses to mothers were equivalent to 0, 0.15, and 0.67 ng/kg-day. The 0.67 ng/kg-day dose
16 group had reduced reproductive rates in both Cohorts I ($p < 0.001$) and II ($p < 0.025$; Bowman
17 et al., 1989, [543744](#)). The mean number of days of offspring survival ($p < 0.023$) also decreased.
18 No effects on birth weight or growth, or physical evidence of toxicity (Bowman et al., 1989,
19 [543745](#)) were observed. Behavioral effects were observed in the offspring (Cohort I: 7, 6, and
20 0 offspring, respectively; Cohort II: 3, 5, and 0 offspring, respectively; Cohort III: 6, 7, and 3,
21 respectively). In the 0.67 ng/kg-day dose group, the number of offspring was insufficient to
22 form a group in either Cohorts I or II. Offspring in the 0.15 ng/kg-day dose group had alterations
23 in social behavior of the mother-infant pairs (mothers had increased care giving, which appeared
24 to be an effect of the infants and not due to the treatment of the mother) and peer group of the
25 offspring after weaning (Cohort I offspring were more dominant or aggressive and exhibited
26 more self-directed behavior; Bowman et al., 1989, [543745](#)). The performance of learning tasks
27 was inversely related to the level of TCDD in the body fat. Schantz and Bowman (1989,
28 [198104](#)) examined effects using discrimination-reversal learning (RL) and delayed spatial
29 alteration (DSA). RL detected effects in the 0.15 ng/kg-day group as measured by retarded
30 learning of the shape reversal ($p < 0.05$), but DSA did not. Schantz et al. (1986, [088206](#))
31 combined the cohorts and looked at 5, 5, and 3 mother-infant pairs in the 0, 0.15, and

1 0.67 ng/kg-day groups, respectively. They found that TCDD-exposed mother-infant pairs spent
2 more time in close, social contact compared to the controls (mutual ventral contact, $p < 0.025$;
3 nipple contact, $p < 0.01$) and infants had reduced locomotor activity ($p < 0.05$), but the
4 dose-effect was complex. Of note is that the control groups contained fewer males than did the
5 TCDD-exposed groups.

6 In a follow-up study, Rier et al. (2001, [199843](#)) examined the DLC levels of sera
7 collected from some monkeys in this study. They reported that animals in this study had
8 elevated serum PCB77 and PCB126 levels and an increased serum TEQ. In fact, the fractional
9 contribution of serum TCDD levels to total serum TEQ was 30% in treated animals. In this
10 study, it is not possible to determine the contribution of TCDD alone to the developmental effect
11 due to the background contamination; thus, EPA has not developed a TCDD LOAEL from the
12 study.

13

14 **2.4.2.1.2. Franc et al. (2001, [197353](#)).**

15 To study the effects of subchronic, low-dose exposure to TCDD on the regulation and
16 expression of the aryl hydrocarbon receptor (AhR), Franc et al. (2001, [197353](#)) used rodent
17 models with varying sensitivities to TCDD. Female Sprague-Dawley rats, inbred Long-Evans
18 rats, and outbred Han/Wistar rats (8 per dose group) were dosed via oral gavage with 0, 140,
19 420, or 1,400 ng/kg TCDD (>99% purity) dissolved in corn oil once every 2 weeks for 22 weeks
20 (0, 10, 30, and 100 ng/kg-day average daily doses). Animals were sacrificed 10 days after the
21 final dosing. Body weights were recorded biweekly and just before sacrifice. After sacrifice,
22 liver and thymus weights were determined. Liver tissue samples were removed and either frozen
23 for RNA isolation followed by semiquantitative RT-PCR or homogenized and prepared for
24 subcellular fraction analysis. Radioligand binding and immunoblotting techniques were used to
25 measure AhR levels, and RT-PCR analysis was used to assess mRNA levels of AhR, aryl
26 hydrocarbon nuclear receptor (ARNT), and CYP1A1.

27 Long-Evans rats exhibited significant ($p < 0.001$) decreased weight gain over time as
28 compared to Sprague-Dawley and Han/Wistar rats as determined by repeated measures analysis
29 of variance (ANOVA). Because body weight gain varied indirectly with TCDD exposure, liver
30 and thymus tissue weights were normalized to body weight for data analysis. TCDD exposure
31 led to a significant ($p < 0.05$) increase in relative liver weights at all three TCDD doses and in all

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1 three rat strains, compared to the control groups. At the upper end of the TCDD dose range,
2 Sprague-Dawley rats dosed with 100 ng/kg-day showed the greatest increase in relative liver
3 weights (160% of the control values), while relative liver weights in Long-Evans and Han/Wistar
4 rats were similar to each other, and also were elevated above control values by 10–20%. At the
5 30 and 100 ng/kg-day doses, the relative thymus weights were significantly lower ($p < 0.05$) in
6 all rat strains compared to their corresponding controls, but the 10 ng/kg-day dose did not
7 produce a statistically significant effect in any strain. However, absolute thymus weight was
8 higher at all doses in Han/Wistar rats, which also had a higher control thymus weight.

9 Supporting observed differences in baseline TCDD sensitivity among the rat strains, liver
10 AhR levels in the control groups as measured by radioligand binding were similar for Sprague
11 Dawley and Han/Wistar rats, but were approximately two-fold higher for Long-Evans rats. A
12 significant ($p < 0.05$) two-fold, dose-dependent increase in radioligand binding of liver AhR was
13 observed at all TCDD doses relative to the control in Sprague-Dawley rats. At the 30 ng/kg-day
14 dose, the AhR level for Long-Evans rats was significantly ($p < 0.05$) increased to approximately
15 250% of the control level.

16 AhR protein levels measured in the liver cytosol by immunoblotting were highest in the
17 10 and 30 ng/kg-day TCDD dose groups for all three rat strains. Significant ($p < 0.05$) increases
18 in AhR levels were observed in the Sprague-Dawley rats that received 30 ng/kg-day, and in
19 Long-Evans rats that received either 10 or 30 ng/kg-day. A significant ($p < 0.05$) decrease in
20 AhR protein level was observed only at the 100 ng/kg-day dose in Han/Wistar rats. Liver AhR
21 protein was not detectable by immunoblotting in nuclear extracts for any strain or dose. The
22 study authors assert that AhR levels measured in cytosol correspond to measures in whole-tissue
23 lysates as demonstrated in their previous work.

24 Based on RT-PCR analysis, all three rat strains showed similar responses in liver AhR
25 mRNA following TCDD exposure. Liver AhR mRNA levels increased significantly ($p < 0.05$)
26 as compared to control levels in all rat strains at 10 and 30 ng/kg-day and in Long-Evans rats at
27 100 ng/kg-day. The study authors observed that statistically significant increases in AhR mRNA
28 levels in the liver were not always associated with statistically significant increases in AhR levels
29 for a given strain and dose, but that the opposite (increases in AhR levels associated with
30 increases in AhR mRNA levels) was always true. Changes in liver ARNT mRNA levels tended
31 to increase with increasing TCDD dose, and the increases were significant ($p < 0.05$) in the

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1 30 ng/kg-day dose groups of Long-Evans and Han/Wistar rats. At the 100 ng/kg-day TCDD
2 dose, all rat strains showed a decrease in ARNT mRNA in the liver relative to controls with
3 significant ($p < 0.05$) differences for the 100 ng/kg-day TCDD dose groups of Sprague-Dawley
4 and Han/Wistar rats. Liver CYP1A1 mRNA induction was not detectable in control animals. A
5 significant ($p < 0.05$) increase in liver CYP1A1 mRNA was observed in all rat strains
6 administered 10 or 30 ng/kg-day TCDD. Liver CYP1A1 mRNA levels also were significantly
7 ($p < 0.05$) elevated above controls in the 100 ng/kg-day groups although not to the same extent
8 as in the 30 ng/kg-day groups. For all rat strains, the largest up-regulation for AhR and ARNT
9 mRNA levels occurred in the 30 ng/kg-day TCDD dose groups.

10 The NOAEL for TCDD identified in this study is 10 ng/kg-day TCDD. At 10 ng/kg-day
11 TCDD, the change in relative liver weight, while significantly ($p < 0.05$) increased in
12 Sprague-Dawley rats, was determined (from Figure 5 in Franc et al., 2001, [197353](#)) to be less
13 than 10% and judged by EPA not to be biologically relevant. Also, at 10 ng/kg-day TCDD, the
14 change in relative thymus weight, was not statistically significantly decreased in
15 Sprague-Dawley, Han-Wistar or Long-Evans rats. The study LOAEL is 30 ng/kg-day, based on
16 statistically and biologically significant increases in relative liver weight in Sprague-Dawley and
17 Long-Evans rats and statistically and biologically significant decreases in relative thymus weight
18 in Sprague-Dawley, Han-Wistar and Long-Evans rats.

19

20 **2.4.2.1.3. Hochstein et al. (2001, [197544](#)).**

21 Adult female mink (12/treatment group) were administered dietary concentrations of
22 0.0006 (control), 0.016, 0.053, 0.180, or 1.40 ppb TCDD (purity >99.8%) for 132 days
23 (Hochstein et al., 2001, [197544](#)). This dose is estimated to be equivalent to 0.03 (control), 0.8,
24 2.65, 9, and 70 ng/kg-day assuming a food consumption of 5% of body weight per day. Females
25 were mated with unexposed males beginning on treatment day 35. Females were allowed to
26 mate every fourth day during a 29-day mating period or until a confirmed mating. Mated
27 females were presented with a second male either the day after initial mating or 8 days later. In
28 the 70 ng/kg-day group, the treated animals were lethargic after 4 to 5 weeks, with several
29 having bloody (tarry) stools near the end of the trial. Two animals in the 70 ng/kg-day dose
30 group died prior to study termination. These animals had lost a large percentage of their body
31 weight (24–43%), and had pale yellow livers and intestinal hemorrhages. Histopathology from

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1 both mink indicated marked diffuse hepatocellular vacuolation. The mean body weight
2 decreased in all treatment groups including the control (losing an average of 3.29% of initial
3 body weight), compared to a dose-dependent loss of up to 26% in the 70 ng/kg-day group.
4 Mating and reproduction were considered subnormal in all groups. The number of females that
5 gave birth in the 0.03 (control), 0.8, 2.65, 9, and 70 ng/kg-day dose groups were 5/12, 0/12, 3/12,
6 8/12, and 0/11, respectively. The study authors speculated that the subnormal breeding and
7 reproductive performances in the control females likely were due to the indoor environment in
8 which the mink were housed. In the three groups that gave birth, there was a dose-dependent
9 decrease in kit body weight at birth, which was significant ($p < 0.05$) in the 9 mg/kg-day group
10 compared to the controls. The body weight in the kits was not significantly different at 3 or
11 6 weeks after birth. Three-week survival rates of 71, 47, and 11% were recorded for kits in the
12 0.03 (control), 2.65, and 9 ng/kg-day dose groups, respectively. Six-week kit survival rates were
13 62, 29, and 11% in the 0.03 (control), 2.65, and 9 ng/kg-day dose groups, respectively.

14 In the adult females, clinical signs of toxicity were noted in the 70 ng/kg-day group near
15 the end of the study and included alopecia and notably thickened, deformed, and elongated
16 toenails. There was a dose-dependent decrease in plasma total solids, total protein, and
17 osmolality that reached statistical significance ($p < 0.05$) in the two highest exposure groups.
18 Anion gap was significantly decreased ($p < 0.05$) and alanine aminotranferase was significantly
19 increased in the 70 ng/kg-day group compared to the controls. At terminal sacrifice, there was a
20 dose-related decrease in body weight. There was a dose-related increase in liver weight that
21 reached statistical significance ($p < 0.05$) in the 70 ng/kg-day dose group. The brains of 42% of
22 the animals in the 70 ng/kg-day dose group had localized accumulation of lymphatic cells within
23 the meninges with mild extension into the adjacent neuropil and mild gliosis. Of the 10 mink
24 surviving to study termination in the 70 ng/kg-day group, 3 had periportal hepatocellular
25 vacuolation. These same brain and liver lesions were not observed in the control mink.

26 As there were no litters produced in the low-dose group and pregnancy outcomes were
27 not dose related, the 0.8 ng/kg-day exposure level does not inform the choice of NOAEL or
28 LOAEL. Thus, the LOAEL for this study is 2.65 ng/kg-day (132-day maternal exposure
29 duration) based on reduced kit survival (47% of control at 6 weeks). A NOAEL cannot be
30 determined for this study.

31

1 **2.4.2.1.4. *Hutt et al. (2008, [198268](#))*.**

2 Hutt et al. (2008, [198268](#)) conducted a 3-month study investigating changes in
3 morphology and morphogenesis of pre-implantation embryos as a result of chronic exposure to
4 TCDD in female rats. The study authors administered 0 or 50 ng/kg TCDD (>99% purity) in
5 corn oil via oral gavage to groups of 3 pregnant Sprague-Dawley rats on gestation days 14 and
6 21 and on postnatal days 7 and 14. The resulting female pups were divided into groups of 3 and
7 administered 0 or 50 ng/kg TCDD (>99% purity) in corn oil (equivalent TCDD doses of 0 and
8 7.14 ng/kg-day) on postnatal day 21 and weekly thereafter until they reached 3 months of age.
9 Pups were then mated, fertilization was verified, and pre-implantation embryos were harvested
10 4.5 days later. Pre-implantation embryos were examined using immunofluorescence microscopy
11 to determine blastomere abnormalities.

12 No significant difference as compared to the control in pre-implantation embryotoxicity
13 was observed following exposure to TCDD. Morphologically normal pre-implantation embryos
14 were significantly ($p < 0.05$) reduced in 50 ng/kg TCDD exposed rats (15 of 41, 36.6%)
15 compared to the control group (31 of 39, 79.5%). Pre-implantation embryos of TCDD-exposed
16 rats included irregularities in mitotic spindles (13 of 18 were monopolar), chromosome patterns
17 in metaphase, blastomere size and shape, blastomere nuclei shape in interphase, f-actin, and
18 cytokinesis. The study authors concluded that the compaction stage of pre-implantation
19 embryogenesis is the most sensitive following exposure to TCDD.

20 A LOAEL for this study is 50 ng/kg (7.14 ng/kg-day adjusted dose) for a significantly
21 ($p < 0.05$) lower proportion of morphologically normal pre-implantation embryos during
22 compaction stage in female Sprague-Dawley pups weekly for 3 months. A NOAEL cannot be
23 determined for this study.

24

25 **2.4.2.1.5. *Ikeda et al. (2005, [197834](#))*.**

26 Ikeda et al. (2005, [197834](#)) studied the effect of repeated TCDD exposure to F0 dams on
27 the male gonads of F1 generation and sex ratio in the F2 generation. Twelve female Holtzman
28 rats were treated with a single dose of 400 ng/kg TCDD ($\geq 98\%$ purity) orally, via gavage,
29 followed by weekly treatment doses of 80 ng/kg TCDD (16.5 ng/kg-day adjusted for continuous
30 exposure of 10 weeks; specified 2 weeks pre-mating, assumed 1 week for successful mating,
31 3 weeks of gestation, and specified 4 weeks to weaning) during mating, pregnancy, and

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1 lactational periods (total exposure duration approximately 10 weeks). Corn oil served as the
2 control in another group of 12 dams. Four dams were sacrificed on gestation day (GD) 20 to
3 evaluate the in utero toxicity of TCDD. Litter sizes from the remaining eight dams were
4 examined on postnatal day (PND) 2, and some of the F1 offspring were sacrificed to estimate
5 TCDD tissue concentrations. The remaining offspring were weaned on PND 28. Some of the F1
6 (number not specified) offspring were mated with untreated females on PND 98, following
7 which, litter size, sex ratio, weight, and anogenital distance of F2 pups were examined on
8 PND 2. Mated and unmated F1 males were sacrificed and the testes, epididymis, seminal
9 vesicle, and the ventral prostate were weighed; the cauda epididymis was weighed and examined
10 for sperm count.

11 All fetuses in the control and TCDD group as a result of in utero exposure in the
12 F0 generation survived. Litter size, sex ratio, and anogenital distance in the F1 generation on
13 PND 2 were not altered as a result of in utero TCDD exposure. Pup weight was significantly
14 ($p < 0.05$) lower in the TCDD-treated group than in controls. TCDD concentration in the
15 adipose tissue of the F0 dams on GD 20 was significantly ($p < 0.05$) higher than in the liver.
16 Adipose TCDD was significantly ($p < 0.01$) reduced at weaning, however, compared to
17 concentrations on GD 20. F1 pup liver TCDD concentration increased significantly ($p < 0.01$)
18 and was higher on PND 28 than PND2. The liver weight in F1 males increased by 14-fold at
19 PND 28 compared to PND 2, implying a transfer of approximately 850 pg of TCDD from the
20 dam to the F1 pup livers during lactation. TCDD also was detected in pup adipose tissue on
21 PND 28. Body weight of TCDD-exposed F1 males was significantly ($p < 0.001$) lower than
22 control males at weaning (PND 28). No significant differences in testis and cauda epididymis
23 weights were observed between the control and treated groups. Ventral prostate weight in the
24 F1 males exposed to TCDD, however, was approximately 60% lower than controls. No change
25 in weight of the body, brain, testes, cauda epididymis, or seminal vesicle was observed at
26 PND 120. Ventral prostate weight, however, was 16% lower than that of the control group
27 ($p < 0.001$). Sperm count in the cauda epididymis of the F1 males was not affected by TCDD
28 exposure.

29 Examination of F2 generation litters indicated no significant differences in litter size, pup
30 body weight, and anogenital distance between TCDD-treated or vehicle control groups. The
31 percentage of male F2 pups born to maternally and lactationally TCDD-exposed males was

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1 significantly ($p < 0.05$) lower (38%) than those sired by control group males (52%). Every
2 female mated with maternally TCDD-exposed F1 males delivered more female than male pups.

3 A LOAEL for TCDD of 16.5 ng/kg-day for an estimated 10 week exposure duration in
4 F0 rat dams is identified in this study for decreased development of the ventral prostate in the
5 F1 generation (60% lower than controls) and for significantly ($p < 0.05$) altered sex ratio
6 (decreased percentage of males) in the F2 generation. A NOAEL cannot be determined for this
7 study.

8

9 **2.4.2.1.6. *Ishihara et al. (2007, [197677](#))*.**

10 Ishihara et al. (2007, [197677](#)) examined the effect of repeated TCDD exposure of
11 F0 males on the sex ratio of F1 offspring. Seven-week-old male ICR mice ($n = 127$) were
12 divided into three groups and treated via gastric intubation with an initial loading dose of either 2
13 or 2,000 ng TCDD/kg BW or an equivalent volume of sesame oil (vehicle) as control, followed
14 by a weekly maintenance doses of 0, 0.4, or 400 ng/kg until the animals were 12 weeks old.
15 One week after the last exposure, the animals were mated with untreated female mice. On the
16 day a vaginal plug was identified, F0 male mice were sacrificed and major organs including
17 testes, epididymis, and liver were removed and weighed. Organ tissues also were examined for
18 histopathological and immunohistochemical changes. Treatment levels, averaged over the
19 6 week period from start of treatment to mating (five maintenance doses), were 0, 0.095, and
20 950 ng/kg-day for the control, low dose and high dose groups, respectively.

21 All TCDD-treated males successfully impregnated untreated females and yielded viable
22 offspring. Mortality, pup weights, and mating and fertility indices were not affected by TCDD
23 exposure. There were no significant differences in body weights or in relative weights of testes,
24 epididymis, or livers in the TCDD-treated F0 males compared to the control group. The livers of
25 some animals (number not specified) in the high-dose group, however, were larger and heavier
26 than in the controls or the low-dose group. Hence, tissues from the high-dose animals were
27 selected for detailed immunohistochemical examination.

28 General histopathological findings in the TCDD-treated groups showed no changes in
29 cell morphology in germ, Sertoli, and Leydig cells of the testes. Arrangement of the germ cells
30 was normal and there was no difference in the epididymis spermatozoon number in either of the
31 TCDD-treated groups compared to controls. Livers of some of the animals in the high-dose

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1 group however, showed enlarged and vacuolated areas in the centrilobular area when compared
2 to the low-dose group and the control group. Immunohistochemical and quantitative
3 immunohistological findings showed a marked increase in staining intensity for cytochrome
4 P450 (CYP)1A1 in the cytoplasm of the hepatocytes in the centrilobular area of the high-dose
5 TCDD group compared to the cells in the low-dose and the control groups. In addition,
6 proportions of immunoreactive CYP1A1 areas in the liver sections of the high-dose group were
7 higher than in the low-dose and control groups. The proportions of immunoreactive CYP1A1
8 also varied across animals ($n = 33$) in the high-dose group.

9 In addition to the above findings, there was a dose-related decrease in the male/female
10 sex ratio. The proportion of male offspring of the high-dose group was significantly lower
11 ($p < 0.05$) than that observed in controls (46.2% versus 53.1%, respectively). Hepatic
12 immunoreactive CYP1A1 staining levels in individual F0 males were strongly correlated with
13 the sex ratio of their offspring.

14 A LOAEL for TCDD of 950 ng/kg-day for a 6 week exposure duration of F0 male mice
15 is identified for significantly ($p < 0.05$) decreased male/female sex ratio (i.e., higher proportion
16 of female offspring) in the F1 generation. The NOAEL is 0.095 ng/kg-day.

17

18 **2.4.2.1.7. *Latchoumycandane and Mathur (2002, [197498](#)) (and related: *Latchoumycandane****
19 ***et al. (2002, [198365](#); 2002, [197839](#); 2003, [543746](#))).***

20 Latchoumycandane and Mathur (2002, [197498](#)) conducted a study to determine whether
21 treatment with vitamin E protected rat testes from TCDD-induced oxidative stress. Groups of
22 albino male Wistar rats ($n = 6$) were administered an oral dose of 0 (vehicle alone) 1, 10, or
23 100 ng TCDD/kg-day for 45 days, while another group of animals ($n = 6$) was co-administered
24 TCDD at the same doses, along with vitamin E at a therapeutic dose of 20 mg/kg-day for
25 45 days. At study termination, animals were fasted overnight, weighed, and sacrificed. Testis,
26 epididymis, seminal vesicles, and ventral prostate were removed, weighed, and preserved for
27 further examination. The left testis was used to determine daily sperm production, while the
28 right testis was used for biochemical studies. Superoxide dismutase, catalase, glutathione
29 reductase, and glutathione peroxidase activity were measured in the testes, along with production
30 of hydrogen peroxide and lipid peroxidation.

1 Body weights of TCDD-treated rats did not differ significantly from the control group.
2 Testis, epididymis, seminal vesicle, and ventral prostate weights in the TCDD-treated groups,
3 however, decreased significantly ($p < 0.05$) when compared to controls. None of these changes
4 were observed in the TCDD-exposed groups receiving vitamin E. There was a dose-related
5 decrease in daily sperm production ($p < 0.05$) in all three TCDD-treated groups when compared
6 to the control group. In contrast, the TCDD treatment groups that also received vitamin E did
7 not show any significant changes in daily sperm production compared to the controls. The
8 TCDD-treated groups also showed significantly ($p < 0.05$) lower activities of the antioxidant
9 enzymes (superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase) than
10 the control group. Levels of hydrogen peroxide and lipid peroxidation increased significantly
11 ($p < 0.05$) in the testes of the rats treated with TCDD compared to the corresponding controls.
12 The TCDD-treated groups that had been co-administered vitamin E show no difference in
13 antioxidant enzyme activities or in reactive oxygen species production when compared with
14 controls.

15 A LOAEL for TCDD of 1.0 ng/kg-day for a 45-day exposure duration in rats is identified
16 in this study for significantly ($p < 0.05$) reduced sperm production and significantly ($p < 0.05$)
17 decreased reproductive organ weights. A NOAEL cannot be determined for this study.

18
19 **2.4.2.1.8. Murray et al. (1979, [197983](#)).**

20 Male (10–16 per treatment) and female (20–32 per treatment) Sprague-Dawley rats were
21 administered diets containing TCDD (purity >99%) to achieve daily concentrations of 1, 10, or
22 100 ng/kg-day through three generations. After 90 days of treatment, F0 rats were mated to
23 produce F1a offspring. Thirty-three days after weaning of the last F1a litter, the F0 rats were
24 mated again to produce F1b offspring. Some F0 rats were mated a third time for a cross-mating
25 study. The F1b and F2 rats were mated at about 130 days of age to produce the F2 and
26 F3 generations. No clinical signs of toxicity or changes in body weight and food consumption
27 were observed in F0 rats during the 90 days of treatment before mating. The 100 ng/kg-day
28 group was discontinued due to the lack of offspring. In the three surviving offspring (all males),
29 no changes in appearance, body weight, or food consumption occurred. A dose of 10 ng/kg-day
30 caused a consistent decreased body weight in both sexes of F1 and F2 rats, which was associated
31 with decreased food consumption. A significant ($p < 0.05$) decrease in fertility in F1 and F2 rats

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1 occurred, but not in F0 rats, administered 10 ng/kg-day. The number of live pups and gestational
2 survival index were significantly ($p < 0.05$) decreased in the 100 ng/kg-day F0 rats and in the
3 10 ng/kg-day F1 and F2 rats. The gestational survival index also was significantly ($p < 0.05$)
4 decreased in F2 rats administered 1 ng/kg-day. Postnatal survival was significantly ($p < 0.05$)
5 reduced only in F2 rats administered 10 ng/kg-day. Growth (as measured by body weight) was
6 affected at 10 ng/kg-day only in the third generation. In the 10 ng/kg-day group, a significant
7 ($p < 0.05$) decrease in relative thymus weight and increase in liver weight also occurred in F₃ rats
8 (weights were not measured in F2 rats). Additionally, mating 100 ng/kg-day TCDD-treated
9 females with untreated males increased the percent of implants resorbed as assessed by uterine
10 histopathology.

11 The reproductive LOAEL is 10 ng/kg-day, based on a significant ($p < 0.05$) decrease in
12 fertility (33–37% lower than controls); decrease in the number of live pups (18–27% lower than
13 controls); decrease in gestational survival (10–11% lower than controls); decrease in postnatal
14 survival (32% lower than controls); and decreased postnatal body weight (14–19% lower than
15 controls at weaning) in one or more generations. The reproductive NOAEL is 1 ng/kg-day.

16

17 **2.4.2.1.9. Rier et al. (1993, [199987](#); 1995, [198566](#)).**

18 Reir et al. (1993, [199987](#); 1995, [198566](#)) examined the impact of chronic TCDD
19 exposure on endometriosis in monkeys. Female rhesus monkeys (eight animals per treatment
20 group) were exposed to 0, 5, or 25 ppt TCDD (purity not specified) in feed for 4 years.
21 Previously, Bowman et al. (1989, [543745](#)) determined that these dietary concentrations were
22 equivalent to 0, 0.15, and 0.67 ng/kg-day, respectively. Ten years after termination of TCDD
23 treatment, the presence of endometriosis was determined via laparoscopic surgical procedure,
24 and the severity of the disease was assessed. The study authors reported that three monkeys in
25 the 0.67 ng/kg-day exposure group died at 7, 9, and 10 years after termination of TCDD
26 treatment. Autopsy results attributed the deaths to widespread and severe peritoneal
27 endometriosis (all three monkeys) along with obstruction of the colon (one monkey) and
28 blockage of the jejunum (one monkey). Other deaths also occurred in the control group (1 death
29 from birthing complications and another from an unknown cause); in the 0.15 ng/kg-day dose
30 group (1 death due to natural causes with no endometriosis), and in the 0.67 ng/kg-day dose
31 group (1 death due to a breeding fight with no incidence of endometriosis). At study

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1 termination, 17 live animals plus the 3 that had previously died of endometriosis were evaluated
2 (total $n = 20$).

3 Incidence of endometriosis was significantly ($p < 0.05$) higher than in the control group
4 with 71 and 86 % incidence rates in the 0.15 and 0.67 ng/kg-day dose groups, respectively,
5 compared to 33% in the control group. Severity of endometriosis was also significantly
6 ($p < 0.001$) correlated with TCDD dose. Staging by rAFS indicated that untreated control
7 animals had either minimal or no incidence of endometriosis. In comparison, endometriosis was
8 absent in 2 of the 7 monkeys in the 0.15 ng/kg-day dose group, while only 1 of the 7 animals in
9 the high dose group was disease free. Moderate-to-severe disease was observed in 3 of the
10 7 animals in the 0.15 ng/kg-day dose group and 5 of the 7 animals in the 0.67 ng/kg-day dose
11 group. Moderate-to-severe disease was not observed in the control group. The authors also
12 compared the incidence and severity of endometriosis in TCDD-exposed animals with
13 304 normal, non-neutered females with no dioxin exposure and reported that the disease was not
14 present in monkeys that were less than 13 years of age, while the disease rate was 30% among
15 animals 13 years of age or older. The study authors report that these findings are in agreement
16 with human and rhesus studies demonstrating that the prevalence of detectable endometriosis can
17 increase with advanced age.

18 As noted previously, in a follow-up study, Rier et al. (2001, [198776](#)) examined the DLC
19 levels of sera collected from some monkeys in this study. They reported that animals in this
20 study had elevated serum PCB77 and PCB126 levels and an increased serum TEQ; the fractional
21 contribution of serum TCDD levels to total serum TEQ was 30% in treated animals. They also
22 reported that the severity of the endometriosis corresponded to the serum PCB77 concentrations
23 rather than total TCDD. In this study, it is not possible to determine the contribution of TCDD
24 alone to the endometriosis due to the background contamination; thus, EPA has not developed a
25 TCDD LOAEL from the study.

26

27 **2.4.2.1.10. Shi et al. (2007, [198147](#)).**

28 Pregnant Sprague-Dawley rat dams (3 per treatment group) were administered 0, 1, 5, 50,
29 or 200 ng/kg TCDD (purity >99%) in corn oil by gavage on GD 14 and GD 21 and on PND 7
30 and PND 14 for lactational exposure to pups (Shi et al., 2007, [198147](#)). Ten female pups per
31 treatment were selected and administered TCDD weekly at the same dose levels through their

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1 reproductive lifespan (approximately 11 months). The corresponding equivalent daily TCDD
2 doses are 0, 0.14, 0.71, 7.14, and 28.6 ng/kg-day. Vaginal opening was slightly but significantly
3 ($p < 0.05$) delayed in 28.6 ng/kg-day females. Vaginal opening was also delayed, but not
4 significantly, in the 0.14 and 7.14 ng/kg-day groups. Reproductive senescence with normal
5 cyclicity was significantly ($p < 0.05$) accelerated beginning at 9 months in 7.14 and
6 28.6 ng/kg-day females. Serum estradiol concentrations were decreased at all time points across
7 the estrous cycle in a dose-dependent manner with a statistically significant decrease ($p < 0.05$)
8 in all but the lowest dose group. TCDD exposure, however, did not affect the number or size
9 distribution of ovarian follicles; responsiveness of the pituitary gland to gonadotropin-releasing
10 hormone, or serum profiles of FSH, LH, or progesterone.

11 A LOAEL for TCDD of 0.71 ng/kg-day for an 11-month exposure duration was
12 identified in this study based on significantly ($p < 0.05$) decreased estradiol levels in offspring.
13 The NOAEL for this study is 0.14 ng/kg-day.

14

15 **2.4.2.1.11. Yang et al. (2000, [198590](#)).**

16 Yang et al. (2000, [198590](#)) studied the impact of TCDD exposure on the incidence and
17 severity of endometriosis in female rhesus monkeys. Groups of 7- to 10-year old nulliparous
18 cynomolgus monkeys were treated with 0 ($n = 5$), 1, 5, or 25 ($n = 6$ per group) ng/kg BW TCDD
19 5 days per week via gelatin capsules for 12 months. Because the monkeys received one capsule
20 5 days per week, the doses adjusted for continuous exposure were 0, 0.71, 3.57, and
21 17.86 ng/kg-day. Prior to TCDD administration, all animals had endometriosis induced during
22 days 12–14 of the menstrual cycle by auto-transplantation of endometrial-strips in multiple
23 abdominal sites. All TCDD-treated and control groups were laparoscopically examined during
24 months 1, 3, and 6 to monitor the survival of endometrial implantations and to obtain peritoneal
25 fluid to determine the concentration and immunotype of endometrial growth regulator cytokines
26 interleukin-6 (IL-6) and interleukin-6 soluble receptor (IL-6sR). Because insufficient peritoneal
27 fluids were present in the treated and control monkeys, however, the study authors collected
28 blood samples at 6 and 12 months during laparoscopy for routine hematology and to assess the
29 circulating levels of IL-6 and IL-6sR. All animals were sacrificed at 12 months, and circulating
30 levels of gonadal steroids also were measured at the time of necropsy.

1 No changes were observed among treatment levels in general toxicological endpoints
2 such as body weight changes, food consumption, hematological endpoints, general activity
3 levels, and caretaker interaction. In addition, TCDD did not impact circulating levels of gonadal
4 steroids measured during necropsy. Similarly, there were no differences in the number of
5 menstrual cycles, the length of the menstrual cycle, and bleeding intervals. Endometrial implants
6 were found in at least one site in all TCDD-treated and control monkeys during the
7 first laparoscopic examination. Follow-up laparoscopies revealed that there was a continuous
8 loss of endometrial implants over time in each dose group. At the 1-, 3-, and 6-month
9 examination, the number of endometrial losses was not significantly different among different
10 dose groups. At the 12-month examination, however, a significantly ($p < 0.05$) higher rate of
11 survival of endometrial implants was observed in the 3.57 and 17.86 ng/kg-day dose groups
12 compared to the control group. The highest rate of endometrial implant survival was observed in
13 the ovaries regardless of the dose group. In contrast, all lesions disappeared from the left broad
14 ligament, whereas two on the right broad ligament and one on the uterine fundus survived.
15 There was a dose-dependent divergence in the growth response of endometrial implants
16 following TCDD exposure. Both the maximum and minimum implant diameters in the
17 17.86 ng/kg-day dose group were significantly ($p < 0.05$) larger compared to controls. In
18 contrast, the maximum and minimum implant diameters in the 0.71 ng/kg-day dose group were
19 significantly ($p < 0.05$) smaller compared to controls. TCDD did not impact implant diameters
20 in the 3.57 ng/kg-day dose group when compared to controls. Histological examinations
21 revealed that endometrial glands and stromal cells were present in all surviving implants.
22 Sections examined in the 17.86 ng/kg-day of TCDD possessed cystic endometrial glands that
23 were more frequently observed in this dose group compared to other groups including controls.
24 In addition, circulating levels of IL-6 were significantly ($p < 0.05$) lower in monkeys exposed to
25 17.86 ng/kg-day TCDD both at 6 and 12 months compared to the control group. In contrast,
26 circulating levels of IL-6sR were significantly ($p < 0.05$) higher in animals treated with 3.57 and
27 17.86 ng/kg-day TCDD at 6 months, while the levels were higher only in the 17.86 ng/kg-day
28 TCDD group at 12 months.

29 A LOAEL for TCDD of 17.86 ng/kg-day for a 1 year exposure duration was identified in
30 this study for significantly ($p < 0.05$) increased endometriosis induced by endometrial implant
31 survival, significantly ($p < 0.05$) increased maximum and minimum implant diameters, and

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1 growth regulatory cytokine dysregulation (as assessed by significantly decreased IL-6 levels,
2 $p < 0.05$). A NOAEL of 3.57 ng/kg-day is identified in this study.

3 4 **2.4.2.2. Developmental Studies**

5 **2.4.2.2.1. Amin et al. (2000, [197169](#)).**

6 Amin et al. (2000, [197169](#)) studied the impact of in-utero TCDD exposure on the
7 reproductive behavior in male pups. Groups of pregnant Harlan Sprague-Dawley rats ($n = 108$
8 divided into 4 cohorts; number of animals in the TCDD treatment group is ~3 per dose group)
9 were dosed via gavage with 0, 25, or 100 ng/kg-day TCDD (purity >98%) in corn oil on GDs
10 10–16. On the day of birth (PND 0), pups were examined for gross abnormalities and the
11 number of live pups, their weights, and sex were recorded from each litter. Litters consisting of
12 more than eight pups were reduced to eight, comprised of four males and four females when
13 possible. Litters consisting of fewer than five pups were excluded from the study to minimize
14 between-litter differences in growth rate, maternal behavior, and lactational exposure. After this
15 exclusion, approximately 10 to 11 litters per exposure group remained. All pups were weaned
16 on day 21 and one male and one female were retained to assess reproductive development, play
17 behavior, reproductive behavior, and saccharin preference behavior. Both male and female pups
18 were tested for saccharin preference between 189 and 234 days of age. A saccharin preference
19 test was conducted for 8 days. For the first 4 days, rats were provided bottles containing tap
20 water, and on days 5 and 6 the animals were provided a bottle containing water and a bottle
21 containing 0.25% saccharin solution. On days 7 and 8, the animals were provided water and a
22 bottle containing 0.50% of saccharin solution. A 0.50% saccharin solution was used because
23 previous studies have reported that male rats exhibited a greater reduction in preference for this
24 saccharin concentration compared to females, hence the sex difference in preference is more
25 marked at this saccharine dose.

26 None of the treated dams exhibited any signs of toxicity as a result of exposure to TCDD.
27 Gestational body weight, liver weight, litter size and percent live births were all comparable to
28 the corresponding control group. Birth rate and weaning weight of the pups also were not
29 affected by TCDD exposure. Sex-related water consumption, however, was significantly
30 ($p < 0.001$) affected during the first 4 days with female pups drinking more water per 100 g of
31 body weight compared to the respective male counterparts. Saccharin consumption was

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1 significantly ($p < 0.001$) affected, with females consuming greater amounts of saccharin solution
2 per 100 g body weight compared to the corresponding males. Additionally, both male and
3 female pups drank significantly ($p < 0.001$) more of the 0.25% saccharin solution compared to
4 the 0.50% saccharin solution. Females of all exposure groups consumed less of both the 0.25
5 and 0.50% saccharin solution compared to the same-sex control group. Comparisons of each
6 exposure group to the control group indicated that only the high TCDD exposure group
7 (100 ng/kg-day) differed significantly ($p < 0.05$) compared to control in the consumption of
8 0.25% saccharin solution. In contrast, for the 0.50% saccharin solution, both the low and high
9 TCDD dose groups differed significantly ($p < 0.05$ and $p < 0.01$, respectively) compared to the
10 control group. The saccharin preference of TCDD-exposed male rats did not differ from that of
11 the male control group. The TCDD-exposed females' preference for saccharin solution,
12 however, was significantly reduced in both the 25 ($p < 0.05$) and the 100 ng/kg-day ($p < 0.005$)
13 dose group compared to that of the female controls. The study authors state that the reduction in
14 saccharin consumption and preference in females could be due to the anti-estrogenic action of
15 TCDD and that recent research reports suggest that TCDD can decrease the level of estrogen
16 receptor (ER) mRNA by blocking the ability of ER to transactivate from the estrogen response
17 element.

18 A LOAEL for TCDD of 25 ng/kg-day for 7 days of gestational exposure is identified for
19 significantly ($p < 0.05$) decreased preference in the consumption of 0.25% saccharin solution. A
20 NOAEL cannot be determined for this study.

21

22 **2.4.2.2.2. Bell et al. (2007, [197041](#)).**

23 Bell et al. (2007, [197041](#)) examined the reproductive effects of TCDD in rats exposed
24 during development. Female CRL:WI (Han) rats were treated with TCDD (99% purity;
25 dissolved in acetone) in the diet at concentrations of 0 (acetone alone; $n = 75$), 28, 93, or
26 530 ($n = 65$ /group) ng TCDD/kg diet, which provided average doses of 0, 2.4, 8, or
27 46 ng/kg-day, respectively. Rats were exposed to TCDD 12 weeks prior to mating, during
28 mating, and through pregnancy. Dams were switched to the control diet after parturition. Litters
29 from pregnant dams were reduced to a maximum size of eight on PND 4 and to five males (if
30 possible) on PND 21. These males were left untreated until sacrificed (25/group, one/litter) on
31 PND 70, while all remaining animals were sacrificed on PND 120. All sacrificed animals were

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1 necropsied and received a seminology examination. Prior to sacrifice, during weeks 12 and 13,
2 20 animals from each dose group were tested for learning ability and motor activity, and were
3 also administered a functional observation battery. During postnatal week 16, groups of 20 male
4 F1 rats from each treatment group were paired with untreated virgin females for 7 days, and
5 mated females were killed on GD 16 and examined for terminal body weights, pregnancy status,
6 number of corpora lutea, and number of intrauterine implantations.

7 The study authors found no evidence of direct maternal toxicity from exposure to TCDD.
8 In the high-dose groups, 8 of 27 dams suffered complete litter loss compared to 3 dams in the
9 control group, but the difference was not statistically significant. Pup survival at PND 4 was also
10 lower in the high-dose group, but the difference again was not statistically significant.

11 A dose-related decrease in mean pup body weight was observed on PND 1, and this trend
12 continued throughout the lactation period. High-dose male pups had lower body weights when
13 compared to controls at PND 21, with this trend continuing over the course of the study.
14 Balanopreputial separation (BPS) was significantly ($p < 0.05$) delayed compared to controls in
15 all three treatment groups by 1.8, 1.9, and 4.4 days in the low-, medium-, and high-dose groups,
16 respectively. The study authors reported that adjustment for lower body weights observed at
17 PND 21 and PND 42 did not affect the estimate of delay in BPS. No adverse effects from
18 maternal treatment were observed on learning or in functional observational battery performance.
19 Offspring in the high-dose group exhibited less activity when compared to controls ($p < 0.05$)
20 when they were subjected to a test of motor activity for 30 minutes.

21 The median precoital time was 2–3 days for all 20 F1 males that were mated during
22 postnatal week 16. The uterine and implantation data were similar in all dose groups and there
23 were no significant differences in the proportion of male offspring between groups. Epididymal
24 sperm counts and sperm motility did not differ significantly between dose groups in animals
25 sacrificed during postnatal week 10. The mean number of spermatids was significantly lower
26 (14%; $p < 0.05$) and the proportion of abnormal sperm was significantly ($p < 0.05$) higher in the
27 high-dose group when compared to controls on PND 70. These effects, however, were not seen
28 in animals sacrificed on PND 120.

29 Terminal body weights were significantly ($p < 0.05$) decreased in the high-dose group
30 (6.9 %) compared to controls on PND 120, while the depression in body weight in the
31 medium-dose group (5.5%) was not statistically significant. At PND 70, the relative and

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1 absolute testis weight of the high-dose group was less than the controls (12 and 18%,
2 respectively). Absolute spleen weight in the high-dose group was significantly higher (8%) on
3 PND 70, and increased significantly ($p < 0.05$) by 1–3% on PND 120 in all dose groups
4 compared to controls. Kidney weight in the low and medium-dose groups was significantly
5 ($p < 0.05$) greater than in controls (~2%) at PND 120. In addition to these organs, ventral
6 prostate (9.4%) and relative liver (~4.5%) weights were significantly ($p < 0.05$) higher than
7 controls on PND 120 in the medium- and low- and high-dose groups, respectively. On
8 PND 120, absolute brain weight was significantly ($p < 0.05$) less than the control in the
9 medium-dose group, while relative brain weight was significantly ($p < 0.05$) higher than the
10 control in the low- and high-dose group. Histological examination revealed no unusual findings.

11 A LOAEL for TCDD of 2.4 ng/kg-day following an estimated 17 week exposure duration
12 of dams was identified in this study for significantly ($p < 0.05$) delayed BPS. A NOAEL was not
13 identified in this study.

14

15 **2.4.2.2.3. *Franczak et al. (2006, [197354](#))*.**

16 Franczak et al. (2006, [197354](#)) examined the impact of chronic TCDD exposure on the
17 onset of reproductive senescence in female rats. Pregnant Sprague-Dawley rats
18 ($n = 2$ -3/dose group) were fed 50 or 200 ng/kg TCDD (>99% purity) or corn oil vehicle
19 (4 mL/kg) orally on GD 14 and 21 and PND 7 and 14 to provide in utero and lactational
20 exposure to TCDD. On PND 21, female pups ($n = 7$ /dose group) were weaned and were
21 subsequently given weekly doses of 50 or 200 ng/kg-week TCDD by gavage (7.14 or
22 28.6 ng/kg-day adjusted for continuous exposure; administered doses divided by 7) or corn oil
23 vehicle. Exposure continued for up to 8 months, and animals were observed for changes in
24 estrus cycle at 4, 6, and 8 months. Rats were sacrificed at 8 months of age when the
25 TCDD-treated animals had entered the transition to reproductive senescence. Following
26 sacrifice, diestrus concentrations of serum LH, FSH, progesterone, and estradiol were measured,
27 and the ovaries were collected for examination.

28 Estrus cycles at 4 months exhibited normal cyclicity in both TCDD-exposed groups and
29 did not differ significantly from the control group. At 6 months, however, there was a tendency
30 ($p < 0.1$) toward loss of normal estrus cyclicity in animals treated with TCDD. At the 8 month
31 observation, estrus cyclicity was significantly ($p < 0.05$) different in both dioxin-exposed groups

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1 compared to controls (cumulative TCDD exposure is reported as 1.7 and 8 µg/kg for the 50 and
2 200 ng/kg dose groups, respectively). The study authors noted that although the low-dose
3 animals showed an increased prevalence of prolonged cycles, persistent estrus or diestrus was
4 observed in only 10% of the rats. Conversely, approximately 50% of the rats exhibited loss of
5 cyclicity in the high-dose group. There were no changes in the number and size distribution of
6 ovarian follicles or the number of corpora lutea at either dose. Progesterone levels at 8 months
7 tended to be higher ($p < 0.08$) in animals receiving either 7.14 or 28.6 ng/kg-day TCDD
8 compared to controls, while serum estradiol concentrations were significantly ($p < 0.03$) lower at
9 diestrus. Serum LH levels in TCDD-treated animals were comparable to those in the control
10 group, while FSH levels were elevated in rats receiving 7.14 ng/kg-day TCDD, but not in the
11 28.6 ng/kg-day dose group.

12 A LOAEL for TCDD of 7.14 ng/kg-day for an 8-month exposure duration was identified
13 for significantly ($p < 0.03$) decreased serum estradiol levels. A NOAEL cannot be determined
14 for this study.

15

16 2.4.2.2.4. *Hojo et al. (2002, [198785](#)) (and related: Zareba et al. (2002, [197567](#))).*

17 Hojo et al. (2002, [198785](#)) studied the impact of prenatal exposure to TCDD on sexually
18 dimorphic behavior in rats. Thirty-six pregnant Sprague-Dawley rats were assigned according to
19 a randomized block design to groups receiving 0, 20, 60, or 180 ng/kg TCDD (98% purity) on
20 GD 8. Litters from pregnant dams were culled to 5 females and 5 males on PND 4 and allowed
21 to wean normally, at which time 5, 5, 6, and 5 litters from the 0, 20, 60, and 180 ng/kg TCDD
22 treatment groups, respectively, were maintained for examination of behavioral response.
23 Offspring were exposed to TCDD (from a single maternal exposure) for about 35 days through
24 gestation and lactation. After weaning at PND 21, offspring were fed ad libitum until PND 80, at
25 which time a fixed amount of food was supplied daily to maintain constant body weights. At
26 90 days old, the rats in these treatment groups were trained to press a lever to obtain food pellets
27 using two operant behavior procedures. Initially, each lever press was reinforced. The
28 fixed-ratio (FR) requirement was then increased every fourth session from the initial setting of 1
29 to values between 6 and 71. The responses for 30 days were studied under a multiple schedule
30 combining FR 11 and another schedule requiring a pause of at least 10 sec between responses
31 (differential reinforcement of low rate, or DRL 10-sec)

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1 Pup and dam body weights were not affected by TCDD exposure, and all pups were
2 successfully trained in the lever-press response within 3–4 days. Analyses of the FR procedure
3 data indicated that the male pups responded at a lower rate at all TCDD doses when compared to
4 the control group. In case of female pups, all TCDD-treated groups responded at a higher rate
5 than controls. None of these results were, by themselves, however, statistically significant.
6 Examination of the FR 11 and DRL 10-second data indicated that when considering the FR
7 component of this multiple procedure, males from all three treatment groups responded at lower
8 rates when compared to the controls. Conversely, all female pups responded at a higher rate than
9 controls. In addition, the treatment-by-sex interaction was significant ($p = 0.036$), with the
10 60 ng/kg female pups responding at a higher rate than the 60-ng/kg male pups. Examination of
11 the delayed response component in the multiple FR 11 and DRL 10-sec procedures indicated that
12 almost all TCDD treatment groups were affected. Like the FR component, male pups at all
13 TCDD dose groups responded at a lower rate compared to controls, while female pups at all dose
14 groups responded at a higher rate than controls. There was also a significant ($p = 0.001$)
15 sex-by-treatment interaction for the DRL 10-sec similar to the FR component. Following
16 behavioral testing, the animals were sacrificed and cortical depth measurements were taken in
17 selected right and left brain regions. Reduced cortical thickness and altered brain morphometry
18 were observed in both male and female offspring in the 180-ng/kg exposure group when
19 compared to controls (reported in a separate article; Zareba et al., 2002, [197567](#)).

20 A nominal LOAEL for TCDD of 20 ng/kg for a single exposure on GD 8 is established
21 for this study based on abrogation of sexually dimorphic neurobehavioral responses. A NOAEL
22 cannot be derived for this study.

23

24 **2.4.2.2.5. Kattainen et al. (2001, [198952](#)).**

25 Pregnant Line A, B, and C rats derived from Han/Wistar and Long-Evans rats
26 (4–8 pregnant dams/strain/treatment group) were administered a single gavage dose of 0, 30,
27 100, 300, or 1,000 ng/kg TCDD (purity >99%) in corn oil on GD 15 (Kattainen et al., 2001,
28 [198952](#)). On PND 1, the litters were culled to three males and three females. Offspring were
29 weaned on PND 28. Female pups were sacrificed on PND 35 and male pups were sacrificed on
30 PND 70. TCDD treatment did not affect body weight or cause clinical signs of toxicity in the
31 dams. In Line B offspring, body weights in the 1,000 ng/kg group were slightly decreased

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1 during PND 1–7, while Line C offspring had slightly decreased body weights throughout the
2 study period (data were not provided). The development of the third molar was affected the
3 most in Line C offspring. In 5 of 10 Line C females and 6 of 10 Line C males treated with
4 1,000 ng/kg TCDD, the lower third molar did not develop. In comparison, 1 of 19 Line A
5 females and 1 of 18 Line B females administered 1,000 ng/kg TCDD lacked the third molar at
6 sacrifice. Third molars were present in all the controls and all male Line A and B offspring
7 administered 1,000 ng/kg. Due to the lack of eruption of the third molar in the majority of
8 Line B and C control females (only 30% erupted), however, the effects of TCDD on third molar
9 eruption could only be evaluated in Line A female offspring (with 94% eruption). There was a
10 dose-dependent decrease in the eruption of the lower third molar in Line A female offspring with
11 a significant ($p < 0.05$) decrease observed in the 300 and 1,000 ng/kg dose groups. In the male
12 offspring, any third molar that developed erupted by PND 70. The mesiodistal length of the
13 existing lower third molar was reduced in a dose-dependent manner in both genders of all
14 three rat lines. In Line A and C females, the decrease was significant ($p < 0.05$) at all doses. The
15 size of the second molars was also significantly decreased with 1,000 ng/kg ($p < 0.05$) in all but
16 Line C males.

17 A developmental LOAEL for TCDD of 30 ng/kg for maternal exposure on GD 15 is
18 established for this study, based on impaired tooth development (significantly reduced
19 mesiodistal length of the lower third molar by approximately 12% to 38% [$p < 0.05$]). A
20 NOAEL could not be determined.

21

22 **2.4.2.2.6. Keller et al. (2007, [198526](#); 2008, [198531](#); 2008, [198033](#)).**

23 Keller et al. (2007, [198526](#); 2008, [198531](#); 2008, [198033](#)) conducted three separate
24 experiments to assess the impact of TCDD on molar tooth development using different mouse
25 strains. In Experiment 1, Keller et al. (2007, [198526](#)) used six inbred mouse strains (C57BL/6J,
26 BALB/cByJ, A/J, CBA/J, C3H/HeJ, and C57BL/10J) known to possess high affinity ligand-
27 binding aryl hydrocarbon receptor alleles (*b*), two with *b1* alleles (C57BL/6J and CBA/J), and
28 four with *b2* alleles (BALB/cByJ, A/J, C3H/HeJ, and CBA/J). Females (number not specified)
29 from each strain were mated with males of the same strain. On GD 13, each pregnant female
30 was assigned to one of the four dose groups and treated with 0, 10, 100, or 1,000 ng TCDD/kg
31 BW via oral gavage. The control group received corn oil. GD 13 was chosen for dosing because

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1 the first morphological signs of tooth development occur on GD 11. The first visible signs of the
2 M1 (molar) occur on GDs 13–14 followed by final cuspal morphology, which is determined on
3 GD 15. The F1 offspring of females from each strain were weaned and separated by sex at PND
4 28 and were euthanized at PND 70. Each F1 mouse was examined for the presence or absence
5 of both maxillary (M^3) and mandibular third molars (M_3) on both the left and right sides. In
6 addition, all mice were scored as either normal or variant in M_1 morphology for both molar rows.

7 In Experiment 2 (Keller et al., 2008, [198531](#)), dams from six inbred mouse strains
8 (C57BL/6J, BALB/cByJ, A/J, CBA/J, C3H/HeJ, and C57BL/10J) were orally dosed on GD 13
9 with 0, 10, 100, or 1,000 ng TCDD/kg BW in corn oil. GD 13 was used as the dosing day
10 because it coincided with the formation of Meckel's cartilage (a major signal center) in the
11 mouse mandible that is followed shortly by intramembranous bone formation on GD 15. The
12 A/J mouse strain was abandoned because the authors had difficulty rearing the offspring from
13 this strain. All offspring ($n = 4$ or 5 per treatment group) from the remaining strains were
14 euthanized at 70 days of age. Mandible size and shape from all selected offspring were
15 examined using geometric morphometric methods to assess the impact of TCDD exposure.

16 In Experiment 3 (Keller et al., 2008, [198033](#)), dams from six inbred mouse strains
17 (C57BL/6J, BALB/cByJ, A/J, C3H/HeJ, CBA/J, and C57BL/10J) were treated with a single oral
18 dose of 0, 10, 100, or 1,000 ng TCDD/kg-BW in corn oil. GD 13 was chosen as the dosing day
19 because the first visible signs of the first molar (M_1) occurs on GDs 13–14 and the final cuspal
20 morphology (the pattern of projections on the chewing surface of the tooth) is not determined
21 until after GD 15. Similar to Experiment 2, the A/J mouse strain was abandoned due to
22 difficulty in rearing offspring. All offspring ($n = 107$ – 110 in each of the five strains for all
23 treatment groups) were euthanized at 70 days of age and their molar size, shape, and asymmetry
24 traits were examined using geometric morphometric methods.

25 In Experiment 1, all four M_3 s were present in all dose groups in mice from C57BL/6J,
26 BALB/cByJ, and C57BL/10J strains. A similar response was observed in the A/J strain mice
27 with only 3 of 51 F1 mice exhibiting missing third molars. Approximately one-third of the mice
28 from the CBA/J and C3H/HeJ strains, however, were missing at least one M^3 or M_3 molar. The
29 numbers of CBA/J mice missing one or both M_3 or M^3 molars were 0/29, 2/21, 6/29, and 30/30
30 in the 0, 10, 100, and 1,000 ng/kg groups, respectively. In the C3H/HeJ animals, the numbers
31 missing one or both molars were 1/24, 3/28, 1/26, and 30/36, respectively.

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1 Maternal TCDD exposure was also found to affect the frequency of M₁ variants, but only
2 in the C57BL/10J strain, and the dose-response relationship was nonmonotonic. The proportions
3 of variants observed in the 0, 10, 100, and 1,000 ng/kg dose groups were 33, 68, 59, and 58%,
4 respectively.

5 A LOAEL for TCDD of 10 ng/kg maternal exposure on GD 13 is identified for this study
6 for increased incidence (33%) of the M₁ variant in the C57BL/10J mouse strain. A NOAEL
7 cannot be determined in this study.

8 In Experiment 2 TCDD exposure of dams did not affect offspring survival or 10-week
9 body weight in any of the inbred mouse strains used. Analysis of variance (ANOVA) indicated
10 that although mandible size in both male and female offspring varied significantly ($p < 0.0001$)
11 among strains, it was not affected by TCDD exposure. In contrast, analysis of covariance
12 indicated that TCDD exposure significantly ($p = 0.0033$) decreased the mandible size in male
13 offspring in the C3H/HeJ strain at all treatment groups. The mean mandible size was similar
14 across all treatment groups in both sexes in all strains with male offspring exhibiting larger
15 mandibles compared to females. Males in the C3H/HeJ strain exhibited a significant (level not
16 reported) downward trend in mandible size throughout all treatment groups. Females in the
17 C3H strain also showed a similar trend in mandible size, but the trend was not significant.
18 ANOVA on mandible shape indicated that males had significantly ($p < 0.0001$) different
19 mandible shape in strain \times treatment groups. In contrast, in female offspring, although the
20 mandible shape was significantly ($p < 0.0001$) different due to strains, treatment groups, and
21 litter, the strain \times treatment interaction was not significant. Male offspring from the C3H/HeJ
22 and C57BL/6J mouse strains appear to be more sensitive to TCDD than BALB/cByJ or
23 CBA/J mice, with the C57BL/10J strain exhibiting intermediate sensitivity. In addition to these
24 analyses, Procrustes distance analysis also indicated that C3H/HeJ mice had the greatest
25 response to the highest dose of TCDD, followed by the C57BL/6J strain. Female offspring in the
26 C3H/HeJ and C57BL/6J strains also exhibited the largest change in Procrustes distance with
27 TCDD exposure. This trend, however, was not statistically significant ($p = 0.29$).

28 A LOAEL for TCDD of 10 ng/kg maternal exposure on GD 13 was identified for this
29 study for significantly ($p = 0.0033$) decreased mandible shape and size in male C3H/HeJ mice.
30 A NOAEL cannot be determined in this study.

1 In Experiment 3, effect of TCDD exposure on offspring survival or body weight was not
2 reported. Three-way ANOVA results showed significant ($p < 0.0001$) differences in molar size
3 among strains, sexes, and litters, but not between treatment groups. Molar size difference in
4 sex \times strain interaction was significant ($p = 0.03$), whereas differences in sex \times treatment and
5 sex \times strain \times treatment were not significant. Additionally, molar size in treatment \times strain
6 interaction also was not statistically significant. Based on these results, the authors reported that
7 molar size varied significantly ($p < 0.0001$) among all five strains tested, with all strains
8 exhibiting similar trends in all four treatment groups. Strain differences in molar size were more
9 apparent in male offspring. A hormesis-like trend in molar size was observed in all strains
10 (except in BALBc/ByJ) and sexes with an increase at the 100 ng/kg dose and a decrease in the
11 1,000 ng/kg dose. In addition to lack of difference in molar size for all treatment groups in all
12 strains, fluctuating asymmetry in molar size also did not increase with increasing doses of
13 TCDD.

14 In contrast to these results on molar size, the Procrustes ANOVA indicated that molar
15 shape was significantly ($p < 0.0001$) affected by strain, sex, treatment, and litter size. Molar
16 shape in sex \times strain and sex \times strain \times treatment interactions was also highly significant
17 ($p < 0.0001$). Based on these results, the authors concluded that differences between males and
18 females varied based on the strain, and that the effect of TCDD exposure on each strain also
19 differed for male and female offspring. Because molar shape in treatment \times strain interaction
20 was significant ($p < 0.0001$), differences in molar shape between the three treatment groups and
21 the control group were analyzed for each strain using nonorthogonal contrasts. In male
22 offspring, contrasts between the control group and 1,000 ng/kg were statistically significant only
23 in the C3H/HeJ ($p < 0.0001$) and CBA/J ($p < 0.03$) strains. These results suggest that these
24 two strains are most susceptible to TCDD effect on molar shape, and similar results were
25 observed in female offspring of these two strains. The contrast in molar shape between the
26 control and the 100 ng/kg treatment group for the female C57BL/6J mice also was statistically
27 significant ($p = 0.0096$). On the whole, when considering Procrustes distance results for molar
28 shape, the C3H/HeJ male offspring had the largest response at the low and high doses, while the
29 female offspring had the largest response at low and mid doses. This observation in male
30 C3H/HeJ mice is consistent with that of TCDD-induced changes in mandible size from Keller
31 et al. (2008, [198531](#)).

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1 A LOAEL for TCDD of 10 ng/kg maternal exposure on GD 13 is identified for this study
2 for significant ($p < 0.0001$) differences in molar shape in male C3H/HeJ mice. A NOAEL
3 cannot be determined in this study.
4

5 **2.4.2.2.7. *Kuchiiwa et al. (2002, [198355](#))*.**

6 Kuchiiwa et al. (2002, [198355](#)) studied the impact of in utero and lactational TCDD
7 exposure on serotonin-immunoreactive neurons in raphae nuclei on F1 male mouse offspring.
8 Twenty-one adult female ddY mice (seven per treatment group) were administered TCDD
9 (99.1% purity) by oral gavage once a week for 8 weeks at doses of 0, 4.9, or 490 ng/kg (0, 0.7, or
10 70 ng/kg-day average daily dose; administered doses divided by 7) or an equivalent volume of
11 olive oil vehicle (6.7 mL/kg) by gavage. Immediately following the final treatment, the mice
12 were housed with untreated male mice for mating. At approximately 20–21 days after mating,
13 3 female mice from each dose group, including the control group gave birth to 10–12 offspring.
14 One day after birth, each litter was culled to 10 offspring to accommodate similar lactational
15 TCDD exposure. On PND 28, the offspring were weaned, and three offspring from each TCDD
16 exposed group and the control group were selected for an immunocytochemical examination at
17 42 days of age. Following sacrifice of these offspring, the brain of each animal was removed
18 and every second serial section of the brain was processed for immunocytochemistry. In
19 addition to the serial sections of the brain, cells from 18 offspring (6 males per treatment group)
20 were used to assess the number of cells in the dorsal and median raphe nucleus, the
21 supralemniscal area, and the Nucleus raphe magnus.

22 Examination of external morphology, birth, and postnatal body weights indicated that
23 there were no differences between the male TCDD-exposed offspring and the control male
24 offspring. TCDD-exposed males, however, were aggressive toward other normal mice and were
25 also hypersensitive to soft touch.

26 Serotonin-immunoreactive neurons were found to be distributed throughout the entire
27 brainstem in 42-day-old males, and the general pattern in the TCDD-exposed animals was
28 consistent with those observed in control male offspring. Serotonergic neurons were identified
29 and counted in the caudal linear nucleus, the median and dorsal raphe nucleus, Nucleus raphe
30 pontis, interpeduncular nucleus, supralemniscal area, pedunculopontine segmental nuclei, deep
31 mencephalic nucleus, Nucleus raphe magnus, pallidus, and obscurus, dorsal and medial to the

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1 facial nucleus and the ventrolateral medulla. Results from computerized cell counts ($n = 6$)
2 showed an average of 1,573.3 immunoreactive neurons in the raphe nuclei from the control
3 group versus 716.3 and 419.8 neurons in the low- and high-dose offspring, respectively. The
4 numbers of immunoreactive neurons in the individual raphe nuclei (dorsalis, medianus, magnus,
5 and B9) from the TCDD-exposed offspring were significantly ($p < 0.01$) lower than control
6 values, with the degree of reduction being dose-related.

7 In the absence of other relevant neurotoxicity endpoints, reduced serotonin is not an
8 adverse endpoint of toxicological significance in and of itself, thus, neither a NOAEL nor a
9 LOAEL can be established for this study. A lowest-observed-effect level (LOEL) of
10 0.7 ng/kg-day for an 8-week exposure duration is identified in this study for a significantly
11 ($p < 0.01$) lower number of serotonin-immunoreactive neurons in the raphe nuclei of male
12 offspring. A no-observed-effect level (NOEL) cannot be determined for this study.

14 **2.4.2.2.8. *Li et al. (2006, [199059](#))***

15 Pregnant and pseudopregnant (obtained by mating normal estrous female mice with
16 vasectomized male mice) NIH mice (10 per treatment group) were exposed to 0, 2, 50, or
17 100 ng/kg-day of TCDD (purity 99%) during early gestation (GDs 1–8), preimplantation
18 (GDs 1–3), or peri-implantation to postimplantation (GDs 4–8) (Li et al., 2006). On GD 9,
19 animals were evaluated. The two highest TCDD doses (50 and 100 ng/kg-day) caused
20 significant ($p < 0.05$) early embryo loss independent of gestational exposure time. At
21 100 ng/kg-day, however, the embryo loss was greater when administered during GDs 1–8 or
22 GDs 1–3 compared to GDs 4–8 ($p < 0.01$). Uterine weight was significantly decreased in the
23 pseudopregnant mice when administered 50 or 100 ng/kg-day TCDD during GDs 1–8
24 ($p < 0.001$) or 1–3 ($p < 0.01$), but was only decreased at 100 ng/kg-day in pseudopregnant mice
25 when administered during GDs 4–8 ($p < 0.01$). Estradiol levels were increased at all TCDD
26 treatment levels (100% at the lowest dose), but statistical significance was not indicated. All
27 doses at all treatment times resulted in a significant reduction ($p < 0.01$) in serum progesterone
28 levels, with a 45% decrease at the lowest dose. Because the hormone effects were observed
29 following 4 days of treatment, the nominal doses were averaged over the entire test period of
30 8 days prior to measurement. The resulting average daily doses of TCDD were 0, 1, 25, and
31 50 ng/kg-day.

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1 A LOAEL of 2 ng/kg-day administered for 4 to 8 days is established in this study for a
2 significant ($p < 0.01$) decrease in progesterone (45% above control) and an approximate 2-fold
3 increase in estradiol levels (significance not indicated). A NOAEL cannot be determined.
4

5 **2.4.2.2.9. *Markowski et al. (2001, [197442](#))*.**

6 Pregnant Holtzman rats (4–7 per treatment group) were administered a single gavage
7 dose of 0, 20, 60, or 180 ng/kg TCDD (purity not specified) in olive oil on GD 18 (Markowski
8 et al., 2001, [197442](#)). One female rat from each litter (4–7 per treatment group) was assigned to
9 training on a wheel apparatus to respond on a lever for brief opportunities to run. Once animals
10 responded to an FR1 schedule of reinforcement, the requirement for lever pressing was increased
11 to FR2, FR5, FR10, FR20, and FR30 schedules. After each training session, the estrous cycle
12 stage was determined. Maternal body weight, length of gestation, number of pups per litter, and
13 sex distribution within litters were unaffected by treatment. For each of the FR schedules, there
14 was a significant dose-related ($p = 0.0001$) decrease in the number of earned run opportunities,
15 lever response rate, and total number of revolutions in the wheel in the adult female offspring.
16 There was no correlation between estrous cycle and responding for access to wheel running.

17 The developmental LOAEL for this study is a single dose of 20 ng/kg administered on
18 GD 18 for neurobehavioral effects. A NOAEL cannot be determined for this study.
19

20 **2.4.2.2.10. *Miettinen et al. (2006, [198266](#))*.**

21 Miettinen et al. (2006, [198266](#)) administered a single oral dose of 0, 30, 100, 300, or
22 1,000 ng/kg TCDD (purity >99%) in corn oil on GD 15 to pregnant Line C rats. The offspring
23 (24–32 per treatment group) were assigned to a sugar-rich cariogenic diet (via feed and drinking
24 water) and were orally inoculated three separate times with fresh cultures of *Streptococcus*
25 *mutans*. Three control groups varied with regard to TCDD exposure and administration of a
26 cariogenic diet. Two of the control groups received no TCDD, and the offspring were either
27 maintained on a normal diet without inoculation with *S. mutans* (C1; $n = 48$) or were given the
28 cariogenic diet with *S. mutans* inoculation (C2; $n = 42$). The final control group was maternally
29 exposed to 1,000 ng/kg TCDD with offspring fed a normal diet without *S. mutans* inoculation
30 (C3; $n = 12$). TCDD did not affect the maternal or offspring body weight. Survival of the
31 offspring was reduced in the 1,000 ng/kg dose group (50–58% survival compared to 83–95% in

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1 C1 and C2, respectively). All offspring administered 1,000 ng/kg were missing all lower
2 third molars. Two animals (8%) in the 100 ng/kg group were missing one of their lower
3 third molars. All doses, except the 100 ng/kg dose, caused a significant ($p < 0.05$) increase in the
4 number of caries lesions compared to group C2 (60, 79, 76, 83, and 91% in the C2, 30, 100, 300,
5 and 1,000 ng/kg groups, respectively). Group C3 (1,000 ng/kg TCDD exposure, normal diet)
6 animals also had increased caries lesions compared to C1 (8% versus 0%, respectively). There
7 were no changes in tooth mineral composition that could explain the increase in caries
8 susceptibility.

9 The developmental LOAEL from this study is a single dose of 30 ng/kg administered on
10 GD 15 based on the significant ($p < 0.05$) increase in dental caries in pups (30% above control).
11 A NOAEL cannot be determined from this study.

12

13 **2.4.2.2.11. Nohara et al. (2000, [200027](#)).**

14 Pregnant Holtzman rats were administered 0, 12.5, 50, 200, or 800 ng/kg TCDD in corn
15 oil by gavage on GD 15 (Nohara et al., 2000, [200027](#)). On PND 2, five males were randomly
16 selected from each litter and dose group. TCDD was detected in the thymus, spleen, and bone
17 marrow of the male pups on PND 21 and PND 49. TCDD was still detected in the thymus and
18 spleen on PND 120 but the levels decreased over time. The TCDD concentration was highest in
19 the thymus at all time points. There were no changes in the body, thymus, or spleen weights of
20 the male offspring on PND 5, PND 21, PND 49, or PND 120. On PND 5, there was a 200-fold
21 increase in CYP1A1 in the thymus of the high-dose male pups. CYP1A1 was only slightly
22 increased in the spleen. This induction decreased through PND 49. There was a slight (not
23 statistically significant) dose-dependent decrease in thymus cellularity in the male offspring at
24 PND 120. Spleen cellularity at PND 49 decreased in a dose-dependent manner (15–50% of the
25 control), with a statistically significant ($p < 0.05$) decrease observed in the high-dose group. A
26 slight but not significant reduction in spleen cellularity was noted in the high-dose group at
27 PND 21. The same effect was not observed at PND 120, nor was there any change in the percent
28 of B or T cells in the spleen. No changes in cytokine levels were observed in the 800-ng/kg
29 group.

30 Although a change in spleen cellularity on PND 49 (puberty) was observed, this effect
31 was transient and there were no coexisting changes in the percentage of splenic lymphocytes,

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1 spleen weight, and cytokine levels. Therefore, a developmental NOAEL of a single dose of
2 800 ng/kg administered on GD 15 is identified for this study. A LOAEL is not established.

3
4 **2.4.2.2.12. Ohsako et al. (2001, [198497](#)).**

5 Pregnant Holtzman rats (6 per treatment group) were administered 0, 12.5, 50, 200, or
6 800 ng/kg TCDD (purity >99.5%) in corn oil by gavage on GD 15 (Ohsako et al., 2001,
7 [198497](#)). On PND 2, five males were randomly selected from each litter. Two male offspring
8 from each litter were sacrificed on PND 49 and PND 120. Neither maternal nor male offspring
9 body weight was affected by TCDD treatment. TCDD was detected in both fat and testes at all
10 dose levels (including controls) with highest levels found in fat. There were no apparent
11 treatment-related effects on testicular weight, epididymal weight, daily sperm production, cauda
12 epididymal sperm reserves, luteinizing hormone, follicle stimulating hormone, or testosterone
13 levels. There was, however, a clear dose-dependent decrease in urogenital complex weight and
14 ventral prostate weight at both PND 49 and PND 120. For male offspring, statistically-
15 significant ($p < 0.05$) decreases were noted in urogenital complex weight at PND 120 in the 200
16 and 800 ng/kg groups, in ventral prostate weight at PND 49 in 800 ng/kg group, and at PND 120
17 in the 200 and 800 ng/kg groups. There was also a dose-dependent decrease in anogenital
18 distance (the length between the base of the genital tubercle and the anterior edge of the anus);
19 the decrease was not statistically significant at PND 49. At PND 120, however, male offspring
20 in all but the lowest dose group had significantly ($p < 0.05$) reduced anogenital distance
21 compared to the control animals. There was also a dose-dependent increase in 5 α R-II mRNA
22 expression in the ventral prostate on PND 49 with significant increases ($p < 0.05$) in the 200 and
23 800 ng/kg animals. There was a significant ($p < 0.01$) decrease in the androgen receptor mRNA
24 in the ventral prostate on PND 49 at all doses tested. Similar effects were not observed on
25 PND 120 or in the caput epididymis on PND 49.

26 The developmental LOAEL for this study is a single dose of 50 ng/kg administered on
27 GD 15 for significantly ($p < 0.01$) reduced anogenital distance in male offspring (approximately
28 14%). The NOAEL for this study is 12.5 ng/kg.

1 **2.4.2.2.13. Schantz et al. (1996, [198781](#)).**

2 Schantz et al. (1996, [198781](#)) studied the impact of in utero TCDD exposure on spatial
3 learning in male and female pups. Groups of pregnant Harlan Sprague-Dawley rats ($n = 108$,
4 divided into 4 cohorts; number of animals in each TCDD group approximately 4 per treatment
5 group) were dosed via gavage with 0, 25, or 100 ng/kg-day TCDD (purity >98%) in corn oil on
6 GDs 10–16. On the day of birth (post natal day [PND] 0), the pups were examined for gross
7 abnormalities and the number of live pups, weight, and sex were recorded for each litter. On
8 PND 2, litters were culled to eight animals and were balanced to include four males and
9 four females whenever possible. To minimize litter-size effects, litters with fewer than five pups
10 were excluded from the study. The exclusion of these litters resulted in 10–11 litters per
11 treatment group. Pups were weaned on PND 21 and one male and one female pup from each
12 litter were maintained for the learning tests. Pups were tested 5 days per week for spatial
13 learning and memory in a radial arm maze and a T-maze. A radial arm maze working memory
14 test and a T-maze DSA task were used a part of the testing process.

15 TCDD treatment did not affect dam gestational weight gain, dam liver weight, gestation
16 length, litter size, percentage of live births, birth weight, or postnatal growth of the pups
17 observed during the course of the study. Exposed pups, however, exhibited some signs of
18 toxicity in all exposure groups. Thymus weight was decreased and liver weight was increased in
19 the 100 ng/kg-day TCDD dose group. Also, liver microsomal 7-ethoxyresorufin-O-deethylase
20 (EROD) activity was markedly induced in pups from both the 25 and 100 ng/kg-day dose
21 groups. In the radial maze test, rats from all TCDD exposure groups displayed a significant
22 ($p < 0.01$) learning behavior as shown by progressively fewer errors from the first block of
23 sessions through the fourth session. The treatment by sex and treatment by session block
24 interactions were not significant. Comparisons between the average number of errors per session
25 block in the TCDD-exposed and control group indicated that both the 25 and the 100 ng/kg-day
26 dose groups made significantly ($p < 0.05$ and $p < 0.001$, respectively) fewer errors compared to
27 the control group. TCDD did not significantly affect adjacent arm selection behavior as
28 measured by C statistic; hence the reduction in errors observed did not appear to be accounted
29 for by an increased tendency to run into adjacent arms. Female pups had a significant ($p < 0.05$)
30 shorter radial arm maze latency, however, compared to the male pups. In the T-maze test,
31 TCDD did not significantly affect the percent of correct performance. All exposure groups

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1 performed best at the shortest delay, which showed a decline as the length of the intertrial delay
2 interval was increased. Additionally, all treated groups improved their performance over a
3 three-block session period. This finding indicated that animals in all groups could learn the task.
4 These observations were confirmed by a highly significant main effect of delay ($p < 0.001$) and
5 highly significant main effect of session blocks ($p < 0.001$). At the shortest 15-second delay,
6 average percent correct performance increased from 75 to 92%, while at the longest 40-second
7 delay, the average percent correct performance increased from 62 to 82%. A significant
8 ($p < 0.05$) main effect of exposure was evident in latency to respond in the T-maze.
9 Comparisons of the exposed group to control group, however, indicated that none of the
10 individual exposure groups differed significantly from the controls. Because no clear pattern
11 was observed in the various exposure groups, differences in latency to respond had no impact on
12 learning of the task.

13 Based on these results, the study authors state that the fact TCDD seems to have a
14 facilitatory effect on radial arm maze learning in rats should be interpreted with caution and
15 needs further evaluation using different and more varied learning tasks. No toxicologically
16 adverse endpoints were concurrently examined. Thus, a LOAEL and a NOAEL cannot be
17 determined for this study.

18

19 **2.4.2.2.14. *Seo et al. (1995, [197869](#))*.**

20 To study developmental effects of TCDD on thyroid hormone levels, time-mated female
21 Sprague-Dawley rat dams ($n = 10-14$ /treatment group) were administered 25 or 100 ng/kg-day
22 of TCDD (>98% pure) in corn oil via gavage from GDs 10-16. Vehicle controls received
23 equivalent amounts of corn oil. The study also investigated PCB treatment outcomes. At birth,
24 pups were weighed and grossly examined for abnormalities. At 2 days of age, litters with fewer
25 than 5 pups were excluded from the analysis and the remaining litters were culled to 4 males and
26 4 females. Each treatment group contained 10 or 11 litters. Pups remained with the dams until
27 weaning. At weaning, 4-6 pups were retained for neurobehavioral tests (which were not
28 reported as part of this study). The remaining offspring were sacrificed, which provided
29 5-9 litters per treatment group. Data were collected from one male and one female where
30 possible. No signs of toxicity were evident in the dams; measurements on dams included
31 gestational weight gain, liver weight, litter size, and live births. Pup birth weight and weaning

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1 weight were unaffected by treatment. In pups sacrificed at weaning (21 days old), a significant
2 ($p < 0.05$) decrease occurred in thymus weight for the high-dose group, but not in thyroid, liver,
3 or brain weight. A significant ($p < 0.05$) decrease (20.4%) was observed in T4 in high-dose
4 females. Thyroid stimulating hormone and T₃ were unaffected by treatment. Uridine
5 diphosphate (UDP)-glucuronosyl transferase activity towards 4-nitrophenol significantly
6 ($p < 0.05$) increased in both treatment groups over control values, and the increase in the
7 high-dose group was significantly ($p < 0.05$) greater than in the low-dose group. Liver
8 microsomal EROD activity was significantly ($p < 0.05$) increased in both treatment groups, but
9 is considered to be an adaptive response and not adverse.

10 A LOAEL of 100 ng/kg-day for decreased thymus weights and decreased thyroxine is
11 identified for this study. A NOAEL of 25 ng/kg-day is established.

12 13 **2.4.2.2.15. *Simanainen et al. (2004, [198106](#))*.**

14 Simanainen et al. (2004, [198106](#)) studied the impact of in utero and lactational TCDD
15 exposure on the male reproductive system in three rat lines that are differentially sensitive to
16 TCDD. Groups of 5 to 8 pregnant Line A, B, and C C57BL/6N CYP1A2 dams were given a
17 single dose of 0, 30, 100, 300, or 1,000 ng/kg of TCDD (purity >99%) in corn oil on GD 15 via
18 oral gavage. Control animals were similarly dosed with a corn oil vehicle. One day after birth,
19 litters were randomly culled to include three males and three females to allow uniform postnatal
20 exposure. Offspring were weaned on PND 28. Dam and pup viabilities were monitored
21 throughout the study. Pup body weights were determined on PNDs 1, 4, 7, 14, and 28.
22 Anogenital distance and crown-rump length were measured on PNDs 1 and 4. On day 70, pups
23 were sacrificed and trunk blood was collected. Serum was collected for testosterone analysis.
24 The testes, cauda of the right epididymis, ventral prostate, seminal vesicles, and thymus was
25 dissected and weighed. Absolute and relative organ weights were determined, and cauda
26 epididymis and testes were also preserved for sperm count analysis.

27 TCDD caused no mortality or overt signs of toxicity to the dams. Pup survival from
28 implantation to the day after birth also was not affected by TCDD exposure. Survival from the
29 day of implantation to the day after birth, however, was uncharacteristically lower in control
30 Line B rats (41%), resulting in a significant difference compared with the two lowest doses (30
31 and 100 ng/mg TCDD). The average survival percentage in the controls for Line A, B, and C

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1 rats was 85% (range 80–86%); 64% (41–86%); and 74% (63–85%); respectively. Percentage of
2 male pup survival in each line between PND 1 and PND 28 was 99% except for Line B males
3 exposed to 30 ng/kg TCDD and Line C males exposed to 30 or 100 ng/kg, where male survival
4 rate averaged 81% (range 81–83%). On PND 70, a significant ($p < 0.05$) reduction in body
5 weight was observed only in Line B and C rats at 1,000 ng/kg. In pups exposed to 1,000 ng/kg
6 TCDD, both absolute and relative weight of the ventral, anterior, and dorsolateral prostrate
7 decreased in all three lines at most postnatal time points measured. The change was most
8 consistent and significant ($p < 0.05$) in the ventral lobe. Animals exposed to 1,000 ng/kg TCDD
9 had an average decrease in absolute weight of the anterior prostrate of 37, 32, and 34% in
10 Lines A, B and C, respectively. Additionally, the average dorsolateral prostrate weight was also
11 decreased by 34, 28, and 39% in Lines A, B, and C, respectively. The effect on the ventral
12 prostrate was reversible with the only significant ($p < 0.05$) decrease in weight observed in
13 Line B rats at PND 70 in the 1,000 ng/kg TCDD dose group. The authors reported that TCDD
14 had no consistent effects on the weight of seminal vesicles. The absolute weights of the testis
15 and epididymis showed a significant ($p < 0.05$) increase on PNDs 28–49, but the relative testis,
16 epididymis, and cauda epididymis weights remained unchanged. In pups exposed to
17 1,000 ng/kg TCDD, severe malformation, including small caput and cauda and degeneration of
18 corpus epididymis, was observed. Malformations in the epididymis were observed in 6 of
19 44 Line C male rat offspring and 3 of 47 Line A male rat offspring. In Line A, B, and C rats at
20 PND 70 in the 1,000 ng/kg TCDD dose group, daily sperm production was reduced by 9, 25, and
21 36% and cauda epididymal sperm reserves were reduced by 18, 42, and 49%, respectively.
22 Daily sperm reduction (17%) was significant ($p < 0.05$) in Line C rats at a TCDD dose of
23 300 ng/kg and in Line B and C rats at 1,000 ng/kg. A reduction in cauda epididymal sperm
24 reserves (25%) was significant ($p < 0.05$) in Line C rats at 300 and 1,000 ng/kg TCDD.

25 A LOAEL for TCDD of 300 ng/kg is identified for reduction in daily sperm production
26 and cauda epididymal sperm reserves in Line C rats. A NOAEL of 100 ng/kg is identified for
27 this study.

28

29 **2.4.2.2.16. Sugita-Konishi et al. (2003, [198375](#)).**

30 Sugita-Konishi et al. (2003, [198375](#)) examined the immunotoxic effects of lactational
31 exposure to TCDD in newborn mice. Eight pregnant female C57BL/6NC_{ji} mice were

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1 administered 0, 1.8, or 18 ng/L of TCDD via drinking water from parturition to weaning of the
2 offspring (for a total of 17 days). Based on an average water intake of 14–16 mL/day, the
3 average daily intake of TCDD for the dams was 1.14 and 11.3 ng/kg-day in the low- and
4 high-dose groups, respectively. In male offspring sacrificed at weaning (21 days after birth),
5 there was a statistically-significant ($p < 0.05$) decrease in relative spleen weight and a
6 statistically-significant ($p < 0.005$) increase in thymic CD4+ cells in the high-dose group. The
7 changes in relative spleen weight and thymic CD4+ cells were dose related, but effects in the
8 low-dose group did not achieve statistical significance. Changes in spleen weight and CD4+ cell
9 numbers were not observed in the female offspring. In a separate experiment, offspring infected
10 with *Listeria monocytogenes* following lactational TCDD exposure exhibited a statistically
11 significant increase in serum tumor necrosis factor alpha (TNF- α) 2 days after infection in both
12 sexes in the low- ($p < 0.05$) and high-dose ($p < 0.005$) groups. There was also a statistically
13 significant increase in serum interferon gamma in *Listeria*-infected high-dose females ($p < 0.05$).
14 The number of bacteria in the spleen was also significantly increased ($p < 0.05$) 2 days after
15 infection in the high-dose females compared to the controls, but not in males. *Listeria* levels in
16 the spleen returned to control levels by 4 days after infection in both sexes.

17 Based on these results, a LOAEL for TCDD of 11.3 ng/kg-day following a 17 day
18 exposure to dams was identified for significantly ($p < 0.05$) decreased spleen weight (in male
19 pups), a significant ($p < 0.005$) increase in thymic CD4+ cells (in male pups), and for increased
20 susceptibility to *Listeria monocytogenes* (in male and female pups). The NOAEL for this study
21 is 1.14 ng/kg-day.

22

23 **2.4.2.3. Acute Studies**

24 **2.4.2.3.1. Burleson et al. (1996, [196998](#)).**

25 Burleson et al. (1996, [196998](#)) studied the impact of TCDD exposure on mice that were
26 challenged with the influenza virus 7 days after treatment with TCDD. Groups of 8-week-old
27 female B6C3F1 mice ($n = 20$, 2 replicate groups) were treated one time with 0, 1, 5, 10, 50, 100,
28 or 6,000 ng/kg TCDD (purity >99%, dissolved in corn oil) via oral gavage. In addition to the
29 treated groups, randomly selected animals were assigned as a sentinel group and screened for
30 numerous pathogens. Results of all tests performed on this sentinel group were negative.

31 Seven days after TCDD treatment, all animals were lightly anesthetized and infected intranasally

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1 with a highly lethal influenza A/Hong Kong/8/68 virus (H3N1; passage 14). The animals were
2 infected with sufficient H3N1 virus to achieve a 30% mortality rate in the control animals.
3 Animals were observed for mortality and morbidity for 21 days following viral infection.
4 Six mice from each treatment group were sacrificed on days 3, 9, and 12 postinfection, and body,
5 thymus, and wet lung weights were recorded. Influenza viral titers were examined by sacrificing
6 eight mice each at 2 hours and at 1, 4, 6, 7, 8, 9, 10, and 11 days post infection.

7 Exposure to TCDD resulted in significantly ($p < 0.05$) increased mortality in the 10, 50,
8 and 100 ng/kg dose groups. No statistically significant difference in the percentage alive was
9 observed between these dose groups. TCDD doses of 1 and 5 ng/kg did not alter mortality in
10 influenza infected animals. A time-related increase in the wet weights of the lungs in infected
11 mice as a result of increased edema also was reflected in an increase in the lung weight-to-body
12 weight ratio. The study authors stated that this ratio was not altered as a result of TCDD
13 exposure. TCDD-only exposures at 1, 10, or 100 ng/kg did not affect thymus weight. Similarly,
14 animals infected with the influenza virus following TCDD exposure also showed no loss in
15 thymic weight. Enhanced mortality in TCDD-treated animals was not correlated with an
16 increase in influenza virus titers. Additionally, animals treated with 1, 10, 100, or 1,000 ng/kg
17 did not affect pulmonary viral titer assays on days 6, 7, and 8 postinfection. The authors also
18 concluded that TCDD did not alter Hong Kong virus replication or clearance.

19 Although these results support immunotoxic effects induced by TCDD, the findings were
20 not reproduced by Nohara et al. (2002, [199021](#)) using the identical study design, and the
21 translation of these findings to humans is dubious. Thus, no LOAEL/NOAEL was established.
22 A LOEL for TCDD of 10 ng/kg for a single exposure is identified for significantly ($p < 0.05$)
23 increased mortality in mice infected 7 days later with the influenza virus. The NOEL for this
24 study is 5 ng/kg.

25 26 **2.4.2.3.2. Crofton et al. (2005, [197381](#)).**

27 Crofton et al. (2005, [197381](#)) studied the impact of TCDD exposure in addition to the
28 impact of mixtures of thyroid disrupting chemicals and PCBs on serum total thyroxine (TT4)
29 concentration. Groups of female Long-Evans rats were dosed via oral gavage with 0, 0.1, 3, 10,
30 30, 100, 300, 1,000, 3,000, or 10,000 ng/kg-day TCDD (purity >99%) in corn oil ($n = 14, 6, 12,$
31 $6, 6, 6, 6, 6, 6,$ and 4, respectively) for 4 consecutive days. On the day following the last dose,

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1 animals were sacrificed, trunk blood was collected, and serum obtained via centrifugation was
2 assayed for TT4 concentration using standard radioimmunoassay methods.

3 No visible signs of toxicity or changes in animal body weight as a result of TCDD
4 exposure were observed. Serum T4 levels showed a dose-dependent decrease, with the levels
5 dropping sharply beginning at 100 ng/kg-day dose. Percent serum T4 levels were 96.3, 98.6,
6 99.8, 93.3, 70.9, 62.5, 52.7, 54.7, and 49.1% in the 0.1, 3, 10, 30, 100, 300, 1,000, 3,000, and
7 10,000 ng /kg-day groups, respectively.

8 A LOAEL for TCDD of 100 ng/kg-day for 4 consecutive days of exposure is identified in
9 this study for a reduction in serum T4 levels (70.9% compared to 100% in controls). The
10 NOAEL for this study is 30 ng/kg-day.

11

12 **2.4.2.3.3. *Kitchin and Woods (1979, [198750](#))***

13 Female Sprague-Dawley rats (nine per control and four per treatment group) were
14 administered a single dose of 0, 0.6, 2, 4, 20, 60, 200, 600, 2,000, 5,000, or 20,000 ng/kg TCDD
15 (purity >99%) in corn oil. Animals were sacrificed 3 days after treatment and CYP level and
16 benzo(a)pyrene hydroxylase activity in the liver were measured. A significant ($p < 0.05$)
17 increase in cytochrome P450 levels occurred with doses of 600 ng/kg or greater and in
18 benzo(a)pyrene hydroxylase activity with doses of 2 ng/kg or greater. Cytochrome P450 was
19 significantly ($p < 0.05$) higher 1 month after a single exposure of 2,000 ng/kg (the only dose
20 measured), but not after 3 or 6 months. Aryl hydrocarbon hydralase (AHH; $p < 0.05$) and EROD
21 ($p < 0.01$) were both significantly increased through 3 months after treatment, and although
22 elevated at 6 months, the results were not significant.

23 CYP induction alone is not considered a significant toxicologically adverse effect given
24 that CYPs are induced as a means of hepatic processing of xenobiotic agents. Thus, no LOAEL
25 or NOAEL was established for this study because adverse endpoints (e.g., indicators of
26 hepatotoxicity) were not measured. The acute LOEL, however, is 2 ng/kg based on a significant
27 ($p < 0.05$) increase in benzo(a)pyrene hydroxylase activity (37% above control). The NOEL is
28 0.6 ng/kg.

29

1 **2.4.2.3.4. *Li et al. (1997, [199060](#))***

2 Female Sprague-Dawley rats (22 days old; 10 per treatment) were administered a single
3 oral dose of TCDD (>98% pure) in corn oil via gavage at doses of 3, 10, 30, 100, 300, 1,000,
4 3,000, 10,000, or 30,000 ng/kg. Vehicle controls received equivalent amounts of corn oil, while
5 naïve controls were sham-treated only. In a preliminary time-course study, animals received a
6 single dose of 10,000 ng/kg and were sacrificed at 1, 2, 4, 8, 16, 24, 48, and 72 hours. The
7 time-course study showed two peaks in LH and FSH levels at 1 hour and 24 hours, with a
8 decrease to control values by 48 hours. Thus, in the dose-response study, animals were
9 sacrificed at 1 or 24 hours after treatment, blood was collected, and serum FSH and LH were
10 measured. The dose-response study demonstrated that the peak at 1 hour was related to the
11 vehicle as the peak also occurred in the vehicle controls, but did not occur in the naïve controls.
12 At 24 hours, FSH was increased at 10 ng/kg and higher (>4-fold increase at 10 ng/kg). Doses of
13 10 to 1,000 ng/kg showed similar increases (not all reached statistical significance; $p < 0.05$). A
14 dose-dependent increase occurred for doses ≥ 3000 ($p < 0.05$) with a maximum increase of
15 20-fold over the vehicle control. At 24 hours, the LH response significantly ($p < 0.05$) increased
16 only for doses ≥ 300 ng/kg with a maximum increase of 15-fold above the vehicle control. The
17 study authors calculated an ED₅₀ of 500 ng/kg for gonadotropin increase. The dose-dependent
18 release of LH was confirmed in in vitro studies, but did not occur with the same magnitude. The
19 increase did not occur in calcium-free medium and was unrelated to gonadotropin releasing
20 hormone.

21 Based on the increase in serum FSH, the LOAEL was 10 ng/kg and the NOAEL was
22 3 ng/kg.

23

24 **2.4.2.3.5. *Lucier et al. (1986, [198398](#))***

25 Adult female Sprague-Dawley rats (six per treatment) were administered a single gavage
26 dose of TCDD (purity not specified) in either corn oil or contaminated soil at doses of 15, 40,
27 100, 200, 500, 1,000, 2,000, 5,000 (corn oil), or 5,500 (contaminated soil) ng/kg. Animals were
28 sacrificed 6 days later and livers were removed for analysis. No clinical signs of acute toxicity
29 or changes in body weight were observed at any dose. AHH increased in a dose-dependent
30 manner with significant ($p < 0.05$) increases observed at 15 ng/kg or greater in corn oil or
31 40 ng/kg or greater in contaminated soil. Cytochrome P450 was significantly ($p < 0.05$)

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1 increased with doses of 1,000 ng/kg or greater in corn oil or 500 ng/kg or greater in contaminated
2 soil. A dose-dependent increase was observed for UDP glucuronyltransferase (significance of
3 individual doses not reported), with the results twice as high with corn oil than with
4 contaminated soil. The authors state that the results indicate bioavailability from soils is 50%.

5 Because the association between AHH activity and TCDD-mediated hepatotoxicity is
6 unknown and no adverse endpoints were measured, a LOAEL or NOAEL was not determined
7 for this study. The acute LOEL for this study is 15 ng/kg, based on the significant ($p < 0.05$)
8 increase (80% above control) in AHH. No NOEL is established.

9
10 **2.4.2.3.6. *Nohara et al. (2002, [199021](#))*.**

11 Male and female B6C3F1 (C57BL/6 × C3H), BALB/c, C57BL/6N, and DBA2 mice
12 (10–40 per treatment group) were administered a single dose of 0, 5, 20, 100, or 500 ng/kg
13 TCDD in corn oil via gavage. Seven days following TCDD treatment, mice were infected with a
14 mouse-adapted strain of influenza (A/PR/34/8; H1N1) at a plaque forming unit dose designed to
15 target approximately 30% mortality in each strain. TCDD did not affect the body weight or
16 survival in any of the infected mouse strains at any dose.

17 Therefore, no LOAEL is established in this study. The NOAEL is 500 ng/kg.

18
19 **2.4.2.3.7. *Simanainen et al. (2003, [198582](#))*.**

20 Simanainen et al. (2003, [198582](#)) studied the short-term effects of TCDD exposure to
21 determine the efficacy and potency relationships among three differentially susceptible rat lines.
22 The three rat lines used were A, B, and C, which were selectively bred from TCDD-resistant
23 Han/Wistar and TCDD-sensitive Long-Evans rats. The study authors reported that Line A rats
24 were most resistant to TCDD acute lethality followed by Line B and C. Groups of five or
25 six randomly selected rats (sex not specified) were treated with a single oral dose of TCDD
26 (purity >99%) in corn oil by oral gavage. The dose of TCDD was reported to range between
27 30 ng/kg and 3,000 µg/kg for Line A, 30 ng/kg and 1,000 µg/kg in Line B, and 30 ng/kg and
28 100 µg/kg for Line C. Control animals were similarly dosed with a corn oil vehicle. Rats were
29 sacrificed on day 8 postexposure, and trunk blood was collected and serum separated. Liver and
30 thymus were removed and weighed, and liver samples were collected and preserved. Liver

1 EROD activity, serum aspartate aminotransferase (ASAT) activity, free fatty acid (FFA)
2 concentration, and total bilirubin concentration were determined. Teeth were also examined.

3 Relative thymus weights were reduced 25% at 300 ng/kg relative to controls in Line B
4 rats. Liver enzyme (CYP1A1) induction, as measured by EROD activity, was evident at all
5 exposure levels; CYP induction is considered to be an adaptive effect and not adverse in itself.
6 No other endpoints were affected below 1 µg/kg in any of the three rat lines.

7 A LOAEL for TCDD of 300 ng/kg is identified for decreased relative thymus weight in
8 Line B rats. A NOAEL of 100 ng/kg is identified for this study.

9 10 **2.4.2.3.8. *Simanainen et al. (2002, [201369](#))*.**

11 To study the short-term effects of TCDD on hormone levels, adult female Long-Evans
12 (TCDD-sensitive) and Han/Wistar (TCDD-resistant) rats ($n = 9-11/\text{treatment}$) were administered
13 a single dose of TCDD (>99% pure) in corn oil via gavage at doses ranging from 30 ng/kg to
14 100 µg/kg. Vehicle controls received an equivalent amount of corn oil. The study also
15 examined other polychlorinated dibenzo-*p*-dioxins outcomes. Rats were sacrificed on day 8
16 postexposure, and trunk blood was collected and serum separated. Liver and thymus were
17 removed and weighed, and liver samples were collected and preserved. Liver EROD activity,
18 serum ASAT activity, FFA concentration, and total bilirubin concentration were determined.
19 Teeth were also examined.

20 Neither FFA or ASAT levels in Han/Wistar rats showed a dose-response relationship. In
21 Long-Evans rats, however, a significant ($p < 0.05$) dose-dependent increase in FFA occurred at
22 300 ng/kg TCDD. Serum ASAT sharply increased in Long-Evans rats between 3,000 and
23 10,000 ng/kg. Body weight change and relative thymus weights were significantly decreased
24 ($p < 0.05$) in Han/Wistar rats with doses $\geq 10,000$ ng/kg and in Long-Evans rats with doses
25 $\geq 1,000$ ng/kg. Liver EROD activity was significantly ($p < 0.05$) increased with all doses in both
26 strains. Serum T4 was significantly ($p < 0.05$) decreased in Long-Evans rats at concentrations
27 ≥ 300 ng/kg, but were not significantly affected in Han/Wistar rats. Serum bilirubin was
28 significantly ($p < 0.05$) increased with doses $\geq 10,000$ ng/kg in Long-Evans rats and
29 $\geq 30,000$ ng/kg in Hans/Wistar rats. Both strains of rat showed a dose-dependent increase in
30 mean severity of incisor tooth defects. The results indicate that TCDD was the most potent
31 congener tested in both rat strains.

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1 A LOAEL of 300 ng/kg for decreased T4 in the Long-Evans rat is identified for this
2 study. A NOAEL of 100 ng/kg is established.

3
4 **2.4.2.3.9. *Smialowicz et al. (2004, [110937](#))***

5 Smialowicz et al. (2004, [110937](#)) examined the impact of TCDD exposure on
6 immunosuppression in mice. Groups of female (number not specified) C57BL/6N CYP1A2
7 (+/+) wild-type mice were administered a single dose of 0, 30, 100, 300, 1,000, 3,000, or
8 10,000 ng/kg TCDD (purity >99%) in corn oil via oral gavage. Control animals were similarly
9 dosed with a corn oil vehicle. To assess immune function, 7 days after TCDD administration, all
10 mice were immunized with sheep red blood cells (SRBCs) via injection into the lateral tail vein.
11 Five days after immunization, mice were sacrificed, blood was collected, and enzyme-linked
12 immunosorbant assays were performed. Additionally, spleen, thymus, and liver weights also
13 were measured.

14 Body and spleen weights of the wild-type mice were unaffected by the TCDD exposure.
15 A decrease in thymus weights of the mice appeared to be dose related. Only mice treated with
16 10,000 ng/kg TCDD, however, showed a statistically significant ($p < 0.05$) decrease in thymus
17 weights compared to corresponding controls. Liver weights also showed a dose-related increase
18 with only animals treated with 3,000 and 10,000 ng/kg TCDD showing statistical significance
19 ($p < 0.05$) compared to the control group. The antibody response to SRBCs indicated a
20 dose-related suppression in the wild-type mice, with animals treated with 1,000, 3,000, and
21 10,000 ng/kg TCDD showing statistically significant ($p < 0.05$) suppression compared to the
22 controls.

23 A LOAEL for TCDD of 1,000 ng/kg is identified in female C57BL/6N CYP1A2 (+/+)
24 wild-type mice for significant ($p < 0.05$) suppression of SRBCs. The NOAEL for this study is
25 300 ng/kg.

26
27 **2.4.2.3.10. *Vanden Heuvel et al. (1994, [197551](#))***

28 Vanden Heuvel et al. (1994, [197551](#)) examined the dose-response relationship between
29 TCDD exposure and induction of hepatic mRNA. Groups of 10-week-old female
30 Sprague-Dawley rats were administered TCDD (purity ~99%) in corn oil once at 0, 0.1, 0.05, 1,
31 10, 100, 1,000, or 10,000 ng/kg-BW. Four days after TCDD treatment, animals were sacrificed

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1 and livers were excised and preserved. Total hepatic RNA was extracted using guanidine
2 thiocyanate and DNA was removed using standard phenol-chloroform-isoamyl alcohol
3 partitioning procedures. Quantitative competitive RNA-PCR method was used to analyze
4 CYP1A1, UDP-glucuronosyltransferase I (UGT1), plasminogen activator inhibitor 2 (PAI2),
5 β -actin, and transforming growth factor α (TGF α). In addition to hepatic mRNA levels,
6 microsomal protein was assayed for EROD activity and livers were tested for TCDD
7 concentration.

8 CYP1A1 mRNA induction levels in the TCDD-treated groups were low in the low-dose
9 region and sharply increased to plateaus at higher doses. The lowest dose that showed a
10 statistically significant ($p < 0.05$) difference compared to controls was the 1 ng/kg dose, which
11 showed a three-fold increase in CYP1A1 mRNA levels. In contrast, a 130-fold increase
12 occurred at 100 ng/kg and a 4,000- and 7,000-fold increase occurred at 1,000 and 10,000 ng/kg,
13 respectively. A slight increase in the CYP1A1/ β -actin levels was observed in the 0.1 ng/kg
14 group, but this increase was not significant. EROD activity exhibited a pattern similar to
15 CYP1A1 activity. EROD activity, however, was approximately 100-fold less sensitive
16 compared to mRNA levels in TCDD-treated groups. Statistical significance (p -value not
17 provided) in CYP1A1 level was observed at the 100 ng/kg dose compared to the 1 ng/kg dose.
18 The study authors reported that, despite this difference in CYP1A1 and EROD activity, the
19 correlation between CYP1A1 enzyme activity and mRNA levels was good. Dose-response
20 relationships for the induction of UGT1, PAI2, and TGF α mRNA differed from what had been
21 observed for CYP1A1 mRNA. UGT1 mRNA was induced, but at the much higher dose of
22 1,000 ng/kg. Additionally, the five-fold maximum induction of UGT1 mRNA was much less
23 than the 7,000-fold induction observed for CYP1A1 mRNA at the 10,000 ng /kg dose. The
24 authors state that this could be a result of the constitutive level of UGT1, which is much higher
25 than CYP1A1, which makes detecting induction of UGT1 in the low dose regions more difficult.
26 PAI2 and TGF α mRNA were not affected by TCDD in rat liver in the dose range tested. These
27 results indicate that dioxin-inducible genes have a quite dissimilar dose-response relationship.

28 Induction of CYP1A1 expression is not considered an adverse effect, as the role of
29 CYP1A1 in TCDD-mediated hepatotoxicity is unsettled. Therefore, in the absence of other
30 indicators of hepatotoxicity, a NOAEL/LOAEL cannot be determined for this study. A LOEL

1 for TCDD of 1 ng/kg for a single exposure was identified for statistically significant ($p < 0.05$)
2 increase in CYP1A1 mRNA levels. The NOEL for this study is 0.1 ng/kg.

4 **2.4.2.4. Subchronic Studies**

5 **2.4.2.4.1. Chu et al. (2001, [521829](#)).**

6 Adult female Sprague-Dawley rats (five per treatment group) were administered TCDD
7 (purity >99%) in corn oil by gavage at doses of 0, 2.5, 25, 250, or 1,000 ng/kg-day for 28 days
8 (Chu et al., 2001, [521829](#)). The 1,000 ng/kg-day dose of TCDD caused a significant ($p \leq 0.05$)
9 decrease in body weight gain (36% lower than the control), increase in relative liver weight (40%
10 greater than the control), and decrease in relative thymus weight (50% lower than the control).
11 There was a significant ($p \leq 0.05$) increase in EROD activity, methoxy resoufin-O-deethylase
12 (MROD) activity, and UDP-glucuronosyl transferase (UDPGT) activity in the liver of female
13 rats receiving 250 or 1,000 ng/kg-day TCDD. In addition, significant ($p \leq 0.05$) increases in
14 serum cholesterol were observed in the 250 and 1,000 ng/kg-day dose groups, and liver ascorbic
15 acid (AA) also was significantly increased in the 1,000 ng/kg-day dose group. There was
16 ~1.5-fold increase in liver glutathione-S-transferase (GST), which was not statistically
17 significant. Other significant ($p \leq 0.05$) findings for the 1,000 ng/kg-day group included a
18 decrease in liver vitamin A (51% lower than the control), an increase in kidney vitamin A
19 (15.5-fold increase above the control), an increase in liver benzyloxy resoufin-O-deethylase
20 (BROD, 30-fold increase above control), a decrease in liver pentoxyresoufin-O-deethylase
21 (PROD, 37% lower than the control), increase in serum albumin (18% above the control), and a
22 decrease in mean corpuscular hemoglobin (MCH, 7% below the control) and mean corpuscular
23 volume (MCV, 7% below the control).

24 Based on the numerous significant ($p \leq 0.05$) liver-related biochemical changes and
25 significant ($p \leq 0.05$) increased relative liver weight, as well as significantly decreased body
26 weight and relative thymus weight, the LOAEL for 28 days of exposure in this study is
27 1,000 ng/kg-day and the NOAEL is 250 ng/kg-day.

29 **2.4.2.4.2. Chu et al., 2007.**

30 Chu et al. (2007) examined the potential impact of TCDD on various organs and the
31 toxicological impacts as a result of interactions between TCDD and PCBs in rats. Groups of

1 female Sprague-Dawley rats ($n = 5$ per treatment group) were treated daily for 28 days via
2 gavage with 0, 2.5, 25, 250, or 1,000 ng /kg-day TCDD (purity not specified) dissolved in corn
3 oil. Body weights were determined three times per week, and clinical observations were made
4 daily. At study termination, all animals were sacrificed and blood was analyzed for various
5 biochemical and hematological parameters. Liver, spleen, heart, thymus, brain, and kidneys
6 were removed and weighed. A small portion of the liver was homogenized and assayed for
7 BROD; EROD; MROD; and PROD. UDPGT, GST, and ascorbic acid levels also were
8 measured. Vitamin A levels in the liver, kidney, and lungs were analyzed as free retinol
9 (vitamin A), and histopathological analysis was conducted on various tissues.

10 Growth rate and thymic weights in rats treated with 1,000 ng/kg-day TCDD were
11 significantly ($p \leq 0.05$) inhibited compared to the control group. Enzyme analysis indicated that
12 measured levels of TCDD in the liver correlated with hepatic microsomal enzyme activity. The
13 authors reported that liver microsomal EROD and MROD activities were significantly ($p < 0.05$
14 for EROD activity, significance level for MROD not reported) increased in the 250 and
15 1,000 ng/kg-day TCDD dose groups compared to the control group. UDPGT levels were
16 significantly (significance level not reported) increased in the 250 and 1,000 ng/kg-day TCDD
17 dose groups compared to the controls. Serum albumin levels were significantly ($p < 0.05$)
18 increased in the 1,000 ng/kg-day TCDD dose group compared to the control group. Serum
19 cholesterol levels were significantly (level not reported) increased compared to the control group
20 at 250 ng/kg-day TCDD dose, while liver ascorbic acid concentrations were significantly (level
21 not reported) increased in the 1,000 ng/kg-day dose group. Hematological analysis indicated that
22 hemoglobin, packed cell volume, MCH, MCV, and platelet values were decreased in the
23 1,000 ng/kg-day TCDD dose group. Significant ($p \leq 0.05$) differences were observed only in
24 MCH and MCV levels compared to the control. Vitamin A levels in the liver and kidney were
25 significantly ($p < 0.05$) lower in the 1,000 ng/kg-day TCDD group compared to the control
26 group. Histopathological evaluation of various tissues indicated that liver, thyroid, and thymus
27 were the target organs. No TCDD-related affects were found in other tissues. A dose-dependent
28 alteration in the thymus consisted of reduced thymic cortex and increased medullar volume with
29 more animals exhibiting these changes at the 250 and 1,000 ng/kg-day dose level compared to
30 the control group. Alterations in thyroid included reduced follicles, reduced colloid density, and
31 increased epithelial height. A dose-dependent change in the thyroid was observed, with the

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1 highest impact evident in reduced follicles and reduced colloid density beginning at a dose of
2 25 ng/kg-day TCDD. Changes in liver were characterized by accentuated hepatic zones,
3 anisokaryosis of hepatocytes, increased cytoplasmic density, and vacuolation. These changes
4 were also dose dependent, with more animals exhibiting these histopathological changes with
5 increasing TCDD dose. Based on these results, the study authors concluded that exposure to
6 TCDD resulted in a wide range of adverse effects with the thyroid proving to be most sensitive.

7 A LOAEL for TCDD of 25 ng/kg for a 28-day exposure is identified for alterations in
8 thyroid, thymus, and liver histopathology. The NOAEL for this study is 2.5 ng/kg-day.
9

10 **2.4.2.4.3. DeCaprio et al. (1986, [197403](#)).**

11 Hartley guinea pigs (10 per sex per dose) were administered TCDD (purity not specified)
12 in the diet for 90 days at concentrations of 0, 2, 10, 76, or 430 ppt (equivalent to 0, 0.12, 0.61,
13 4.9, and 26 ng/kg-day in males and 0, 0.12, 0.68, 4.86, and 31 ng/kg-day in females calculated by
14 the study authors using food consumption and body weights). Other animals were administered
15 the high-dose diet (i.e., 430 ppt) for 11, 21, or 35 days and then administered the control diet
16 (i.e., no exposure) for the remainder of the 90 days for recovery analysis. Four high-dose males
17 died and two were sacrificed moribund by day 45; the remaining four animals were sacrificed on
18 day 46 for necropsy. Four high-dose females also died and two were sacrificed moribund by day
19 55 with the remaining females sacrificed on day 60 for necropsy. Animals in the 76- and
20 430-ppt groups had significantly ($p < 0.05$) reduced body weights. Organ weights were not
21 obtained in the 430-ppt group due to the early sacrifice, but in the 76-ppt group a significant
22 decrease in relative thymus weight ($p < 0.05$) was observed, and relative liver ($p < 0.01$) and
23 brain ($p < 0.05$) weights in males increased. Although a similar trend occurred in the females,
24 the results were not statistically significant. Males administered 76 ppt in the diet also had a
25 53% increase in triglycerides ($p < 0.05$). The same increase was observed in females, but was
26 not statistically significant. In the recovery groups, mortality during the recovery period after 11
27 or 21 days of treatment was 10% and after 35 days of treatment was 70%. Animals lost weight
28 during the treatment period. Although the body weight increased during the recovery period, the
29 body weight remained low compared to the control for the study duration.

1 The LOAEL from this study is 4.9 ng/kg-day for 90 days of exposure, based on
2 decreased body weight (12–15%; $p < 0.05$) and changes in organ weights (10–30%, significant
3 only in the males). The NOAEL is 0.61 ng/kg-day.

4 5 **2.4.2.4.4. *Devito et al. (1994, [197278](#))*.**

6 Female B6C3F1 mice (5 per treatment) were administered 0, 1.5, 4.5, 15, 45, or
7 150 ng/kg TCDD (98% pure) in corn oil via gavage, 5 days a week for 13 weeks. This dose is
8 equivalent to 0, 1.07, 3.21, 10.7, 32.1, 107 ng/kg-day (adjusted for continuous exposure,
9 administered dose multiplied by 5 and divided by 7). Body weight was recorded weekly and
10 animals were sacrificed 3 days after the last treatment. Examinations were performed on the
11 lung, skin, uterus, and liver. No differences were observed in the liver or uterus weights or in the
12 estrogen receptor levels in these two tissues. A dose-dependent increase in EROD activity (an
13 indicator of CYP1A1 [CYP] induction) in the lung, skin, and liver was observed, with significant
14 ($p < 0.05$) increases even at the lowest dose. The TCDD doses used did not achieve maximal
15 EROD induction. A significant ($p < 0.05$) increase in liver acetanilide-4-hydroxylase (ACOH;
16 an indicator of CYP1A2 induction) also was observed with all doses. A maximum induction of
17 ACOH occurred with doses of 3.21 ng/kg-day and greater. A dose-dependent increase in
18 specific phosphotyrosyl protein (pp) levels also was observed. Levels of pp34 and pp38 were
19 significantly ($p < 0.05$) increased even at the lowest dose, while pp32 reached statistical
20 significance ($p < 0.05$) with doses of 4.5 ng/kg-day and above.

21 The role of CYPs and phosphorylated pp32, pp34, and pp38 in TCDD-mediated toxicity
22 is unknown, and changes in the activity or function of these proteins are not considered adverse
23 Therefore, no LOAEL or NOAEL is established. The 13-week LOEL is 1.07 ng/kg-day, based
24 on a significant ($p < 0.05$) increase in EROD, ACOH, pp34, and pp38 levels (all increased by at
25 least 2-fold). No NOEL is established for this study.

26 27 **2.4.2.4.5. *Fattore et al. (2000, [197446](#))*.**

28 Fattore et al. (2000, [197446](#)) examined TCDD-induced reduction of hepatic vitamin A
29 levels in a subchronic rat bioassay on Sprague-Dawley rats. Four experiments were conducted;
30 Experiments 1, 2, and 3 were conducted in both male and female rats, while Experiment 4 was
31 conducted only in female rats. The dosing regimens for each experiment were as follows

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1 **Experiment 1:** Groups of six Iva:SIV 50 rats (male and female) were maintained on a diet
2 consisting of 0, 200, 2,000, or 20,000 ng TCDD/kg diet and 3- μ g vitamin A/kg diet for
3 13 weeks. Assuming food consumption of 10% of body weight per day, the average daily
4 doses are 0, 20, 200, and 2,000 ng/kg-day TCDD.

5 **Experiment 2:** Groups of six male and female rats were treated with 0 or
6 200 ng TCDD/kg-day and 3 μ g vitamin A/kg diet for 13 weeks.

7 **Experiment 3:** Groups of six male and female rats were fed 0, 200, or
8 1,000 ng TCDD/kg-day and 3 μ g vitamin A/kg diet for 13 weeks.

9 **Experiment 4:** Groups of female rats (number not specified; IVA;SIV 50 Sprague-Dawley
10 strain) were treated with TCDD for 26 and 39 weeks in addition to a 13-week dietary
11 treatment with 0 or 100 ng TCDD/kg-day and 3 μ g vitamin A/kg diet for 13 weeks.
12

13 For a 13-week exposure duration employed in all four experiments, male and female rats
14 were treated at 0, 20, 100 (females only), 200, 1,000, or 2,000 ng/kg-day. In all
15 four experiments, liver from control and treated animals was analyzed at termination for free
16 retinol content to determine hepatic vitamin A levels.
17

18 **Results:**

19 **Experiment 1:** Liver and body weights in both treated males and females were significantly
20 affected at all but the lowest dose tested (20 ng/kg-day). Liver injury was severe, particularly
21 in female rats treated with 2,000 ng TCDD/kg-day. Dietary intake of vitamin A in male rats
22 was comparable to intake in controls, except in the 2,000 ng/kg-day group, which showed a
23 reduction of 16% in the dietary intake of vitamin A compared to controls. There was no
24 effect of TCDD on vitamin A intake in female rats. Hepatic vitamin A levels showed a
25 dose-dependent reduction with levels dropping sharply in the 200 and 2,000 ng/kg-day dose
26 groups, particularly in treated females. The reduction was significant at 200 ng/kg-day
27 ($p < 0.05$) and 2,000 ng/kg-day ($p < 0.01$) in males, and at 200 ng/kg-day ($p < 0.5$) and
28 2,000 ng/kg-day ($p < 0.001$) in females. The reductions ranged from 68–99% in males and
29 72–99% in females when compared to corresponding controls.

30 **Experiment 2:** Changes in liver and body weights were not reported. Hepatic vitamin A
31 level in males and females were reduced by 70% and 99%, respectively, compared to
32 controls, in rats receiving 20 ng/kg-day (significance level in females: $p < 0.01$).

33 **Experiment 3:** Similar to the results of Experiments 1 and 2, a dose-related trend of
34 significantly ($p < 0.001$) reduced hepatic vitamin A level was observed in both males and
35 females, with males exhibiting a particularly sharp drop at the 1,000 ng/kg-day dose
36 compared to controls.

37 **Experiment 4:** Females treated with 100 ng/kg-day showed significant reductions in hepatic
38 vitamin A levels ($p < 0.05$ – 0.001) at all three treatment durations (13, 26, and 39 weeks).
39

1 A LOAEL for TCDD of 20 ng/kg-day for a 13-week subchronic exposure was identified
2 in this study for decreased hepatic vitamin A levels (27 and 24 % lower than the corresponding
3 control in female and male rats, respectively). This LOAEL is determined using data from
4 Experiment 1. A NOAEL was not identified in this study.

5 **2.4.2.4.6. *Fox et al. (1993, [197344](#))*.**

6 Sprague-Dawley rats (6 per sex per dose) were gavaged with TCDD (purity not
7 specified) in corn oil using a dose-loading regime to achieve and maintain steady-state levels of
8 0.03, 30, or 150 ng/g in the liver. The regime consisted of an initial loading dose of 5, 2,500, or
9 12,000 ng/kg followed every 4 days with a maintenance dose of 0.9, 600, or 3,500 ng/kg.
10 Averaging the doses over the 14 days provides average daily doses of 0.55, 307, and
11 1,607 ng/kg-day (e.g., 5 ng/kg-day on day 1 and 0.9 ng/kg-day on days 5, 9, and 13 is $5 + 0.9$
12 $+ 0.9 + 0.9/14 = 0.55$ ng/kg-day). Body weight, liver weight, and liver gene expression were
13 measured at 7 and 14 days. A significant ($p < 0.05$) decrease in body weight occurred in
14 high-dose males (at 14 weeks only) and females (at 7 and 14 days). A significant ($p < 0.05$)
15 increase in absolute and relative liver weights was observed in mid- and high-dose males and
16 females at both 7 and 14 days. Although the liver of treated animals indicated moderate
17 vacuolization and swelling, there was no indication of necrosis. An increase in gene expression
18 (clone 1, CYP1A1, CYP1A2, and albumin) was observed in the mid- and high-dose groups. A
19 significant ($p < 0.05$) decrease in labeling index (indication of cell proliferation) occurred in both
20 females (all doses) and males (high-dose only) during week 1, but not during week 2.

21 The 14-day LOAEL is 307 ng/kg-day for significant ($p < 0.05$) increases in absolute and
22 relative liver weights (25–34%). The NOAEL is 0.55 ng/kg-day.

23 **2.4.2.4.7. *Hassoun et al. (1998, [136626](#))*.**

24 Female B6C3F1 mice (number not specified) received TCDD (>98% pure) in corn oil
25 5 days per week for 13 weeks via gavage at doses of 0, 0.45, 1.5, 15, or 150 ng/kg (equivalent to
26 0, 0.321, 1.07, 10.7, and 107 ng/kg-day adjusted for continuous exposure; administered dose
27 multiplied by 5 and divided by 7). Three days after the final dose, animals were sacrificed and
28 brains were removed for oxidative stress testing. Biomarkers for oxidative stress included
29 production of superoxide anion, lipid peroxidation, and DNA single-strand breaks. A significant

1 ($p < 0.05$) increase was observed in superoxide anion production, lipid peroxidation as measured
2 by thiobarbituric acid-reactive substances (TBARS), and DNA single-strand breaks with all
3 doses tested.

4 No other indicators of brain pathology were assessed, and it is unfeasible to link the
5 markers of oxidative stress to a TCDD-induced toxicological outcome in the brain. Thus, no
6 LOAEL/NOAEL was established. The subchronic (13-week) LOEL is 0.32 ng/kg-day, based on
7 significant ($p < 0.05$) increases in superoxide anion production (80% above control); lipid
8 peroxide production (25% above the control); and DNA single-strand breaks (2-fold over the
9 control). No NOEL is established.

11 **2.4.2.4.8. Hassoun et al. (2000, [197431](#)).**

12 Hassoun et al. (2000, [197431](#)) examined the effect of subchronic TCDD exposure on
13 oxidative stress in hepatic and brain tissues. Groups of 8-week-old female Harlan Sprague-
14 Dawley rats (6 rats/group) were administered TCDD (98% purity, dissolved in 1% acetone in
15 corn oil) via gavage at 0, 3, 10, 22, 46, or 100 ng/kg-day, 5 days/week for 13 weeks (0, 2.14,
16 7.14, 15.7, 32.9, or 71.4 ng/kg-day adjusted for continuous exposure; administered doses were
17 multiplied by 5 and divided by 7 days/week). Animals were sacrificed at the end of the study
18 period, and brain and liver tissues were collected and used to determine the production of
19 reactive oxygen species, lipid peroxidation, and DNA single-strand breaks (SSBs).

20 A dose-dependent effect was observed in both the liver and brain tissue as a result of
21 TCDD treatment. Based on the maximal induction of superoxide anion by various doses, more
22 production of superoxide anion was observed in the liver tissue when compared to the brain
23 tissue with an observed increase of 3.1- and 2.2-fold respectively, when compared to the control
24 group. A similar dose-dependent effect was observed in the induction of lipid peroxidation in
25 TCDD-treated animals with an approximately 1.8-fold increase in lipid peroxidation in both
26 tissues relative to the corresponding controls. A dose-dependent relationship was also observed
27 for DNA SSBs in both the hepatic and brain tissues at all TCDD-treated doses compared to
28 controls. Increases were statistically significant ($p \leq 0.05$) beginning at the lowest administered
29 dose.

30 Similar to the statement above, because no adverse endpoints were measured, no
31 LOAEL/NOAEL was established. However, a LOEL for TCDD of 2.14 ng/kg-day for a

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1 13-week exposure duration was identified in this study for significant increases ($p \leq 0.05$) in
2 superoxide anion, lipid peroxidation, and DNA SSBs in the liver and brain tissues. A NOEL
3 cannot be determined for this study.

4
5 **2.4.2.4.9. Hassoun et al. (2003, [198726](#)).**

6 Hassoun et al. (2003, [198726](#)) examined the role of antioxidant enzymes in
7 TCDD-induced oxidative stress in various regions of the rat brain after subchronic exposure.
8 Groups of 8-week-old female Harlan Sprague-Dawley rats (12 rats/group) were administered
9 TCDD (98% purity, dissolved in 1% acetone in corn oil) via gavage at 0, 10, 22, or 46 ng/kg-day
10 (0, 7.14, 15.7, or 32.9 ng/kg-day adjusted for continuous exposure; administered doses were
11 multiplied by 5 and divided by 7) daily for 13 weeks. Animals were sacrificed at the end of the
12 study period and the brain was immediately removed and dissected to the following regions:
13 cerebral cortex (Cc), hippocampus (H), cerebellum (C), and brain stem including midbrain, pons,
14 and medulla. Four pooled samples from each region per dose (i.e., 3 animals/pooled sample)
15 were used in the study. Dissected regions were subsequently assayed for lipid peroxidation
16 (thiobarbituric acid reactive substances, or TBARS), superoxide dismutase, catalase, and
17 glutathione peroxidase. Because the cytochrome c reduction method was used to determine
18 superoxide anion (SA) production in brain tissues, superoxide dismutase (SOD) was added to
19 some of the brain tissue samples that had the highest SA production (tissue homogenates from
20 Cc and H from rats treated with 46 ng/kg-day TCDD).

21 A dose-dependent increase in the production of SA was observed in the Cc and H, but
22 significant changes in SA production were not observed in either the C or the mid-brain, pons, or
23 medulla brain stem cells. Similar to SA production, there was a dose-dependent increase in the
24 production of TBARS in the Cc and H regions of the brain, but no significant changes were
25 observed in either the C or the B sections of the brain. The study authors also measured the
26 activities of various enzymes as a result of TCDD treatment and reported a dose-dependent
27 increase in SOD activity in the C and B sections, while there was dose-dependent suppression in
28 SOD activity in Cc and H. In contrast, catalase activity was significantly ($p < 0.05$) increased in
29 H and Cc at the 10 ng/kg-day TCDD dose level compared to controls and the mid- and high-dose
30 animals. Catalase activity also was increased in a dose-dependent manner in the C section, but
31 no significant changes in the activity of this enzyme were observed in the B section at any of the

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1 three TCDD tested doses. The effects of subchronic exposure to different doses of TCDD on
2 glutathione stimulating hormone peroxidase (GSH-Px) showed a different response compared to
3 other enzymes. There was a dose-dependent increase in the activity of this enzyme in the C and
4 B regions of the brain, while a significant increase in the activity of GSH-Px occurred in Cc and
5 H only at the 10 ng/kg-day TCDD dose. In addition, the activity of this enzyme was suppressed
6 in a dose-dependent manner in the Cc and H at 22 and 46 ng/kg-day TCDD doses. Based on
7 these results, the study authors concluded that induction of oxidative stress by TCDD in the rat
8 brain occurs mainly in the Cc and H regions.

9 Similar to the statement above, because no adverse endpoints were measured, no
10 LOAEL/NOAEL was established. However, a LOEL for TCDD of 7.14 ng/kg-day for a
11 13-week exposure duration was identified for this study for increases in superoxide anion and
12 lipid peroxidation production, as well as increased activity in SOD, catalase, and GSH-Px.

13

14 **2.4.2.4.10. Kociba et al. (1976, [198594](#)).**

15 Adult Sprague-Dawley rats (12 per sex per treatment group) were administered TCDD
16 (purity not reported) in corn oil via gavage 5 days per week at doses of 0, 1, 10, 100, or
17 1,000 ng/kg-day (equivalent to 0, 0.71, 7.14, 71.4, or 714 ng/kg-day averaged over 7 days; 5/7 of
18 dose). Five animals per group were sacrificed at the end of treatment, and the remaining animals
19 were observed over 13 weeks post treatment (only initial results for the post-treatment period
20 were provided in the report). Body weights and food consumption were measured semiweekly.
21 Hematology and clinical chemistry were measured after 36–37 or 85–86 days of treatment and
22 59–60 days after termination of treatment. Forty-eight hour urine samples were collected from
23 select rats from 85–89 days of treatment and 52–56 days after cessation of treatment. Gross and
24 histopathological exams were conducted on the tissues.

25 Four high-dose females died during treatment. Two high-dose females and
26 two high-dose males died during the post-treatment period. Animals treated with 714 ng/kg-day
27 were less active during the treatment period, which became less evident during the
28 post-treatment period. Yellow discoloration of the external pinnae also was noted in this group,
29 both during treatment and during the post-treatment period. A significant ($p < 0.05$) reduction in
30 body weight and food consumption was observed in the 71.4 and 714 ng/kg-day groups. The
31 following significant ($p < 0.05$) hematology changes were observed in the high-dose

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1 (714 ng/kg-day) males at all measured time points: decreased packed cell volume, decreased red
2 blood cells, decreased hemoglobin, increased reticulocytes, and decreased thrombocytes.
3 Significant ($p < 0.05$) changes also occurred in the high-dose females, but the only consistent
4 observation was a decrease in thrombocytes and increased leukocytes. Significant changes in
5 clinical chemistry ($p < 0.05$) and urinalysis ($p < 0.05$) were more consistent between the sexes in
6 the high-dose group and included increases in total and direct serum bilirubin; increase in serum
7 alkaline phosphatase; decreased urinary creatinine; and increased urinary coproporphyrin,
8 uroporphyrin, and delta-amino-levulinic. The following significant ($p < 0.05$) changes were
9 observed in the 71.4 ng/kg-day group: decreased packed cell volume (4–9%) in males; decreased
10 red blood cells (2–10%) in males; decreased hemoglobin (2–13%) in males; increased urinary
11 coproporphyrin (2.2-fold increase during treatment) in females; increased urinary
12 delta-amino-levulinic (47% increase during treatment) in females; increased total and direct
13 serum bilirubin (48–61%) in females; and increased serum alkaline phosphatase (2-fold) in
14 females. The following significant ($p < 0.05$) changes in relative organ weights were observed
15 increased brain weight in 714 ng/kg-day males and females; increased liver weight in males
16 (71.4 and 714 ng/kg-day) and females (7.14, 71.4, and 714 ng/kg-day); increased spleen weight
17 in 714-ng/kg-day males and females; decreased thymus weight in 71.4 and 714 ng/kg males and
18 females; and increased testes weight in 714 ng/kg-day males. Microscopic changes were
19 observed in the thymus, and in other lymphoid tissues, and in the liver in rats treated with
20 71.4 ng/kg-day or greater.

21 The subchronic (13-week) LOAEL is 71.4 ng/kg-day, based on the numerous changes
22 noted in body weight, hematology, clinical chemistry, urinalysis, and histopathology. The
23 NOAEL is 7.14 ng/kg-day.

24

25 **2.4.2.4.11. Mally and Chipman (2002, [198098](#)).**

26 Female F344 rats (3 per treatment group) were administered TCDD at concentrations of
27 0, 2.5, 25, or 250 ng/kg in corn oil via gavage for either 3 consecutive days or 2 days per week
28 for 28 days (Mally and Chipman, 2002, [198098](#)). The average daily doses for the 28-day study
29 when adjusted for 7 days a week were 0, 0.71, 7.1, and 71 ng/kg-day (i.e., 2/7 of administered
30 dose). No clinical signs of toxicity were observed. Histological examination of the liver
31 revealed no abnormalities. All doses of TCDD reduced the number of connexin (Cx) 32 plaques

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1 and Cx32 plaque area in the liver, which was considered the target tissue. The reductions were
2 not statistically significant after the 3-day treatment, but were significant after the 28-day
3 treatment ($p < 0.05$). TCDD also caused a reduction in the Cx32 plaque number and area in the
4 thyroid after 28 days, but the results were not statistically significant. Although the reduction in
5 Cx32 plaque number and plaque area in the liver and thyroid occurred at all dose levels, there
6 was no relation to dose. TCDD did not induce hepatocyte proliferation.

7 In the absence of additional indicators of hepatotoxicity, changes in Cx32 plaques are not
8 clearly linked to TCDD-mediated hepatotoxicity, nor are they considered an adverse effect.
9 Additionally, no toxicologically-relevant endpoints were examined. Therefore, a NOAEL or
10 LOAEL cannot be determined. A 28-day LOEL at the lowest dose of 0.71 ng/kg-day for
11 significantly ($p < 0.05$) decreased Cx32 plaque area is evident (approximately 70% of the
12 controls).

14 **2.4.2.4.12. Slezak et al. (2000, [199022](#)).**

15 Slezak et al. (2000, [199022](#)) studied the impact of subchronic TCDD exposure on
16 oxidative stress in various organs of B6C3F1 female mice. Groups of 8- to 10-week-old female
17 B6C3F1 mice (number not specified) were administered TCDD (purity >98%, dissolved in corn
18 oil) via gavage at 0, 0.15, 0.45, 1.5, 15, or 150 ng/kg-day (0, 0.11, 0.32, 1.07, 10.7, or
19 107.14 ng/kg-day adjusted for continuous exposure) 5 days per week for 13 weeks. Three days
20 after the last treatment, the animals were sacrificed and organs were removed for the
21 measurement of oxidative stress indicators including SA, lipid peroxidation (TBARS), and
22 GSH-Px. Tissue TCDD concentrations also were measured.

23 The study authors reported that TCDD dose range resulted in overlapping tissue
24 concentrations for liver, lung, kidney and spleen. Liver had the highest TCDD concentration,
25 with each tissue demonstrating a dose-dependent increase in TCDD concentration. Compared to
26 controls, SA production was significantly ($p < 0.05$) lower at the 0.15 ng/kg-day TCDD dose,
27 while it was significantly ($p < 0.05$) higher at 15 and 150 ng/kg-day. A dose-dependent increase
28 in hepatic TBARS production was observed, although the rate of production was significant
29 ($p < 0.05$) only at the highest TCDD administered dose (150 ng/kg-day) compared to controls.
30 AA also followed the same pattern observed for SA and TBARS with AA production
31 significantly ($p < 0.05$) increased at the 15 and 150 ng/kg-day TCDD doses. Contrary to the SA,

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1 TBARS, and AA responses, GSH levels were decreased at 0.15 ng/kg-day, were increased at
2 0.45 and 150 ng/kg-day, and did not change at 1.5 or 15 ng/kg-day when compared to the control
3 group. Unlike the liver, there was no significant increase in SA production in the lung at any of
4 the TCDD tested doses; a dose dependent reduction, however, was observed at 0.45, 15, and
5 150 ng/kg-day compared to controls. GSH and AA production was decreased at 0.15 ng/kg-day,
6 while AA production was significantly ($p < 0.05$) increased at 15 and 150 ng/kg-day. Kidney
7 SA production showed a statistically significant ($p < 0.05$) increase only at the 15 and
8 150 ng/kg-day doses. GSH, like the liver and the lung, exhibited a decrease in production
9 following treatment at 0.15 ng/kg-day with this trend continuing at 0.45 and 1.5 ng/kg-day. AA
10 levels were significantly ($p < 0.05$) lower at all subchronic doses, except at 1.5 ng/kg-day dose.
11 SA levels in the spleen differed little from the control group at any of the TCDD doses. Total
12 GSH was higher only at the 150 ng/kg-day dose level, while the AA levels were significantly
13 ($p < 0.05$) decreased at 0.15, 1.5, and 150 ng/kg-day.

14 Similar to the statements regarding the Hassoun et al. studies above, because no adverse
15 endpoints were measured, no LOAEL/NOAEL was established. Therefore, a NOAEL or
16 LOAEL cannot be determined. However, a NOEL and LOEL of 1.07 and 10.7 ng/kg-day,
17 respectively, are identified in this study for increases in superoxide anion in the liver.

18

19 **2.4.2.4.13. Smialowicz et al. (2008, [198341](#)).**

20 Female B6C3F1 mice (8–15 per treatment group) were administered TCDD (purity
21 >98%) in corn oil by gavage at doses of 0, 1.5, 15, 150, or 450 ng/kg-day, 5 days a week for
22 13 weeks (1.07, 10.7, 107, or 321 ng/kg-day, adjusted for continuous exposure; i.e., 5/7 of the
23 dose) (Smialowicz et al., 2008, [198341](#)). Mice were immunized 3 days after the final TCDD
24 exposure with an intravenous injection of an optimal concentration of 4×10^7 SRBCs and
25 sacrificed 4 days later. No TCDD-related effects on body weight were observed. There was a
26 dose-related decrease in relative spleen weight (9–19% lower than control values) with
27 statistically significant ($p < 0.05$) decreases at all but the lowest dose. Additionally, there was a
28 statistically significant ($p < 0.05$) increase in relative liver weight (5–21%) in all treatment
29 groups compared to controls. Statistically significant dose-dependent decreases were observed
30 in the antibody response to SRBCs (24–89% lower than control values), as measured by both the
31 number of plaque forming cells per 10^6 cells and plaque forming cells per spleen.

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1 The 13-week LOAEL for this study is 1.07 ng/kg-day based on a significant ($p < 0.05$)
2 increase in relative liver weight (10%) and a significant ($p < 0.05$) decrease in antibody response
3 to SRBCs (24%). A NOAEL cannot be determined for this study.
4

5 **2.4.2.4.14. *Van Birgelen et al. (1995, [197096](#); 1995, [198052](#))***

6 Van Birgelen et al. (1995, [197096](#); 1995, [198052](#)) studied the impact of TCDD exposure
7 on various biochemical endpoints in rats. Groups of 7-week-old female Sprague-Dawley rats
8 ($n = 8$ per treatment group) were treated with 0, 200, 400, 700, 5,000, or 20,000 ng/kg TCDD
9 (purity >99%) in diet for 13 weeks. Daily TCDD intake based on food consumption, diet level,
10 and mean weight was estimated to be 0, 14, 26, 47, 320, or 1,024 ng/kg-day. Blood samples
11 were collected from treated animals and assayed for retinol (vitamin A), triiodothyronine, and
12 total (TT4) and free (FT4) thyroxine. At study termination, the animals were sacrificed and the
13 liver, thymus, spleen, and kidneys were removed and weighed. Parts of the liver were
14 homogenized and assayed to determine EROD; CYP1A1; CYP1A2; and UDPGT activity. Liver
15 samples also were analyzed for retinol content.

16 TCDD-treated animals showed a dose-related decrease in food consumption. Animals
17 treated with 1,024 ng/kg-day TCDD consumed 32% less food compared to controls. Similarly, a
18 dose-related decrease in body weight gain was observed in all animals treated with TCDD.
19 Animals treated with ≥ 47 ng/kg-day of TCDD showed a statistically significant ($p < 0.05$)
20 decrease in body weight gain. Relative liver weights were significantly ($p < 0.05$) increased in
21 the 320 and 1,024 ng/kg-day TCDD dose groups compared to the controls. Absolute and relative
22 thymus weights were significantly ($p < 0.05$) decreased at all TCDD dose groups compared to
23 the control group. Relative kidney and spleen weights were significantly ($p < 0.05$) higher in
24 animals dosed with ≥ 47 ng/kg-day of TCDD compared to the control group, with the greatest
25 increase occurring in animals treated with 1,024 ng/kg-day TCDD (121 and 173% higher than
26 controls for kidney and spleen, respectively). Cytochrome P450 enzymes, including EROD,
27 CYP1A2, CYP1A1, and UDPGT, exhibited statistically significant ($p < 0.05$) increases in
28 activity at all TCDD dose groups compared to the control group. TT4 and FT4 thyroid hormone
29 concentrations were statistically significantly ($p < 0.05$) decreased only at TCDD doses
30 ≥ 47 ng/kg-day. A dose-dependent increase was observed in the plasma retinol concentrations
31 with significant ($p < 0.05$) increases occurring at ≥ 47 ng/kg-day TCDD after a 13-week

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1 exposure. A dose-dependent reduction in liver retinoid levels also was observed after 13 weeks
2 of TCDD exposure with the levels dropping significantly ($p < 0.05$) at all TCDD-treated doses
3 compared to the control group.

4 A LOAEL for TCDD of 14 ng/kg for a 13-week exposure is identified for significantly
5 ($p < 0.05$) decreased absolute and relative thymus weights and significantly ($p < 0.05$) decreased
6 liver retinoid levels. A NOAEL cannot be determined for this study.

7 8 **2.4.2.4.15. Vos et al., (1973, [198367](#)).**

9 Vos et al. (1973, [198367](#)) conducted a study to examine the immune response in
10 laboratory animals treated with TCDD. In one experiment, 10 female Hartley strain guinea pigs
11 were orally treated with 8 weekly doses of 0, 8, 40, 200, and 1,000 ng/kg TCDD in corn oil
12 (purity of TCDD not specified) (0, 1.14, 5.71, 28.6, and 143 ng/kg-day adjusted for continuous
13 exposure; administered dose divided by 7). At study termination, the animals were sacrificed,
14 and heart blood was used to determine total leukocyte and differential leukocyte counts. In
15 another experiment, the effect of TCDD on humoral immunity was determined by injecting
16 0.1 mL of tetanus toxoid into the right hind-foot pad on day 28 (1 left foot tetanus toxoid,
17 aluminum phosphate-adsorbed) and again on day 42 (1 left foot tetanus toxoid, unadsorbed).
18 Blood was collected ($n = 10$) on days 35 and 49, and the serum tetanus-antitoxin concentrations
19 were determined using a modified single radial immunodiffusion technique.

20 All guinea pigs receiving 1,000 ng/kg-day TCDD either died or were killed when
21 moribund between 24 and 32 days. These animals showed severe weight loss, lymphopenia, and
22 depletion of the lymphoid organs, especially the thymus. Microscopic observations revealed
23 severe atrophy of the thymic cortex with substantial destruction of lymphocytes, with the nuclear
24 debris being engulfed by macrophages. Large cystic Hassall bodies, filled with
25 polymorphonuclear leukocytes were observed in the medulla. All animals treated with 0, 8, 40,
26 or 200 ng/kg-day TCDD survived until study termination. Body weight gain was significantly
27 ($p < 0.01$) lower in the 200 ng/kg-day group. Absolute thymus weight was significantly reduced
28 in the 40 and 200 ng/kg-day treatment groups ($p < 0.01$ and $p < 0.05$, respectively). In contrast,
29 relative thymus weight was significantly ($p < 0.01$) reduced only in the 200 ng/kg-day dose
30 group. The absolute weight of the superficial cervical lymph nodes was significantly ($p < 0.05$)
31 decreased in the 200 ng/kg-day group, while the relative adrenal weight was significantly

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1 ($p < 0.05$) increased in the 200 ng/kg-day dose group. Total leukocyte count was significantly
2 ($p < 0.05$) decreased in the 40 ng/kg-day dose group and total lymphocyte count was
3 significantly decreased at 8, 40, and 200 ng/kg-day ($p < 0.01$, $p < 0.05$, and $p < 0.05$,
4 respectively). A significant (p -values not provided) monotonic dose-response relationship was
5 determined for body weight (decrease), relative thymus weight (decrease), relative adrenal
6 weight (increase), and total leukocyte and lymphocyte count (decrease). Microscopic
7 examination of the lymphoid organs and adrenals showed no effects, while slight cortical atrophy
8 of the thymus was observed at the 200 ng/kg-day dose.

9 Animals receiving the tetanus toxoid injection showed a small but significant increase in
10 serum tetanus antitoxin concentrations at the 8 and 40 ng/kg-day dose ($p < 0.05$ and $p < 0.01$,
11 respectively). Measurement at days 49 and 56 indicated that serum antitoxin levels had
12 decreased sharply and the significant ($p < 0.05$ on day 49 and $p < 0.01$ on day 56) effect was
13 seen only at the 200 ng/kg-day dose level.

14 A LOAEL for TCDD of 5.71 ng/kg-day for an 8-week exposure is identified in this study
15 for significantly ($p < 0.01$) reduced absolute thymus weight, significantly ($p < 0.05$) reduced
16 leukocyte and lymphocyte count, and significantly ($p < 0.01$) increased serum tetanus antitoxin
17 concentration. The NOAEL for this study is 1.14 ng/kg-day.

18 19 **2.4.2.4.16. *White et al. (1986, [197531](#))*.**

20 White et al. (1986, [197531](#)) studied the impact of TCDD exposure on serum complement
21 levels. Groups of female (C57BL/6 × C3H)F1(B6C3F1) mice were treated for 14 consecutive
22 days with TCDD in corn oil (purity of TCDD not specified) at doses of 0, 10, 50, 100, 500, 1,000
23 or 2,000 ng/kg-day via gastric intubation ($n = 6-8$). At study termination, blood was collected
24 from anesthetized animals and assayed for serum complement activity and complement
25 component C3 levels.

26 Serum complement activity between the 10 and 100 ng/kg-day doses was between 69 and
27 59% compared to the vehicle control group, with all treatment groups being significantly
28 ($p < 0.05$) low compared to the vehicle control. In contrast, C3 levels were comparable to the
29 vehicle control with levels ranging between 98 and 94% of the control group. The higher doses
30 of 500, 1,000, and 2,000 ng/kg-day, however, produced a marked decrease of the component

1 hemolytic activity (45, 35, and 19% of the vehicle control) and of C3 levels (91, 81, and 74 % of
2 the vehicle control, respectively; significance level at $p < 0.05$).

3 A LOAEL for TCDD of 10 ng/kg-day for a 14-day exposure is identified in this study for
4 significantly ($p < 0.05$) lower serum complement activity. A NOAEL cannot be determined for
5 this study.

6 7 **2.4.2.5. Chronic Studies (Noncancer Endpoints)**

8 **2.4.2.5.1. Cantoni et al. (1981, [197092](#)).**

9 CD-COBS rats (4 per treatment) were orally administered TCDD (purity not specified)
10 dissolved in acetone:corn oil (1:6) at doses of 0 (vehicle alone), 10, 100, or 1,000 ng/kg per week
11 (equivalent to 1.43, 14.3, and 143 ng/kg-day adjusted for continuous exposure, administered
12 dose by dividing the dose by 7) for 45 weeks. Urine was collected several times during
13 treatment and tested for porphyrin excretion. Twenty-four hours after the final dose, animals
14 were sacrificed and their livers, spleens, and kidneys were removed for analysis of total
15 porphyrins. All treatment groups had a significant ($p < 0.05$) increase in coproporphyrin
16 excretion beginning at 6, 3, or 2 months, respectively. Uroporphyrin excretion was significantly
17 ($p < 0.05$) increased in the 14.3 ng/kg-day group at 10 months and in the 143 ng/kg-day group
18 beginning at 6 months. The high-dose group also had a significant ($p < 0.05$) increase in
19 excretion of heptacarboxylic methyl ester beginning at 6 months. The high-dose group had a
20 marked porphyric state beginning at 8 months as indicated by a 70-fold increase above controls
21 in total urinary porphyrin excretion. This group also had a significant ($p < 0.05$) increase in total
22 porphyrins in the liver, kidneys, and spleen.

23 The 45-week LOAEL for this study is 1.43 ng/kg-day, based on a 2- to 3-fold increase in
24 urinary coproporphyrin excretion. No NOAEL was established for this study.

25 26 **2.4.2.5.2. Croutch et al. (2005, [197382](#)).**

27 Croutch et al. (2005, [197382](#)) examined the impact of TCDD exposure on body weight
28 via insulin-like growth factor (IGF) signaling. Female Sprague-Dawley rats were randomly
29 assigned in groups of five to initial loading doses of TCDD (purity >98.5%, dissolved in corn
30 oil) at 0, 12.5, 50, 200, 800, or 3,200 ng/kg-day, followed by treatment with maintenance doses
31 equivalent to 10% of the initial loading dose every third day to maintain a pharmacokinetic

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1 steady state throughout the entire study (equivalent to: 14-day average = 0, 1.25, 5, 20, 80, or
2 320 ng/kg-day; 28-day average = 0, 0.85, 3.4, 13.6, 54.3, or 217 ng/kg-day; 63-day average = 0,
3 0.60, 2.4, 9.5, 38, or 152 ng/kg-day; and 128-day average dose = 0, 0.51, 2.0, 8.1, 32.5, or
4 130 ng/kg-day). Following 2, 4, 8, 16, 32, 64, or 128 days of initial dosing, the animals were
5 sacrificed, livers were removed and weighed, and trunk blood was collected to analyze glucose
6 content. Rat liver phosphoenolpyruvate carboxykinase (PEPCK) mRNA and protein levels also
7 were analyzed, and PEPCK activity was measured.

8 Body weights of TCDD-treated animals decreased after the second week of the
9 3,200 ng/kg-day TCDD loading dose, with significant differences beginning at week 9. There
10 was also a statistically significant ($p \leq 0.05$) difference in body weights at weeks 10, 11, 13, 18,
11 and 19 at the highest loading dose (3,200 ng/kg-day). PEPCK activity in the liver was also
12 decreased in a dose-dependent manner following TCDD administration at approximately
13 16 days. PEPCK inhibition was statistically significant ($p \leq 0.05$) on day 4 in rats treated with
14 either 800 or 3,200 ng/kg-day TCDD when compared to animals treated with a loading dose of
15 200 ng/kg-day. A similar statistically significant change was observed in animals treated with
16 3,200 ng/kg-day on day 16 when compared to the 200 ng/kg-day treatment group. In contrast,
17 differences in PEPCK activity at other doses or time points were not statistically significant. In
18 TCDD-treated animals, there was also a dose-dependent decrease in PEPCK mRNA expression
19 along with a decrease in PEPCK protein levels in the liver. In addition to body weight and
20 PEPCK activity changes, animals treated with 3,200 ng/kg-day TCDD showed a sharp decline in
21 circulating IGF-I levels on day 8 compared to the control group (corn oil) and TCDD-treated
22 animals at lower doses. In the highest dose animals, IGF-I levels continued to decline to 42% of
23 the control group by day 16 of the study. The IGF-I levels at the highest dose plateaued at an
24 average decrease of 66% through day 128 when compared to controls. Beginning at day 8, the
25 decrease in IGF-I was statistically significant at every time point through day 128 compared to
26 the control group, as well as groups treated with either 12.5 or 50 ng/kg-day TCDD. Similar
27 statistically significant decreases also were observed for the 800 ng/kg-day TCDD-treated groups
28 with an initial decrease of 37% on day 16 followed by a further decline to approximately 45%
29 thereafter compared to controls and the 12.5, 50, and 200 ng/kg-day dose groups. In contrast to
30 these results, circulating levels of insulin and glucose were unaffected by TCDD treatment, while

1 the active or phosphorylated form of AMPK- α protein increased with dose as a result of TCDD
2 treatment.

3 A LOAEL for TCDD of 217 ng/kg-day for a 28-day exposure duration (because this
4 represented the most sensitive time for elicitation of effects) was identified in this study for
5 decreased body weight, significant ($p \leq 0.05$) inhibition of PEPCK activity, and reduced IGF-I
6 levels (42% lower than the control group). A NOAEL of 54.3 ng/kg-day was identified in this
7 study.

8

9 **2.4.2.5.3. Hassoun et al. (2002, [543725](#)).**

10 Hassoun et al. (2002, [543725](#)) examined the potential of TCDD and other dioxin-like
11 chemicals to induce oxidative stress in a chronic rat bioassay. Groups of six Harlan
12 Sprague-Dawley female rats were treated with 0, 3, 10, 22, 46, or 100 ng/kg-day TCDD
13 (98% purity), 5 days a week via gavage for 30 weeks. The administered doses adjusted for
14 continuous exposure were 0, 2.14, 7.14, 15.7, 32.9, and 71.4 ng/kg-day, respectively
15 (administered doses were multiplied by 5 and divided by 7). At study termination, hepatic and
16 brain tissues from all treated rats were divided into two portions and examined for the production
17 of reactive oxygen species and SSBs in DNA.

18 When compared to controls, there was a dose-dependent increase in the production of
19 superoxide anion in TCDD-treated animals ranging from 21–998% and 66–257% in hepatic and
20 brain tissues, respectively. Hepatic tissues had statistically significant ($p < 0.05$) increases in
21 superoxide anion production at doses ≥ 7.14 ng/kg-day, while the brain tissue had a statistically
22 significant ($p < 0.05$) increase over controls at all doses. Similarly, increases in lipid
23 peroxidation were observed in hepatic and brain tissues with a 481% increase ($p < 0.05$) at
24 71.4 ng/kg-day in the hepatic tissue when compared to controls. The increase in lipid oxidation
25 in brain tissue ranged from 33–188% ($p < 0.05$) in the 2.14–71.4 ng/kg-day dose groups. DNA
26 SSBs were also observed in both hepatic and brain tissue in all treated groups. When compared
27 to the control group, there was a dose-dependent statistically significant ($p < 0.05$) increase in
28 DNA SSBs ranging from 58–322% and 29–137% in hepatic and brain tissues, respectively.
29 Nonmonotonic dose-response relationships were observed for superoxide production and lipid
30 peroxidation in liver tissues, with greater-than-linear increases in effect between the two highest
31 dose levels.

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1 As stated above, because no adverse endpoints were measured, no LOAEL/NOAEL was
2 established. However, a LOEL for TCDD of 2.14 ng/kg-day for a 30-week exposure duration is
3 identified in this study for significant ($p < 0.05$) increases in superoxide anion, lipid peroxidation
4 production, and DNA SSBs in the liver and brain tissues. A NOEL cannot be determined for this
5 study.

6
7 **2.4.2.5.4. Kociba et al. (1978, [001818](#)).**

8 Sprague-Dawley rats (50 per sex per treatment group) were administered TCDD (purity
9 >99%) in the diet at doses of 0, 1, 10, or 100 ng/kg-day for 2 years. Body weights and food
10 consumption were routinely measured. Hematology, clinical chemistry, and urinalysis were
11 measured after 3, 12, or 23 months of treatment. Animals were routinely palpitated for tumors.
12 Gross and histopathological exams were conducted on the tissues of dead or dying animals or at
13 terminal sacrifice. Specific organs also were weighed.

14 The high-dose females had a statistically significant ($p < 0.05$) increase in mortality
15 compared to the controls during the second half of the study. Mortality changes in males were
16 variable and of questionable toxicological significance. A significant ($p < 0.05$) reduction in
17 body weight occurred in the 100 ng/kg-day males and females beginning at 6 months. Mid-dose
18 females also had reduced body weight, but to a lesser degree during the same time frame. There
19 were no consistent changes in food consumption. The following significant ($p < 0.05$)
20 hematology changes were observed in the high-dose animals: decreased packed cell volume in
21 males after 3 months and in females after 1 year, decreased red blood cells in females after
22 1 year and in males at terminal sacrifice, decreased hemoglobin in males after 3 months and in
23 females after 1 year, and decreased total white blood cell count in females after 1 year. Changes
24 in clinical chemistry ($p < 0.05$) occurred only in high-dose females and consisted of an increase
25 in serum alkaline phosphatase and gamma glutamyl transferase. Significant changes in
26 urinalysis occurred only in females and included increased urinary coproporphyrin in the mid-
27 and high-dose groups, increased urinary uroporphyrin in the mid- and high-dose groups, and
28 increased urinary delta-amino-levulinic acid in the high-dose group. Significant ($p < 0.05$)
29 changes in relative organ weights were observed, including increased liver weight in mid- and
30 high-dose females and decreased thymus weight in high-dose females. Mid- and high-dose rats
31 showed hepatocellular degeneration and inflammatory and necrotic changes in the liver. Thymic

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1 and splenic atrophy were noted in high-dose females. An increase in non-neoplastic lung lesions
2 was noted in mid-dose females and high-dose males and females. High-dose females had an
3 increase in uterine changes. High-dose males had a significant ($p < 0.05$) increase in the
4 incidence of stratified squamous cell carcinomas of the tongue. High-dose males and females
5 had a significant ($p < 0.05$) increase in the incidence of squamous cell carcinomas of the hard
6 palate/turbinates.

7 The chronic (2-year) LOAEL is 10 ng/kg-day, based on the numerous significant
8 ($p < 0.05$) changes noted in coproporphyrin excretion (67% increase above control) and an
9 increase in liver and lung lesions in female rats. The NOAEL is 1 ng/kg-day.

11 **2.4.2.5.5. Maronpot et al. (1993, [198386](#)).**

12 An initiation-promotion study was performed in female Sprague-Dawley rats (8–10 rats
13 per group). Rats were initiated with saline or diethylnitrosamine (DEN), followed 2 weeks later
14 by promotion with biweekly administration of TCDD (purity not specified) in corn oil via
15 gavage for 30 weeks. The doses were stated to be equivalent to 3.5, 10.7, 35.7, or
16 125 ng/kg-day. Rats were sacrificed 7 days after the final treatment. A significant ($p < 0.05$)
17 decrease in body weight occurred in the 125 ng/kg-day group. A significant ($p < 0.05$) increase
18 in relative liver weight occurred in the 35.7 and 125 ng/kg-day groups. There was a significant
19 ($p < 0.05$) increase in the labeling index in the 125 ng/kg-day group, but only with DEN
20 initiation. In the TCDD-alone group, a 2-fold increase in labeling index occurred in the
21 125 ng/kg-day group that did not reach statistical significance. A significant ($p < 0.05$) trend for
22 increased alkaline phosphatase levels was observed in TCDD-treated animals, but despite a
23 50% increase in the highest dose group the increase was not statistically significant. Total
24 cholesterol and triglycerides were significantly ($p < 0.05$) higher in the
25 125 ng/kg-day TCDD-alone group. A significant ($p < 0.05$) increase in 5'-nucleotidase occurred
26 in the 35.7 and 125 ng/kg-day TCDD-alone groups. A dose-dependent increase in the incidence
27 and severity of liver toxicity as measured by microscopic lesions was observed.

28 The 30-week LOAEL is 35.7 ng/kg-day, based on a significant ($p < 0.05$) increase in
29 relative liver weight (12%, accompanied by increases in incidence and severity of liver lesions).
30 The 30-week NOAEL is 10.7 ng/kg-day.

1 **2.4.2.5.6. *National Toxicology Program (1982, [543764](#))***.

2 National Toxicology Program (NTP, 1982, [543764](#)) conducted a carcinogenic bioassay of
3 TCDD on rats and mice. Fifty male and female Osborne-Mendel rats and male and female
4 B6C3F1 mice were treated twice per week with TCDD (purity not specified) in corn oil via oral
5 gavage at doses of 0, 5, 25, or 250 ng/kg for rats and male mice (1.4, 7.1, 71 ng/kg-day adjusted
6 for continuous exposure; administered doses multiplied by 2 and divided by 7) and 0, 20, 100, or
7 1,000 ng/kg for female mice (5.7, 28.6, or 286 ng/kg-day adjusted for continuous dosing;
8 administered doses multiplied by 2 and divided by 7) for 104 weeks. Seventy-five rats and mice
9 of each sex served as vehicle controls. One untreated control group of 25 rats and mice of each
10 sex was present in the TCDD treatment room and one untreated control group consisting of
11 25 rats and mice of each sex were present in the vehicle-control room. Animals surviving until
12 study termination were sacrificed at 105 or 108 weeks. A complete histopathological evaluation
13 was conducted on all animals.

14 Survival rates were not affected by TCDD exposure in rats or mice of either sex. Male
15 rats exhibited a dose-related depression in mean body weight after week 55, while the females
16 exhibited a dose-related body-weight depression after 45 weeks of TCDD exposure. However,
17 the magnitude of the body weight response is not indicated. Mean body weights in male and
18 female mice were comparable to the vehicle control group throughout the bioassay. Noncancer
19 histopathologic findings included increased incidences of liver lesions (termed toxic hepatitis)
20 from TCDD exposure, and were detected in the high-dose rats and high-dose mice of each sex.

21 A LOAEL for TCDD of 1.4 ng/kg-day for a 104-week exposure duration is identified for
22 increased incidences of liver lesions in mice of both sexes. A NOAEL cannot be determined for
23 this study.

24
25 **2.4.2.5.7. *National Toxicology Program (2006, [197605](#))***.

26 Female Sprague-Dawley rats (81 control; 82 treatment group) were administered TCDD
27 (purity >98%) in corn oil:acetone (99:1) via gavage at doses of 0, 3, 10, 22, 46, or
28 100 ng/kg-day, 5 days per week for 105 weeks (0, 2.14, 7.14, 15.7, 32.9, or 71.4 ng/kg-day,
29 adjusted for continuous exposure) (NTP, 2006, [197605](#)). In addition to this primary group, a
30 stop group of 50 animals was administered 100 ng/kg-day TCDD in corn oil:acetone (99:1) via
31 gavage for 30 weeks and then just the vehicle for the remainder of the study. Up to 10 rats per

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1 dose group were sacrificed and evaluated at 14, 31, or 53 ($n = 8$) weeks for biologically
2 noteworthy changes in the incidences of neoplasms or non-neoplastic lesions in the liver, lung,
3 oral mucosa, uterus, pancreas, thymus, adrenal cortex, heart, clitoral gland, ovary, kidney,
4 forestomach, bone marrow, mesentery gland, and pituitary gland. All interim sacrifice animals
5 also received a complete necropsy and microscopic examination, and the following organs were
6 weighed: the left kidney, liver, lung, left ovary, spleen, thymus (14 weeks only), and thyroid
7 gland. Out of 53 control animals and 53 or 54 animals per treatment group not used for interim
8 sacrifice analyses, at study termination the number of surviving animals had declined to 25 in the
9 control group and to 21, 23, 19, 22, and 21 in five treatment groups, respectively, due to
10 accidental deaths, moribund animals, or death due to natural causes.

11 Survival rate was not affected by TCDD treatment. Mean body weights in the high dose
12 primary study group and the 100 ng/kg stop group were less than the vehicle control group after
13 week 13 of the study. The mean body weights of animals in the 46 ng/kg-day group were less
14 than in the vehicle control at study termination (2 years), whereas animals in the 22 ng/kg-day
15 had lower mean body weights compared to controls during the last 10 weeks of study. In
16 addition to body weight changes, liver weights were also impacted as a result of TCDD
17 exposure. Absolute and relative liver weights were significantly (either $p \leq 0.01$ or $p \leq 0.05$)
18 higher in all dose groups compared to controls at the 14- and 31-week evaluation period, whereas
19 the relative liver weights were significantly (either $p \leq 0.01$ or $p \leq 0.05$) higher only at
20 ≥ 10 ng/kg-day at 53 weeks.

21 No clinical findings associated with TCDD treatment were observed. TCDD caused
22 changes in thyroid hormone levels at 14, 31, and 53 weeks. The following changes were
23 statistically significant ($p \leq 0.05$) compared to the vehicle control: decrease in TT4 at doses
24 ≥ 22 ng/kg-day at 14 and 31 weeks and at doses ≥ 46 ng/kg-day at 53 weeks; decrease in FT4 at
25 doses ≥ 22 ng/kg-day at 14 and 31 weeks; increase in total T₃ at doses ≥ 46 ng/kg-day at 14 and
26 31 weeks and at doses ≥ 10 ng/kg-day at 53 weeks; and increase in TSH at doses ≥ 46 ng/kg-day
27 at 14 weeks. There was a statistically-significant ($p \leq 0.05$) increase in hepatocyte proliferation
28 at 14 weeks (22 ng/kg-day group only); 31 weeks (all doses); and 53 weeks (≥ 46 ng/kg-day).
29 There were statistically significant ($p \leq 0.01$) dose-dependent increases in liver (includes EROD
30 [CYP1A1-associated] activity; 7-pentoxoresorufin-O-deethylase [PROD; CYP2B-associated]
31 activity; and acetanilide-4-hydroxylase [CYP1A2-associated] activity) and lung (EROD)

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1 cytochrome P450 enzyme activities in all treatment groups at all three evaluation periods
2 compared to the vehicle control group. The largest effect was an 82-fold induction of hepatic
3 EROD activity in the 46 ng/kg-day group at 31 weeks.

4 TCDD was detected at the greatest concentration in the liver, followed by fat tissue, with
5 tissue concentration increasing in both of these tissues in a dose-dependent manner. TCDD
6 tissue levels generally remained constant after the first measurement at week 14. Pathological
7 examination at week 14 revealed increased incidences of hepatocellular hypertrophy in animals
8 administered ≥ 10 ng/kg-day TCDD. Examinations at weeks 31 and 53 indicated that incidence
9 and or severity of hepatocellular hypertrophy was increased at all treatment doses although
10 incidences were statistically significant ($p \leq 0.05$) only at ≥ 10 ng/kg-day doses. The incidence of
11 non-neoplastic hepatic lesions (including inflammation, necrosis, multiple eosinophilic focus,
12 diffuse fatty change, pigmentation, toxic hepatopathy) in the liver increased at doses
13 ≥ 22 ng/kg-day beginning at 14 weeks. Severity of the lesions increased at 14 weeks at doses
14 ≥ 46 ng/kg-day and were also observed at lower dose levels during later evaluation periods (31
15 and 53 weeks). By terminal sacrifice, numerous non-neoplastic changes were noted in TCDD
16 treated rats, even at the lowest dose tested.

17 Noncancer cardiovascular and pulmonary effects were evident after 2 years of TCDD
18 exposure. Significantly increased incidences of minimal to mild cardiomyopathy were seen in
19 male and female rats at ≥ 10 ng/kg-day. In the lung, there was a significant ($p \leq 0.01$)
20 dose-dependent increase, when compared to the vehicle control, in the incidence of bronchiolar
21 metaplasia of the alveolar epithelium at all dose groups in the primary study.

22 A LOAEL for TCDD of 2.14 ng/kg-day adjusted dose for a 105-week exposure duration
23 is identified in this study for significantly (either $p \leq 0.01$ or $p \leq 0.05$) increased absolute and
24 relative liver weights, increased incidence of hepatocellular hypertrophy, and increased incidence
25 of alveolar to bronchiolar epithelial metaplasia. A NOAEL cannot be determined for this study.

26 27 **2.4.2.5.8. Rier et al. (2001, [198776](#); 2001, [543773](#)).**

28 Female rhesus monkeys (8 per treatment group) were administered 0, 5, or 25 ppt TCDD
29 (purity not specified) in the diet for 4 years. Previously, Bowman et al. (1989, [543745](#))
30 determined that these dietary concentrations were equivalent to 0, 0.15, and 0.67 ng/kg-day,
31 respectively. Thirteen years after termination of TCDD treatment, serum concentrations of

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1 TCDD and dioxin-like polyhalogenated aromatic hydrocarbons (PHAH) were measured in
2 six control monkeys, six monkeys treated with 0.15 ng/kg-day, and three monkeys treated with
3 0.67 ng/kg-day (Rier et al., 2001, [198776](#)). Even after 13 years without treatment, there was
4 significantly ($p < 0.05$) elevated serum levels of TCDD and other dioxin-like compounds in
5 treated monkeys. There was a significant increase in triglycerides and total lipids in the serum of
6 monkeys treated with either 0.15 or 0.67 ng/kg-day, but not in cholesterol or phospholipids. In
7 addition to these 15 animals, 8 other female monkeys (4 treated with 0.67 ng/kg-day TCDD that
8 died 7 to 11 years after treatment and 4 lead-treated animals with no history of PHAH exposure)
9 were evaluated for endometriosis. Elevated serum concentrations of TCDD were not correlated
10 with endometriosis. Increased serum levels of 3,3',4,4'-tetrachlorobiphenyl (TCB), however,
11 were associated with the presence and severity of endometriosis ($p < 0.05$). TCB was found in
12 none of the animals without endometriosis, including TCDD-treated animals, nor was it found in
13 control animals with endometriosis. Animals with elevated serum levels of TCB,
14 pentachlorobiphenyl, and total serum analyte TCDD equivalents (TEQ) had an increased
15 incidence of endometriosis, but severity was associated only with increased levels of TCB. EPA
16 did not develop a LOAEL for TCDD for this study, because of DLC contamination.

17 In a separate study that evaluated the same 15 monkeys 13 years after exposure, Rier
18 et al. (2001, [543773](#)) examined effects on systemic immunity. Peripheral blood mononuclear
19 cells (PBMC) obtained from untreated monkeys secreted no detectable levels of TNF- α in
20 response to T-cell mitogen exposure. There was, however, a significant ($p < 0.05$)
21 dose-dependent increase in TNF- α production in PBMC from the TCDD-treated monkeys.
22 Although PBMC from treated monkeys with endometriosis produced more TNF- α than cells
23 from unexposed controls without the disease (median 128 pg/mL compared to not detected;
24 $p < 0.01$), PBMC from TCDD-treated animals without endometriosis also produced more TNF- α
25 than controls (median 425 pg/mL, $p < 0.067$). TNF- α production from the animals without
26 endometriosis, however, was much more variable and was not statistically significant compared
27 to controls. In addition, there was a dose-related but statistically insignificant decrease in PBMC
28 cytotoxicity against natural killer-sensitive RAJI cells in TCDD-treated animals compared to the
29 unexposed controls. The results were again related to TCDD exposure and not the presence of
30 endometriosis. TCDD alone was not associated with changes in PBMC surface antigen
31 expression, but increased serum levels of TCDD. 1,2,3,6,7,8-Hexachlorodibenzofuran and

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1 3,3',4,4',5-pentachlorobiphenyl were correlated with increased numbers of CD3+/CD25- and
2 CD3-/CD25+ leukocytes, as well as increased secretion of TNF- α in response to T-cell mitogen
3 exposure. Although TNF- α production is considered to be a general indicator of inflammation,
4 relative adversity of increased TNF- α secreted by PBMCs in and of itself cannot be substantiated
5 in the absence of concurrent physiological measurements of an inflammatory response.
6 Therefore, neither a LOAEL nor NOAEL can be determined for this study.

7
8 **2.4.2.5.9. Sewall et al. (1993, [197889](#)).**

9 Sewall et al. (1993, [197889](#)) examined the impact of TCDD exposure on the hepatic
10 epidermal growth factor receptor (EGFR) as a critical effect in hepatocarcinogenicity. In
11 two separate experiments, groups of 6- to 8-week-old female Sprague-Dawley rats were
12 randomly assigned to the following groups: control group, receiving saline and corn oil; a
13 promoted group that received four different doses of TCDD along with saline; a DEN-only
14 initiated control group; and a DEN and TCDD initiated and promoted group that received
15 four different doses of TCDD. DEN was administered via intraperitoneal injection at a dose of
16 175 mg/kg [saline (S) vehicle] as the initiating agent to animals that were 70 days old. The
17 control animals received saline only. In the first experiment, each treatment group (S/TCDD and
18 DEN/TCDD) that included sham-operated or ovariectomized and intact animals were treated
19 with TCDD (purity >98%) at 125 ng/kg-day. In the second dose-response experiment,
20 DEN-initiated and saline control treatment groups (intact animals, 84 days old) were
21 administered TCDD (purity >98%) in corn oil via oral gavage once every 2 weeks for 30 weeks
22 at doses equivalent to 0, 3.5, 10.7, 35.7, or 125 ng/kg-day ($n = 9$). A week after the last
23 treatment, all animals were sacrificed and livers were harvested and fixed for
24 immunohistochemistry. Sections of the fixed liver were tested for EGFR binding, EGFR
25 autophosphorylation, immunolocalization of EGFR, and hepatic cell proliferation.

26 In the first experiment, intact animals treated with 125 ng/kg-day TCDD exhibited a
27 65% reduction in EGFR binding capacity. In contrast, the EGFR equilibrium maximum binding
28 capacity (B_{\max}) of the ovariectomized rats was not statistically different from the ovariectomized
29 control rats, and no changes in the K_d were detected in any treatment group. In the
30 dose-response experiment with intact animals, a significant ($p < 0.05$) TCDD dose-dependent
31 decrease in the B_{\max} of EGFR was shown. A two-factor, five-level ANOVA indicated that the

1 effect of TCDD exposure on EGFR B_{max} was significant ($p = 0.0001$), whereas, the effect of
2 DEN treatment on EGFR B_{max} was not significant. Comparative analysis using Fisher's
3 protected least significant difference indicated that the lowest TCDD dose resulting in a
4 statistically significant ($p < 0.05$) decrease in the EGFR B_{max} was 10.7 ng/kg-day S/TCDD
5 group. At the highest TCDD dose of 125 ng/kg-day, the EGFR B_{max} was reduced by 38%
6 compared to controls in both the DEN initiated and noninitiated groups. A two-factor, five-level
7 ANOVA showed no significant effect on EGFR K_d in either the DEN- or the TCDD-treated
8 groups. The EGFR autophosphorylation assay indicated that, with increasing TCDD dose, the
9 amount of EGFR autophosphorylation in DEN/TCDD-treated animals decreased. The study
10 authors state that this decrease is similar to the dose-response alterations observed for the EGFR
11 B_{max}. Additionally, EGFR autophosphorylation in control and 125 ng/kg-day noninitiated
12 animals was similar to the corresponding dose levels for the DEN-treated animals, suggesting
13 that DEN treatment did not affect the EGFR or the EGFR response to TCDD under the
14 experimental conditions. The immunolocalization assay indicated that staining was more
15 apparent in the centrilobular and midzonal regions of the liver in the DEN initiated control
16 animals, whereas, the amount of hepatocyte plasma membrane staining in DEN/TCDD treated
17 animals substantially decreased. The cell proliferation assay showed a decrease in the cell
18 labeling index in the 3.5 ng/kg-day DEN/TCDD dose group that was statistically less ($p \leq 0.05$)
19 than the labeling index for the control group. In contrast, the labeling index for the
20 125 ng/kg-day DEN/TCDD treatment group was significantly ($p \leq 0.05$) higher compared to
21 controls. Except for the low-dose (3.5 ng/kg-day) group, a clear dose-response trend
22 (two mid-level doses were not statistically significant) was observed in the other three TCDD
23 treated groups.

24 The role of EGFR in TCDD-mediated hepatotoxicity is unknown, and as such, this
25 endpoint cannot be unequivocally linked to TCDD-induced hepatotoxicity nor labeled as
26 adverse. Thus, no LOAEL/NOAEL was established. A LOEL for TCDD of 3.5 ng/kg-day for a
27 30-week exposure duration was identified in this study for a significant ($p = 0.0001$ using
28 ANOVA) decrease in EGFR B_{max} levels. A NOEL cannot be determined for this study.

29

1 **2.4.2.5.10. Sewall et al. (1995, [198145](#)).**

2 Sewall et al. (1995, [198145](#)) studied the dose-response relationship for thyroid function
3 alterations in female rats as a result of TCDD exposure. Groups of female Sprague-Dawley rats
4 were initiated with DEN at 70 days of age at a dose of 175 mg/kg in a saline vehicle via an i.p.
5 injection. DEN was administered as a liver-initiating agent for a concurrent study to determine
6 TCDD promotion of hepatic preneoplastic foci. Saline-treated animals served as controls. At
7 84 days of age, both the DEN-initiated and the saline-noninitiated groups of animals were
8 administered TCDD (purity >98%) or corn oil vehicle via oral gavage once every 2 weeks for
9 30 weeks at dose levels equivalent to 0, 0.1, 0.35, 1.0, 3.5, 10.7, 35.7, or 125 ng/kg-day ($n = 9$
10 per group). One week after the last TCDD treatment, the animals were sacrificed and the thyroid
11 was removed and fixed for further analysis. Blood was drawn from the abdominal aortic vein,
12 and the serum was isolated and preserved for hormone analysis. Liver was also removed and
13 prepped for further analysis. Thyroid hormone analysis was performed to determine serum TSH,
14 T3, and T4 levels using radioimmunoassay kits. Histological examination was conducted on
15 eosin-stained sections of the thyroid tissue. RNA level in the hepatic tissue was determined
16 using a reverse transcription polymerase chain reaction (RT-PCR) technique.

17 TCDD treatment did not affect thyroid weight. A dose-dependent decrease in serum
18 T4 levels was observed in both noninitiated and DEN-initiated animals with T4 levels dropping
19 significantly ($p < 0.05$) at the 35 and 125 ng/kg-day TCDD doses in the noninitiated group.
20 Compared to the noninitiated control group, DEN alone did not significantly affect T4 levels.
21 Serum T3 level in the 125 ng/kg-day treatment group was slightly elevated but was not
22 significantly different from levels in the control group. TSH levels in DEN initiated rats were
23 increased at a dose of 3.5 ng/kg-day. In the noninitiated group, TSH level in the 125 ng
24 TCDD/kg-day group was 3.27 ± 0.34 ng/mL ($n = 9$) compared to 1.3 ± 0.18 ng/mL in the corn
25 oil control group ($n = 7$). This result, in conjunction with the T4 data, demonstrates that TCDD
26 had a similar effect on thyroid hormone levels in both the noninitiated and DEN initiated groups.
27 Histological sections examined for nodular lesions or neoplasms exhibited thyroid follicular
28 adenoma in one DEN/corn oil control animal. The DEN/TCDD-treated animals exhibited
29 diffuse follicular hyperplasia, with the size of colloidal follicles decreasing with TCDD
30 treatment. Other qualitative DEN/TCDD-related changes included increased frequency of
31 abnormally shaped follicles. The study authors reported that image analysis demonstrated a

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1 significant ($p = 0.013$) TCDD dose-related decrease in mean follicle size along with a significant
2 ($p = 0.001$) TCDD dose-related increase in parenchymal area. Additionally, like T4 and TSH
3 levels, DEN treatment alone or in combination with TCDD did not influence thyroid follicular or
4 C-cell morphology.

5 RT-PCR results for UGT1 and CYP1A1 mRNA levels indicated that the amount of
6 UGT1 mRNA at the 125 ng/kg-day dose was approximately 2.5-fold higher compared to the
7 concurrent controls. The study authors also stated that the maximal response for the UGT1
8 mRNA levels was reached at a dose between 1.0 and 3.5 ng TCDD/kg-day. In contrast, the
9 maximum induction of CYP1A1 mRNA was 260-fold higher at the 125 ng/kg-day compared to
10 the concurrent controls.

11 A LOAEL for TCDD of 35 ng/kg-day for a 30-week exposure duration was identified in
12 this study for a significant ($p < 0.05$) decrease in T4 levels. The NOAEL for this study is
13 10.7 ng/kg-day.

15 **2.4.2.5.11. Toth et al. (1979, [197109](#)).**

16 Toth et al. (1979, [197109](#)) examined the impact of TCDD exposure on the formation of
17 liver tumors in male mice. Ten-week-old, outbred Swiss/H/Riop male mice were administered
18 sunflower oil or TCDD (purity not specified; in sunflower oil) at 0, 7, 700 or 7,000 ng/kg (0, 1,
19 100, or 1,000 ng/kg-day adjusted for continuous dosing; administered dose divided by 7; $n = 38$,
20 44, 44, and 43, respectively) once per week via gastric tube for 1 year. Once exposure had
21 ceased, animals were followed for the rest of their lives. After spontaneous death or when mice
22 were moribund, autopsies were performed and all organs were examined histologically.

23 Average life span in the 1,000 ng/kg-day dose group decreased considerably (72%) when
24 compared to the control group. TCDD also caused dose-dependent, severe chronic and ulcerous
25 skin lesions (12, 30, and 58% in the 1, 100, and 1,000 ng/kg-day dose groups, respectively) that
26 was followed by generalized lethal amyloidosis (12, 23, and 40% in the 1, 100, and
27 1,000 ng/kg-day dose groups, respectively).

28 A LOAEL for TCDD of 1 ng/kg-day for 1-year exposure duration was identified in this
29 study for severe chronic and ulcerous skin lesions (12% higher than controls), and generalized
30 lethal amyloidosis (12% higher than controls). A NOAEL cannot be determined for this study.

1 **2.4.2.6. Chronic Studies (Cancer Endpoints)**

2 **2.4.2.6.1. Della Porta et al. (1987, [197405](#)).**

3 Della Porta et al. (1987, [197405](#)) studied the long-term carcinogenic effects of TCDD in
4 B6C3F1 (C57BL/6JDp × C3Hf/Dp) mice. Six-week-old male and female mice (initially about
5 15/sex/dose, and increased by approximately 30 to 40 per group within a few weeks) were
6 administered 0, 2,500, and 5,000 ng/kg TCDD (purity not provided) in corn oil by oral gavage
7 once per week for 52 weeks (0, 357, and 714 ng/kg-day adjusted for continuous exposure). At
8 ages 31 to 39 weeks, 41 male mice and 32 female mice in the 2,500 ng/kg dose group were
9 mistakenly administered a single dose of 25,000 ng/kg TCDD. TCDD treatment for the
10 2,500 ng/kg dose group was halted for 5 weeks (beginning the week after the 25,000 ng/kg dose
11 was administered in error) and resumed until exposure was terminated at 57 weeks. Mortality
12 was observed and body weights recorded at unspecified intervals until 110 weeks of age, when
13 all surviving animals were sacrificed and necropsied. Histopathological analysis was conducted
14 on the following organs and tissues: Harderian glands, pituitary, thyroid, adrenals, tongue,
15 esophagus, and trachea; lungs, liver, pancreas; spleen, kidneys, and bladder; testes, ovaries, and
16 uterus, mesenteric lymph nodes, small intestine, and all other organs with presumed pathological
17 changes.

18 Body weights of both male and female mice exposed to 2,500 and 5,000 ng/kg TCDD
19 were markedly lower than in the corresponding control groups (statistical significance not
20 reported). Relative to the controls, a significant ($p < 0.001$), dose-related decrease in survival
21 occurred in animals treated with either dose of TCDD. In the subset of animals treated
22 inadvertently with a single dose of 25,000 ng/kg TCDD, mortality in male mice increased shortly
23 after this treatment; females, however, did not show a mortality increase following the
24 inadvertent treatment. This mortality in male mice was associated with subcutaneous edema,
25 degenerative hepatocyte changes, and bile duct hyperplasia. The incidence of non-neoplastic
26 lesions (such as amyloidosis of the liver, spleen, adrenals, and pancreas), liver necrosis, and
27 nephrosclerosis, was increased in mice exposed to TCDD compared to controls (statistical
28 significance not reported).

29 The study authors used two statistical tests to analyze tumor incidence. Because of the
30 increased mortality in treated groups compared to controls, one test, which assumes all tumors
31 are fatal, overestimated the differences between the treated and control groups. The second test

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1 assumes that all tumors are incidental and resulted in an underestimation of TCDD effects. Both
2 tests were used to analyze the results for nonthymic lymphomas and hepatic adenomas and
3 carcinomas. Incidence of nonthymic lymphomas (6/45, 4/51, and 3/50 in the 0, 2,500, and
4 5,000 ng/kg dose groups, respectively in males and 17/49, 21/42, and 17/48 in the 0, 2,500, and
5 5,000 ng/kg dose groups, respectively in females) was significantly ($p < 0.05$ in males and
6 $p < 0.01$ in females) higher in TCDD-treated animals compared to the corresponding controls
7 using the fatal tumor test. However, the incidental tumor test showed that this higher incidence
8 was not significant. Similarly, a significantly ($p < 0.001$) higher incidence of hepatocellular
9 adenomas occurred in male mice using the fatal tumor test (10/43, 11/51, and 10/50 in the 0,
10 2,500, and 5,000 ng/kg dose groups, respectively), but the incidence was not significant when
11 assessed using the incidental tumor test. Hepatocellular carcinomas in males were significant
12 ($p < 0.001$) using either the fatal or incidental tumor tests (5/43, 15/51, and 33/50 in the 0, 2,500,
13 and 5,000 ng/kg dose groups, respectively). In female mice, hepatocellular adenomas were
14 significant using both the fatal ($p < 0.01$) and incidental ($p < 0.001$) tumor tests (2/49, 4/42, and
15 11/48 in the 0, 2,500, and 5,000 ng/kg dose groups, respectively). Similar results for female
16 mice were obtained for incidence of hepatocellular carcinomas (1/49, 12/42, and 9/48 in the 0,
17 2,500, and 5,000 ng/kg dose groups, respectively), which also were significant using both the
18 fatal ($p < 0.01$) and incidental ($p < 0.05$) tumor tests. TCDD-related incidences of other tumor
19 types in both sexes were uniformly low and comparable in the treatment and control groups.

20 These results indicate that TCDD is carcinogenic in male and female B6C3F1 mice,
21 causing hepatocellular adenomas and carcinomas in both sexes.

22 In addition to the long term bioassay results in mice described by Della Porta et al. (1987,
23 [197405](#)), carcinogenic effects of TCDD in a neonatal bioassay were reported in the same
24 publication. Briefly, groups of male and female B6C3F1 and B6CF1 (C57/BL6J \times BALB/c)
25 mice were treated with 0, 1000, 30,000 or 60,000 ng/kg BW TCDD via intraperitoneal (i.p.)
26 injection beginning at postnatal day 10. Animals were treated once weekly for 5 weeks and then
27 observed until 78 weeks of age. However, because this study utilized i.p. injection as the route
28 of TCDD exposure, it does not qualify for further consideration based on the study selection
29 criterion that the study design consist of orally administered TCDD.

30

1 **2.4.2.6.2. Kociba et al. (1978, [001818](#)).**

2 As discussed above, Kociba et al. (1978, [001818](#)) conducted a lifetime (2-year) feeding
3 study of male and female Sprague-Dawley rats using doses of 0, 1, 10, and 100 ng/kg-day.
4 There were 50 males and 50 females in each group.

5 With respect to the cancer endpoints examined, the most significant finding was an
6 increase in hepatocellular hyperplastic nodules and hepatocellular carcinomas in female rats.
7 The incidence of hepatocellular carcinomas was significantly elevated above the control
8 incidence at the 100 ng/kg-day dose, whereas increased incidence of hyperplastic nodules was
9 evident in the 10 ng/kg-day dose group.

10 There have been two reevaluations of slides of liver sections from the Kociba et al. study
11 (Goodman and Sauer, 1992, [197667](#); Sauer, 1990, [198829](#); Squire, 1990, [548781](#)). The Squire
12 Review was requested by EPA as an independent review of the slides. The Sauer Review was
13 carried out using refined criteria for the diagnosis of proliferative hepatocellular lesions
14 (Maronpot et al., 1986, [013967](#); Maronpot et al., 1989, [548778](#)). Liver tumor incidences for the
15 three evaluations are compared in Appendix F. Although there are some quantitative differences
16 between the evaluations, the lowest detectable effect for liver tumor incidence is consistently
17 observed at 10 ng/kg-day.

18 In the 10 ng/kg-day dose group, significant increases in the incidence of hyperplastic
19 nodules of the liver were observed in female rats (18/50 in the Kociba evaluation, 27/50 in the
20 Squire evaluation). Two females (2/50) had hepatocellular carcinomas. In the 1990 reevaluation
21 (Goodman and Sauer, 1992, [197667](#); Sauer, 1990, [198829](#)), nine females (9/50) were identified
22 with hepatocellular adenomas and none with carcinomas; thus only one-third of the previously
23 observed “tumors” were identified when using the refined diagnostic criteria. As discussed
24 below, the tumor reclassification of Goodman and Sauer (1992, [197667](#)) was used in the
25 dose-response modeling for the Kociba et al. (1978, [001818](#)) data set.

26 In addition to nodules in the liver, increased incidence of stratified squamous cell
27 carcinoma of the tongue and nasal turbinates/hard palate, and keratinizing squamous cell
28 carcinoma of the lung were also observed in female rats in the 100 ng/kg-day dose group.
29 One possible cause for the induction of lung tumors in the Kociba feeding study may have been
30 the aspiration of dosed feed into the lungs. However the promotion of lung tumors has been
31 observed in mice treated systemically by intraperitoneal (i.p.) injections of TCDD (Beebe et al.,

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1 1995, [548754](#)). In addition the induction of hyperplastic and metaplastic lesions in rats has been
2 observed following chronic oral gavage treatment with TCDD (Tritscher et al., 2000, [197265](#)).
3 More recently, chronic oral exposure to HCDD resulted in the induction of lung tumors in treated
4 female rats (Rozman, 2000, [548758](#)). These data indicate that the induction of lung tumors in
5 the Kociba was most likely primarily the result of systemic chronic dietary exposure to TCDD
6 rather than due to a localized exposure to aspired dosed feed.

7 There was no detectable increase in liver tumor incidences in male rats in any of the dose
8 groups. The mechanism responsible for dioxin-mediated sex specificity for
9 hepatocarcinogenesis in rats is not clear, but may involve ovarian hormones (Lucier et al., 1991,
10 [199007](#)).

11 Although there was no increase in liver tumors in male rats in this study, in the
12 100 ng/kg-day group, there was an increased incidence of stratified squamous cell carcinoma of
13 the hard palate/nasal turbinate, stratified squamous cell carcinoma of the tongue, and adenoma of
14 the adrenal cortex.

15 Kociba et al. (1978, [001818](#)) had reported that chemically related increases in
16 preneoplastic or neoplastic lesions were not found in the 1 ng/kg-day dose group. However,
17 Squire identified two male rats in the 1 ng/kg-day dose group with squamous cell carcinoma of
18 the nasal turbinates/hard palate, and one of these male rats had a squamous cell carcinoma of the
19 tongue. These are both rare tumors in Sprague-Dawley rats, and these sites are targets for
20 TCDD, implying that 1 ng/kg-day may not represent a NOEL. However, no dose-response
21 relationships were evident for tumors at these sites (Huff et al., 1991, [197981](#))

22 There is considerable controversy concerning the possibility that TCDD-induced liver
23 tumors are a consequence of cytotoxicity. Goodman and Sauer (1992, [197667](#)) have extended
24 the reevaluation of the Kociba slides to include liver toxicity data and have reported a correlation
25 between the presence of overt hepatotoxicity and the development of hepatocellular neoplasms in
26 female rats. With the exception of two tumors in controls and one each in the low- and mid-dose
27 groups, all liver tumors occurred in livers showing clear signs of toxicity. However, male rat
28 livers exhibit cytotoxicity in response to high TCDD doses, yet they do not develop liver tumors.
29 Moreover, both intact and ovariectomized female rats exhibit liver toxicity in response to TCDD,
30 yet TCDD is a more potent promoter in intact but not ovariectomized rats (Lucier et al., 1991,
31 [199007](#)). Therefore, if cytotoxicity is playing a role in liver tumorigenesis, other factors must

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1 also be involved. Also, there is little information on the role of cytotoxicity in TCDD-mediated
2 cancer at other sites such as the lung and thyroid.

3
4 **2.4.2.6.3. Toth et al. (1979, [197109](#)).**

5 In a study of 10-week-old outbred male Swiss/H/Riop mice, Toth et al. (1979, [197109](#))
6 administered oral gavage TCDD doses of 0, 7, 700, and 7,000 ng/kg-day in sunflower oil weekly
7 for 1 year (0, 1, 100, or 1,000 ng/kg-day adjusted for continuous dosing; see details above). All
8 mice (100/group) were followed for their entire lives. The study authors identified the effective
9 number of mice in each group to be the number of surviving animals when the
10 first tumor-bearing animal was identified. The average lifespan of the control, low, mid and high
11 dose groups was 588, 649, 633, and 424 days, respectively.

12 In the 100 ng/kg-day dose group, liver tumor incidence was twice that of the control
13 group and was statistically significant ($p < 0.01\%$). A dose-related increase in liver tumor
14 incidence was observed (18, 29, 48, and 30% in the control and three TCDD-treated groups,
15 respectively) in all treated mice. Increases were not statistically significant, however, at 1 and
16 1,000 ng/kg-day. The study authors also stated that spontaneous and induced liver tumors were
17 not histologically different. Additionally, the ratio of benign hepatomas to hepatocellular
18 carcinomas in the control group was not affected by treatment and an increase was observed only
19 in the absolute number of liver tumors. Cirrhosis was not observed with the tumors.

20
21 **2.4.2.6.4. NTP (1982, [543764](#)).**

22 As discussed above, the NTP (1982, [543764](#)) study was conducted using
23 Osborne-Mendel rats and B6C3F1 mice (NTP, 1982, [543764](#)). Groups of 50 male rats,
24 50 female rats, and 50 male mice received TCDD as a suspension in corn oil:acetone (9:1) by
25 gavage twice each week at doses of 0, 5, 25, or 250 ng/kg-day (daily averaged doses of 0, 1.4,
26 7.1, or 71 ng/kg-day for rats and male mice and doses of 0, 5.7, 28.6, or 286 ng/kg-day for
27 female mice.

28 There were no statistically significant dose-related decreases in survival in any
29 sex-species group. TCDD-induced malignant liver tumors occurred in the high-dose female rats
30 and in male and female mice. These can be considered to result from TCDD exposure because
31 they are relatively uncommon lesions in control Osborne-Mendel rats (male, 1/208; female,

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1 3/208), are seen in female rats and mice of both sexes, and their increasing incidence with
2 increasing dose is statistically significant (Cochran-Armitage trend test, $p = 0.004$). Because
3 liver tumors were increased in both sexes of mice, this effect is not female-specific as was
4 observed in rats. Interestingly, liver tumor incidences were decreased in female rats in both the
5 NTP and Kociba low doses (not statistically significant compared with controls). For example,
6 the combined control incidence data were 11/161 (7%) compared with 4/99 (4%) in the low-dose
7 group.

8 The incidences of thyroid gland (follicular cell) tumors were increased in all three dose
9 groups in male rats. Because the responses in the two highest dose groups are highly significant,
10 the statistically significant elevation of incidence in the lowest dose group (Fisher exact
11 p -value = 0.042) is considered to be caused by exposure to TCDD, suggesting that thyroid tumor
12 incidence may be the most sensitive site for TCDD-mediated carcinogenesis. Because
13 71 ng/kg-day is above the maximum tolerated dose (MTD) (Huff et al., 1991, [197981](#)), thyroid
14 tumors occur at doses more than 50 times lower than the MTD.

15 TCDD-induced neoplasms of the adrenal gland were observed in the 7.1 ng/kg-day/dose
16 group in male rats and in high-dose female rats. Fibrosarcomas of the subcutaneous tissue were
17 significantly elevated in high-dose female mice and female rats. One additional tumor type,
18 lymphoma, was seen in high-dose female mice. Lung tumors were elevated in high-dose female
19 mice; the increase was not statistically significant when compared with concurrent controls, but
20 the increase was dose related (Cochran-Armitage trend test, $p = 0.004$).

21 Huff (1992, [548757](#)) concluded, based on the NTP bioassay results, that TCDD was a
22 complete carcinogen and induced neoplasms in rats and mice of both sexes. As was observed in
23 the Kociba study (1978, [001818](#)), liver tumors were observed with greater frequency in treated
24 female rats, but in male rats the thyroid appears to be the most sensitive (increased tumor
25 incidence at doses as low as 1.4 ng/kg-day).

26

27 **2.4.2.6.5. NTP (2006, [197605](#)).**

28 As discussed above, female Sprague-Dawley rats (53 control; 53 or 54 animals per
29 treatment group) were administered TCDD (purity >98%) in corn oil:acetone (99:1) via gavage
30 at doses of 0, 3, 10, 22, 46, or 100 ng/kg-day, 5 days per week for 105 weeks (0, 2.14, 7.14, 15.7,
31 32.9, or 71.4 ng/kg-day, adjusted for continuous exposure) (NTP, 2006, [197605](#)). In addition to

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1 this primary group, a stop-dose group of 50 animals was administered 100 ng/kg-day TCDD in
2 corn oil:acetone (99:1) via gavage for 30 weeks and then just the vehicle for the remainder of the
3 study. At study termination, the number of surviving animals had declined to 25 in the control
4 group and to 21, 23, 19, 22, and 21 in five treatment groups, respectively, due to accidental
5 deaths, moribund animals, or death due to natural causes.

6 Incidence of hepatocellular adenomas was significantly ($p < 0.001$) increased in the
7 100 ng/kg-day dose group in the primary study and exceeded incidences seen in historical
8 vehicle control range at study termination. A dose-related increase in the incidence of
9 cholangiosarcoma was seen in the primary study group in animals receiving 22 ng/kg-day or
10 higher doses of TCDD. The high dose group of 100 ng/kg-day had the highest incidence of
11 cholangiosarcoma with a significant ($p < 0.001$) number of animals exhibiting multiple
12 cholangiosarcomas. Such an incidence was not seen in historical vehicle controls. In contrast,
13 only two cholangiosarcomas and hepatocellular adenomas were seen in the 100 ng/kg-day group
14 in the stop-exposure study.

15 In the lung, at 2 years, there was a significantly ($p = 0.002$) increased incidence of cystic
16 keratinizing epithelioma in the 100 ng/kg-day dose group of the primary study, while there were
17 no epitheliomas in the 100 ng/kg-day group of the stop-exposure study. There was also a
18 significant ($p \leq 0.01$) dose-dependent increase, when compared to the vehicle control, in the
19 incidence of bronchiolar metaplasia of the alveolar epithelium at all dose groups in the primary
20 study. Squamous metaplasia was also present in the 46 and 100 ng/kg-day dose groups in the
21 primary study, and was also observed in the 100 ng/kg-day dose group in the stop-exposure
22 study.

23 A positive trend in the incidence of gingival squamous cell carcinoma of the oral cavity
24 was seen at all doses (except 22 ng/kg-day), with the incidence significantly ($p = 0.007$) high in
25 the 100 ng/kg-day dose group. In addition, the occurrence of this lesion in the 46 and
26 100 ng/kg-day group of the primary study and 100 ng/kg-day group of the stop-exposure study
27 exceeded the historical control range. The incidence of gingival squamous hyperplasia was
28 significantly (either $p \leq 0.01$ or $p \leq 0.05$) increased in all dose groups of the primary study as
29 well as the 100 ng/kg-day group of the stop-exposure study.

30 In the uterus, at 2 years, there was a significantly ($p = 0.032$) higher rate of squamous cell
31 carcinoma in the 46 ng/kg-day group compared to vehicle controls. In addition there were

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1 two squamous cell carcinomas in the 100 ng/kg-day group of the stop-exposure study. No
2 squamous cell carcinomas have been reported in historical vehicle controls.

3 These results indicate that TCDD is carcinogenic to female Sprague-Dawley rats and
4 causes tumors at multiple sites.

5

6 **2.4.3. Summary of Key Data Set Selection for TCDD Dose-Response Modeling**

7 To meet the NAS' concerns regarding transparency and clarity in the identification of
8 TCDD studies for dose-response assessment, EPA has, in this section, developed and applied
9 two sets of criteria for animal bioassays and epidemiologic studies. EPA has collected and
10 evaluated these studies, including studies from the 2003 Reassessment and newer studies found
11 via literature searches and through public submissions. Tables 2-4 and 2-5 contain the final lists
12 of key cancer and noncancer studies, respectively, that have met EPA's inclusion criteria for
13 epidemiologic data. Tables 2-6 and 2-7 provide the final lists of key studies that have met EPA's
14 inclusion criteria for animal bioassay data for cancer and noncancer studies, respectively.
15 Collectively, these four tables contain the final set of key studies that EPA has used to develop
16 noncancer and cancer dose-response assessments for TCDD in Sections 4 and 5 of this
17 document, respectively. In Sections 4 and 5, additional evaluations are made to determine which
18 study/endpoint data sets are the most appropriate for development of the RfD and OSF for
19 TCDD, using statistical criteria, dose-response modeling results and decisions regarding
20 toxicological relevance of the endpoints. The approaches taken to select the final candidate
21 study/endpoint data sets are discussed in Sections 4 and 5 and are illustrated in Figures 4-1, 4-2
22 and and 5-3 of those sections.

Table 2-1. Summary of epidemiological cancer studies (key characteristics)

Publication	Length of follow-up	Latency period	Half-life for TCDD	Fraction of TEQs accounted for by TCDD
NIOSH cohort studies				
Fingerhut et al. (1991, 197375)	1942–1987	0, 20 years	N/A	N/A
Steenland et al. (1999, 197437)	1942–1993	0, 15 years	N/A	N/A
Steenland et al. (2001, 197433)	1942–1993	0, 15 years	8.7 years (Michalek et al., 1996, 198893)	TCDD accounted for all occupational TEQ; 10% of background
Cheng et al. (2006, 523122)	1942–1993	0, 10, 15 years	8.7 years (Michalek et al., 1996, 198893), and CADM (Aylward et al., 2005, 197114)	N/A
Collins et al. (2009, 197627)	1942–2003	None	7.2 years (Flesch-Janys et al., 1996, 197351)	N/A
BASF cohort studies				
Thiess et al. (1982, 064999)	1953–1980	None	N/A	N/A
Zober et al. (1990, 197604)	1953–1987	Years since first exposure: 0–9, 10–19, and 20+	N/A	N/A
Ott and Sober (1996, 198101)	1953–1991	None	5.8 years	N/A
Hamburg cohort studies				
Manz et al. (1991, 199061)	1952–1989	None, used duration of employment (<20, >20 years)	N/A	N/A
Flesch-Janys et al. (1995, 197261)	1952–1992	None	7.2 years Flesch-Janys et al. (1994, 197372)	Mean TEQ without TCDD was 155 ng/kg; mean TEQ with TCDD was 296.5 ng/kg
Flesch-Janys et al. (1998, 197339)	1952–1992	None	7.2 years Flesch-Janys et al. (1996, 197351), also used decay rates that were function of age and fat composition	Mean concentration of TCDD was 101.3 ng/kg; for TEQ (without TCDD) mean exposure was 89.3 ng/kg
Becher et al. (1998, 197173)	1952–1992	0, 5, 10, 15 and 20 years	7.2 years Flesch-Janys et al. (1996, 197351) took into account age and fat composition	Not described

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**Table 2-1. Summary of epidemiological cancer studies (key characteristics)
(continued)**

Publication	Length of follow-up	Latency period	Half-life for TCDD	Fraction of TEQs accounted for by TCDD
Seveso cohort studies				
Bertazzi et al. (2001, 197005)	1976–1996	Periods postexposure: 0, 0–4, 5–9, 10–14, 15–19 years	N/A	N/A
Warner et al. (2002, 197489)	1976–1998	None	8 years (Pirkle et al., 1989, 197861)	N/A
Pesatori et al. (2003, 197001)	1976–1996	Period postexposure: 20 years	N/A	N/A
Baccarelli et al. (2006, 197036)	1976–1998	Period postexposure: 22 years	N/A	N/A
Consonni et al. (2008, 524825)	1976–2001	Periods postexposure: 0, 0–4, 5–9, 10–14, 15–19, 20–24 years	N/A	N/A
Chapaevsk cohort studies				
Revich et al. (2001, 199843)	Cross-sectional study (1995–1998)	N/A	N/A	N/A
Ranch Hand cohort studies				
Akhtar et al. (2004, 197141)	1962–1999	None	N/A	N/A
Michalek and Pavuk (2008, 199573)	1962–2004	None, but stratified by period of service	7.6 years	N/A

**Table 2-1. Summary of epidemiological cancer studies (key characteristics)
(continued)**

Publication	Length of follow-up	Latency period	Half-life for TCDD	Fraction of TEQs accounted for by TCDD
New Zealand cohort studies				
t'Mannetje et al. (2005, 197593)	1969–2000 (herbicide producers); 1973–2000 (herbicide sprayers)	N/A	N/A	N/A
McBride (2009, 198490)	1969–2004	None	N/A	N/A
McBride et al. (2009, 197296)	1969–2004	None	7 years	N/A
Dutch cohort study				
Hooiveld et al. (1998, 197829)	1955-1991	Periods postexposure: 0–19 years, >19 years	7.1 years	N/A

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Table 2-2. Epidemiological cancer study selection considerations and criteria

	Methods use to ascertain health outcomes were unbiased, highly sensitive and specific.	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.	Association between TCDD and adverse health effect, with exposure-response relationship.	Exposure assessment methodology clear and adequately characterizes individual-level exposures. Limitations and uncertainties in exposure assessment considered.	Study size and follow-up large enough to yield precise estimates of risk and ensure adequate statistical power.	Published in peer-reviewed literature with appropriate discussion of strengths, limitations.	Exposure primarily TCDD and quantified so that dose-response relationship can be assessed.	Effective dose & oral exposure estimable & consistent w/ current biological understanding. Latency and appropriate window(s) of exposure examined.	Pass for dose-response analyses?
Cancer	Considerations					Criteria			Y/N
NIOSH Cohort Studies									
Fingerhut et al. (1991, 197375) all cancer sites, site-specific analyses	√	X	X	X	√	√	X	√	N
Steenland et al. (1999, 197437) all cancer sites combined, site-specific analyses	√	√	√	√	√	√	√	√	N ^a
Steenland et al. (2001, 197433) all cancer sites combined	√	√	√	√	√	√	√	√	Y
Cheng et al. (2006, 523122) all cancer sites combined	√	√	√	√	√	√	√	√	Y
Collins et al. (2009, 197627) all cancer sites combined, site-specific analyses	√	√	√	√	√	√	√	√	Y
BASF Cohort Studies									
Thiess et al. (1982, 064999) all cancer sites combined, site-specific analyses	√	X	X	X	X	√	X	X	N
Zober et al. (1990, 197604) all cancer sites combined, site-specific analyses	√	√	X	X	X	√	X	X	N

Table 2-2. Epidemiological cancer study selection considerations and criteria (continued)

	Methods use to ascertain health outcomes were unbiased, highly sensitive and specific.	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.	Association between TCDD and adverse health effect, with exposure-response relationship.	Exposure assessment methodology clear and adequately characterizes individual-level exposures. Limitations and uncertainties in exposure assessment considered.	Study size and follow-up large enough to yield precise estimates of risk and ensure adequate statistical power.	Published in peer-reviewed literature with appropriate discussion of strengths, limitations.	Exposure primarily TCDD and quantified so that dose-response relationship can be assessed.	Effective dose & oral exposure estimable & consistent w/ current biological understanding. Latency and appropriate window(s) of exposure examined.	Pass for dose-response analyses?
Cancer	Considerations					Criteria			Y/N
Ott and Zober (1996, 198101) all cancer sites combined	√	√	√	√	√	√	√	√	Y
Hamburg Cohort									
Manz et al. (1991, 199061) all cancer sites combines, site-specific analyses	√	√	√	√	√	√	X	√	N
Flesh-Janys et al. (2006, 197621) all cancer sites combined	√	√	√	√	√	√	√	X	N
Flesh-Janys et al. (1998, 197339) all cancer sites combined, site-specific analyses	√	√	√	√	√	√	√	√	N ^b
Becher et al. (1998, 197173) all cancer sites combined	√	√	√	√	√	√	√	√	Y
Seveso Cohort									
Bertazzi et al. (2001, 197005) all cancer sites combined, site-specific analyses	√	√	√	X	√	√	X	X	N
Pesatori et al. (2003, 197001) all cancer sites combined, site-specific analyses	√	√	X	X	√	√	X	X	N

Table 2-2. Epidemiological cancer study selection considerations and criteria (continued)

	Methods use to ascertain health outcomes were unbiased, highly sensitive and specific.	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.	Association between TCDD and adverse health effect, with exposure-response relationship.	Exposure assessment methodology clear and adequately characterizes individual-level exposures. Limitations and uncertainties in exposure assessment considered.	Study size and follow-up large enough to yield precise estimates of risk and ensure adequate statistical power.	Published in peer-reviewed literature with appropriate discussion of strengths, limitations.	Exposure primarily TCDD and quantified so that dose-response relationship can be assessed.	Effective dose & oral exposure estimable & consistent w/ current biological understanding. Latency and appropriate window(s) of exposure examined.	Pass for dose-response analyses?
Cancer	Considerations					Criteria			Y/N
Consonni et al. (2008, 524825) all cancer sites combined, site-specific analyses	√	√	√	X	√	√	X	X	N
Seveso Cohort–Women’s Health Study									
Baccarelli et al. (2006, 197036) site specific analysis	√	√	X	√	√	√	√	√	N ^c
Warner et al. (2002, 197489) breast cancer incidence	√	√	√	√	√	√	√	√	Y
Chapaevsk Study									
Revich et al. (2001, 199843) all cancer sites combined, site-specific analyses	X	X	X	X	√	X	X	X	N
Ranch Hands Cohort									
Akhtar et al. (2004, 197141) all cancer sites combined, site-specific analyses	√	X	√	√	√	√	X	√	N
Michalek and Pavuk (2008, 199573) all cancer sites combined	√	X	√	√	√	√	X	√	N

Table 2-2. Epidemiological cancer study selection considerations and criteria (continued)

	Methods use to ascertain health outcomes were unbiased, highly sensitive and specific.	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.	Association between TCDD and adverse health effect, with exposure-response relationship.	Exposure assessment methodology clear and adequately characterizes individual-level exposures. Limitations and uncertainties in exposure assessment considered.	Study size and follow-up large enough to yield precise estimates of risk and ensure adequate statistical power.	Published in peer-reviewed literature with appropriate discussion of strengths, limitations.	Exposure primarily TCDD and quantified so that dose-response relationship can be assessed.	Effective dose & oral exposure estimable & consistent w/ current biological understanding. Latency and appropriate window(s) of exposure examined.	Pass for dose-response analyses?
Cancer	Considerations					Criteria			Y/N
Others									
Hooiveld et al. (1998, 197829) all cancer sites combined, site-specific analyses	√	√	√	√	X	√	√	X	N
t'Mannetje et al. (2005, 197593) all cancer sites combined, site-specific analyses	√	X	√	√	√	X	X	X	N
McBride et al. (2009, 197296) all cancer sites combined, site-specific analyses	√	X	X	√	X	√	X	X	N
McBride et al. (2009, 198490) all cancer sites combined, site-specific analyses	√	√	X	√	X	√	√	√	N ^d

^aThis study has been superseded and updated by Steenland et al. (2001, [197433](#)).

^bBecher et al. (1998, [197173](#)) assessed this same cohort taking cancer latency into account, thereby superseding this study.

^cIt is unknown whether the frequency of t(14;18)translocations in lymphocytes relates specifically to an increased risk of non-Hodgkin's lymphoma. Given this lack of obvious adverse effect, dose-response analyses for this outcome were not conducted.

^dNo dose-response associations were noted.

√ = Consideration/criteria satisfied; X = Consideration/criteria not satisfied.

Table 2-3. Epidemiological noncancer study selection considerations and criteria

	Methods use to ascertain health outcomes were unbiased, highly sensitive and specific.	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.	Association between TCDD and adverse health effect, with exposure-response relationship.	Exposure assessment methodology clear and adequately characterizes individual-level exposures. Limitations and uncertainties in exposure assessment considered.	Study size and follow-up large enough to yield precise estimates of risk and ensure adequate statistical power.	Published in peer-reviewed literature with appropriate discussion of strengths, limitations.	Exposure primarily TCDD and quantified so that dose-response relationships can be assessed.	Effective dose & oral exposure estimable & consistent w/ current biological understanding. Latency and appropriate window(s) of exposure examined for a Nonfatal endpoint.	Pass for dose-response analyses?
Noncancer	Considerations					Criteria			Y/N
NIOSH Cohort									
Steenland et al. (1999, 197437) mortality (noncancer) -ischemic heart disease	√	X	√	√	√	√	X	X	N
Collins et al. (2009, 197627) mortality (noncancer)	√	√	X	√	√	√	√	X	N
BASF Cohort									
Ott and Zober (1996, 198101) mortality (noncancer)	√	√	X	√	√	√	√	X	N
Hamburg Cohort									
Flesch-Janys et al. (1995, 197261) mortality (noncancer)	√	√	√	√	√	√	√	X	N
Seveso Cohort–Women’s Health Study									
Eskenazi et al. (2002, 197168) menstrual cycle characteristics	√	√	√	√	√	√	√	√	Y
Eskenazi et al. (2002, 197164) endometriosis	X	X	X	√	X	√	√	X	N
Eskenazi et al. (2003, 197158) birth outcomes	X	X	X	√	√	√	√	X	N

Table 2-3. Epidemiological noncancer study selection considerations and criteria (continued)

	Methods use to ascertain health outcomes were unbiased, highly sensitive and specific.	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.	Association between TCDD and adverse health effect, with exposure-response relationship.	Exposure assessment methodology clear and adequately characterizes individual-level exposures. Limitations and uncertainties in exposure assessment considered.	Study size and follow-up large enough to yield precise estimates of risk and ensure adequate statistical power.	Published in peer-reviewed literature with appropriate discussion of strengths, limitations.	Exposure primarily TCDD and quantified so that dose-response relationships can be assessed.	Effective dose & oral exposure estimable & consistent w/ current biological understanding. Latency and appropriate window(s) of exposure examined for a Nonfatal endpoint.	Pass for dose-response analyses?
Noncancer	Considerations					Criteria			Y/N
Warner et al. (2004, 197490) age at menarche	√	√	X	√	√	√	√	X	N
Eskenazi et al. (2005, 197166) age at menopause	√	√	X	√	√	√	√	X	N
Warner et al. (2007, 197486) ovarian function	√	√	X	√	√	√	√	X	N
Eskenazi et al. (2007, 197170) uterine leiomyoma	√	√	√	√	√	√	√	X	N ^a
Seveso Cohort–Other Studies									
Bertazzi et al. (2001, 197005) mortality (noncancer)	√	√	X	X	√	√	X	X	N
Consonni et al. (2008, 524825) mortality (noncancer)	√	√	X	X	√	√	X	X	N
Mocarelli et al. (2000, 197448) sex ratio	√	√	√	√	√	X	√	X	N ^b

Table 2-3. Epidemiological noncancer study selection considerations and criteria (continued)

	Methods use to ascertain health outcomes were unbiased, highly sensitive and specific.	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.	Association between TCDD and adverse health effect, with exposure-response relationship.	Exposure assessment methodology clear and adequately characterizes individual-level exposures. Limitations and uncertainties in exposure assessment considered.	Study size and follow-up large enough to yield precise estimates of risk and ensure adequate statistical power.	Published in peer-reviewed literature with appropriate discussion of strengths, limitations.	Exposure primarily TCDD and quantified so that dose-response relationships can be assessed.	Effective dose & oral exposure estimable & consistent w/ current biological understanding. Latency and appropriate window(s) of exposure examined for a Nonfatal endpoint.	Pass for dose-response analyses?
Baccarelli et al. (2002, 197062 ; 2004, 197045) immunological effects	√	√	√	√	√	√	√	X	N
Landi et al. (2003, 198362) gene expression	√	√	X	√	X	√	X	X	N
Alaluusua et al. (2004, 197142) oral hygiene	√	√	√	√	√	√	√	√	Y
Baccarelli et al. (2005, 197053) chloracne	√	√	√	√	√	√	√	√	N ^c
Baccarelli et al. (2008, 197059) neonatal thyroid function	√	√	√	X	√	√	√	√	Y
Mocarelli et al. (2008, 199595) semen quality	√	√	√	√	√	√	√	√	Y
Chapaevsk Study									
Revich et al. (2001, 199843) mortality (noncancer) and reproductive health	√	X	X	X	√	√	X	X	N
Ranch Hands Cohort									
Michalek and Pavuk (2008, 199573) diabetes	√	X	√	√	√	√	X	√	N

Table 2-3. Epidemiological noncancer study selection considerations and criteria (continued)

	Methods use to ascertain health outcomes were unbiased, highly sensitive and specific.	Risk estimates are not susceptible to biases from confounding exposures or from study design or statistical analysis.	Association between TCDD and adverse health effect, with exposure-response relationship.	Exposure assessment methodology clear and adequately characterizes individual-level exposures. Limitations and uncertainties in exposure assessment considered.	Study size and follow-up large enough to yield precise estimates of risk and ensure adequate statistical power.	Published in peer-reviewed literature with appropriate discussion of strengths, limitations.	Exposure primarily TCDD and quantified so that dose-response relationships can be assessed.	Effective dose & oral exposure estimable & consistent w/ current biological understanding. Latency and appropriate window(s) of exposure examined for a Nonfatal endpoint.	Pass for dose-response analyses?
Other									
Ryan et al. (2002, 198508) sex ratio	X	X	X	X	√	√	X	X	N
Kang et al. (2006, 199133) long-term health consequences	X	X	X	√	√	√	X	X	N
McBride et al. (2009, 198490) mortality (noncancer)	X	X	X	√	X	√	√	X	N
McBride et al. (2009, 197296) mortality (noncancer)	X	√	X	√	X	√	X	X	N

^aCategorical measures of TCDD suggest an inverse association between TCDD exposure and uterine fibroids. The observed direction of the reported associations precluded quantitative dose-response modeling.

^bThe somewhat arbitrary cut off age of 19 for statistically significant exposure associations results in a highly uncertain critical exposure window. It is difficult to determine whether effects are a consequence of the initial high exposure during childhood or a function of the cumulative exposure for this entire exposure window. The differences between these two dose estimates are quite large.

^cChloracne is recognized to occur following high TCDD exposure levels. This study provides limited relevance to TCDD RfD development, as exposure levels observed in the general population are much lower.

√ = Consideration/criteria satisfied. X = Consideration/criteria not satisfied.

Table 2-4. Epidemiological studies selected for TCDD cancer dose-response modeling

Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases/deaths	Effect Measure/ RR (95% CI)	Risk factors	Comments	Reference
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	Cumulative serum lipid TCDD concentrations (CSLC) 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	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.	Cheng et al. (2006, 523122)
Mortality from all cancers	USA, 1942–1993	NIOSH cohort including 3,538 male workers, 256 cancer deaths	CSLC 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	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, 537122)	Steenland et al. (2001, 197433)

Table 2-4. Epidemiological studies selected for TCDD cancer dose-response modeling (continued)

Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases/deaths	Effect Measure/ RR (95% CI)	Risk factors	Comments	Reference
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	Cumulative TCDD serum lipid concentrations based on area under curve (in µg/kg years); back-extrapolation to date of last employment took into account age and percent 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	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)	Available: year of entry, age of entry, duration of employment, birth cohort, β-HCH; TEQ other than TCDD	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)	Becher et al. (1998, 197173)
				Continuous exposure TCDD (µg/kg years)	124	β = 0.0089, p = 0.0047			

Table 2-4. Epidemiological studies selected for TCDD cancer dose-response modeling (continued)

Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases/deaths	Effect Measure/ RR (95% CI)	Risk factors	Comments	Reference
Mortality and incidence for all cancers combined, as well as for specific cancer sites	Ludwigshafen, 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	Cumulative TCDD serum lipid concentrations 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.	<i>Internal cohort analysis</i>	Date of 1 st TCDD exposure 1.22 (95% CI: 1.00–1.50)	Available: age, BMI, smoking status and history of occupational exposure to aromatic amines and asbestos	Included in U.S. EPA (2003, 537122) Positive associations noted for digestive cancer, but not for respiratory cancer	Ott and Zober (1996, 198101)
				External comparisons exposure categories: <0.1, 0.1–0.99, 1.0–1.99 >2 µg/kg	31 cancer deaths 47 incident cancers <i>External cohort analyses</i>	1.11 (95% CI: 0.91–1.35) SMRs 8 0.8 (0.4–1.6) 8 1.2 (0.5–2.3) 8 1.4 (0.6–2.7) 7 2.0 (0.8–4.0)			

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Table 2-4. Epidemiological studies selected for TCDD cancer dose-response modeling (continued)

Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases/deaths	Effect Measure/ RR (95% CI)	Risk factors	Comments	Reference
Breast cancer incidence	Italy 1976–1998	981 women from zones A and B with available archive serum samples, 15 breast cancer cases	TCDD serum lipid concentrations (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.	<20 ppt	Cases	1.0	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, 537122)	Warner et al. (2002, 197489)
				20.1–44 ppt	1	1.0 (0.1–10.8)			
				44.1–100 ppt	2	4.5 (0.6–36.8)			
				>100 ppt	7	3.3 (0.4–28.0)			
				Log ₁₀ TCDD also modeled as continuous variable	5	2.1 (1.0–4.6)			

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Table 2-4. Epidemiological studies selected for TCDD cancer dose-response modeling (continued)

Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases/deaths	Effect Measure/ RR (95% CI)	Risk factors	Comments	Reference
Mortality from all cancers and specific cancer types	Midland, Michigan, 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	Cumulative serum lipid TCDD concentrations 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, fatal lung, fatal prostate, fatal leukemias and fatal non-Hodgkin lymphomas 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.	Collins et al. (2009, 197627)

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Table 2-5. Epidemiological studies selected for TCDD noncancer dose-response modeling

Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases/deaths	Effect Measure/ RR (95% CI)	Risk factors	Comments	Reference
b-TSH measured 72 hours after birth from a heel pick (routine screening for all newborns in the region)	Italy, 1976; children, 1994–2005	<i>Population-based study:</i> 1,041 singletons (56 from zone A, 425 from zone B and 533 from reference) born between Jan. 1, 1994–June 30, 2005. <i>Plasma dioxin study:</i> 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).	<i>Population-based study:</i> Reference Zone B Zone A <i>Plasma dioxin study:</i> Continuous maternal plasma TCDD	 533 births 425 births 56 births	<i>Population-based study</i> Mean b-TSH 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 $\beta = 0.75$ ($p < 0.001$)	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.	Baccarelli et al. (2008, 197059)

Table 2-5. Epidemiological studies selected for TCDD noncancer dose-response modeling (continued)

Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases/deaths	Effect Measure/ RR (95% CI)	Risk factors	Comments	Reference
Sperm conc. (million/mL) Progressive motility (%) Serum E ₂ (pmol/L)	Italy, 1976, 1998	135 exposed (from zone A) and 184 nonexposed men aged 1–26 in 1976 were included. These subjects were selected from the cohort of 257 exposed and 372 unexposed people.	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.	TCDD quartiles		Mean values were compared between the exposed and comparison groups for sperm concentration, volume, motility and count, FSH, E ₂ , LH, and Inhibin B.	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).	Mocarelli et al. (2008, 199595)

Table 2-5. Epidemiological studies selected for TCDD noncancer dose-response modeling (continued)

Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases/deaths	Effect Measure/ RR (95% CI)	Risk factors	Comments	Reference
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; 130 subjects recruited from the non-ABR region (unexposed)	Serum TCDD (ng/kg) from 1976 samples for those who resided in Zone ABR; no serum levels for non-ABR residents (unexposed). TCDD exposure represent levels as of 1976 (after accident)	Non-ABR Zone	10/39	Dental defect %	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%).	Alaluusua et al. (2004, 197142)
				31–226 ng/kg	1/10	26%			
				238–592 ng/kg	5/11	10%			
				700–26000 ng/kg	9/15	45%			
			<5 years of age at time of accident	25/75					
				Non-ABR Zone or 31–226 ng/kg serum TCDD		Odds Ratios (among those <5 years of age at time of accident)			
				238-26,000 ng/kg serum TCDD		1.0		Also assessed hypodontia and other dental and oral aberrations, but these were too rare to allow modeling by ABR zone.	
						2.4 (1.3–4.5)			

Table 2-5. Epidemiological studies selected for TCDD noncancer dose-response modeling (continued)

Health outcome	Location, time period	Cohort description	Exposure assessment	Exposure measures	No. of cases/deaths	Effect Measure/ RR (95% CI)	Risk factors	Comments	Reference
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, A positive association found among women who were pre-menarcheal at the time of accident (n = 134)	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.		Eskenazi et al. (2002, 197168)

Table 2-6. Animal bioassays selected for cancer dose-response modeling

Species/strain	Sex exposure route/duration	<i>n</i>	Average daily dose levels (ng/kg-day)	Cancer types	Statistical significant tumors (pairwise with controls or trend tests)	Reference
Mouse/B6C3F1	Male/Female Oral gavage once per week; 52 weeks	Approximately 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)	Della Porta et al. (1987, 197405)
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	Kociba et al. (1978, 001818); (Female liver tumors analysis updated in Goodman and Sauer, 1992, 197667)
Mouse/B6C3F1	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 (1982, 543764)
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	NTP (1982, 543764)

Table 2-6. Animal bioassays selected for cancer dose-response modeling (continued)

Species/strain	Sex exposure route/duration	<i>n</i>	Average daily dose levels (ng/kg-day)	Cancer types	Statistical significant tumors (pairwise with controls or trend tests)	Reference
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	NTP (2006, 197605)
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	Toth et al. (1979, 197109)

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Table 2-7. Animal bioassay studies selected for noncancer dose-response modeling

Species/ strain	Exposure protocol	Sex (exposure group)	<i>n</i>	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Reproductive toxicity studies									
Monkey/ Rhesus	Daily dietary exposure in female monkeys (3.5–4 years)	F (F0, F1, F2, F3)	3 to 7 (F1)	0, 0.15, or 0.67	0.15	0.67	Reproductive and developmental effects	Neurobehavioral effects (e.g., discrimination- reversal learning affected)	Bowman et al.(1989, 543744 ; 1989, 543745); Schantz and Bowman (1989, 198104); Schantz et al. (1986, 088206)
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	Franc et al. (2001, 197353)
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	Hochstein et al (2001, 197544)
Rat/Sprague- Dawley	Oral gavage (GD 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	Hutt et al. (2008, 198268)

Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	<i>n</i>	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Reproductive toxicity studies (continued)									
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 prostate (F1), decreased sex ratio (percentage of males) (F2)	Ikeda et al. (2005, 197834)
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)	Ishihara et al. (2007, 197677)
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	Latchoumycandane and Mathur (2007, 197298) and related Latchoumycandane et al. (2002, 198365 ; 2002, 197839 ; 2003, 543746)

Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	<i>n</i>	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Reproductive toxicity studies (continued)									
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	Murray et al. (1979, 197983)
Monkey/Rhesus	Daily dietary exposure (4 years)	F	8	0, 0.15, or 0.67	None	0.15	Reproductive effects	Increased incidence of endometriosis (disease ranged from moderate to severe)	Rier et al. (1993, 199987 ; 1995, 198566)
Rat/Sprague-Dawley	Maternal corn oil gavage (weekly on GD 14 and 21; PND 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)	Shi et al. (2007, 198147)
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	Yang et al. (2000, 198590)

Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	<i>n</i>	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Developmental toxicity studies									
Rat/Harlan Sprague- Dawley	Corn oil gavage (GD 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)	Amin et al. (2000, 197169)
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)	Bell et al. (2007, 197041)
Rat/Sprague- Dawley	Maternal corn oil gavage (GD 14 and 21; PND 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)	Franczak et al. (2006, 197354)

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Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	<i>n</i>	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Developmental toxicity studies									
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)	Hojo et al. (2002, 198785) and related Zareba et al. (2002, 197567)
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)	Kattainen et al. (2001, 198952)
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)	Keller et al. (2007, 198526 ; 2008, 198531 ; 2008, 198033)
Mouse/ddY	Maternal olive oil gavage (weekly for 8 weeks prior to mating)	F (F0) M (F1)	7 (F0) 3 (F1 immunocytochemical 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)	Kuchiiwa et al. (2002, 198355)

Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	<i>n</i>	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Developmental toxicity studies									
Mouse/NIH (pregnant and pseudo- pregnant)	Maternal sesame oil gavage daily for 8 days (GD 1–8)	F	10	0, 2, 50, or 100	None	2	Developmental effects	Decreased progesterone and increased serum estradiol levels	Li et al. (2006, 199059)
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)	Markowski et al. (2001, 197442)
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)	Miettinen et al. (2006, 198266)
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)	Nohara et al. (2000, 200027)
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)	Ohsako et al. (2001, 198497)
Rat/Harlan Sprague- Dawley	Maternal corn oil gavage (GD 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)	Schantz et al. (1996, 198781)
Rat/Sprague- Dawley	Maternal corn oil gavage (GD 10–16)	F and M (F1)	~15 (F0); 5–9 (F1)	0, 25, or 100	25	100	Developmental effects	Decreased thymus weight	Seo et al. (1995, 197869)

Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	<i>n</i>	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Developmental toxicity studies									
Rat/TCDD-resistant Han/Wistar bred with TCDD-sensitive Long-Evans	Maternal corn oil gavage (GD 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	Simanainen et al. (2004, 198106)
Mouse/C57/6 NCJi	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 <i>Listeria</i> (F1 males and females); increase in thymic CD4+ cells (F1 males); decreased spleen weight (F1 males)	Sugita-Konishi et al. (2003, 198375)
Acute toxicity studies									
Mouse/B6C3F1	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	Burleson et al. (1996, 196998)
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	Crofton et al. (2005, 197381)
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)	Kitchin and Woods (1979, 198750)

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Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	<i>n</i>	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Acute toxicity studies (continued)									
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	Li et al. (1997, 199060)
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)	Lucier et al. (1986, 198398)
Mouse/B6C3F1 (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	Nohara et al. (2002, 199021)
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	Simanainen et al. (2002, 201369)

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Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	<i>n</i>	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Acute toxicity studies (continued)									
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	Simanainen et al. (2003, 198582)
Mouse/C57BL/6N CYP1A2 (+/+) wild-type	Corn oil gavage (single dose)	F	Not specified	0, 30, 100, 300, 1000, 3000, or 10,000	300	1,000	Immunotoxicity	Decreased antibody response to SRBCs	Smialowicz et al. (2004, 110937)
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	Vanden et al. (1994, 197551)
Subchronic toxicity studies									
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. (2001, 521829)
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	Chu et al., 2007
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)	DeCaprio et al. (1986, 197403)

Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	<i>n</i>	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Subchronic toxicity studies (continued)									
Mice/B6C3F1	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	DeVito et al. (1994, 197278)
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	Fattore et al. (2000, 197446)
	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					
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	Fox et al. (1993, 197344)
Mouse/B6C3F1	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. (1998, 136626)

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Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	<i>n</i>	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Subchronic toxicity studies (continued)									
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. (2000, 197431)
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	Hassoun et al. (2003, 198726)
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	Kociba et al. (1976, 198594)
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	Mally and Chipman (2002, 198098)
Mouse/ B6C3F1	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	Slezak et al. (2000, 199022)
Mouse/ B6C3F1	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	Smialowicz et al. (2008, 198341)

Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	<i>n</i>	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Subchronic toxicity studies (continued)									
Rat/Sprague-Dawley	TCDD in diet (13 weeks)	F	8	0, 14, 26, 47, 320, or 1,024	None	14	Multiple endpoints	Decreased absolute and relative thymus weights, decreased liver retinoid levels	Van Birgelen (1995, 197096 ; 1995, 198052)
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	Vos et al. (1973, 198367)
Mouse/B6C3F1	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	White et al. (1986, 197531)
Chronic toxicity studies									
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	Cantoni et al. (1981, 197092)
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	Croutch et al. (2005, 197382)
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	Hassoun et al. (2002, 543725)

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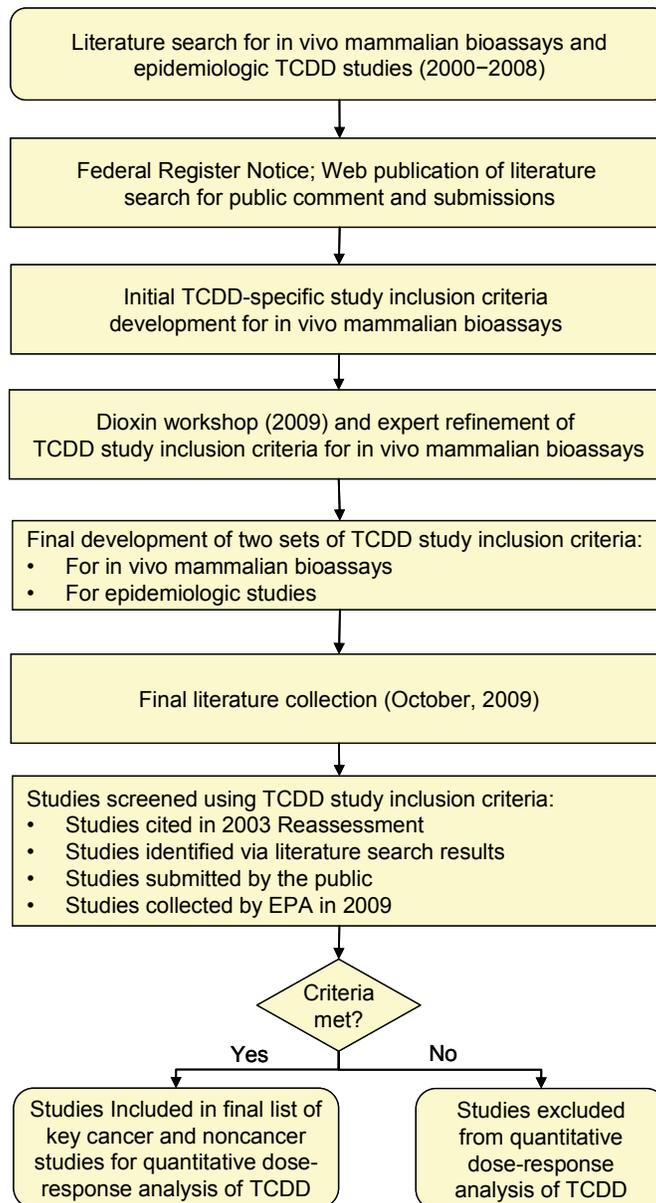
Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	<i>n</i>	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Chronic toxicity studies (continued)									
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	Kociba et al. (1978, 001818)
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	Maronpot et al. (1993, 198386)
Mouse/B6C3F1; 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 (1982, 543764)
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	NTP (2006, 197605)
Monkey/Rhesus	Daily dietary exposure (4 years)	F	8	0, 0.15, or 0.67	None	0.15	General toxicological endpoints and reproductive effects	Elevated serum triglycerides and total lipids	Rier et al. (2001, 198776 ; 2001, 543773)

Table 2-7. Animal bioassay studies considered for noncancer dose-response modeling (continued)

Species/ strain	Exposure protocol	Sex (exposure group)	<i>n</i>	Average daily dose levels (ng/kg-day)	NOAEL (ng/kg-day)	LOAEL (ng/kg-day)	Endpoint(s) examined	LOAEL/NOAEL Endpoint(s)	Reference
Chronic toxicity studies (continued)									
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. (1993, 197889)
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	Sewall et al. (1995, 198145)
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	Toth et al. (1979, 197109)

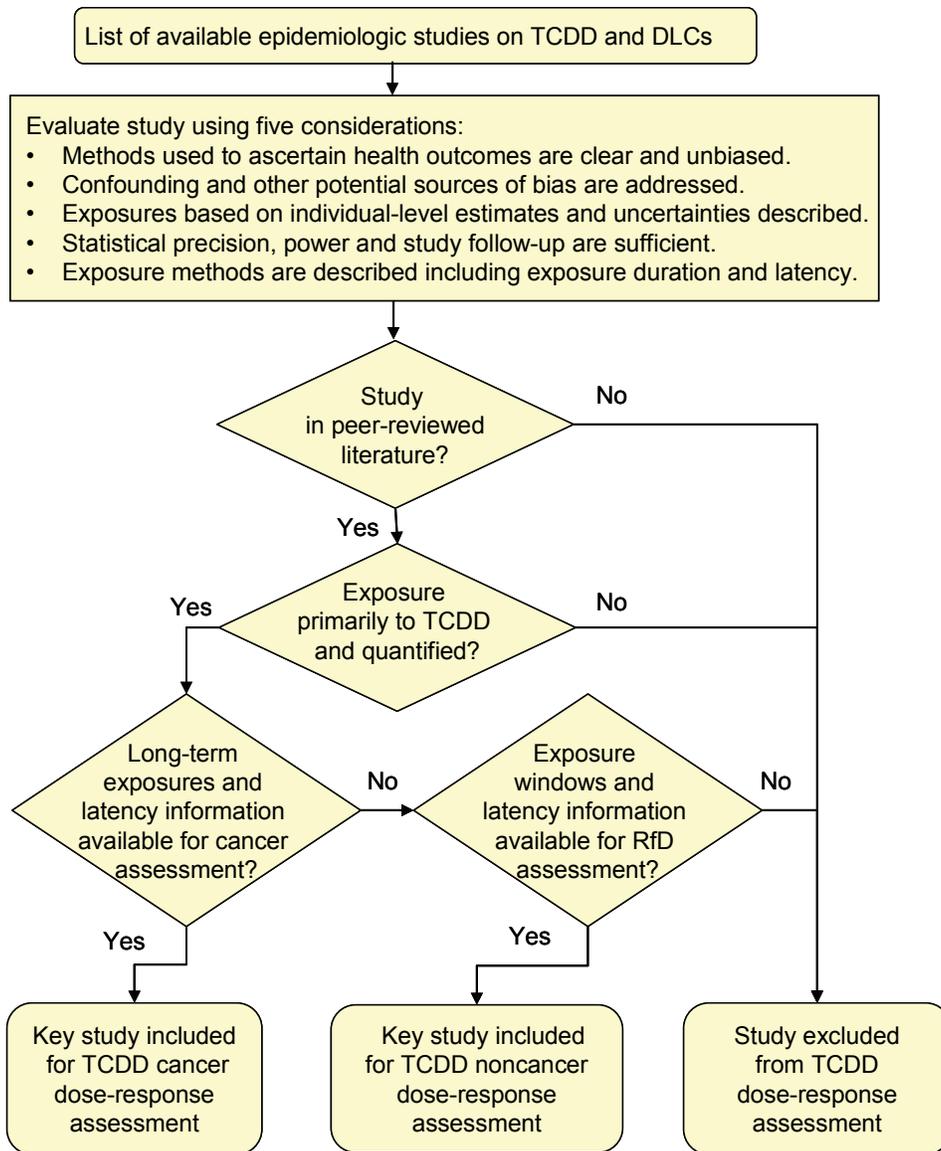
ND = not determined.



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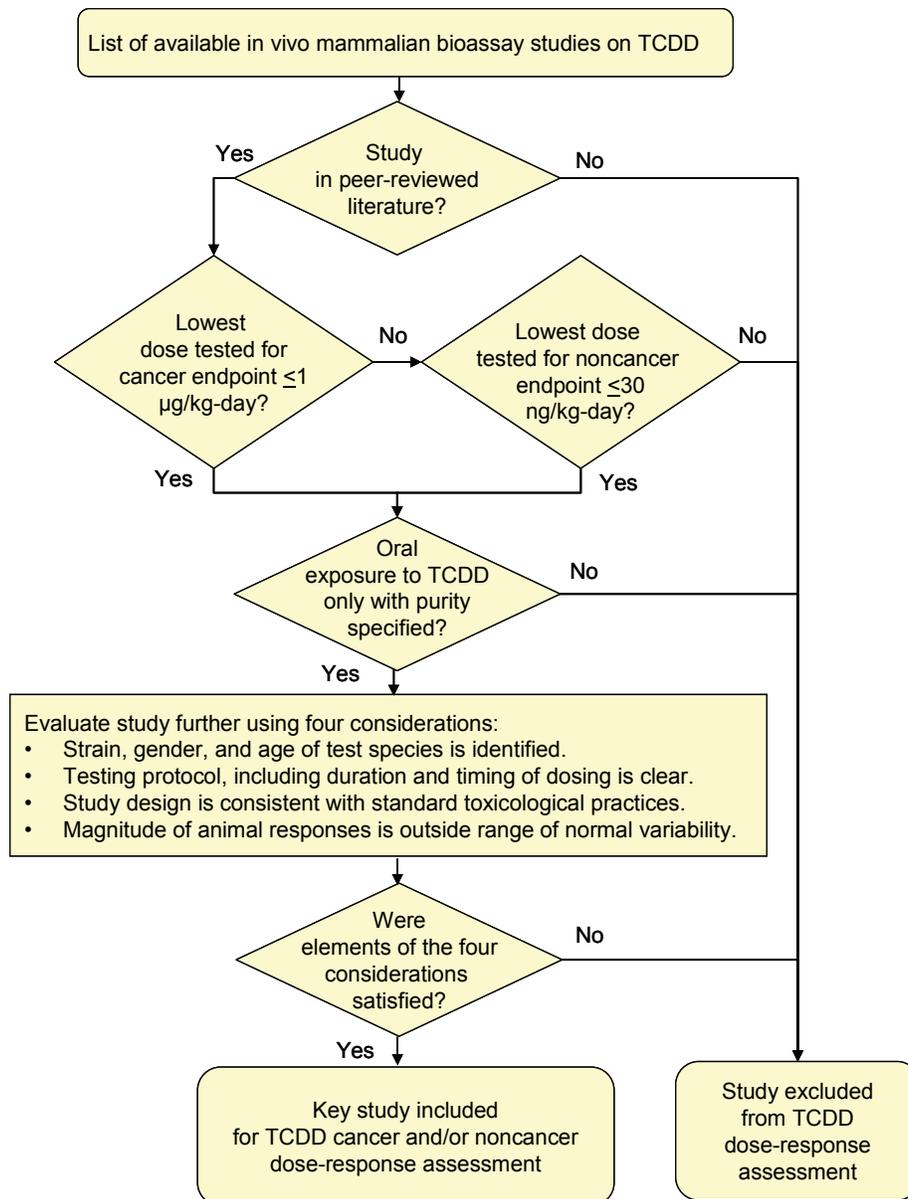
Figure 2-1. EPA’s process to select and identify in vivo mammalian and epidemiologic studies for use in the dose-response analysis of TCDD. EPA first conducted a literature search to identify studies published since the 2003 Reassessment. Results were published and additional study submissions were accepted 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.

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3 **Figure 2-2. EPA’s process to evaluate available epidemiologic studies using**
4 **study inclusion criteria for use in the dose-response analysis of TCDD.** EPA
5 applied its TCDD-specific epidemiologic study inclusion criteria to all studies published
6 on TCDD and DLCs. The studies were initially evaluated using five considerations
7 regarded as providing the most relevant kind of information needed for quantitative
8 human health risk analyses. For each study that was published in the peer-reviewed
9 literature, EPA then examined whether the exposures were primarily to TCDD and if the
10 TCDD exposures could be quantified so that dose-response analyses could be conducted.
11 Finally, EPA required that the effective dose and oral exposure be estimable: (1) for
12 cancer, information is required on long-term exposures, (2) for noncancer, information is
13 required regarding the appropriate time window of exposure that is relevant for a specific,
14 nonfatal health endpoint, and (3) for all endpoints, the latency period between TCDD
15 exposure and the onset of the effect is needed. Only studies meeting these criteria were
included in EPA’s TCDD dose-response analysis.

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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. Next, to ensure working in the low-dose range for TCDD dose-response analysis, EPA applied dose requirements to the lowest tested average daily doses in each study, with specific requirements for cancer ($\leq 1 \mu\text{g}/\text{kg}\text{-day}$) and noncancer ($\leq 30 \text{ ng}/\text{kg}\text{-day}$) studies. Third, EPA required that the animals were exposed via the oral route to only TCDD and that the purity of the TCDD was specified. Finally, the studies were evaluated using four considerations regarded as providing the most relevant kind of information needed for quantitative human health risk analyses from animal bioassay data. Only studies meeting all of these criteria and considerations were included in EPA’s TCDD dose-response analysis.

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1 Although the NAS basically agreed with EPA’s use of body burden as a dose metric in
2 the 2003 Reassessment (e.g., see NAS, 2006, [198441](#), p. 7), the NAS was concerned about the
3 limitations of first order kinetic models, such as the one used in the 2003 Reassessment, to
4 estimate TCDD body burdens.

5
6 TCDD, other dioxins, and DLCs act as potent inducers of CYP, a property that
7 can affect both the hepatic sequestration of these compounds and their half-lives.
8 Hepatic sequestration of dioxin may influence the quantitative extrapolation of the
9 rodent liver tumor results because the body-burden distribution pattern in highly
10 dosed rats would differ from the corresponding distribution in humans subject to
11 background levels of exposure. EPA should consider the possible quantitative
12 influence of dose-dependent toxicokinetics on the interpretation of animal
13 toxicological data (NAS, 2006, [198441](#), p. 129).

14
15 The NAS also asked EPA to evaluate the impact of kinetic uncertainty and variability on
16 dose-response assessment. The NAS committee asked EPA to use TK models to examine both
17 interspecies and human interindividual differences in the disposition of TCDD, which would
18 better justify EPA dose-response modeling choices.

19
20 The Reassessment does not adequately consider the use of a PBPK model to
21 define species differences in tissue distribution in relation to total body burden for
22 either cancer or noncancer end points (NAS, 2006, [198441](#), p. 62).

23
24 EPA ...should consider physiologically based pharmacokinetic modeling as a
25 means to adjust for differences in body fat composition and for other differences
26 between rodents and humans (NAS, 2006, [198441](#), p. 10).

27
28 The Reassessment does not provide details about the magnitudes of the various
29 uncertainties surrounding the decisions EPA makes in relation to dose metrics
30 (e.g., the impact of species differences in percentage of body fat on the
31 steady-state concentrations present in nonadipose tissues). The committee
32 recommends that EPA use simple PBPK models to define the magnitude of any
33 differences between humans and rodents in the relationship between total body
34 burden at steady-state concentrations (as calculated from the intake, half-life,
35 bioavailability) and tissue concentrations. The same model could be used to
36 explore human variability in kinetics in relation to elimination half-life. EPA
37 should modify the estimated human equivalent intakes when necessary (NAS,
38 2006, [198441](#), p. 73).

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

3
4 EPA makes a number of assumptions about the appropriate dose metric and
5 mathematical functions to use in the Reassessment's dose-response analysis ...
6 but does not adequately comment on the extent to which each of these
7 assumptions could affect the resulting risk estimates...EPA did not quantitatively
8 describe how this particular selection affected its estimates of exposure and
9 therefore provided no overall quantitative perspective on the relative importance
10 of the selection (NAS, 2006, [198441](#), p. 51).

11 12 **3.2. OVERVIEW OF EPA'S RESPONSE TO THE NAS COMMENTS ON THE USE OF** 13 **TOXICOKINETICS IN DOSE-RESPONSE MODELING APPROACHES FOR** 14 **TCDD**

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

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

21

22 **3.3. PHARMACOKINETICS (PK) AND PK MODELING**

23 **3.3.1. PK Data and Models in TCDD Dose-Response Modeling: Overview and Scope**

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

1 humans have been reviewed (NAS, 2006, [198441](#); U.S. EPA, 2003, [537122](#); van Birgelen and
2 van, 2000, [523248](#)).

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

- 5
6 ■ **TCDD is very highly lipophilic** and thus is more soluble in fat or other relatively
7 nonpolar organic media than in water. The *n*-octanol/water partition coefficient is a
8 commonly-used measure of lipophilicity equal to the equilibrium ratio of a substance's
9 concentration in *n*-octanol (a surrogate for biotic lipid) to the substance's concentration
10 in water (Leo et al., 1971, [019600](#)). For TCDD, this coefficient is on the order of
11 10,000,000 or more (ATSDR, 1998, [197033](#)). It follows that the solubility of TCDD in
12 the body's lipid fraction, i.e., the fatty portions of various tissues, including adipose,
13 organs, and blood, is extremely high.
- 14 ■ **TCDD is very slowly metabolized** compared to many other organic compounds, with an
15 elimination half life in humans on the order of years following an initial period of
16 distribution in the body (Carrier et al., 1995, [197618](#); Michalek et al., 2002, [199579](#)).
17 Most laboratory animals used for toxicologic testing tend to eliminate TCDD much more
18 quickly than people, although even in animals TCDD is eliminated much more slowly
19 than most other chemicals.
- 20 ■ **TCDD induces binding proteins in the liver** that have the effect of sequestering some
21 of the TCDD. The ability of TCDD to alter gene expression and the demonstration that
22 the induction of CYP1A2 is responsible for hepatic TCDD sequestration suggest that
23 both pharmacokinetic and pharmacodynamic events must be incorporated for a
24 quantitative description of TCDD disposition (Santostefano et al., 1998, [200001](#)). The
25 induction of these proteins implies that TCDD tends to be eliminated more rapidly in the
26 early years following short-term, high-level exposures than it is after those initial levels
27 have declined. Leung et al. (1988, [198815](#)) and Andersen et al. (1993, [196991](#)), in their
28 PBPK modeling, had taken into consideration the issue of liver protein binding. Recent
29 efforts of pharmacokinetic modeling have supported the concentration-dependent
30 elimination of TCDD in animals and humans (Aylward et al., 2005, [197014](#); Emond et
31 al., 2006, [197316](#)).

32
33 Sections 3.3.2 and 3.3.3 present the salient features of TCDD pharmacokinetics in
34 animals and humans, with particular focus on mechanisms and data of relevance to interspecies
35 and intraspecies variability. Section 3.3.4 describes the various dose metrics for the
36 dose-response modeling of TCDD and the characteristics of pharmacokinetic models potentially
37 useful for estimating these metrics. Finally, Sections 3.3.5 and 3.3.6 summarize the results of
38 application of pharmacokinetic models to derive dose metrics as well as the uncertainty
39 associated with the predictions of dose metrics used in dose-response modeling. Dose metrics

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1 derived via PBPK modeling approaches are utilized in Sections 4 and 5 of this document for
2 noncancer and cancer TCDD dose-response modeling, respectively.

3 4 **3.3.2. PK of TCDD in Animals and Humans**

5 **3.3.2.1. Absorption and Bioavailability**

6 When administered via the oral route in the dissolved form, TCDD appears to be well
7 absorbed. Animal studies indicate that oral exposure to TCDD in the diet or in an oil vehicle
8 results in the absorption of >50% of the administered dose (Nolan et al., 1979, [543785](#); Olson et
9 al., 1980, [197976](#)). Human data from Poiger and Schlatter (1986, [197336](#)) indicate that >87% of
10 the oral dose (after ingestion of 105 ng [³H]-2,3,7,8-TCDD [1.14 ng/kg BW] in 6 mL corn oil)
11 was absorbed from the gastrointestinal tract. Lakshmanan et al. (1986, [548729](#)), investigating
12 the oral absorption of TCDD, suggested that it is absorbed primarily by the lymphatic route and
13 transported predominantly by chylomicrons.

14 Oral absorption is generally less efficient when TCDD is more tightly bound in soil
15 matrices. Based on experiments in miniature swine, Wittsiepe et al. (2007, [548736](#)) reported an
16 approximately 70% reduction in bioavailability when TCDD was administered in the form of
17 contaminated soil, relative to TCDD after extraction from the same soil matrix with solvents.
18 Working with soil from the prominent contamination site at Times Beach, Missouri, Shu et al.
19 (1988, [548739](#)) reported an oral bioavailability of approximately 43% based on experiments in
20 rats. Percent dose absorbed by the dermal route is reported to be less than the oral route, whereas
21 absorption of TCDD by the transpulmonary route appears to be efficient (Banks and Birnbaum,
22 1991, [548742](#); see, for example; Banks et al., 1990, [548741](#); Diliberto et al., 1996, [143712](#);
23 Nessel et al., 1992, [548743](#); Roy et al., 2008, [548747](#); U.S. EPA, 2003, [537122](#)).

24 25 **3.3.2.2. Distribution**

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

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

6 Column 1 in Table 3-1 presents the tissue: blood partition coefficients for TCDD (Emond
7 et al., 2005, [197317](#); Wang et al., 1997, [104657](#)). Column 3 of this table lists the physical
8 volume of each tissue, scaled to a person weighing 60 kg. The last column shows the
9 implications of the tissue volumes and tissue: blood partition coefficients for the effective
10 volumes of distribution for each tissue and for the body as a whole. It can be seen that, purely on
11 the basis of solubility space, the fat should be expected to contain about 94% of the TCDD in the
12 body, and that the body as a whole behaves as if it is about 1,200 liters in terms of
13 blood-equivalents (i.e., approximately 22-fold larger than its physical volume).

14 Maruyama et al. (2002, [198448](#)) have published another set of tissue/ blood partition
15 coefficients for TCDD and other dioxin congeners based in part on observations of tissue
16 concentrations measured in autopsy specimens from eight Japanese people without known
17 unusual exposures to TCDD. Their estimates of TCDD partition coefficients seem to be rather
18 large and variable, with a fat: blood value of 247 ± 78 (standard deviation [SD]), a liver: blood
19 value of 9.8 ± 5.7 and a muscle: blood value of 18 ± 10.6 . Depending on time of autopsy, tissue
20 samples may not be an accurate source of information on observed, in vivo partition coefficients
21 because weight loss is likely to occur pre and post mortem. In particular, a decline in fat stores
22 volume could lead to an increased concentration of dioxin in fat in autopsy specimens relative to
23 what would be observed in vivo.

24 The calculations shown in Table 3-1 do not include the additional amount that will be
25 bound to induced proteins in the liver. That induction and binding will tend to increase the
26 contribution of the liver on the effective volume of distribution (Birnbaum, 1986, [548749](#)).

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

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

$$t_{1/2} \text{ for tissue perfusion loss} = \frac{\ln(2)}{\text{Rate constant for loss}} \quad (\text{Eq. 3-2})$$

$$= \frac{\ln(2) \times \text{Tissue volume (liters)} \times \text{Tissue/Blood Partition Coefficient}}{\text{Blood flow (liters/hour)}}$$

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. (2005, [197317](#); 2006, [197316](#)).

Despite the high lipid bioconcentration potential of TCDD, the adipose tissue does not always have the highest concentration (Abraham et al., 1988, [199510](#); Geyer et al., 1986, [064899](#); Poiger and Schlatter, 1986, [197336](#)). 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, [199510](#)) 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 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

1 high dose to low dose extrapolations. This behavior is essentially a result of dose-dependent
2 hepatic processes, as described below.

4 **3.3.2.3. Metabolism and Protein Binding**

5 The metabolism of TCDD is slow, particularly in humans, and it is thought to be
6 mediated by the CYP1A2 enzyme that is inducible by TCDD (Olson et al., 1994, [198008](#);
7 Ramsey et al., 1982, [548750](#); Weber et al., 1997, [548753](#); Wendling et al., 1990, [548751](#)). The
8 low rate of metabolism in combination with sequestration appear to account for the retention of
9 TCDD in liver, and these processes collectively contribute to the long half-life for elimination of
10 TCDD from the body.

11 Dynamic changes in TCDD binding in liver and partitioning to fat have been studied
12 extensively in rats and mice (Diliberto et al., 1995, [197309](#); 2001, [197238](#)). Figure 3-1 shows
13 observations by Diliberto et al. (1995, [197309](#)) of the ratio of liver concentrations to adipose
14 tissue concentrations for mice given doses spread over a 100-fold range and studied at four
15 different times following exposure. It can be seen that even for the lowest dose studied the
16 liver:fat concentration ratio is higher than would be expected based on the lipid contents of the
17 tissues (i.e., 0.06:1, corresponding to the ratio of human liver:blood and fat:blood partition
18 coefficients; see Table 3-1). Moreover, the relative concentration in the liver consistently rises
19 with dose, with the steepest rise observed during the first two weeks after dosing. If the
20 distribution of TCDD were governed solely by passive partitioning into fat, there should be no
21 such change in relative concentrations with dose. However, data presented in Figure 3-1
22 illustrate that at longer time points, the ratio of TCDD in the liver to TCDD in fat decreases,
23 indicating that a redistribution of the chemical occurs as time goes on for each applied dose. The
24 redistribution of TCDD tissue levels from liver to fat with increasing time suggests that binding
25 of the chemical in the liver (including via induction of CYP1A2) is an important kinetic
26 consideration at early exposure points with relatively high applied doses.

27 Experiments with CYP1A2 “knock-out” mice (i.e., congenic strains differing in only a
28 single gene that is “knocked out” in one of the strains) indicate that the inducible binding of
29 TCDD is attributable to CYP1A2 (Diliberto et al., 1997, [548755](#); 1999, [143713](#)). As noted
30 previously, this enzyme is believed to make an important contribution to metabolism of TCDD.
31 Given the critical role of CYP1A2 induction in the kinetics of TCDD, dose- and time-dependent

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1 induction of this protein in rats has been examined and modeled (Emond et al., 2004, [197315](#);
 2 Emond et al., 2006, [197316](#); Santostefano et al., 1998, [200001](#); Wang et al., 1997, [104657](#)).
 3 Accordingly, the amount of CYP1A2 in the liver can be computed as the time-integrated product
 4 of inducible production and a simple first-order loss process (Wang et al., 1997, [104657](#)):

$$5 \quad \frac{dCYP_{2A1}}{dt} = S(t)K_0 - K_2C_{A2t} \quad (\text{Eq. 3-3})$$

7
 8 where CYP_{2A1} is the concentration of the enzyme, K_2 is the rate constant for the first order loss,
 9 C_{A2t} is the concentration of CYP1A2 in the liver, K_0 is the basal rate of production of CYP1A2 in
 10 the liver, and $S(t)$ is a multiplicative stimulation factor for CYP1A2 production in the form of a
 11 Hill-type function:

$$12 \quad S(t) = 1 + \frac{In_{A2}(C_{Ah-TCDD})^h}{(IC_{A2})^h + (C_{Ah-TCDD})^h} \quad (\text{Eq. 3-4})$$

14
 15 where IC_{A2} corresponds to the concentration of the aryl hydrocarbon (Ah)-TCDD complex at
 16 which half of the maximum fold stimulation of CYP2A production is reached, and h , the Hill
 17 exponent, determines the curvature of the stimulation in relation to concentration of the
 18 Ah-TCDD complex at relatively low doses. A value of 0.6 as the Hill exponent has been used by
 19 Wang et al. (1997, [104657](#); 2000, [198738](#)) and Emond et al. (2004, [197315](#); 2005, [197317](#); 2006,
 20 [197316](#)), indicative of a negative cooperation, i.e., the curve is convex-upward (supralinear),
 21 depicting a faster increase in the low-dose region compared to a straight line. Additional
 22 parameters in this expression include In_{A2} , the maximum fold increase in the CYP1A2 synthesis
 23 rate over the basal rate that can occur at high levels of TCDD, and $(C_{Ah-TCDD})$, the concentration
 24 of TCDD bound to the aryl hydrocarbon receptor (AhR). This concentration in turn depends on
 25 the concentration of TCDD in the liver (C_{Lif}), the concentration of the AhR (Ah_{Li}) in liver, and
 26 the dissociation constant for the Ah-TCDD receptor complex, K_{DAh} :

$$27 \quad C_{Ah-TCDD} = \frac{Ah_{Li} \times C_{Lif}}{K_{DAh} + C_{Lif}} \quad (\text{Eq. 3-5})$$

28
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1 **3.3.2.4. Elimination**

2 Elimination half-lives (i.e., the time taken for the concentration to be reduced to one-half
3 of its initial level) of TCDD range from 11 days in the hamster to 2,120 days in humans
4 (U.S. EPA, 2003, [537122](#)). Hepatic metabolism and binding processes, fecal excretion, and
5 accumulation in adipose tissue collectively determine the dose-dependent elimination half-lives
6 in various species. Aylward et al. (2005, [197114](#)) depicted the relationship between the
7 elimination rate versus initial level of lipid-corrected TCDD in serum for 36 people (see
8 Figure 3-2). Even though this analysis was done using the initial TCDD level, rather than the
9 geometric mean or midpoint level in the decline for each person, it indicated a
10 concentration-dependency of the half-life and elimination of TCDD in exposed individuals.
11

12 **3.3.2.5. Interspecies Differences and Similarities**

13 Among the pharmacokinetic determinants of TCDD, some are known to vary markedly
14 between species whereas others are not characterized sufficiently in this regard. Overall, the
15 qualitative determinants of the body burden and elimination half-lives appear to be similar across
16 species. Based on empirical observations for TCDD as well as with other PCDFs, Carrier et al.
17 (1995, [197618](#); 1995, [543780](#)) argued that in rats, monkeys, and humans, the dose-dependent
18 changes in the fraction contained in liver and adipose tissue follow a similar pattern across
19 species. The authors suggested that the half-saturation body burden is around 100 ng/kg and the
20 plateau of liver dose (as fraction of body burden) appears to occur around 1,000 ng/kg.
21 Literature also indicates that AhR is conserved phylogenetically (Fujii-Kuriyama et al., 1995,
22 [543727](#); Harper et al., 2002, [198124](#); Nebert et al., 1991, [543728](#)) and is present in mammalian
23 species, including experimental animals and humans (Lorenzen and Okey, 1991, [198397](#);
24 Manchester et al., 1987, [198054](#); Okey et al., 1994, [548759](#); Roberts et al., 1985, [198706](#);
25 Roberts et al., 1986, [198780](#)). These qualitative similarities in pharmacokinetic determinants and
26 outcome support the use of animal data to infer general patterns of the pharmacokinetic behavior
27 of TCDD in humans. However, quantitative differences in determinants, including
28 physiological, physicochemical, and biochemical, need to be taken into account. Even though
29 species-specific physiological parameters can be obtained from the literature, key data on
30 species-specific biochemical parameters (particularly binding constants, maximal capacity,
31 induction rates, and other parameters) are not available for humans at this time. However, these

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1 can be inferred by using a pharmacokinetic model fit to in vivo data on the rate of TCDD
2 elimination from specific compartments in humans (Aylward et al., 2005, [197014](#); Carrier et al.,
3 1995, [197618](#); Carrier et al., 1995, [543780](#); Emond et al., 2004, [197315](#); Emond et al., 2005,
4 [197317](#); Emond et al., 2006, [197316](#)).

6 **3.3.3. PK of TCDD in Humans: Interindividual Variability**

7 TCDD pharmacokinetics and tissue doses vary across the human population as a function
8 of the interindividual variability of the key kinetic determinants. Because the NAS comments
9 focused on health effects associated with chronic, lifetime exposure, the key kinetic determinants
10 for such exposures include clearance, binding, and temporal changes in volume of distribution.
11 When considering the interindividual variability in pharmacokinetics and dose metrics of TCDD,
12 it is important to recognize that the elevated lipid-corrected serum concentrations in highly
13 exposed persons are associated with greater elimination rates, probably due to greater degrees of
14 induction of CYP1A2 in the liver and possibly other related metabolic enzymes (Abraham et al.,
15 2002, [197034](#); Aylward et al., 2005, [197014](#); Emond et al., 2006, [197316](#); Grassman et al., 2000,
16 [548762](#)).

17 The interindividual variability in fat content is a critical parameter in pharmacokinetic
18 models given the characteristics of TCDD (see Section 3.3.2). Both metabolic elimination and
19 elimination via the GI tract depend on the fraction of TCDD in the body that is available outside
20 of adipose tissue. As body fat content rises, a smaller portion of the total body TCDD will be
21 contained in the relatively available fraction outside of the adipose tissue. Because elimination
22 of TCDD by both metabolism and fecal excretion depends on the small proportion of TCDD that
23 exists outside of fat tissue, people with larger proportions of body fat—including many older
24 people—will tend to require longer times to reduce TCDD levels by a given proportion than
25 leaner people (Emond et al., 2006, [197316](#); Rohde et al., 1999, [548764](#); Van der Molen et al.,
26 1998, [548765](#); Van der Molen, et al., 1996, [548768](#)).

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

1 **3.3.3.1. Life Stage and Gender**

2 The influence of the variability of fat content in human population on the distribution and
3 clearance of TCDD has been evaluated by several investigators. There are data showing an
4 inverse dependency of TCDD elimination rate on percent body fat. Figure 3-3 shows this
5 relationship in a study in which TCDD elimination via feces was measured in six people in
6 relation to their body fat content (Rohde et al., 1999, [548764](#)). Observations of TCDD
7 elimination rates in a small number of men and women in the Seveso cohort (Aylward et al.,
8 2005, [197114](#)) provide a modest opportunity to compare TCDD elimination rates with actual
9 human data. Based on the partition coefficients reported by Emond et al. (2006, [197316](#)), the
10 elimination rates for the men in the sampled group are expected to be greater than the elimination
11 rates in the women. Taking into consideration calculations similar to those shown in Table 3-2,
12 and fat proportions inferred from body mass indices using the equations of Lean et al. (1996,
13 [548770](#)), the Seveso men studied are expected to have an overall average of about 3.92% of their
14 TCDD body burden outside of fat, whereas the women are expected to have an average of only
15 2.36% outside of fat. On this basis, the TCDD elimination rates in the men are expected to be
16 $3.92/2.36 = 1.66$ times faster than the elimination rates in the women. By comparison, Michalek
17 et al. (2002, [199579](#)) reported observed elimination rates in men and women that result in a
18 slightly lower ratio:

19

$$20 \frac{\text{men:}0.111 \text{ year}^{-1} \pm 0.010 \text{ (std.error)}}{\text{women:}0.071 \text{ year}^{-1} \pm 0.010 \text{ (std.error)}} = 1.56 \quad (\text{Eq. 3-6})$$

21

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

24 A further point of comparison can be derived using the observed body mass index
25 (BMI)¹² and TCDD elimination rate of each of the male Ranch Hand military veterans, whose
26 TCDD elimination rates were observed between 9 and 33 years after their time in Vietnam. The
27 average BMI over that time was 29.44 (based on 287 measurements for the 97 veterans,
28 tabulated in three periods by Michalek et al., 2002, [199579](#)), and their average age was about

¹²The body mass index, or BMI, is calculated as the body weight in kilograms divided by the square of the height in meters.

1 44.5 for the measurements. Based on these data, the corresponding average estimated percent
2 body fat is 29.7% using the Lean et al. (1996, [548770](#)) formula for men. The observed average
3 TCDD elimination rate constant for these men for the period was $0.092 \text{ year}^{-1} \pm 0.004$ (standard
4 error), corresponding to a half life of 7.5 years. This half life is slightly longer than the central
5 estimate of the half life of 6.2 years (i.e., $\ln(2)/0.111$) for the smaller group of Seveso males with
6 their slightly smaller estimated percent body fat. Figure 3-4 shows a simple plot of these data
7 and a fitted unweighted regression line characterizing the relationship between estimated fat
8 content and TCDD elimination rates. Variation in metabolic enzyme activities and other routes
9 of loss is also likely to be important, but there is little human quantitative information available
10 on these issues.

11 More recently, Kerger et al. (2006, [198651](#)) estimated the slope of the relationship
12 between half-life and age to be 0.12 years (95% confidence interval, 0.10–0.14), which
13 corresponds to the rate of increase in TCDD half-life for each year of age. The authors
14 speculated that although age explained most of the variance in the individual half-life trends, it
15 was also correlated with TCDD concentration, BMI, and body fat mass. The regression model
16 developed by these authors discriminated between the high and low TCDD exposures or
17 concentrations. Thus, after accounting for the TCDD (concentration \times age) term's effect on the
18 slope of age, the final model for TCDD concentration ≤ 700 ppt was

19

$$20 \quad t_{1/2} = 0.35 + 0.12 \times \text{Age} \quad (\text{Eq. 3-7})$$

21 For TCDD concentration >700 ppt, the final model was:

22

$$23 \quad t_{1/2} = 0.35 + 0.088 \times \text{Age} \quad (\text{Eq. 3-8})$$

24

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

27

28 **3.3.3.1.1. Prenatal period.**

29 Data to estimate TCDD elimination rates for fetuses are not available. Levels of TCDD
30 in fetal tissues for rats were experimentally estimated at different gestational periods and utilized
31 in a developmental model by Emond et al. (2004, [197315](#)). There is information on body

1 composition that is relevant to prediction of TCDD dose to fetus. These data, summarized as
2 part of the radiation dosimetry model of the International Commission on Radiological
3 Protection, are consistent with the idea that early fetuses are nearly all water and less than
4 1% lipid, and lipid levels rise toward parity with protein near the time of normal delivery.

5 Bell et al. (2007, [197050](#)) reported that the disposition of TCDD into the fetus shows
6 dose dependency, with a greater proportion of the dose reaching the fetus at lower doses of
7 TCDD. Further, both CYP1A1 and CYP1A2 are highly inducible (~103-fold) in fetal liver,
8 whereas CYP1A2 shows much lower induction (10-fold) in maternal liver. It has been
9 speculated that this is due to the lower basal levels of CYP1A2 in fetal liver, as compared to
10 maternal liver (Bell et al., 2007, [197050](#)). The greater relative disposition to the fetus at low
11 doses may be the result of higher bioavailability due to less hepatic sequestration and elimination
12 in the mother.

13 14 **3.3.3.1.2. *Infancy and childhood.***

15 Hattis et al. (2003, [548773](#)) describe the general pattern of change of body fat content
16 with age in children. Central tendency values for percent body fat begin at about 12% at birth
17 and rise steeply to reach about 26% near the middle of the first year of life. Fat content then falls
18 to reach a minimum of approximately 15% at 5–8 years of age, followed by a sex-dependent
19 “adiposity rebound” that takes females to about 26% body fat while the males remain near
20 16–17% on average by age 20. The interindividual variability distributions about these central
21 values are complex, as some children experience the “adiposity rebound” earlier than others, and
22 this creates patterns that are not simply interpretable as unimodal normal distributions. Hattis et
23 al. (2003, [548773](#)) did find it possible to fit distributions of body fat content inferred from
24 NHANES skin fold measures to mixtures of two normal distributions for children between age 5
25 and 18.

26 At least two groups of authors have published PBPK modeling results indicating
27 generally more rapid clearance of TCDD in children than in adults, a trend that is consistent with
28 the generally lower fat content of children (Kreuzer et al., 1997, [198088](#); Leung et al., 2006,
29 [548779](#); Van der Molen et al., 2000, [548777](#)). The rapid expansion of the adipose tissue
30 compartment can contribute, in part, to the reduced apparent half-life in children (Clewell et al.,

1 2004, [056269](#)). This reduction may also be due to varying rates of metabolism and/or fecal lipid
2 excretion (Abraham et al., 1996, [548782](#); Kerger et al., 2007, [548784](#)).

3 Furthermore, very young children have different modes and quantities of exposure
4 compared to adults. Lakind et al. (2000, [198094](#)) characterize distributions of milk intake for
5 nursing infants to characterize distributions of TCDD exposure. This is also a corresponding
6 route of loss of TCDD stores for lactating women, as described in Section 3.3.3.2 below.

7 8 **3.3.3.1.3. Adulthood and old age.**

9 The fraction of fat in relation to body weight in adulthood and old age can be computed
10 as a function of the BMI and age (e.g., Lean et al., 1996, [548770](#)):

$$11 \quad \% \text{ Body Fat (males)} = 1.33 \times \text{BMI} + 0.236 \times \text{Age} - 20.2 \quad (\text{Eq. 3-9})$$

$$12 \quad \% \text{ Body Fat (females)} = 1.21 \times \text{BMI} + 0.262 \times \text{Age} - 6.7 \quad (\text{Eq. 3-10})$$

13
14
15 The above equations are the result of analysis of data based on underwater weighing of
16 63 men and 84 women (age range 16.8–65.4). The salient observation with respect to TCDD for
17 these data is that age and BMI-dependent variability in fat content have implications for the
18 variability in TCDD elimination rates and internal dose among adults.

19 20 **3.3.3.2. Physiological States: Pregnancy and Lactation**

21 Data on body fat content in pregnant women at various stages of gestation (Pipe et al.,
22 1979, [548786](#)) have potential implications for TCDD elimination rates during pregnancy, even
23 though the relationship between these parameters has not been formally analyzed.

24 Lactation is viewed as an additional route of elimination for some chemicals such as
25 TCDD. According to a recent study, a breast-feeding woman expels through lactation an
26 estimated 8.76 kg fat per year [q_f (kg/day), 0.8 kg milk/day with an average 3% lipid], and the
27 partition coefficient between blood lipid and milk fat (K_{BM}) for TCDD is 0.92 (Milbrath et al.,
28 2009, [198044](#); Wittsiepe et al., 2007, [548736](#)). The estimated rate of elimination of TCDD due
29 to breast-feeding (k_{bfed}) can then be computed as follows (Milbrath et al., 2009, [198044](#)):

1
$$k_{bfed} = \frac{q_f \times \Delta t_{bfed}}{K_{BM} \times \frac{pbf_i}{100} \times BW_i} \quad (\text{Eq. 3-11})$$

2
3 where

4 Δt_{bfed} (unitless) = the fraction of the year during which the woman was actively breast-
5 feeding;

6 pbf_i = woman's percent body fat; and

7 BW = woman's body weight in kg.

8
9 Assuming no interaction between breast-feeding and other half-life determinants
10 Milbrath et al. (2009, [198044](#)), the authors predicted a half-life of 4.3 years for TCDD in a
11 30-year-old, nonsmoking woman with 30% body fat if she did not breast-feed that year, and a
12 half-life of 1.8 years if she breast-fed for 6 months.

13 3.3.3.3. *Lifestyle and Habits*

14 One of the factors related to lifestyle and habits that could influence TCDD kinetics is
15 smoking. Smoking has been reported to enhance the elimination of dioxin and dioxin-like
16 compounds (Ferriby et al., 2007, [548789](#); Flesch-Janys et al., 1996, [197351](#)). Milbrath et al.
17 (2009, [198044](#)) accounted for interindividual variation in body composition as well as smoking
18 habits in an empirical model. The predicted half-life (years) for an individual i as a function of
19 age, smoking status, and percent body fat i was as follows

20
21
$$t_{1/2}(age, smoke, pbf)_i = [\beta_{(0age)} + \beta_{(age)} \times age_i] \times SF_i \times \frac{pbf_i}{pbf_{ref(age_i)}} \quad (\text{Eq. 3-12})$$

22
23 where

24 $\beta_{(0age)}$ = intercept constant derived from regressed data;

25 $\beta_{(age)}$ = slope constant derived from regressed data;

26 age_i = specific age i (years);

27 pbf_i = individual percent body fat;

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1 $pbf_{ref(age_i)}$ = reference percent body fat; and
2 SF_i = the unitless, multiplicative smoking factor.
3

4 **3.3.3.4. Genetic Traits and Polymorphism**

5 One particular genetic locus that is potentially related to TCDD pharmacokinetics and
6 tissue dose is the gene for the AhR. Eight candidate AhR polymorphisms have been identified to
7 date (Connor and Aylward, 2006, [197632](#); Harper et al., 2002, [198124](#)). Given the role of AhR
8 in regulating the induction of CYP1 isozymes (Baron et al., 1998, [548791](#); Connor and Aylward,
9 2006, [197632](#); Toide et al., 2003, [548792](#)), the polymorphism might lead to interindividual
10 differences in metabolic clearance, the significance of which would depend upon the dose, fat
11 content, and exposure scenario. In this regard, it should be noted that the inducibility of aromatic
12 hydrocarbon hydroxylase in human tissues has been reported to be highly variable, up to
13 100–fold (Connor and Aylward, 2006, [197632](#); Smart and Daly, 2000, [548794](#); Wong et al.,
14 1986, [548795](#)).

15 Finally, the scientific literature contains values of K_d (the dissociation constant of the
16 TCDD–AhR complex) ranging from about 1 to much higher values (corresponding to lower
17 binding affinity) (reviewed in Connor and Aylward, 2006, [197632](#)). This provides suggestive
18 evidence for a heterogeneous human AhR, with functionally important polymorphisms (Micka et
19 al., 1997, [548797](#); Roberts et al., 1986, [198780](#)), even though some of the range may be
20 attributed to experimental procedural differences and to other factors (Connor and Aylward,
21 2006, [197632](#); Harper et al., 2002, [198124](#); Lorenzen and Okey, 1991, [198397](#); Manchester et
22 al., 1987, [198054](#)).

23 The various pharmacokinetic processes and determinants (see Sections 3.3.2 and 3.3.3),
24 individually or together, might influence the dose metrics of relevance to the dose-response
25 modeling of TCDD.
26

27 **3.3.4. Dose Metrics and Pharmacokinetic Models for TCDD**

28 **3.3.4.1. Dose Metrics for Dose-Response Modeling**

29 The **dose metric** related to a toxicologic endpoint can range from the maximal
30 concentration, the area under a time-course curve (AUC), or the time-averaged concentration of

1 the toxic moiety in the body, blood, or target tissue, to an appropriate measure of the resulting
2 interactions in the target tissue (e.g., receptor occupancy or functional biomarkers related to
3 specific effects). A single dose metric, however, is unlikely to be sufficient for all endpoints and
4 exposure durations. Further, the ideal dose metric chosen on the basis of the mode of action
5 (MOA) may not be the dose metric for which model predictions can be obtained with a high
6 level of confidence. Consideration of these issues is critical to the selection of the dose metrics
7 of relevance to dose-response modeling of TCDD.

8 Figure 3-5 lists a range of alternative dose metrics for TCDD in terms of their relevance
9 based on considerations of pharmacokinetic mechanisms and MOA. The **administered dose** or
10 daily intake (ng/kg-day) is the least relevant dose metric for dose-response modeling of TCDD.
11 This dose adjusts only for body weight differences between species. The administered dose,
12 when used with an uncertainty factor for kinetics (or kinetic adjustment factor, such as $BW^{3/4}$)
13 and an uncertainty factor for dynamics, can also account for allometrically-predicted
14 pharmacokinetic (clearance) and pharmacodynamic differences between species in deriving the
15 human equivalent dose (HED). In effect, the use of kinetic and dynamic adjustment or
16 uncertainty factors facilitates the computation of HED. Such a calculation of HED is associated
17 with the steady-state blood concentration of parent chemical in rats by accounting for species
18 differences in metabolic clearance. This is generally done by relating to body surface area or
19 metabolic rates, with no corresponding temporal changes in the volume of distribution (see, for
20 example, Krishnan and Andersen, 1991, [548799](#)). Such calculations of HED for TCDD may not
21 be appropriate given that (1) steady-state was not attained in all critical toxicological studies
22 chosen for the assessment, (2) the clearance is mainly due to enzyme(s) and processes whose
23 levels/rates do not necessarily vary across species or life stages as a function of body surface
24 differences, and (3) there is a likelihood of change in volume of distribution over time.
25 Furthermore, the use of administered dose does not explicitly account for the dose-dependent
26 elimination of TCDD from tissues as demonstrated in multiple studies (reviewed in
27 Sections 3.3.2 and 3.3.4). The use of administered dose in TCDD dose-response modeling is
28 unlikely to facilitate the characterization of the true relationship between the response and the
29 relevant measures of internal dose that are influenced by dose-dependent elimination and binding
30 processes. Additionally, the use of administered dose to extrapolate across species or life stages

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

3 Dose metrics for TCDD may include absorbed dose, body burden, serum or whole blood
4 concentration, tissue concentration, and possibly functional-related metrics of relevance to the
5 MOA (e.g., receptor occupancy, change in protein levels). These measures can be calculated as
6 a current (terminal), average (over a defined period), or integral quantity. The applicability of
7 the integral measures, such as the AUC (i.e., the area under the curve of a plot of blood or
8 plasma concentration vs. time), traditionally used for analyzing chronic toxicity data, is
9 questionable in the case of TCDD. This is because of differences in lifespan and uncertainties
10 regarding the appropriateness of the duration to be specified for averaging the AUC in
11 experimental animals and humans for certain critical effects (NAS, 2006, [198441](#)).

12 Among the alternative dose metrics, the **absorbed dose** accounts for differences in body
13 weight as well as species-specific differences in bioavailability. Thus, the **absorbed dose** is
14 equivalent to **body burden**. **Body burden**, or more appropriately the body concentration,
15 represents the amount of TCDD per kg body weight. TCDD body burdens, like other dose
16 measures, can be determined as the peak, the average over the period of the bioassays, or the
17 level at the end of the experiments. Thus, the terminal or average body burdens can be obtained
18 either using data or pharmacokinetic models and used in dose-response modeling. The body
19 burden is a measure of TCDD dose that reflects the net impact of bioavailability, uptake,
20 distribution, and elimination processes in the organism. It is essentially a function of the volume
21 of distribution and clearance processes, and as such it does take into account the temporal
22 changes in volume of distribution as well as the concentration-dependent clearance. These are
23 phenomena that are critical to the understanding of TCDD dose to the target. However, the body
24 burden may not accurately reflect the tissue dose (NAS, 2006, [198441](#)), and as such does not
25 allow for analysis of species-specific differences in target organ sensitivity to TCDD. In
26 essence, the body burden represents only an “overall average” of TCDD concentration in the
27 body, without regard to the differential partitioning and accumulation in specific tissues,
28 including the target tissue(s).

29 **Serum (or blood) concentration** of TCDD is a dose metric that reflects both the body
30 burden and the dose to target tissues. Serum or blood concentration, at steady-state, would be
31 reflective of the impact of clearance processes, and expected to be directly proportional to the

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1 tissue concentrations of TCDD (NAS, 2006, [198441](#)). This dose metric for lipophilic chemicals
2 such as TCDD is often expressed as a lipid-normalized value, to adjust for varying serum lipid
3 content (e.g.; DeKoning and Karmaus, 2000, [548801](#); Niskar et al., 2009, [548802](#)) (Patterson et
4 al., 2009), particularly in human biomonitoring studies, thus of relevance to dose-response
5 modeling; however, the serum lipid-normalized concentrations of TCDD are not routinely
6 collected and reported in animal toxicologic studies. Serum lipid-adjusted of TCDD
7 concentration is calculated as the ratio of serum TCDD content over serum lipid content per unit
8 volume. Alternatively, TCDD serum lipid-normalized calculation can be estimated by using the
9 formula $TL = (2.27 \times TC) + TG + 62.3$ mg/dL where the total lipid (TL) content of each sample
10 is estimated from its total cholesterol (TC) and triglyceride (TG) (Patterson et al., 2009). The
11 lipid-adjusted serum concentration, however, would be reflective of the lipid-adjusted
12 concentration of TCDD in other organs (reviewed in Aylward et al., 2008, [197068](#)) depending
13 upon the extent of steady-state attained and the similarity of lipid composition across tissues in
14 each species. In essence, the serum lipid-normalized measure is representative of the amount of
15 TCDD per specified volume of total lipids, whereas the whole blood measure will be reflective
16 of the ensemble of free, lipid-bound and protein-bound TCDD in plasma and erythrocytes, which
17 may be species-specific. Even though these dose metrics are thought to be more closely and
18 directly related to the tissue concentrations associated with an effect, a less direct association
19 might occur at increasing doses when nonlinear processes dominate the kinetics and distribution
20 of TCDD into organs such as the liver.

21 **Tissue concentration** of TCDD, as free, bound, or total TCDD, is a more relevant
22 pharmacokinetic measure of dose, given that it provides a measure of exposure of the target cells
23 to the chemical. In this regard, the CYP1A2-bound fraction may be considered as a relevant
24 dose metric for certain toxic effects; however, the available data contain mixed results regarding
25 the mechanistic linkage of this dose metric to toxicity and carcinogenicity (reviewed in Budinsky
26 et al., 2006, [594248](#)). In such cases, the use of alternative dose metrics (e.g., bound
27 concentration as well as the serum concentration) in dose-response modeling could be
28 considered. Other function-related biomarkers and dose metrics could facilitate the additional
29 consideration of pharmacodynamic aspects reflecting tissue- and species-specific sensitivity.
30 These metrics represent the most relevant measures of tissue exposure and sensitivity to TCDD.

1 Empirical time-course data on the alternative dose metrics of TCDD associated with
2 epidemiologic and experimental (animal) studies are not available, requiring the use of
3 pharmacokinetic models to obtain estimates of these dose metrics. These models may be simple,
4 based on first order kinetics (see Section 3.3.4.2), or more complex based on physiochemical,
5 biochemical, and physiological parameters for simulating uptake, distribution (including
6 sequestration to proteins), and clearance of TCDD (see Section 3.3.4.3). Receptor occupancy
7 and functional biomarkers as dose metrics for TCDD require a clear understanding of mode of
8 action of TCDD and availability of relevant data. In the absence of such information, these
9 possible dose metrics can not be utilized at the present time.

11 3.3.4.2. *First-Order Kinetic Modeling*

12 Figure 3-6 illustrates the process of estimating a human-equivalent TCDD oral exposure
13 from an experimental animal-administered dose, based on the assumption that body burden is the
14 effective dose metric for TK equivalence across species. The primary assumption is that the
15 time-weighted average (TWA) TCDD body burden over some critical time period is the
16 proximate toxicokinetically-effective dose eliciting a toxicologic effect.¹³ The process consists
17 of estimating the effective average body burden in the experimental animal over some time t_A
18 (generally the experimental duration) using a TK model, then “back-calculating” a daily human
19 exposure level that would result in that average body burden over some time t_H (the human
20 equivalent to t_A).

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

$$24 \quad BB(t) = BB(0) + \frac{d(1 - e^{-kt})fa}{k} \quad (\text{Eq. 3-13})$$

25 where
26

27 $BB(t)$ = the body burden at time t (ng/kg);

28 $BB(0)$ = the initial body burden (ng/kg);

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

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

¹³The conversion depicted in Figure 3-6 does not account for toxicodynamic differences between species.
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1 t = the time at which the body burden is determined (days); and
 2 fa = the fraction of oral dose absorbed (unitless).

3

4 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
 5 humans, this parameter is $BB_H(t) = BB_H(0)e^{-k_H t_H} + \frac{d_H(1 - e^{-k_H t_H})fa_H}{k_H}$.

6

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

8

9
$$BB_H(0)e^{-k_H t_H} + \frac{d_H(1 - e^{-k_H t_H})fa_H}{k_H} = BB_A(0)e^{-k_A t_A} + \frac{d_A(1 - e^{-k_A t_A})fa_A}{k_A} \quad (\text{Eq. 3-14})$$

10

11 Rearranging yields the general solution for d_H .

12

13
$$d_H = d_A \frac{k_H}{k_A} \frac{fa_A}{fa_H} \frac{(1 - e^{-k_A t_A})}{(1 - e^{-k_H t_H})} + BB_A(0)e^{-k_A t_A} - BB_H(0)e^{-k_H t_H} \quad (\text{Eq. 3-15})$$

14

15 Assuming that initial body burdens are very small compared to $BB(t)$ and that the fraction of
 16 TCDD absorbed is the same for humans and experimental animals, and using the relationship

17 $k = \frac{\ln(2)}{t_{1/2}}$, where $t_{1/2}$ is the whole-body half-life, a simplified solution for d_H is obtained.

18

19
$$d_H = d_A \frac{t_{1/2A}}{t_{1/2H}} \frac{(1 - e^{-k_A t_A})}{(1 - e^{-k_H t_H})} \quad (\text{Eq. 3-16})$$

20

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

1 half-lives. The latter approach was used in the 2003 Reassessment for conversion of animal
 2 cancer slope factors to the human equivalent, where only lifetime exposures are relevant.¹⁴

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

$$7 \quad BB(t) = BB(0) \frac{1}{t} \int_0^t e^{-k\tau} d\tau + d \frac{fa}{k} \frac{1}{t} \int_0^t (1 - e^{-k\tau}) d\tau = BB(0) \frac{(1 - e^{-kt})}{kt} + d \frac{fa}{k} \left[1 - \frac{(1 - e^{-kt})}{kt} \right] \quad (\text{Eq. 3-17})$$

9
 10 On the basis of average body burden as given in Eq. 3-17, is transformed again assuming
 11 minimal initial body burden ($BB(0) \sim 0$), as follows:

$$12 \quad d_H = d_A \frac{t_{1/2A}}{t_{1/2H}} \frac{\left[1 - \frac{(1 - e^{-k_A t_A})}{k_A t_A} \right]}{\left[1 - \frac{t_{H0}}{t_H} - \frac{(e^{-k_H t_{H0}} - e^{-k_H t_H})}{k_H t_H} \right]} \quad (\text{Eq. 3-18})$$

14
 15 where t_{H0} is the initial human exposure time.

16 The value of t_A is the duration of the experimental exposure period. For some gestational
 17 exposures, if a critical exposure window is defined, t_A will be the duration of the critical
 18 exposure window. The value of t_H is the human-equivalent duration corresponding to t_A .
 19 However, for t_A less than lifetime (less than 2 years in rodents) and no defined susceptible life
 20 stage, t_H cannot begin at 0 (because typically animal experiments do not begin at age 0), but must
 21 end at 25,550 days (70 years) to include the terminal (pseudo) steady-state level, at which the
 22 $BB_H(t): d_H$ ratio is highest. Otherwise, starting t_H at 0 would not be protective for less-than-
 23 lifetime effects that could be manifest at any age in humans; the average is determined from the
 24 terminal end of the human exposure period because the daily exposure achieving the target blood
 25 concentration is smaller than for the same exposure period beginning at birth (i.e., d_H would be

¹⁴No conversions to human-equivalent exposures were attempted for other effects in the 2003 Reassessment.
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1 higher for earlier exposure periods) and is health protective for effects occurring after
2 shorter-term exposure.¹⁵ Figure 3-7 depicts the relationship of daily dose to TWA body burden
3 graphically for several exposure duration scenarios. For shorter durations occurring later in life,
4 the average body burden over the exposure period does not differ substantially from the
5 steady-state value. Even for half-lifetime exposures, the deviation of the average from steady
6 state is minimal. Only for lifetime exposures does the difference become more marked, but only
7 by about 15%. Note that in the 2003 Reassessment, a constant value of 3,000 was used for
8 $BB_H(t):d_H$, based on the relationship of continuous exposure to theoretical steady-state body
9 burden ($t = \text{lifetime}$, $t_{1/2} = 2,593$ days); this approach, while conservative, does not account for
10 exposure scenarios of different durations and does not strictly reflect the average body burden
11 dose metric.

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

21 Application of first order kinetics for the risk assessment of TCDD can only be used to
22 estimate total body burdens or back-calculate administered dose from experimental data. Body
23 burden calculations using first order kinetics is based on the assumption of a first order decrease
24 in the levels of administered dose as function of time. In that sense, any loss of TCDD from the
25 body is described by using a rate constant that is not specific to any biological process. This
26 constant is usually estimated from estimates of half-life of TCDD. Assuming a constant half-life
27 value for the clearance for long-term or chronic TCDD exposure is not biologically supported
28 given the observed data indicating early influence of CYP1A2 induction and binding to TCDD
29 and later redistribution of TCDD to fat tissue. Abraham et al. (1988, [199510](#)) found that the
30 liver:adipose tissue concentration ratio in female Wistar rats exposed to a subcutaneous TCDD

¹⁵See the following section (3.3.4.3) for a more detailed discussion of this concept.

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1 dose of 300 ng/kg decreased from 10.3 at 1 day postexposure to 0.5 at 91 days postexposure.
2 Consequently, using half-life estimates based on observed steady-state levels of TCDD will not
3 account for the possibility of accelerated dose-dependent clearance of the chemical at the early
4 stages and thus would result in estimation of lower administered levels of the chemical. The
5 dynamic change in half-life due to dose-dependent elimination at the early stages of TCDD
6 exposure and its later redistribution to fat tissues for steady-state levels is better described using
7 biologically-based models, such as the PBPK models and concentration- and age-dependent
8 elimination (CADM) models (Aylward et al., 2005, [197014](#); Carrier et al., 1995, [197618](#); Carrier
9 et al., 1995, [543780](#); Emond et al., 2004, [197315](#); Emond et al., 2005, [197317](#); Emond et al.,
10 2006, [197316](#)). Additionally, these models provide estimates for other dose metrics (e.g., serum
11 or tissue levels) that are more biologically relevant to response than administered dose or total
12 body burden (see Section 3.3.4.3).

13

14 **3.3.4.3. *Biologically-Based Kinetic Models***

15 The development and evolution of biologically-based kinetic models for TCDD have
16 been reviewed by EPA (2003, [537122](#)) and Reddy et al. (2005, [594251](#)). The initial PBPK
17 model of Leung et al. (1988, [198815](#)) was developed with the consideration of TCDD binding to
18 CYP1A2 in the liver. The next level of PBPK models by Andersen et al. (1993, [196991](#)) and
19 Wang et al. (1997, [104657](#)) used diffusion-limited uptake and described protein induction by
20 interaction of DNA binding sites. The models of Kohn et al. (1993, [198601](#)) and Andersen et al.
21 (1997, [197172](#)) further incorporated extensive hepatic biochemistry and described zonal
22 induction of CYP by TCDD. TCDD PBPK models have evolved to include detailed descriptions
23 of gastrointestinal uptake, lipoprotein transport, and mobilization of fat, as well as biochemical
24 interactions of relevance to organ-level effects (Kohn et al., 1996, [022626](#); Roth et al., 1994,
25 [198063](#)). Subsequently, developed PBPK models either used constant hepatic clearance rate
26 (Maruyama et al., 2002, [198448](#); Wang et al., 1997, [104657](#); Wang et al., 2000, [198738](#)) or
27 implemented varying elimination rates as an empirical function of body composition or dose
28 (Andersen et al., 1993, [196991](#); Andersen et al., 1997, [197172](#); Kohn et al., 1996, [022626](#);
29 Van der Molen et al., 1998, [548765](#); Van der Molen et al., 2000, [548777](#)). The more recent
30 pharmacokinetic models explicitly characterize the concentration-dependent elimination of
31 TCDD (Aylward et al., 2005, [197014](#); Carrier et al., 1995, [197618](#); Carrier et al., 1995, [543780](#);

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1 Emond et al., 2004, [197315](#); Emond et al., 2005, [197317](#); Emond et al., 2006, [197316](#)). The
2 biologically-based pharmacokinetic models describing the concentration-dependent elimination
3 (i.e., the pharmacokinetic models of Aylward et al. (2005, [197014](#)) and Emond et al. (2005,
4 [197317](#); 2006, [197316](#)) are relevant for application to simulate the TCDD dose metrics in
5 humans and animals exposed via the oral route. The rationale for considering the application of
6 Aylward et al. (2005, [197014](#)) and Emond et al. (2004, [197315](#); 2005, [197317](#); 2006, [197316](#))
7 models for estimating dose metrics for possible application to TCDD risk assessment is based on
8 the following considerations.

- 10 • Both models represent research results from the more recent peer-reviewed publications.
- 11 • Both models are relatively simple and less parameterized than earlier kinetic models for
12 TCDD. The Aylward et al. (2005, [197014](#)) model is based on two-time scale TCDD
13 kinetics described by Carrier et al. (1995, [197618](#)), and the Emond et al. (2004, [197315](#);
14 2005, [197317](#); 2006, [197316](#)) PBPK models are reduced versions of earlier complex
15 PBPK models. Although simple, both the Aylward et al. (2005, [197014](#)) and Emond et
16 al. (2004, [197315](#); 2005, [197317](#); 2006, [197316](#)) models are still inclusive of important
17 kinetic determinants of TCDD disposition.
- 18 • Both models are uniquely formulated with dose-dependent hepatic elimination consistent
19 with the physiological interpretations commonly accepted by the scientific community.
- 20 • Both models and extrapolated human versions were tested against human data collected
21 in a variety of human exposure scenarios (Aylward et al., 2005, [197014](#); Emond et al.,
22 2005, [197317](#)).
- 23 • Both models are capable of deriving one or more of the candidate dose-metrics that are of
24 interest to EPA’s dose-response assessment of TCDD.

26 **3.3.4.3.1. CADM model.**

27 **3.3.4.3.1.1. Model structure.**

28 The pharmacokinetic model of Aylward et al. (2005, [197014](#)), referred to as the CADM
29 model in this report, is based on an earlier model developed by Carrier et al. (1995, [197618](#);
30 1995, [543780](#)) that describes the dose-dependent elimination and half-lives of polychlorinated
31 dibenzo-*p*-dioxins and furans. This model describes the TCDD levels in blood (body), liver, and
32 adipose tissue. Blood itself is not characterized physically as a separate compartment within the
33 model, and the distribution of TCDD to tissues other than adipose tissue and liver (usually less
34 than 4%) is not accounted for by the model. The original structure of the Carrier et al. (1995,

1 [197618](#); 1995, [543780](#)) model was modified by Aylward et al. (2005, [197014](#)) to include TCDD
2 elimination through partitioning from circulating lipids across the lumen of the large intestine
3 into the fecal content (see Figure 3-8). The most recent version of the Carrier model (Aylward et
4 al., 2005, [197014](#); 2008, [197068](#)) includes fecal excretion of TCDD from two routes:
5 (1) elimination from circulating blood lipid through partitioning into the intestinal lumen; and
6 (2) elimination of unabsorbed TCDD from dietary intake.

7 A basic assumption of this model is that metabolic elimination of TCDD is a function of
8 its current concentration in the liver. The current concentration of TCDD in the liver increases
9 with increasing body burden in a nonlinear fashion as a result of the induction of (and binding of
10 TCDD to) specific proteins (i.e., CYP1A2). Consequently, the fraction of TCDD body burden
11 contained in the liver increases nonlinearly (with a corresponding decrease in the fraction
12 contained in adipose tissues) with increasing body burden of TCDD (Aylward et al., 2005,
13 [197114](#); Carrier et al., 1995, [197618](#)).

14 Of particular note is that the adipose tissue compartment of the model is considered to
15 represent the lipid contained throughout the body. It then assumes that the concentrations of
16 TCDD in lipids of plasma and various organs is essentially equivalent to that of adipose tissue,
17 and as such these concentrations are included in the adipose compartment of the model. Even
18 though this approximation is fairly reasonable given the available data, there is some concern
19 that the adipose compartment of this model also includes the lipid content of the liver to some
20 unknown extent. Removal of lipid volume from the liver would mathematically alter total
21 hepatic concentration and therefore would affect the estimated levels of the chemical available
22 for binding to proteins.

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

1 **3.3.4.3.1.2. Mathematical representation.**

2 The CADM model describes the distribution to tissues (including liver and adipose
3 tissue) based on exchange from blood at time intervals of one month. The model is based on
4 quasi-steady-state-approximation, and thus it is also based on the consideration that the
5 intertissue processes reach their equilibrium values “quasi-instantaneously.” In this regard,
6 absorption and internal distribution reflective of kinetics at the cellular level (e.g., diffusion,
7 receptor binding, and enzyme induction) likely occur on a relatively fast time scale (a few hours
8 to a few days). However, the overall body concentration (i.e., body burden) varies slowly with
9 time such that it remains virtually unchanged during short time intervals.

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

19
20 **3.3.4.3.1.3. Parameter estimation.**

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

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

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1 **3.3.4.3.1.4. Model performance and degree of evaluation.**

2 The CADM model was not evaluated for its capabilities in predicting data sets not used
3 in its parameterization. In other words, one or more of the key input parameters (f_{hmin} , f_{hmax} , k_e ,
4 K) was or were obtained essentially by fitting to the species-specific pharmacokinetic data, such
5 that there was no “external” validation data set to which the model was applied. Despite the lack
6 of emphasis on the “external” validation aspect, the authors (Aylward et al., 2005, [197114](#));
7 (Carrier et al., 1995, [197618](#); Carrier et al., 1995, [543780](#)) have demonstrated the ability of the
8 model to describe multiple data sets covering a range of doses and species.

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

13 The pharmacokinetic data sets for TCDD that were used to calibrate/evaluate the CADM
14 model by Aylward et al. (2005, [197114](#); Carrier et al., 1995, [197618](#); Carrier et al., 1995,
15 [543780](#)) included the following:

- 16
- 17 • Adipose tissue and liver concentrations of TCDD following a single oral dose of 1 $\mu\text{g}/\text{kg}$
18 in monkeys (McNulty et al., 1982, [543782](#));
 - 19 • Percent dose retained in liver for a total dose of 14 ng in hamsters (Van den Berg et al.,
20 1986, [543781](#));
 - 21 • Elimination kinetics of TCDD in female Wistar rats following a single subcutaneous dose
22 of 300 ng/kg (data from Abraham et al., 1988, [199510](#));
 - 23 • Liver and adipose tissue concentrations (terminal measurements) in Sprague–Dawley rats
24 given 1, 10 or 100 ng TCDD/kg bw during 2 years (Kociba et al., 1978, [001818](#)); and
 - 25 • Serum lipid concentrations of TCDD over a period of several years in 54 adults (29 men
26 and 25 women) from Seveso and in three Austrian patients (Aylward et al., 2005,
27 [197114](#)).
- 28

29 For illustration purposes, Figure 3-9 shows model simulations of rat data from Carrier et
30 al. (1995, [197618](#)). Figure 3-2 (see Section 3.3.2.4) depicts the human data that were used by the
31 authors to support the concentration-dependent elimination concept; the model was
32 parameterized to fit approximately to these data (Aylward et al., 2005, [197114](#)).

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

3
4 **3.3.4.3.1.5. Confidence in CADM model predictions of dose metrics.**

5 A qualitative level of confidence associated with the predictability and reliability of
6 absorbed dose and body burden for oral exposures in humans (as well as several animal species)
7 by this model can be ranked as high (see Table 3-6). This model, however, does not account for
8 the differential solubility of TCDD in serum lipids and adipose tissue lipids, nor does it account
9 for the diffusion-limited uptake by adipose tissue. Due to these limitations, the confidence
10 associated with the predictions of the serum lipid concentration of TCDD is considered medium,
11 particularly when it is not documented that steady-state is reached during the critical toxicologic
12 studies and human exposures. Furthermore, the CADM model does not facilitate the
13 computation of TCDD concentrations in specific internal organs (other than liver and adipose
14 tissue). The reliability of this model for simulating the liver concentration (free, bound, or total)
15 of TCDD at low doses is considered to be low. This low confidence level is a result of the
16 uncertainty associated with the key parameter f_{hmin} . This parameter needs to be re-calibrated for
17 each study/species/population to effectively represent the free fraction of TCDD in liver and the
18 amount of TCDD contained in the hepatic lipids and bound to the liver proteins (whose levels
19 might be reflective of background exposures of various sources; see Carrier et al., 1995,
20 [197618](#)). The uncertainty related to the numerical value of this parameter in animals and
21 humans—particularly at very low exposures—raises concern regarding the use of this model to
22 predict TCDD concentration (free, bound, or total) in liver as the dose metric for dose-response
23 modeling. Although the use of the parameter f_{hmax} permits the prediction of the dose to liver at
24 high doses, it does not specifically facilitate the simulation of the amount bound to the protein or
25 level of induction in liver. Because the CADM model is not capable of simulating enzyme
26 induction based on biologically-relevant parameters, its reliability for predicting the
27 concentration of TCDD bound specifically to the AhR is not known. Finally, due to the lack of
28 parameterization or verification with kinetic data in pregnant, lactating, or developing animals or
29 humans, the CADM model is unlikely to be reliable in the current form for use in *predicting*
30 potential dose metrics in these subpopulations or study groups that might form the basis of points
31 of departure (PODs) for the assessment.

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1 **3.3.4.3.2. PBPK model.**

2 **3.3.4.3.2.1. Model structure.**

3 Emond et al. (2004, [197315](#); 2006, [197316](#)) simplified the eight-compartment rat model
4 of Wang et al. (1997, [104657](#)) to a four-compartmental model (liver, fat, rest of body and
5 placenta with fetal transfer) (Emond et al., 2004, [197315](#)), and later to a three-compartment adult
6 model (liver, fat, rest of the body) (Emond et al., 2006, [197316](#)) (see Figures 3-10 and 3-11).
7 Their rationale for simplification of the model was based on evaluating, critiquing, and
8 improving all earlier PBPK models by Wang et al. (1997, [104657](#)). In general, the main reason
9 for the simplification was that extrapolation of a PBPK model to humans with these many (i.e.,
10 eight compartments) compartments would be problematic due to the limited availability of
11 relevant human data for validation (Emond et al., 2004, [197315](#)). One major difference from
12 earlier models, repeatedly emphasized by Emond et al. (2005, [197317](#); 2006, [197316](#)), was their
13 description (included in their simplified PBPK models) of the dose-dependent, inducible
14 elimination of TCDD. The rationale for including TCDD binding and induction of CYP1A2 into
15 the model was earlier described by Santostefano et al. (1998, [200001](#)).

16 The most recent version of the rat and human PBPK models developed by Emond et al.
17 (2006, [197316](#)) describes the organism as a set of three compartments corresponding to real
18 physical locations—liver, fat, and rest of the body—interconnected by systemic circulation (see
19 Figure 3-10). The liver compartment includes descriptions of CYP1A2 induction, which is
20 critical for simulating TCDD sequestration in liver and dose-dependent elimination of TCDD. In
21 this model, the oral absorption of TCDD from the GI tract accounts for both the lymphatic (70%)
22 and portal (30%) systems.

23 The biological relationship between TCDD “sequestration” by liver protein and its
24 “elimination” by the liver is not entirely clear. TCDD is metabolized slowly by unidentified
25 enzymes. CYP1A2 is known to metabolize TCDD based on studies in CYP1A2 KO mice
26 (Diliberto et al., 1997, [548755](#); 1999, [143713](#)), in which the metabolic profile is different
27 compared to wild-type mice. However, since several metabolites appear in the feces of CYP1A2
28 knock out mice, it is assumed that there are other enzymes involved in TCDD metabolism.
29 TCDD binds to the AhR and induces not only CYP1A2, but also CYP1A1, CYP1B1, and several
30 UGTs and transporters (Gasiewicz et al., 2008, [473406](#)). Both hydroxylated and glucuronidated
31 hydroxyl metabolites are found in the feces of animals treated with TCDD (Hakk et al., 2009,

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1 [594256](#)). Because the exact enzymes involved with TCDD are unknown and yet the metabolism
2 is induced by TCDD, an assumption of increased the elimination rate of TCDD in proportion to
3 the induction of CYP1A2 is made. In the PBPK model, CYP1A2 is needed because TCDD
4 binds to rat, mouse, and human CYP1A2 (Diliberto et al., 1999, [143713](#); Staskal et al., 2005,
5 [198276](#)). Thus CYP1A2 induction is necessary to describe TCDD pharmacokinetics due to
6 TCDD binding. Hence, CYP1A2 can be used as a marker of Ah-receptor induction of “TCDD
7 metabolizing enzymes.” Other models use AhR occupancy as a marker of induction of “TCDD
8 metabolizing enzymes” (Andersen et al., 1997, [197172](#); Kohn et al., 2001, [198767](#)).

9 Figure 3-11 depicts the structure of the rat developmental-exposure PBPK model (Emond
10 et al., 2004, [197315](#)). This model was developed to describe the relationship between maternal
11 TCDD exposure and fetal TCDD concentration during critical windows of susceptibility in the
12 rat. In formulating this PBPK model, Emond et al. (2004, [197315](#)) reduced the original
13 8-compartment model for TCDD in adult rats by Wang et al. (1997, [104657](#)) to a 4-compartment
14 (i.e., liver, fat, placenta, and rest of the body) model for maternal rat. Activation of the placental
15 compartment and a separate fetal compartment occurs during gestation (Emond et al., 2004,
16 [197315](#)).

17

18 **3.3.4.3.2.2. Mathematical representation.**

19 The key equations of the PBPK model of Emond et al. (2004, [197315](#)) are reproduced in
20 Text Boxes 3-1 and 3-2, whereas those from Emond et al. (2005, [197317](#); 2006, [197316](#)) are
21 listed in Table 3-7. The rate of change of TCDD in the various tissue compartments is modeled
22 on the basis of diffusion limitation considerations. Accordingly, mass balance equations are
23 used to compute the rate of change in the tissue (i.e., intracellular compartment) and tissue blood
24 (i.e., extracellular compartment). The membrane transfer of TCDD is computed using a
25 permeation coefficient-surface area cross product (PA) for each tissue. Metabolism and binding
26 of TCDD to the AhR and inducible hepatic protein (CYP1A2) are described in the liver. The
27 total mass in the liver was then apportioned between free dioxin (C_{lf}) and bound forms of TCDD
28 (see Figure 3-12). The dose- and time-dependent induction of hepatic CYP1A2 in the liver is
29 described per Wang et al. (1997, [104657](#)) and Santostefano et al. (1998, [200001](#)). Accordingly,
30 the amount of CYP1A2 in the liver was computed as the time-integrated product of inducible
31 production and a simple first-order loss process (Wang et al., 1997, [104657](#)):

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$$\frac{dCYP_{1A2}}{dt} = S(t)K_0 - K_2C_{A2t} \quad (\text{Eq. 3-19})$$

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):

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

where, $S(t)$ is the stimulation function, In_{A2} is the maximum fold of CYP1A2 synthesis rate over the basal rate, $C_{Ah-TCDD}$ 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:

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

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. Furthermore, the numerator in Eq. 3-21 should more appropriately be $CYP1A2_{induced}$ rather than $[CYP1A2_{induced} - CYP1A2_{Basal}]$ to avoid the problem of lower levels of induction at low doses resulting in a lower

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1 than basal rate of synthesis of *CYP1A2*. The above equation, however, does not describe
 2 changes in elimination rate in direct proportionality with the *CYP1A2* levels; also, the *K_{el}* value
 3 by itself does not reflect a scalable basal metabolic rate. Rather, these two terms collectively
 4 describe the outcome related to the TCDD elimination processes, based on fitting to observations
 5 in rats (Santostefano et al., 1998, [200001](#)). The impact of *CYP1A2* induction and sequestration
 6 on binding and elimination of TCDD is simulated using the Emond et al. (2004, [197315](#)) model.

7 The gestational model consisted of a fetal compartment, and the transfer of TCDD
 8 between the placental and fetal compartments was described as a diffusion-limited (rather than a
 9 perfusion-limited) process (see Text Boxes 3-1 and 3-2).¹⁶

10

Text Box 3-1.

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

Cardiac Output: $Q_c(mL/h) = Q_{cc} \times 60 \left(\frac{BW_{mother}}{1,000} \right)^{0.75}$

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:

$C_b(nmol/mL) =$

$$\frac{((Q_f \times C_{fb}) + (Q_{re} \times C_{reb}) + (Q_{li} \times C_{lib}) + (Q_{pla} \times C_{plab}) + Lymph) - (C_b \times Cl_{ru})}{Q_c}$$

11

¹⁶Diffusion 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.

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Text Box 3-2.**Placenta Tissue Compartment**

(a) Tissue-blood subcompartment

$$\frac{dA_{plab}}{dt} (\text{nmol} / \text{h}) = Q_{pla}(C_a - C_{plab}) + PA_{pla}(C_{plab} - C_{plafree})$$

$$C_{plab} = \frac{A_{plab}}{W_{plab}}$$

(b) Tissue cellular matrices

$$\frac{dA_{pla}}{dt} (\text{nmol} / \text{h}) = PA_{pla}(C_{plab} - C_{plafree}) - \frac{dA_{pla_fet}}{dt} + \frac{dA_{fet_pla}}{dt}$$

$$C_{pla}(\text{nmol} / \text{mL}) = \frac{A_{pla}}{W_{pla}}$$

Free TCDD Concentration in Placenta

$$C_{plafree}(\text{nmol} / \text{mL}) = C_{pla} - \left[(C_{plafree} \times P_{pla} + \left(\frac{Plab_{max} \times C_{plafree}}{K_{dpla} + C_{plafree}} \right)) \right]$$

Dioxin Transfer from Placenta to Fetuses

$$\frac{dA_{pla_fet}}{dt} (\text{nmol} / \text{h}) = Cl_{pla_fet} \times C_{pla}$$

Dioxin Transfer from Fetuses to Placenta

$$\frac{dA_{fet_pla}}{dt} (\text{nmol} / \text{h}) = Cl_{pla_fet} \times C_{fet}V$$

Fetal Dioxin Concentration (Fetuses 5 = Per Litter)

$$\frac{dA_{fet}}{dt} (\text{nmol} / \text{h}) = \frac{dA_{pla_fet}}{dt} - \frac{dA_{fet_pla}}{dt}$$

$$C_{fet}(\text{nmol} / \text{h}) = \frac{A_{fet}}{W_{fet}}$$

$$C_{fet}V(\text{nmol} / \text{mL}) = \frac{C_{fet}}{P_{fet}}$$

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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. (2005, [197317](#); 2006, [197316](#)). 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, [104657](#)) except that the value of affinity constant for CYP1A2 was changed from 0.03 to 0.04 nmol/mL to get better fit to experimental data (Emond et al., 2004, [197315](#)) and the variable elimination parameter (*K_{el}v*) was obtained by optimization of model fit to kinetic data from Santostefano et al. (1998, [200001](#)) and (Emond et al., 2005, [197317](#); Emond et al., 2006, [197316](#); Wang et al., 1997, [104657](#)). Wang et al. (1997, [104657](#)) used measured tissue weights whereas the tissue blood flows and tissue blood weights were obtained from International Life Sciences Institute (ILSI, 1994, [046436](#)). The partition coefficients (which were similar to those of Leung et al., 1988, [198815](#); 1990, [192833](#)), the permeability x 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, [104657](#)). 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, [104657](#)). The receptor concentrations and dissociation constant of TCDD bound to AhR were obtained by fitting the model to TCDD tissue concentration combining with enzyme data reported by Santostefano et al. (1998, [200001](#)) whereas the basal CYP1A2 in liver was based on literature data (Wang et al., 1997, [104657](#)).

The parameters for the human PBPK model were primarily based on the rat model (Emond et al., 2005, [197317](#); Emond et al., 2006, [197316](#); Wang et al., 1997, [104657](#)). 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 *K_{el}v* was estimated by fitting to human data (Emond et al., 2005, [197317](#)).

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

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1 based on existing data. Exponential equations for the growing compartments were used (see
2 Figure 3-13), except for adipose tissue for which a linear increment based on literature data was
3 specified. While physiological parameters for the pregnant rat were obtained from the literature,
4 all other input parameters were set equal to that of nonpregnant rat (obtained from Wang et al.,
5 1997, [104657](#)), see Tables 3-7 and 3-8. The current version of the rat gestational model contains
6 parameters for variable elimination from Emond et al. (2006, [197316](#); Table 3-8), and still
7 provides essentially the same predictions as the original publication (Emond et al., 2004,
8 [197315](#)).

10 **3.3.4.3.2.4. Model performance and degree of evaluation.**

11 The PBPK model of Emond et al. (2004, [197315](#); 2005, [197317](#); 2006, [197316](#)) had
12 parameters estimated by fitting to kinetic data, such that the resulting model consistently
13 reproduced the kinetic data. The same model structure with a single set of species-specific
14 parameters could reproduce the kinetics of TCDD following various doses and exposure
15 scenarios not only in the rat but also in humans. The simulations of the PBPK model of Emond
16 et al. (2006, [197316](#)) have been compared with two sets of previously published rat data: blood
17 pharmacokinetics following a single dose of 10 µg/kg (the dose corresponding to the mean
18 effective dose for induction of CYP1A2) (Santostefano et al., 1998, [200001](#)) (see Figure 3-14);
19 and hepatic TCDD concentrations during chronic exposure to 50, 100, 500, or 1,750 ng/kg
20 (Walker et al., 1999, [198615](#)) (see Figure 3-15). It is relevant to note that the PBPK model of
21 Emond et al. (2004, [197315](#); 2006, [197316](#)) is essentially a reduced version of the Wang et al.
22 (1997, [104657](#)) model, and it therefore provides simulations of liver and fat concentrations of
23 TCDD that deviated by not more than 10–15% of those of Wang et al. (1997, [104657](#)). The
24 nongestational model of Emond et al. (2004, [197315](#)) simulated the kinetic data in liver, fat,
25 blood and rest of body of female Sprague-Dawley rats given a single dose of 10 µg TCDD/kg
26 (data from Santostefano et al., 1996, [594258](#)) and in liver and fat of male Wistar rats treated with
27 a loading dose of 25 ng/kg followed by a weekly maintenance dose of 5 ng TCDD/kg by gavage
28 (data from Krowke et al., 1989, [198808](#)).

29 The gestational rat PBPK model simulated the following PK data sets (Emond et al.,
30 2004, [197315](#)):

- 1 • TCDD concentration in blood, fat, liver, placenta, and fetus of female Long–Evans rats
2 given 1, 10, or 30 ng/kg, 5 days/week, for 13 weeks prior to mating followed by daily
3 exposure through parturition (Hurst et al., 2000, [198806](#));
- 4 • TCDD concentration in tissues (liver, fat), blood, placenta and fetus determined on
5 gestation day (GD) 16 and GD 21 following a single dose of 0.05, 0.8, or 1 µg/kg given
6 on GD 15 to pregnant Long Evans rat (Hurst et al., 2000, [199045](#));
- 7 • Maternal and fetal tissue concentrations on GD 9, GD 16 and GD 21 after a single dose
8 of 1.15 µg TCDD/kg given to Long–Evans rats on GD 9 or GD 15 (Hurst et al., 1998,
9 [134516](#)); and
- 10 • Fetal TCDD concentrations determined on GD 19 and GD 21 in rats exposed to
11 5.6 µg TCDD/kg on GD 18 (Li et al., 2006, [199059](#)).

12
13 Furthermore, the scaled rat model was shown to be capable of simulating human data
14 from the Austrian and Seveso subjects (see Figures 3-16 and 3-17). In this regard, it is useful to
15 note that the computational version of the PBPK model of Emond et al. (2005, [197317](#); 2006,
16 [197316](#)) also contained the necessary equation to transform the model output of blood
17 concentration into serum lipid adjusted concentration of TCDD.

18 The human model of Emond et al. (2005, [197317](#); Emond model) has advantages for
19 improving the TCDD dosimetry used in existing human epidemiological studies because the
20 model predicts the redistribution of TCDD within the body (to stores in fat and liver) based on
21 physiological principles. However, because the dose-dependency of metabolic elimination in the
22 Emond model was not calibrated to human data, it is important to review the predictions of this
23 model using a database of human observations that is as extensive as possible and a spread of
24 internal TCDD concentrations that is as wide as possible. Thus, presented below is a
25 juxtaposition of modeled elimination rates from the Emond model with observations for
26 two highly exposed Austrian patients (severe intoxication of “unknown origin” (Geusau et al.,
27 2001, [197444](#))) and nine of 10 Ranch Hand veterans¹⁷ used for the original “validation”
28 comparisons presented in the Emond et al. (2005, [197317](#)).

29 Figure 3-18 shows the time course of the declines in TCDD serum concentrations in
30 two highly-exposed Austrian subjects compared with the Emond model results. The comparison
31 in Figures 3-17 and 3-18 indicates that the Emond model adequately describes the rate of TCDD

¹⁷In preliminary comparisons, the simulation run for the 10th Ranch Hand veteran appeared anomalous and was therefore excluded from this summary.

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1 elimination for the more highly exposed Austrian patients, but predicts a somewhat faster rate of
2 decline than that observed for the less heavily exposed patient.

3 Figure 3-19 shows the results of combining the simulated and observed rates of loss for a
4 group of Austrian and Ranch Hand subjects evaluated by Emond et al. (2005, [197317](#)), counting
5 only one data point per person. The X-axis in this figure is the TCDD serum concentration at the
6 midpoint of the observations for each subject. The error bars in the figure represent ± 1 standard
7 error. The results of this figure illustrate two points: (1) the Emond model simulation (open
8 squares) are generally very close to the actual data (solid circles) for the nine Ranch hands
9 (clustered toward lower left corner) and one of the the two Austrian patients (upper right corner);
10 and (2) both the Emond model simulation results and the actual data show a linear trend and
11 linear regression lines were plotted, respectively, as shown in Figure 3-19.

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

19 Overall, the conclusion from the above analysis is that the Emond model is reasonable to
20 use, but the model might be improved by (1) include the two nondose-dependent pathways of
21 elimination documented in the Geusau papers (GI elimination via the feces and loss via the
22 sloughing of skin cells), and (2) reducing the extent of loss via the dose-dependent metabolism
23 pathway from the liver (Geusau et al., 2002, [594259](#); Harrad et al., 2003, [197324](#)) so that overall
24 loss rates for the average elimination rates from the Ranch Hand veterans is maintained.

25 A sensitivity analysis of inputs used to estimate inducible elimination rate for a single
26 oral dose of 0.001 to 10 $\mu\text{g}/\text{kg}$ in the rat indicated that the number of key parameters ranged from
27 seven at the low dose region to 12 at the high dose (see Figure 3-20)(Emond et al., 2006,
28 [197316](#)). The sensitive parameters identified included the oral absorption parameters (KABS),
29 volumes of liver and adipose tissue (WLIO, WFO), adipose tissue:blood partition coefficient
30 (PF), and the basal CYP1A2 level (CYP1A2 1A2). At high doses, the most sensitive parameters

1 also included those related to the maximal induction of CYP1A2 and AhR binding capacity (see
2 Figure 3-20) (Emond et al., 2006, [197316](#)).

3 The gestational rat model described in Emond et al. (2004, [197315](#)), upon
4 reparameterization, could simulate the kinetics of TCDD in mice. The initial changes to the rat
5 model parameters included: rest of the body:blood partition coefficient (PRE), basal
6 concentration (CYP1A2_1A2), delay in induction time (CYP1A2_1TAU) and adipose tissue
7 permeability coefficient (PAFF), in accordance with Wang et al. (2000, [198738](#)) (see Table 3-8).
8 Subsequently, four parameters (adipose tissue:blood partition coefficient, CYP1A2 affinity
9 parameter, GI tract elimination transit constant (hour^{-1}) and the interspecies metabolic parameter
10 *Kelv* (hour^{-1}) were re-estimated based on visually fit of model simulations to the PK data from
11 Diliberto et al. (2001, [197238](#)), following an oral dose 150 ng TCDD/kg/day, 5 days/week for
12 17 weeks (see Table 3-7). The resulting mouse model is capable of reproducing the kinetics of
13 TCDD in the adult (see Figures 3-21 through 3-27), as well as, to a very limited extent, the
14 kinetics during gestation (see Figure 3-28).

15

16 **3.3.4.3.2.5. Confidence in PBPK model predictions of dose metrics.**

17 The PBPK model facilitates prediction of absorbed dose, body burden, and blood
18 concentration of TCDD for oral exposures in adult humans and rats (adult and developing) with
19 high confidence (see Table 3-10). The model output of blood concentration can be normalized to
20 lipid content representative of the study group (species, sex, age, lifestage, and diet). However,
21 the PBPK model of Emond et al. (2004, [197315](#); 2005, [197317](#); 2006, [197316](#)) does not simulate
22 plasma and erythrocyte TCDD concentrations separately, and it predicts tissue concentrations on
23 the basis of tissue:whole blood partition coefficients and not on the basis of serum
24 lipid-normalized values.

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

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1 cannot be directly obtained in the system (e.g., bound concentrations in the liver of exposed
2 humans). For these dose measures (i.e., bound concentration and total liver concentration), the
3 level of confidence can be further improved or diminished by the outcome of sensitivity analysis.
4 In this regard, the results of a focused sensitivity analysis indicate that the most sensitive
5 parameters of the human model are among the most uncertain (i.e., those parameters for which
6 estimates were not obtained in humans) with respect to prediction of liver TCDD concentration,
7 contrary to the animal model (see Section 3.3.6).

8 With respect to the mouse model, however, the level of confidence is low to medium,
9 given that it has not been verified extensively with blood, body burden, or tissue concentration
10 time-course or dose-response data. However, the mouse PBPK model, based on the rat model
11 that has been evaluated with several PK data sets, has been shown to reproduce well the limited
12 mouse liver kinetic data (see Figures 3-21 through 3-28; Boverhoff et al., 2005, [594260](#)). The
13 same model structure has been used for simulating kinetics of TCDD in humans successfully.
14 Overall, the adult mouse model, given its biological basis combined with its ability to simulate
15 TCDD kinetics in multiple species, is considered to exhibit a medium level of confidence for
16 simulating dose metrics for use in high to low dose extrapolation and interspecies (mouse to
17 human) extrapolation. Even though similar considerations are applicable to gestational model in
18 mice, the confidence level is considered to be low since very limited comparison with empirical
19 data has been conducted (see Figure 3-28). Despite the uncertainty in these predictions, the
20 scaled rat gestational model, given its biological and mechanistic basis, might be of use in
21 predicting dose metrics in these groups that might form the basis of PODs in certain key studies.

23 **3.3.4.4. *Applicability of PK Models to Derive Dose Metrics for Dose-Response Modeling of*** 24 ***TCDD: Confidence and Limitations***

25 Both the CADM and PBPK models describe the kinetics of TCDD following oral
26 exposure to adult animals and humans by accounting for the key processes affecting kinetics,
27 including hepatic sequestration phenomena, induction, and nonlinearity in elimination, and
28 distribution in adipose tissue and liver. Both models can be used for estimating body burdens
29 and serum lipid adjusted concentrations of TCDD. However, there are several differences
30 between these two models. The PBPK model calculates the free and bound concentrations of
31 TCDD in the intracellular subcompartment of tissues. The total or receptor-bound

1 concentrations in liver are unambiguous and more easily interpretable with the PBPK model than
2 with the CADM model. In addition, the PBPK model computes bound and total concentrations
3 as a function of the free concentration in the intracellular compartment of the tissue. By contrast,
4 the CADM model simulates the total concentration based on empirical consideration of hepatic
5 processes. Consequently, the amount of TCDD bound to AhR or CYP1A2 cannot be simulated
6 with the CADM model. The CADM model computes only the total TCDD concentration in
7 liver, and describes TCDD elimination through partitioning from circulating lipids across the
8 lumen of the large intestine into the feces, while the PBPK model accounts for this process
9 empirically within its hepatic elimination constant. Elimination of TCDD via skin, a minor
10 process, is not described by either model. Thus, dose-response modeling based on body burden
11 of TCDD in adult animals and humans can be conducted with either of the models, provided the
12 duration of the experiment is at least one month, due to limitations in the CADM model. As
13 shown in Figure 3-29, the predicted slope and body burden over a large dose range are quite
14 comparable (generally within a factor of two).

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

24 The CADM model is simple and based on fewer parameters than the PBPK model.
25 Because the CADM model is constructed by fitting to data, its performance is likely to be
26 reliable for the range of exposure doses, species, and life stages from which the parameter
27 estimates were obtained. On the other hand, the PBPK model structure and parameters are
28 biologically-based and can be adopted for each species and life stage. Accordingly, the PBPK
29 model has been adopted to simulate the kinetics of TCDD in the fetus and in pregnant rats, as
30 well as in adult humans and rats (Emond et al., 2004, [197315](#); Emond et al., 2005, [197317](#);
31 Emond et al., 2006, [197316](#)). The time step for calculation and dosing in the CADM model

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1 corresponds to 1 month. This requirement represents a constraint in terms of the use of this
2 model to simulate a variety of dosing protocols used in animal toxicity studies. This
3 requirement, however, is not a constraint with the PBPK models. So, simulating the body
4 burden and serum lipid concentrations for a longer duration of exposure, either model would
5 appear to be useful; but the PBPK model would be the tool of choice for simulating alternative
6 dose metrics of TCDD (e.g., blood concentration, total tissue concentration, bound
7 concentration) for various exposure scenarios (including single dose studies), routes and life
8 stages in the species of relevance, to TCDD dose-response assessment, particularly, mice, rats,
9 and humans.

10 Two minor modifications, to enhance the biological basis, were made to the PBPK model
11 of Emond et al. (2006, [197316](#)), before its use in the computation of dose metrics for TCDD.
12 The first one involved the recalculation of the volume of the rest of the body as follows:

13

$$14 \quad WRE0 = (0.91 - (WLIB0 \times WLI0 + WFB0 \times WF0 + WLI0 + WF0)) / (1 + WREB0) \quad (3-22)$$

15

16 where

17 $WRE0$ = weight of cellular component of rest of body compartment (as fraction of
18 body weight);

19 $WLI0$ = weight of cellular component of liver compartment (as fraction of body
20 weight);

21 WFO = weight of cellular component of fat compartment (as fraction of body
22 weight);

23 $WREB0$ = weight of the tissue blood component of the rest of body compartment (as
24 fraction of body weight);

25 $WLIB0$ = weight of the tissue blood component of the liver compartment (as fraction
26 of body weight); and

27 $WFB0$ = weight of the tissue blood component of the fat compartment (as fraction of
28 body weight).

29

30 In the original code, the weight of the rest of body compartment was calculated as the
31 difference between 91% of body weight and the sum total of the fractional volumes of blood,
32 liver tissue (intracellular component), and adipose tissue (intracellular component). The blood
33 compartment in the PBPK model is not explicitly characterized with a volume; as a result, the

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1 total volume of the compartments is less than 91%. The recalculations shown above were used
2 to address this problem. Given the very low affinity of TCDD for blood and rest of the body,
3 reparameterizing the model resulted in less than a 1% change in output compared to the
4 published version of the PBPK model for chronic exposure scenarios (Emond et al., 2006,
5 [197316](#)).

6 The second minor modification related to the calculation of the rate of TCDD excreted
7 via urine. The original model code computed the rate of excretion by multiplying the urinary
8 clearance parameter with the concentration in the rest of the body compartment. Instead, the
9 code was modified to use the blood concentration in this equation. This resulted in the
10 re-estimation of the urinary clearance value in the rat and human models but it did not result in
11 any significant change in the fit and performance of the original model.

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

15 **3.3.4.5. Recommended Dose Metrics for Key Studies**

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

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

29 The confidence in the ability of PK models to simulate blood concentration as a dose
30 metric is high, given that the models have been shown to consistently reproduce whole blood (or
31 serum lipid-normalized) TCDD concentration profiles in both humans and rats. Considering the

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1 facts that the PBPK models simulate whole blood rather than the serum lipid-normalized
2 concentrations of TCDD and that the study-specific values of serum lipid content are not known
3 with certainty, it is preferable to rely on TCDD blood concentrations as the dose metric. The
4 blood concentrations, if intended, can be normalized on the basis of appropriate total lipid levels.
5 However, based on mechanistic considerations, the confidence in their use would be somewhat
6 lower for hepatic effects. This conclusion reflects the concern regarding the inconsistent
7 relationship between the two variables with increasing dose levels and the fraction of
8 steady-state attained at the time of observation. For other systemic effects related to tissue
9 concentrations, the confidence in the use of TCDD serum or blood concentration is high,
10 particularly for chronic exposures, given the absence of data on organ-specific nonlinear
11 mechanisms. In general, the tissue concentration typically cannot be calculated as a reliable dose
12 metric with either the CADM or the Emond models. One exception is the use of the Emond
13 PBPK models to estimate levels in liver, a metric that is relevant based on MOA considerations.
14 However, it is noted that the hepatic TCDD level encompasses free and bound TCDD and it is a
15 highly complex entity for dose metric considerations. Finally, the AhR-bound concentration
16 may be evaluated for receptor-mediated effects. This dose metric can be obtained by PBPK
17 models, although uncertainties associated with lack of data for this dose metric renders it to be of
18 low confidence (see Table 3-10). The alternative dose metrics for dose-response modeling of
19 TCDD selected on the basis of MOA and PK modeling considerations are summarized in
20 Tables 3-11 and 3-12.

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

28

1 **3.3.5. Uncertainty in Dose Estimates**

2 **3.3.5.1. Sources of Uncertainty in Dose Metric Predictions**

3 **3.3.5.1.1. Limitations of available PK data.**

4 **3.3.5.1.1.1. Animal data.**

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

12

13 **3.3.5.1.1.2. Human data.**

14 The human data on potential dose metrics are restricted to the serum lipid-adjusted
15 TCDD concentrations associated with mostly uncharacterized exposures (see Sections 3.3.2 and
16 3.3.3). While these data are useful in estimating half-lives in exposed human individuals, they
17 do not provide estimates of hepatic clearance or reflect target organ exposure. Some autopsy
18 data have been used to infer the partition coefficients; however, these data were collected
19 without quantification of the temporal nature of TCDD uptake (see Section 3.2). Despite the
20 limitations associated with the available human data, there has been some success in using these
21 data to infer the half-lives and elimination rates in humans using pharmacokinetic models
22 (Aylward et al., 2005, [197014](#); Carrier et al., 1995, [197618](#); Emond et al., 2006, [197316](#)).

23

24 **3.3.5.1.2. Uncertainties associated with model specification.**

25 Uncertainty associated with model specification should be viewed as a function of the
26 specific application, such as interspecies extrapolation, intraspecies variability, or high dose to
27 low dose extrapolation. Because the use of pharmacokinetic models in this assessment is limited
28 to interspecies extrapolation and high dose to low dose extrapolation, it is essential to evaluate
29 the confidence in predicted dose metrics for these specific purposes. For interspecies
30 extrapolation, the PBPK and CADM models calculate differences in dose metric between an
31 average adult animal and an average adult human. Both models have a biologically and

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1 mechanistically-relevant structure along with a set of parameters with reasonable biological
2 basis, and reproduce a variety of pharmacokinetic data on TCDD in both rodents and humans.
3 These models possess low uncertainty with respect to body burden, blood, and TCDD/serum
4 (lipid) concentration for the purpose of conducting rat to human extrapolation. However, for
5 other dose metrics, such as free, total, or bound hepatic concentrations, the uncertainty is higher
6 in the CADM model compared to the PBPK model due to model specification differences related
7 to the mechanisms of sequestration and induction in the liver (see Section 3.3.3).

8 For the purpose of high dose to low dose extrapolation in experimental animals,
9 confidence in both models is high with respect to a variety of dose metrics (see previous
10 discussion). The high confidence results from the use of the PBPK models to reproduce a
11 number of data sets covering a wide range of dose levels in rodents (rats, mice) including the
12 dose ranges of most of the key toxicological studies. Given that the TCDD levels during and at
13 the end of exposures were not measured in most of the key studies, use of the PBPK models is
14 preferred because these models account for dose-dependent elimination, induction, and
15 sequestration. Despite the empirical nature of the specification of these key processes in PBPK
16 models, they essentially reproduce the dose-dependent behavior in rodents, supporting their use
17 in deriving dose metrics for dose-response modeling of TCDD. Overall, the confidence in the
18 use of the alternative dose metrics (identified in Table 3-10) is greater than the confidence in the
19 use of administered dose for TCDD, for relating to the concentration within tissues to produce an
20 effect. The administered dose does not take into account interspecies differences in the volume
21 of distribution and clearance or the complex nonlinear processes determining the internal dose.

22 The PBPK model of Emond et al. (2006, [197316](#)) could benefit from further refinement
23 and validation, including a more explicit consideration of nondose-dependent elimination
24 pathways. As indicated in Section 4, there is some uncertainty associated with the way the
25 elimination of TCDD is described in the existing human PBPK model. The current model
26 essentially treats all TCDD elimination as related to dose dependent metabolism in the liver. In
27 this regard, the classical and more recent PK data on TCDD may be useful in further improving
28 the confidence in their predictions. However, it is likely that there is nondose-dependent
29 elimination of TCDD via feces and, to a lesser extent skin; juxtaposition of available elimination
30 rate data with the PBPK model predictions suggests that the current PBPK model modestly
31 overestimates the dose dependency of overall TCDD elimination. (The central estimate of the

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1 slope of the relationship between the log of the TCDD elimination rate and the log of the TCDD
2 level is only about three-fourths of that expected using the unmodified PBPK model). Emond et
3 al. (2005, [197317](#)) acknowledge that the model did not describe the elimination of TCDD from
4 the blood into the intestines, but it indirectly accounted for this phenomenon with the use of the
5 optimized elimination rate.

7 **3.3.5.1.3. *Impact of human interindividual variability.***

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

18 **3.3.5.2. *Qualitative Discussion of Uncertainty in Dose Metrics***

19 The usefulness of the CADM and PBPK models for conducting dose-response modeling
20 (rodent bioassays), interspecies (rodent to human) and intraspecies (high-dose to low-dose)
21 extrapolations is determined by their reliability in predicting the desired dose metrics. The
22 confidence in the model predictions of dose metrics is dictated by the extent to which the model
23 has been verified with empirical data relevant to the dose metric, supplemented by sensitivity
24 and uncertainty analyses. Analysis of sensitivity or uncertainty has not been conducted with the
25 CADM model. For the PBPK model, Emond et al. (2006, [197316](#)) published the initial results
26 from sensitivity analyses of acute exposure modeling (see Section 3.3.3). One of the objectives
27 of a sensitivity analysis that is of highest relevance to this assessment is the identification of the
28 most critical model parameters with respect to the model output (i.e., dose metric).

29 If the model simulations have only been compared to entities that do not correspond to
30 the moiety representing the dose metric, or if the comparisons have only been done for some but
31 not all relevant dose levels, routes, and species, then the reliability in the predictions of dose

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1 metric can be an issue. The extent to which model results are uncertain will depend largely upon
2 the extent to which the dose metric is measurable (e.g., serum concentrations of TCDD) or
3 inferred (e.g., AhR-bound TCDD concentration).

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

19 Sensitivity analysis for the Emond rat PBPK model predictions of liver TCDD
20 concentration indicated that hepatic CYP1A2 concentration is the most sensitive parameter
21 (Emond et al., 2006, [197316](#)). For the Emond human PBPK model, the absorption parameters,
22 basal concentration of CYP1A2, and adipose tissue: blood partition coefficients were identified as
23 highly-sensitive parameters.

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

29 With regard to the predictability of body burden, the absorption and excretion parameters
30 were among the sensitive parameters in the rat. Several other parameters were also identified as
31 being sensitive in humans. Despite the sensitivity to these parameters and the uncertainty

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1 associated with individual parameter estimates, the overall confidence in the model predictions
2 of body burden appears to be high given the reproducibility of empirical data on tissue burdens
3 and blood concentrations of TCDD in various experiments by both models. Similar conclusions
4 can be drawn for blood concentration of TCDD predicted by the PBPK model, except that the
5 assigned value of blood (serum) lipid content will have additional impact on this dose metric to
6 the extent that the calibration data were in terms of LASC. Variability of total lipid levels and
7 variability of the contribution of phospholipids and neutral lipids to the total lipid pool across
8 species, lifestage and study groups is to be expected (Bernert et al., 2007, [594270](#); Poulin and
9 Theil, 2001, [594269](#)).

10 Both conceptual (biological) relevance and prediction uncertainty are important in the
11 choice of dose metric for dose-response modeling and interspecies extrapolation. Conceptual
12 relevance has to do with how “close” the metric is to the observed effect, taking into account
13 both the target tissue and the MOA. In this context, a greater degree of confidence is held for
14 dose metrics that are more proximate to the event (i.e., specific effect). Prediction uncertainty
15 reflects the lack of confidence in the model predictions of dose metrics. Tables 3-13 and 3-14
16 provide a qualitative ranking of the importance and magnitude of each dose metric with respect
17 to these two sources of uncertainty. Conceptual relevance is low for the use of administered
18 dose in dose-response modeling because known (non-linear) physiological processes are ignored;
19 conversely, conceptual uncertainty is much lower for use of internal dose metrics more proximal
20 to the affected organs.

21 Table 3-13 presents a cross-walk of relevance, uncertainty and overall confidence
22 associated with the use of various dose metrics for dose-response modeling of TCDD. As shown
23 in Table 3-13, blood/serum levels have the highest overall confidence (medium) followed by
24 body burden (medium to low) for application in dose-response modeling. When using the mouse
25 PBPK model along with the human model (see Table 3-14), the contribution of the prediction
26 uncertainty to the overall uncertainty increases due to the limited comparison of the mouse
27 model simulations with empirical data.

29 **3.3.6. Use of the Emond PBPK Models for Dose Extrapolation from Rodents to Humans**

30 EPA has selected the Emond et al. (2004, [197315](#); 2005, [197317](#); 2006, [197316](#)) PBPK
31 models, as modified by EPA for this assessment, for establishing toxicokinetically-equivalent

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1 exposures in rodents and humans.¹⁸ The 2003 Reassessment (U.S. EPA, 2003, [537122](#))
2 presented a strong argument for using the relevant tissue concentration as the effective dose
3 metric. However, no models exist for estimation of all relevant tissue concentrations. Therefore,
4 EPA has decided to use the concentration of TCDD in blood as a surrogate for tissue
5 concentrations, assuming that tissue concentrations are proportional to blood concentrations.
6 Furthermore, because the RfD and cancer slope factor are necessarily expressed in terms of
7 average daily exposure, the blood concentrations are expressed as averages over the relevant
8 period of exposure for each endpoint. Specifically, blood concentrations in the model
9 simulations are averaged from the administration of the first dose to the administration of the last
10 dose plus one dosing interval (time) unit in order to capture the peaks and valleys for each
11 administered dose. That is, for daily dosing, 24 hours of TCDD elimination following the last
12 dose is included in the average (the modeling time interval is one hour); for a weekly dosing
13 protocol, a full week is included. In addition, because of the accumulation of TCDD in fat and
14 the large differences in elimination kinetics between rodent species and humans, exposure
15 duration plays a much larger role in TK extrapolation across species than for rapidly-eliminated
16 compounds. Because of these factors, EPA is using discrete exposure scenarios that relate
17 human and rodent exposure durations. The use of discrete exposure scenarios was introduced
18 previously in Section 3.4.4.2 describing first-order kinetic modeling and is further described in
19 the following paragraphs. This section concludes with a quantitative evaluation of the impact of
20 exposure duration on the rodent-to-human TK extrapolation from both the human and rodent
21 “ends” of the process.

22 Figure 3-30 shows the TCDD blood concentration-time profile for continuous exposure
23 at 0.01 ng/kg-day, as predicted by the Emond human PBPK model, and the target TCDD
24 concentrations corresponding to the three discrete exposure scenarios used by EPA in this
25 document. The target concentrations are those that would be identified in the animal bioassay
26 studies that correspond to a particular POD (no-observed-adverse-effect level, lowest-observed-
27 adverse-effect level, or benchmark dose lower confidence bound) established for that bioassay.
28 That is, the target concentrations represent the toxicokinetically-equivalent internal exposure to
29 be translated into an equivalent human intake (or HED).

¹⁸The 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).

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

6 For the gestational exposure scenario, the effective TCDD blood concentration (usually
7 the peak) determined for the particular POD in a particular developmental study is matched to
8 the average TCDD blood concentration over the gestational portion of the human gestational
9 exposure scenario. The HED is determined as the continuous daily intake, starting from birth
10 that would result in that average blood concentration over the 9-month gestational period for a
11 pregnancy beginning at 45 years of age. The choice of 45 years as the beginning age of
12 pregnancy is health protective of the population in that the daily exposure achieving the target
13 blood concentration is smaller than for earlier pregnancies. A table for converting average
14 gestational blood concentrations and other internal dose metrics to human intake for the 45-year-
15 old pregnancy scenario is presented in Appendix C.4. Also, a comparison of the 45-year old
16 pregnancy scenario to one beginning at age 25 is presented in Table 3-15. Using the 25 year-old
17 pregnancy scenario increases the HED by 30 to 60% for typical animal bioassay PODs (3 to
18 30 ng/kg).

19 For a less-than-lifetime exposure, the average TCDD blood concentration over the
20 exposure period in the animal bioassay associated with the POD is matched to the average over
21 the 5-year period that includes the peak concentration (58 years for an intake of 0.01 ng/kg-day).
22 The HED is determined as the continuous daily intake that would result in the target
23 concentration over peak 5-year period. The use of the peak is analogous to the approach in the
24 2003 Reassessment, where the terminal steady-state body burden played the same role. The
25 5-year average over the peak is taken to smooth out sharp peaks and more closely approximate a
26 plateau. The choice of peak is health protective because humans of any age must be protected
27 for short-term exposures, and the daily intake achieving a given TCDD blood concentration is
28 smallest when matched to the peak exposure as opposed to an average over shorter durations.
29 Thus, target concentrations for any exposure duration of less-than-lifetime must be averaged
30 backwards from the end of the lifetime scenario, rather than from the beginning. The only
31 exception would be if the short-term endpoints evaluated in the animal bioassay were associated

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1 with a specific life stage (such as for the gestational scenario). Note that this scenario lumps all
2 exposures from 1 day to over 1 year in rodents into the same less-than-lifetime category.
3 Conceptually, duration-specific scenarios could be constructed by defining equivalent rodent and
4 human exposure durations. However, for the most part, defining duration equivalents across
5 species is a somewhat arbitrary exercise, not generally based on physiologic or toxicologic
6 processes, but relying primarily on fraction-of-lifetime conversions. EPA defines “lifetime”
7 exposure as 2 years and 70 years for rodents and humans, respectively. So, a half-lifetime
8 equivalence of 1 year in rodents and 35 years in humans is defined easily. Also, considering a
9 subchronic exposure to be 10–15% of lifetime, leads to an equivalence of 90 days in rodents and
10 7–10 years in humans. However, in the practical sense with respect to the Emond human PBPK
11 model predictions, the difference in the dose-to-target-concentration ratios are not significantly
12 different from the peak 5-year average scenario, differing by less than 5%. A table for
13 converting less-than-lifetime average blood concentrations and other internal dose metrics to
14 human intake is presented in Appendix C.4.

15 The net effect of using three different scenarios for estimating the HED from rodent
16 exposures is that, for the same target concentration, the ratio of administered dose (to the rodent)
17 to HED will be larger for short-term exposures than for chronic exposures. Figure 3-31 is
18 similar to Figure 3-30, except that it shows the relationship of daily intake to a fixed target
19 TCDD blood concentration level. Figure 3-31 shows that, for human intakes of approximately
20 0.01 ng/kg-day, the difference in the defined scenarios is 40% or less, with a lifetime-scenario
21 daily intake of 0.014 ng/kg-day required to reach the same target concentration for a shorter-term
22 exposure of 0.01 ng/kg-day. The corresponding daily intake for the gestational scenario is
23 0.011 ng/kg-day. Because of the nonlinearities in the Emond human PBPK model, the
24 magnitude of the difference between the lifetime and less-than-lifetime exposure scenarios
25 increases at lower intake levels, but not to a substantial degree.

26 The differential effect of short- and long-term exposures is much more accentuated at the
27 rodent end of the exposure kinetic modeling. Analogous to the processes described in the
28 previous section for first-order body burden (see Section 3.4.2.2), the TCDD blood concentration
29 for single exposures is essentially the immediate absorbed fraction of the administered dose,
30 which will be somewhat lower than the administered dose, while for chronic exposure, the
31 TCDD blood concentration will reflect the long-term accumulation from daily exposure, which

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1 will be very much larger than the administered dose (expressed as a daily intake). Table 3-16
2 shows the overall impact of TK modeling on the extrapolation of administered dose to HED,
3 comparing the Emond PBPK and first-order body burden models. For comparison purposes, the
4 administered dose is fixed at 1 ng/kg-day for all model runs. Large animal-to-human TK
5 extrapolation factors (TK_{EF}) are evident for short-term mouse studies, decreasing in magnitude
6 with increasing exposure duration. The only exception is the slightly lower extrapolation factor
7 for the mouse 1-day exposure, which is the result of the relatively short TCDD half-life (10 days)
8 in mice and the use of the peak TCDD blood concentration as representative of single exposures,
9 compared to the average TCDD blood concentration over the exposure period used for multiple
10 exposures. The TK_{EFS} are lower for rats because of the slower elimination of TCDD in rats
11 compared to mice. Also, because of the nonlinear kinetics inherent in the Emond PBPK model,
12 the span of the HED (13-fold for mice) across these exposure durations is greater than the span
13 of the lipid-adjusted serum concentration (LASC; 4-fold for mice). Because of the dose-
14 dependence of TCDD elimination in the Emond model, the TK_{EF} becomes smaller with
15 decreasing intake. The result of this nonlinearity is that, although Table 3-16 shows much lower
16 TK_{EFS} for the Emond PBPK model than for the first-order body burden metric, at much lower
17 HED levels the two models give much closer predictions.

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

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*	1.210	100.00

4
 5 *The total tissue volume presented here represents only 91% of body weight because some of the weight and
 6 volume of the body is occupied by bone and other structures where TCDD uptake and accumulation do not occur to
 7 a significant extent.
 8

9 Source: Wang et al. (1997, [104657](#)), Emond et al. (2005, [197317](#); 2006, [197316](#)).
 10

11 **Table 3-2. Blood flows, permeability factors and resulting half lives (t_{1/2}) for**
 12 **perfusion losses for humans as represented by the TCDD PBPK model of**
 13 **Emond et al. (2005, [197317](#); 2006, [197316](#))**
 14
 15

Tissue	Permeability (fraction of compartment blood flow)	Rate constant for compartmental elimination (hour⁻¹)	t_{1/2} (hrs)
Fat	0.12	0.0049	143
Liver	0.03	0.77	0.90
Rest of the body	0.35	3.84	0.18

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Table 3-3. Toxicokinetic conversion factors for calculating human equivalent doses from rodent bioassays

Half-life (days) ^a	Mouse	Rat (Wistar)	Rat (other)	Guinea pig
	10	20	25	40
Exposure duration (days)	Conversion factor (CF) ^b $BB_A(t_A):d_A$ given in parentheses			
1	3882 (0.77)	3815 (0.79)	3802 (0.79)	3783 (0.79)
7	1107 (2.71)	1020 (2.94)	1004 (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)

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^aHalf-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).

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Table 3-4. Equations used in the concentration and age-dependent model (CADM; Aylward et al., 2005, [197014](#))^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)	$C_{adipose} = \frac{Q_{body}}{W_a} * (1 - (f_{min} + \frac{(f_{max} - f_{min}) * C_{body}}{K + C_{body}}))$
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	$k_e = k_{e0} - k_{eslope} * Age$

4 ^aFor abbreviations and parameter descriptions, see Table 3-5.

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2
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Table 3-5. Parameters of the Concentration and Age-Dependent Model (CADM; Aylward et al., 2005, [197014](#))

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, 198045)
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

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^aThe 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., 2005, [197114](#); Carrier et al., 1995, [197618](#)).

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

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

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^aConcentration and age-dependent model (Aylward et al., 2005, [197014](#)).
H = high, M = medium, L = low, NA = not applicable.

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Table 3-7. Equations used in the TCDD PBPK model of Emond et al. (2006, 197316)

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	$Qc(mL / hr) = QCCAR \times 60 \left(\frac{BW}{1000} \right)^{0.75}$ <p>A factor of 60 corresponds to the conversion of minutes to hours, and 1,000 is conversion of BW from grams to kilograms.</p>
Blood compartment	$Cb(nmol / mL) = \frac{[(Qf \times Cfb) + (Qre \times Creb) + (Qli \times Clib) + lymph]}{Qc} - \frac{(Cb \times CLURI)}{Qc}$
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	$Ct_{AhRbound}(nmol / mL) = \frac{LIBMAX \times Clifree}{KDLI + Clifree}$ <p>All other induction processes and equations have been described and presented by Wang et al. (1997, 104657).</p>

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Table 3-7. Equations used in the TCDD PBPK model of Emond et al. (2006, 197316) (continued)

Aspect	Equation
Gastrointestinal absorption and distribution of TCDD to the portal lymphatic circulation	
Amount of TCDD remaining in lumen cavity	$\frac{dLumen}{dt} (nmol / hr) = [(KST + KABS) \times lumen] + intake$ Lumen is 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	$\frac{dPortal}{dt} (nmol / hr) = KABS \times lumen \times 0.3$

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Note: Key parameters and abbreviations are defined in Table 3-10.

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 ^b	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 ^c	311.4 ^c
Tissue (intracellular) volumes (fraction of BW)							
Liver	WLI0	Calculated	Calculated	0.0549 ^f	0.0549 ^f	0.036 ^c	0.036 ^c
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

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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

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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
CYP1A2 induction parameters							
Dissociation constant CYP1A2 (nmol/mL)	KDLI2	40 ⁱ	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
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

^aUnits for human nongestational parameters are L rather than mL and kg rather than g where applicable.

^bBody weight varies by study (Emond et al., 2004, [197315](#)).

^cKrishnan and Andersen (2007).

^dUnits are L/kg/hr.

^eWang et al. (1997, [104657](#)).

^fILSI (1994, [046436](#)).

^gFixed.

^hLeung et al. (1990, [192833](#)).

ⁱOptimized.

^jEmond et al. (2004, [197315](#)).

^kWang et al. (2000, [198738](#)).

^lLawrence and Gobas (1997, [199072](#)).

^mCalculated to estimate 87% bioavailability of TCDD in humans (Poiger and Schlatter, 1986, [197336](#)).

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Table 3-9. Regression analysis results for the relationship between log₁₀ serum TCDD at the midpoint of observations and the log₁₀ 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

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Table 3-10. 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

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

Table 3-11. Overall confidence associated with alternative dose metrics for cancer and 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 3-12. Overall confidence associated with alternative dose metrics for cancer and 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.

Table 3-13. 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

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

H = high, M = medium, L = low, NA = not applicable, ? = if relevant to MOA of response.

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1 **Table 3-14. Contributors to the overall uncertainty in the selection and use**
 2 **of dose metrics in the dose-response modeling of TCDD based on mouse and**
 3 **human PBPK models**
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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	MH
Receptor occupancy	L(?)	H

5 H = high, M = medium, L = low, NA = not applicable, ? = if relevant to MOA of response.
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9 **Table 3-15. Comparison of human equivalent doses from the Emond human**
 10 **PBPK model for the 45-year-old and 25-year-old gestational exposure**
 11 **scenarios**
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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

13 ^aDetermined from the Emond rodent PBPK models assuming a single exposure on GD13.
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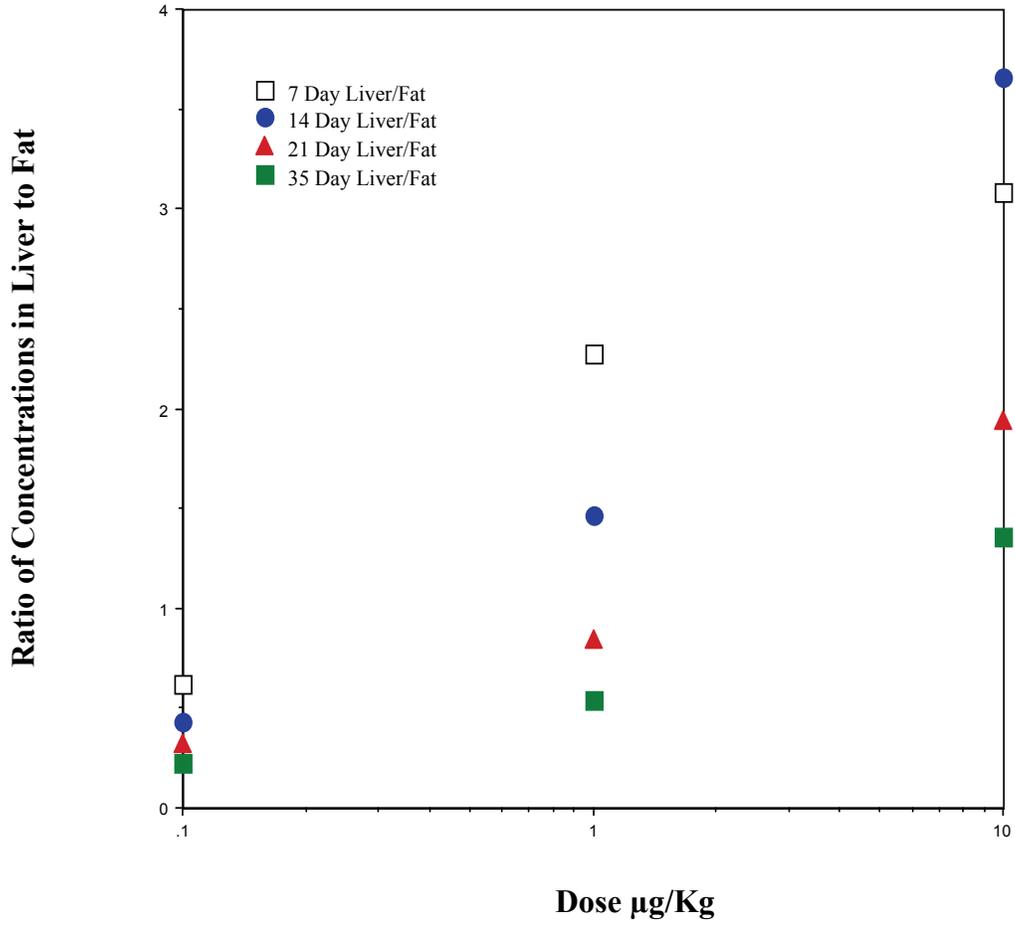
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Table 3-16. Impact of toxicokinetic modeling on the extrapolation of administered dose to HED, comparing the Emond PBPK and first-order body burden models

Exposure duration (days)	1 st -order BB		Emond PBPK		
	HED (ng/kg-day)	TK _{EF}	LASC (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

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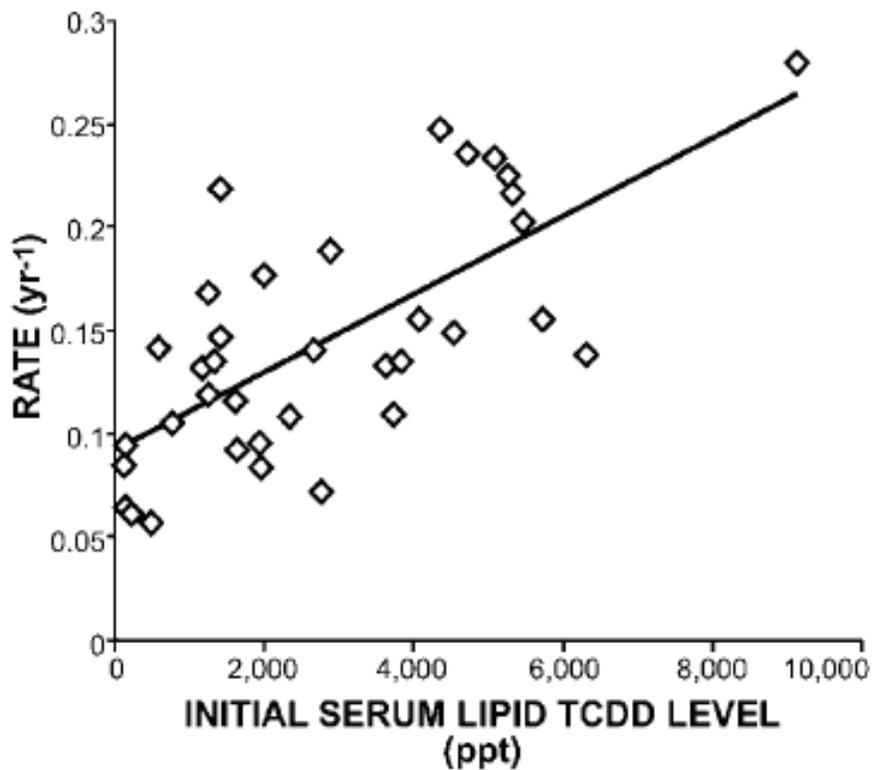
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Figure 3-1. Liver/fat concentration ratios in relation to TCDD dose at various times after oral administration of TCDD to mice.

Source: Dilberto et al. (1995, [197309](#)).

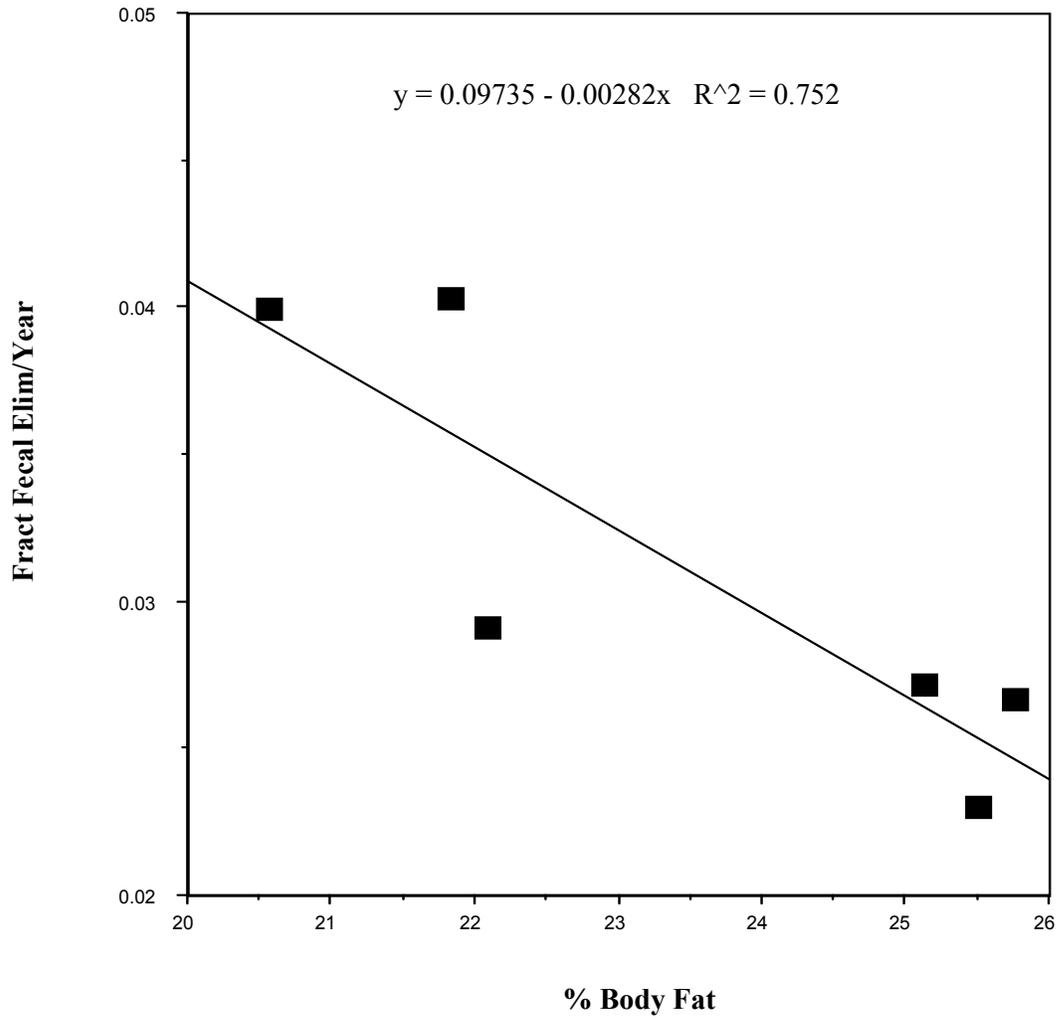


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Figure 3-2. First-order elimination rate fits to 36 sets of serial TCDD sampling data from Seveso patients as function of initial serum lipid TCDD.

Source: Aylward et al. (2005, [197014](#)).

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

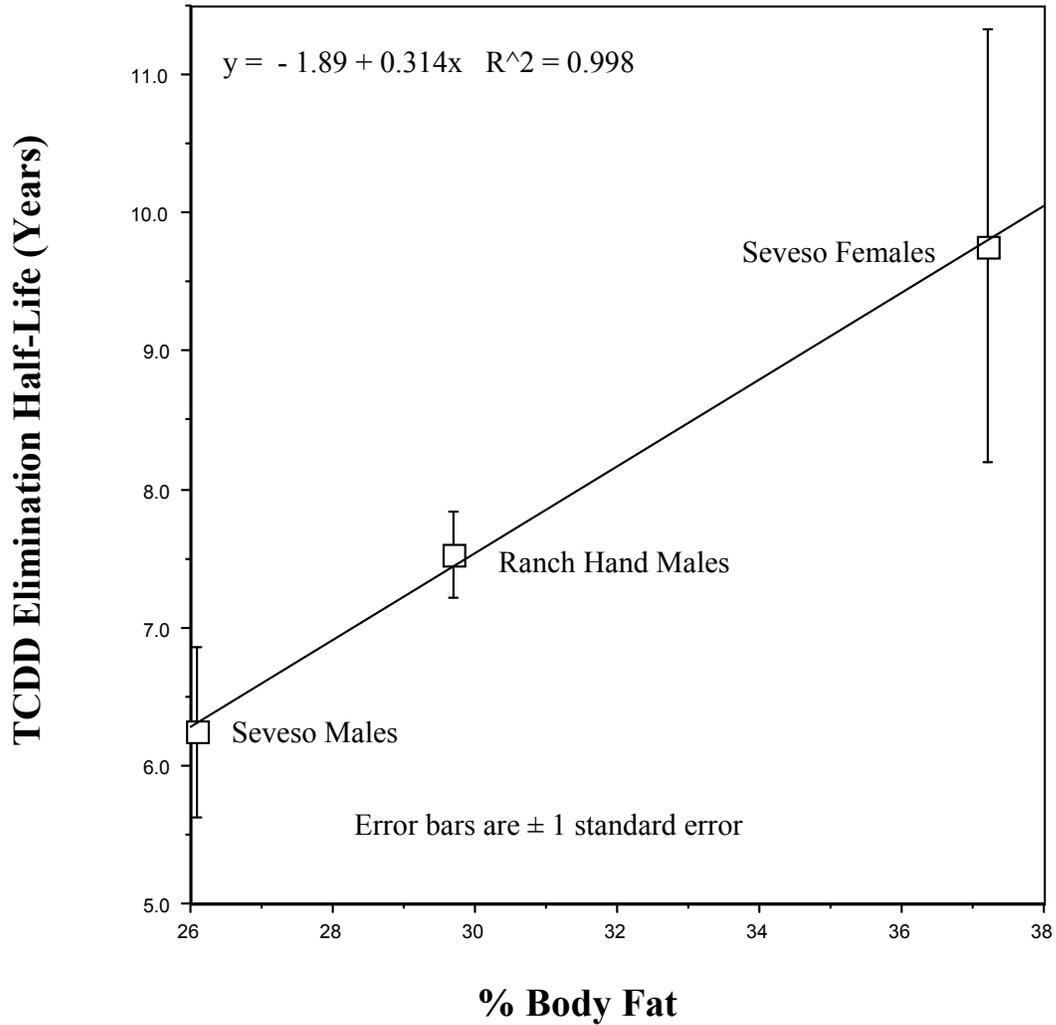
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Source: Rohde et al. (1999, [548764](#)).

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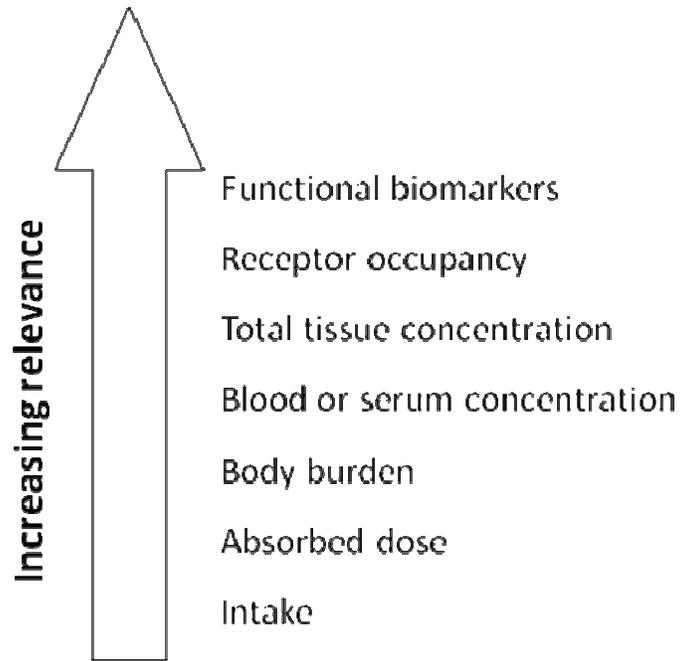
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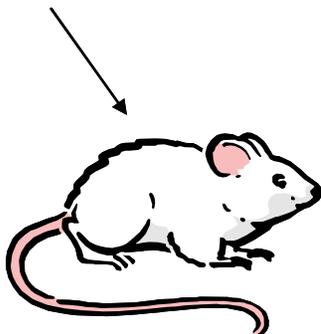
Figure 3-4. Unweighted empirical relationship between percent body fat estimated from body mass index and TCDD elimination half-life—combined Ranch Hand and Seveso observation.



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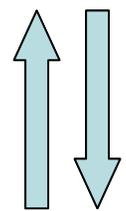
Figure 3-5. Relevance of candidate dose metrics for dose-response modeling, based on mode of action and target organ toxicity of TCDD.

Experimental Applied Dose



$$Body\ Burden_{Rat}(t) = BB(0)e^{-kt} + \frac{d(1-e^{-kt})fa}{k}$$

$Body\ Burden_{Rat}(t)$

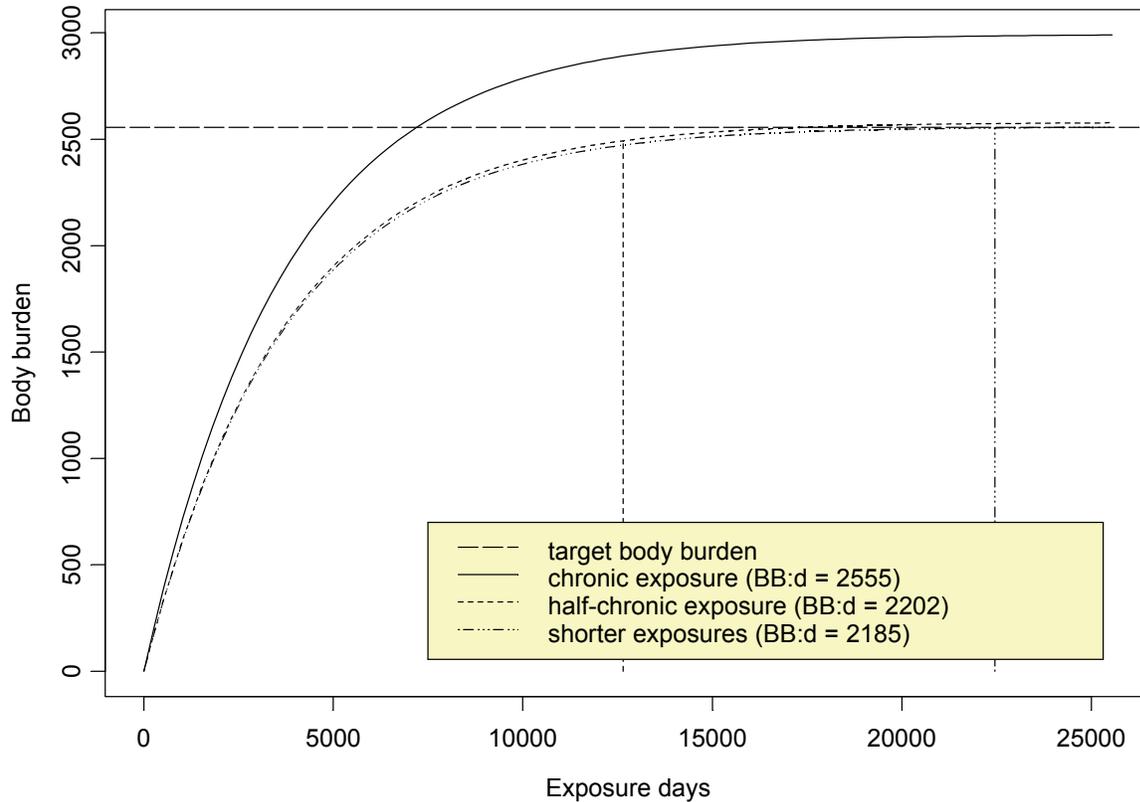


$Body\ Burden_{Human}(t)$

Human Estimated Exposure

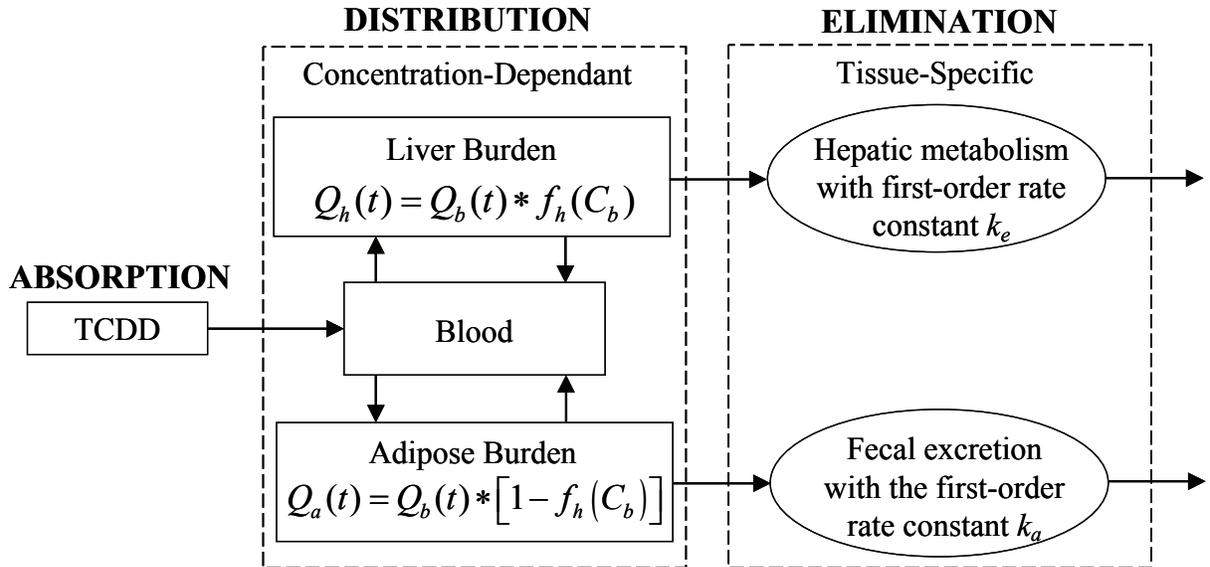
$$d_H = d_A \frac{t_{1/2A} (1 - e^{-k_A t_A})}{t_{1/2H} (1 - e^{-k_H t_H})}$$

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.



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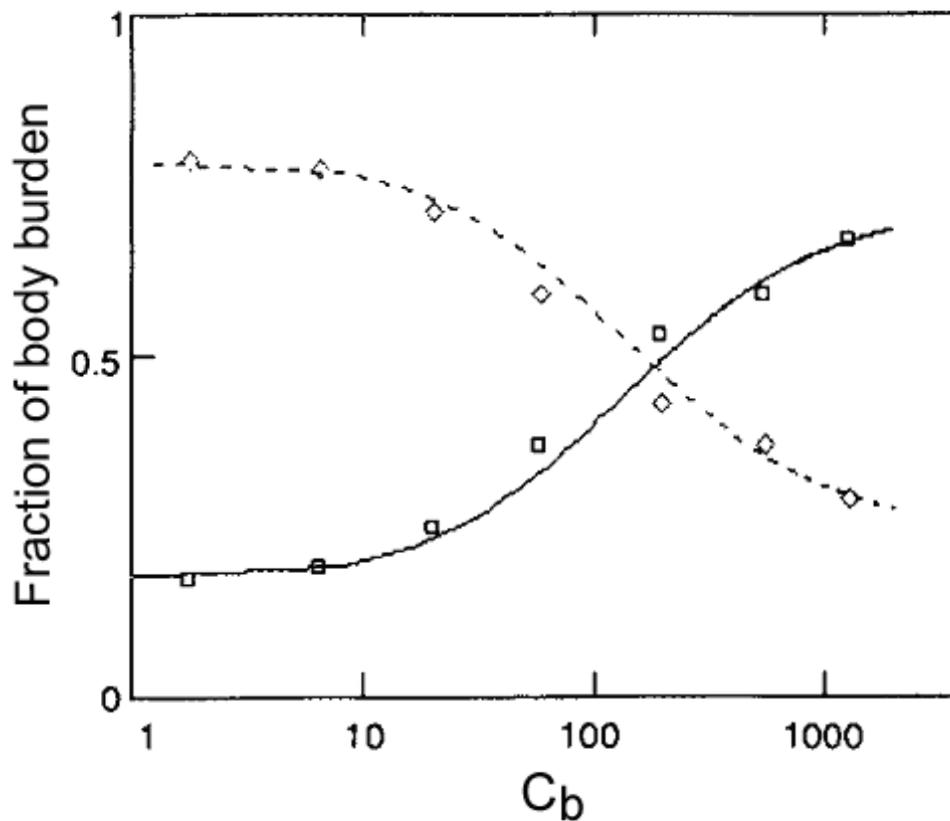
Figure 3-7. Human body burden time profiles for achieving a target body burden for different exposure duration scenarios. $BB:d$ is $BB_H(t_H):d_H$ in Figure 3-6. The curve depicted using the solid line illustrates the increase in the human body burden over time for a hypothetical human administered 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).



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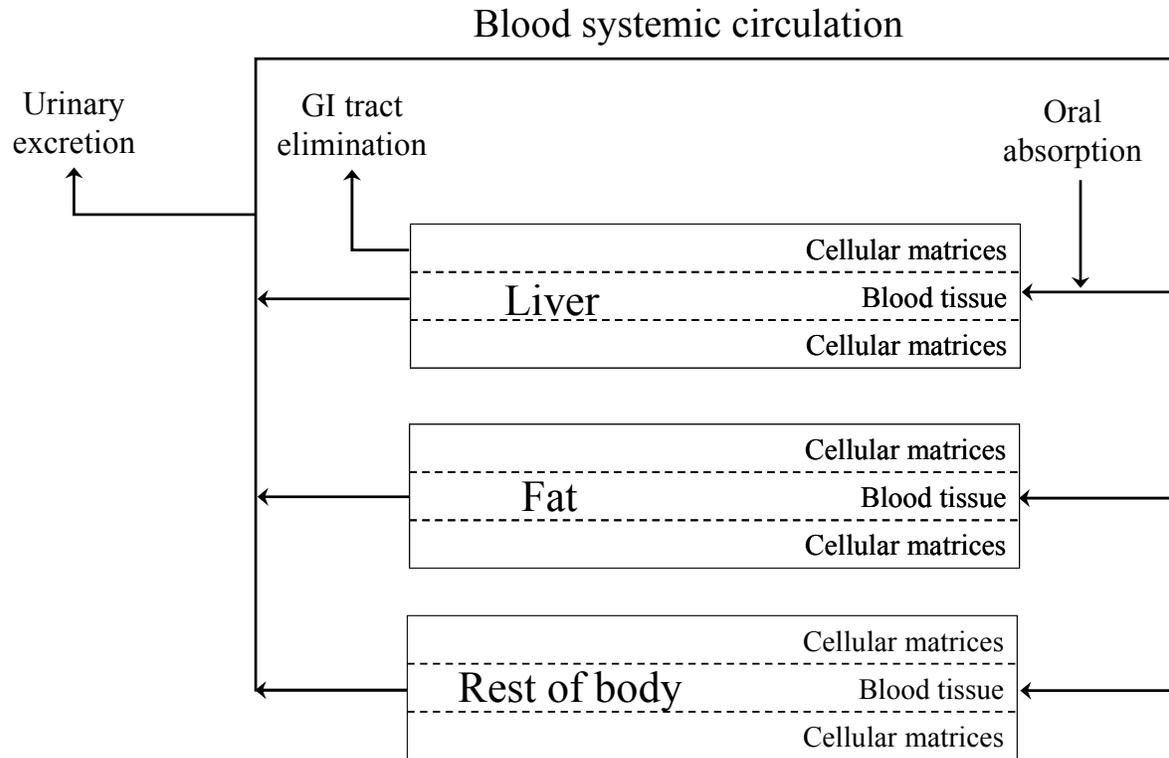
Figure 3-8. Schematic of the CADM structure.

Source: Aylward et al. (2005, [197014](#)).



1 **Figure 3-9. Comparison of observed and simulated fractions of the body**
 2 **burden contained in the liver and adipose tissues in rats.** f_h , fraction contained
 3 in liver (observation) (\square); f_{h-sim} , fraction contained in liver (simulation) (—); f_{at} ,
 4 fraction contained in the adipose tissue (observation) (\diamond); f_{at-sim} , fraction contained
 5 in the adipose tissue (simulation) (---); and C_b , body concentration in ng TCDD/kg
 6 body wt.

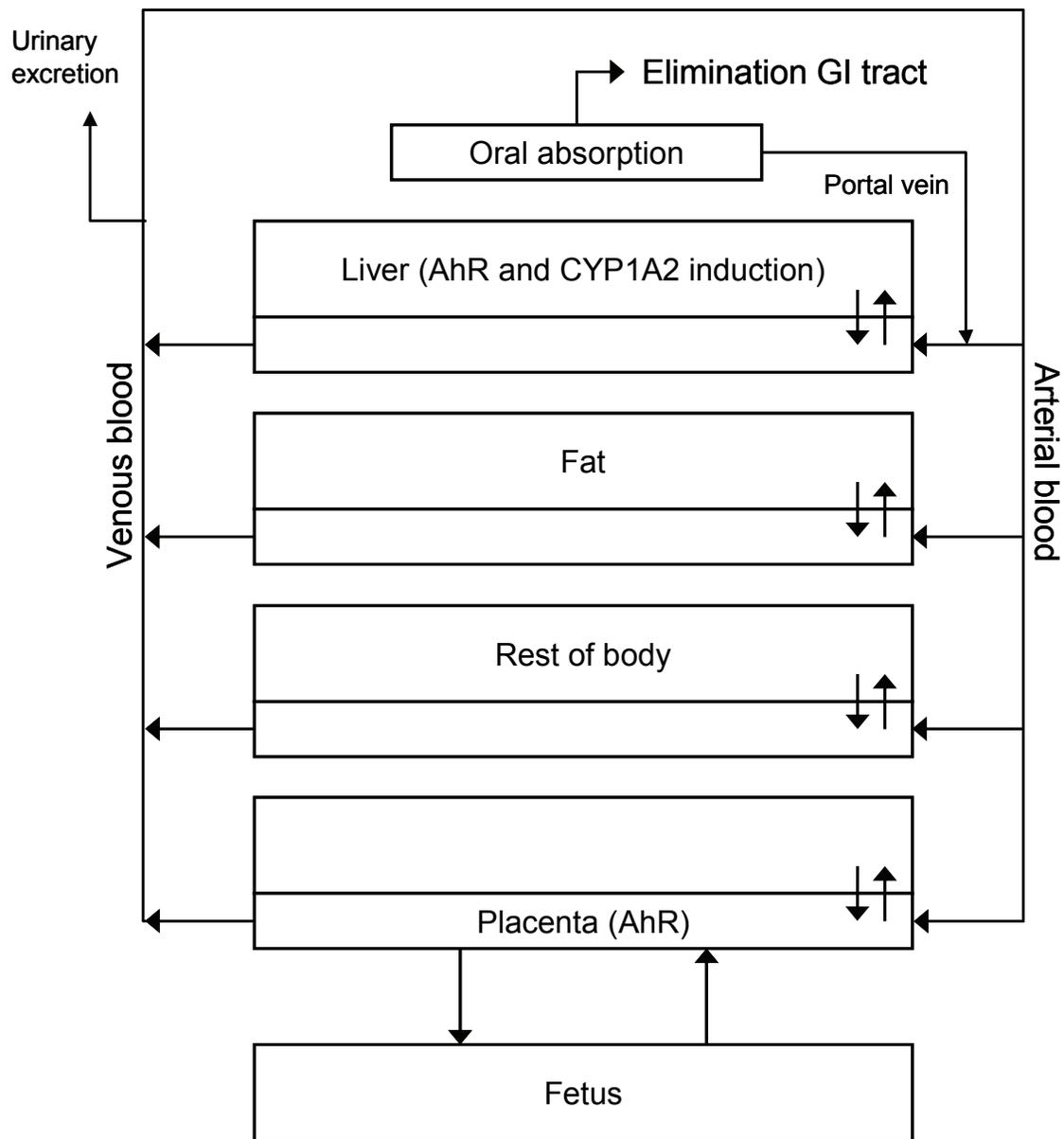
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 8 Source: Carrier et al. (1995, [197618](#)); data from Abraham et al. (1988, [199510](#))
 9 measured 7 days after dosing.



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

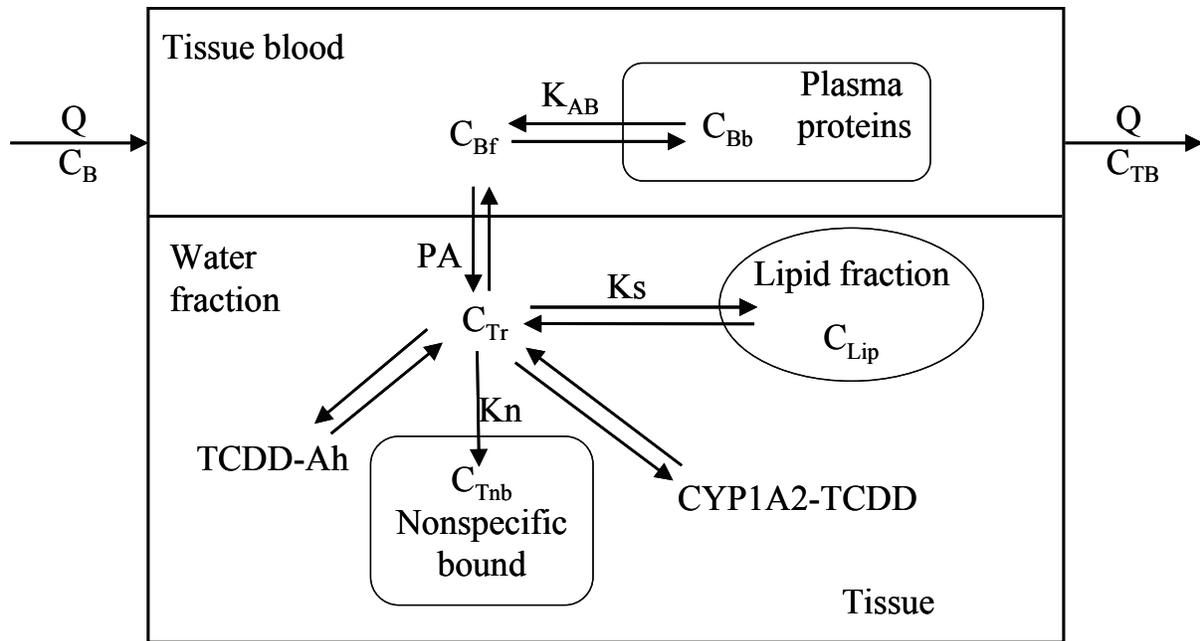
Source: Emond et al. (2006, [197316](#)).



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

Source: Emond et al. (2004, [197315](#)).

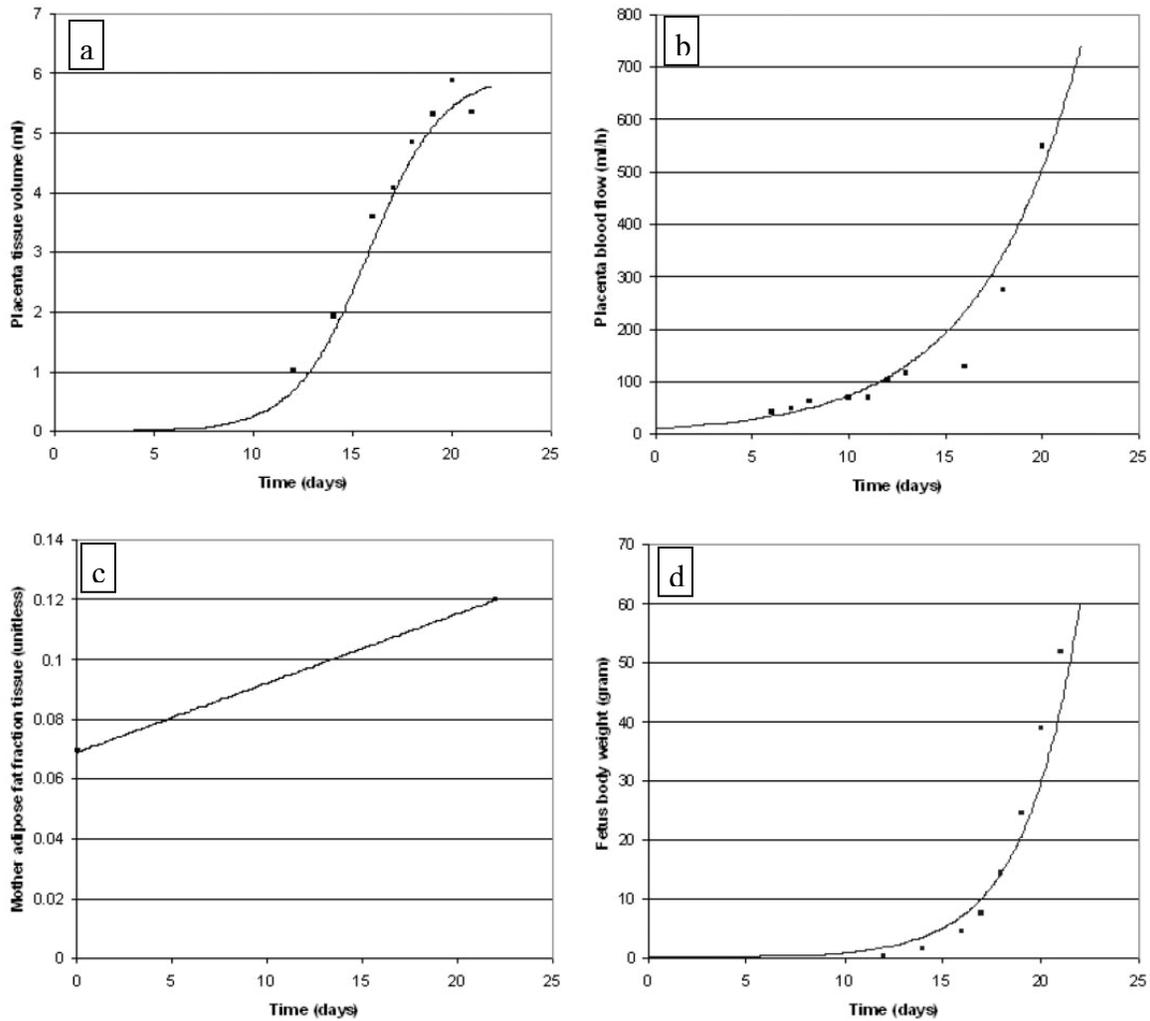


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Figure 3-12. TCDD distribution in the liver tissue.

Source: Wang et al. (1997, [104657](#)).

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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, [594274](#)). (b) Blood flow rate in Placental compartment during gestation. Experimental data from Buelke-Sam et al. (1982, [020478](#); 1982, [020477](#)). (c) Fat fraction of body weight during gestation. Experimental data came from Fisher et al. (1989, [065288](#)), and (d) Fetal growth during gestation. Experimental data obtained from Sikov (1970, [594274](#)).

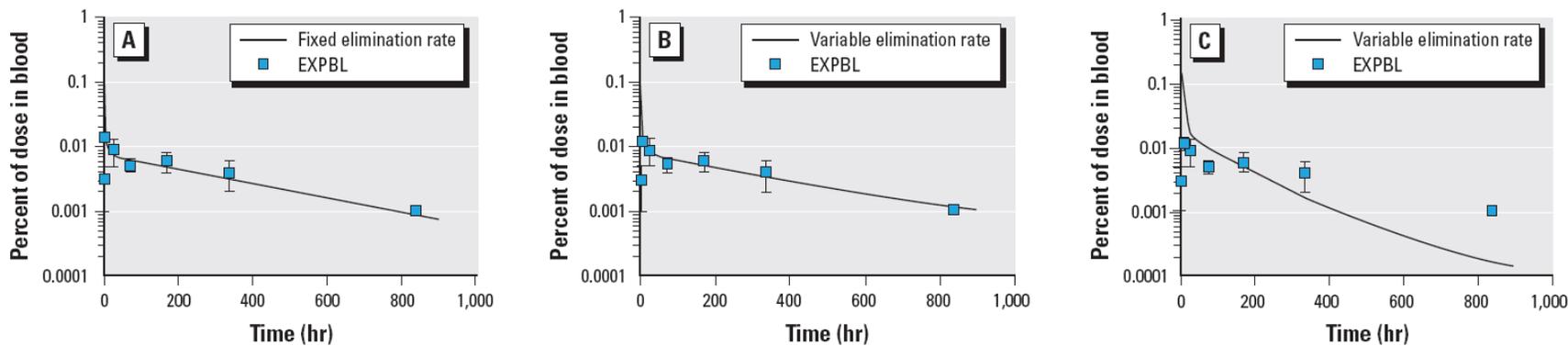
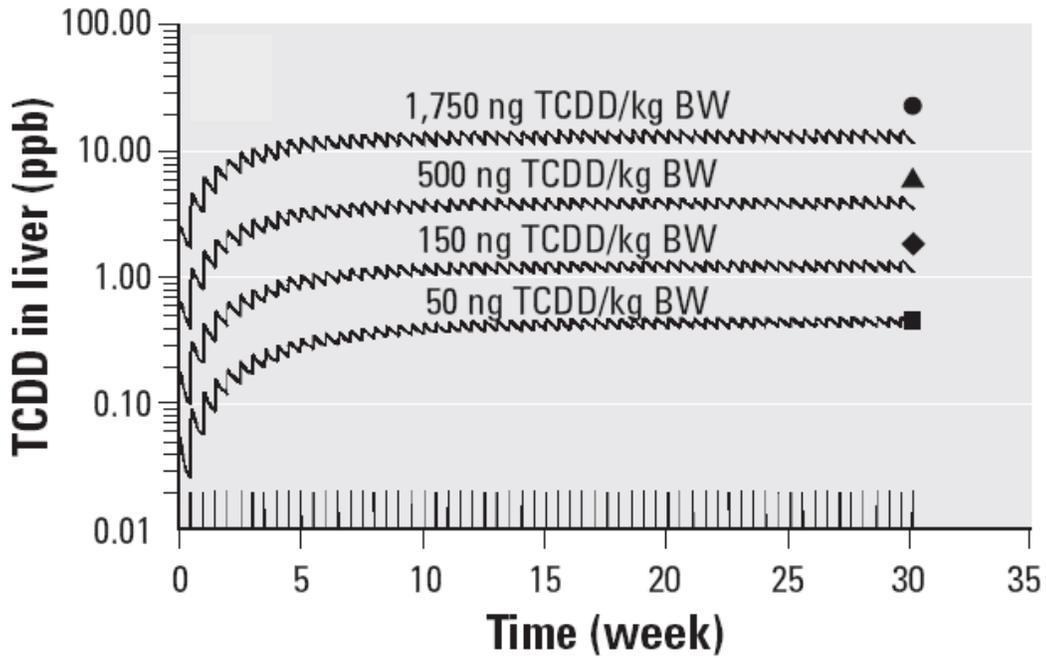


Figure 3-14. Comparisons of model predictions to experimental data using a fixed elimination rate model with hepatic sequestration (A) and an inducible elimination rate model with (B) and without (C) hepatic sequestration. EXBL, experimental blood levels. Model predictions were compared with the data of Santostefano et al. (1998, [200001](#)), where female rats were exposed to a single oral dose of 10 μg of TCDD/kg BW. Error bars are \pm SD.

Source: Edmond et al. (2006, [197316](#)).

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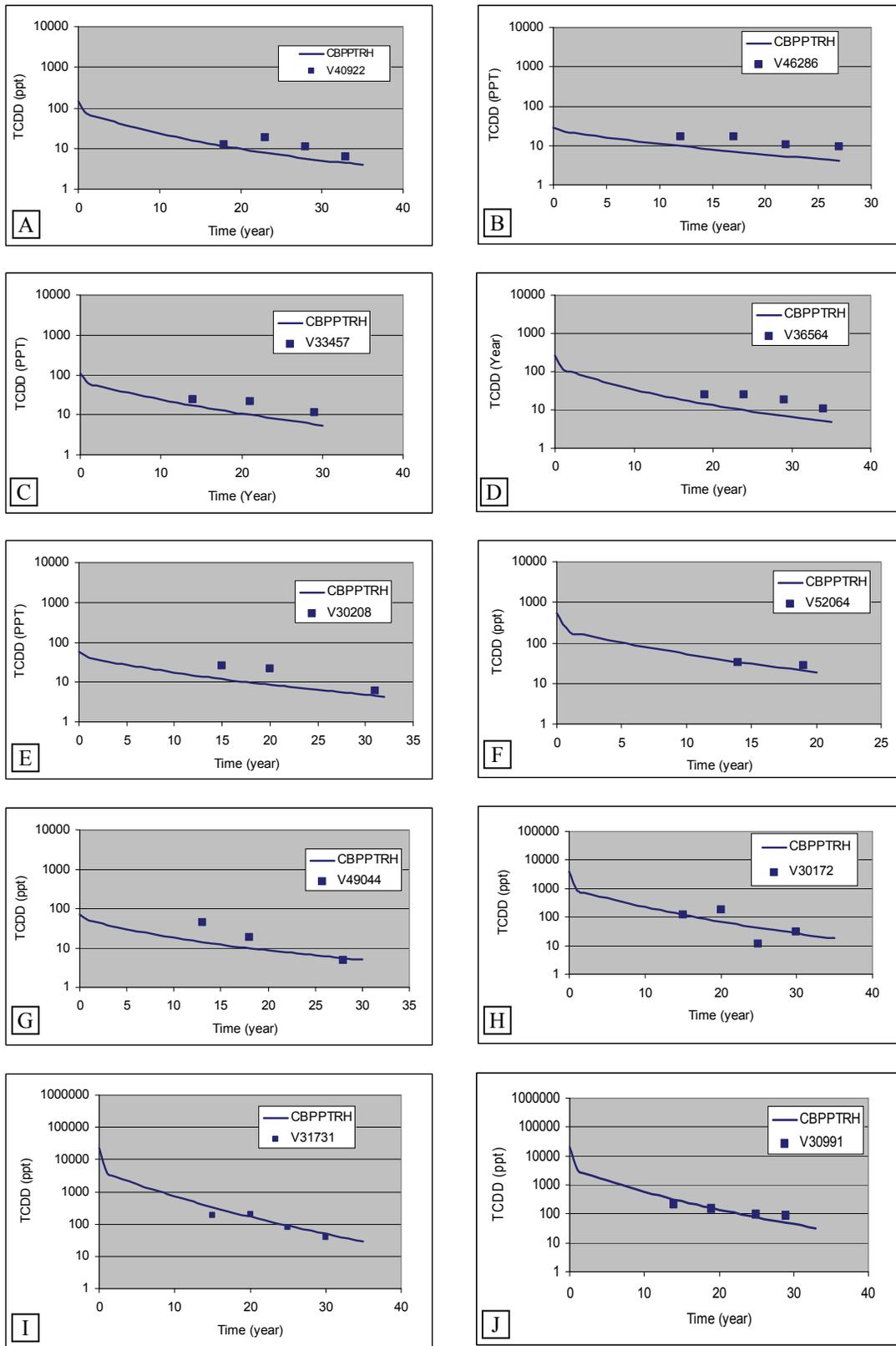


3 **Figure 3-15. PBPK model simulation of hepatic TCDD concentration (ppb)**
4 **during chronic exposure to TCDD at 50, 150, 500, 1,750 ng TCDD/BW using**
5 **the inducible elimination rate model compared with the experimental data**
6 **measured at the end of exposure.**

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Source: Emond et al. (2006, [197316](#)).

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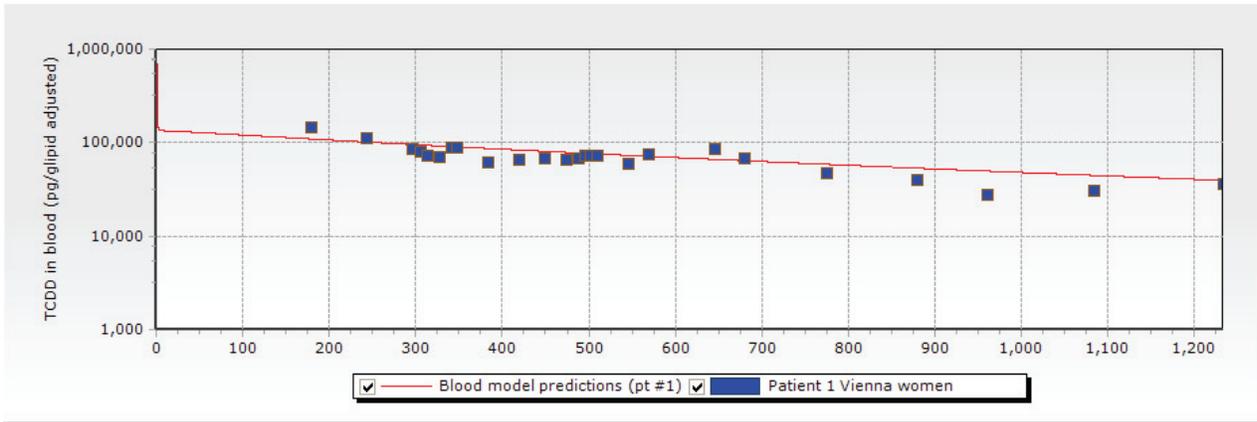
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Figure 3-16. Model predictions of TCDD blood concentration in 10 veterans (A-J) from Ranch Hand Cohort.

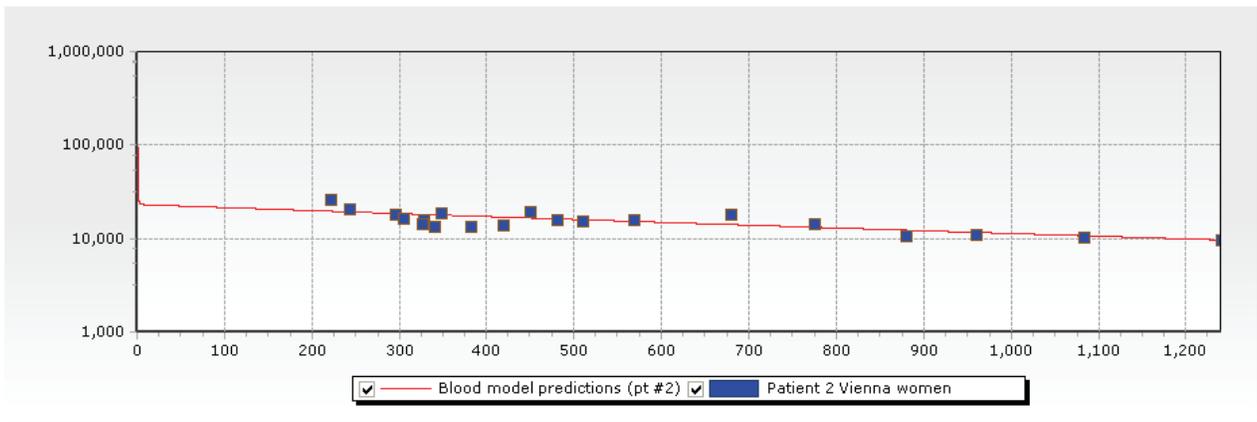
Source: Emond et al. (2005, [197317](#)).

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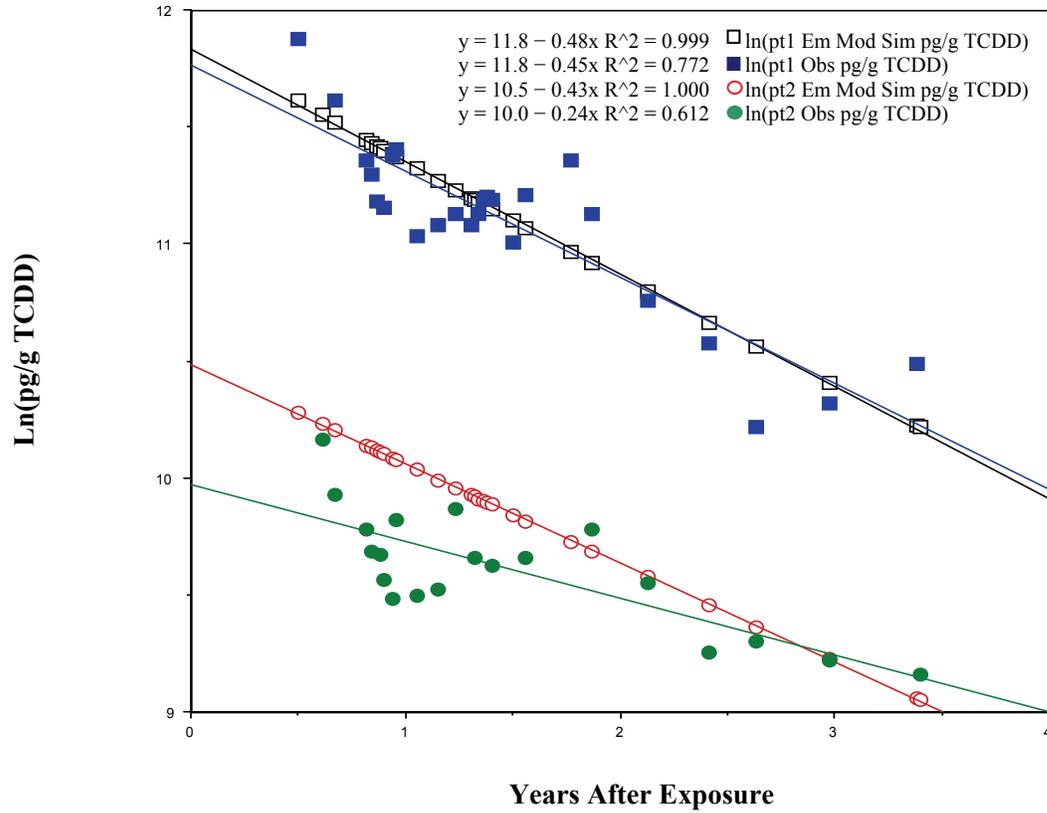


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Figure 3-17. Time course of TCDD in blood (pg/g lipid adjusted) for two highly exposed Austrian women (patients 1 and 2). Symbols represent measured concentrations, and lines represent model predictions. These data were used as part of the model evaluation (Geusau et al., 2002, [594259](#)).

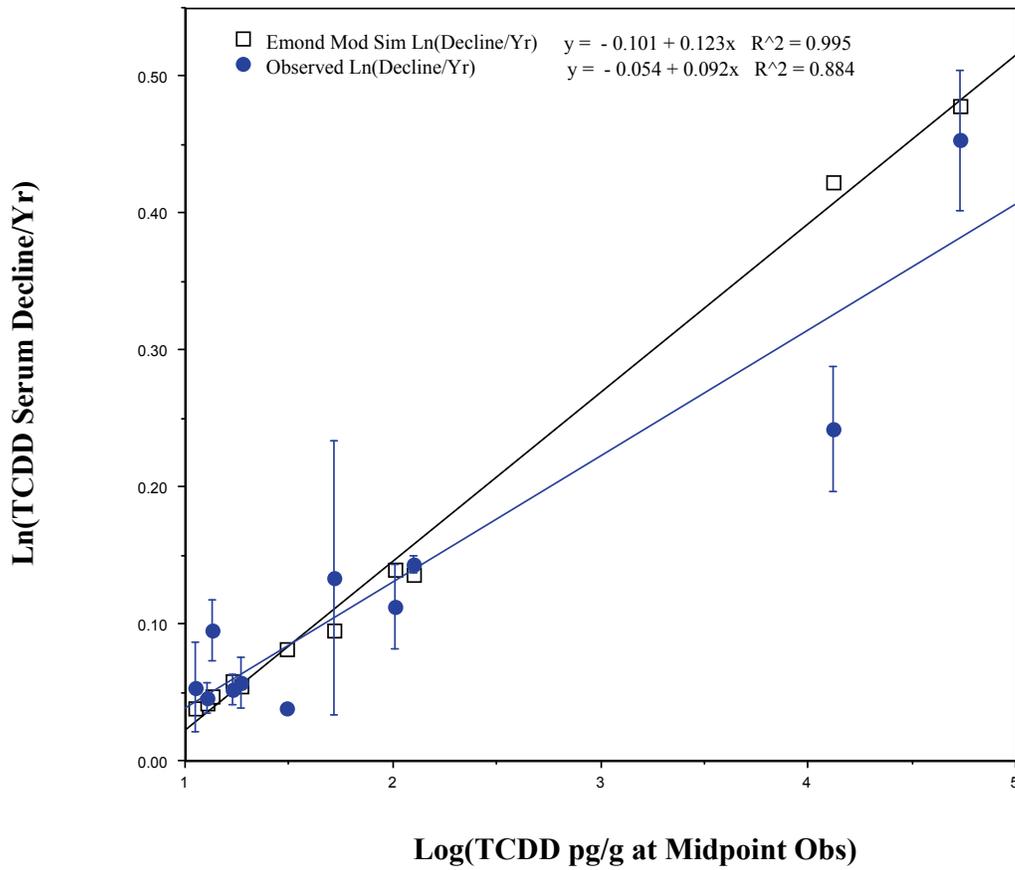
Source: Emond et al. (2005, [197317](#)).

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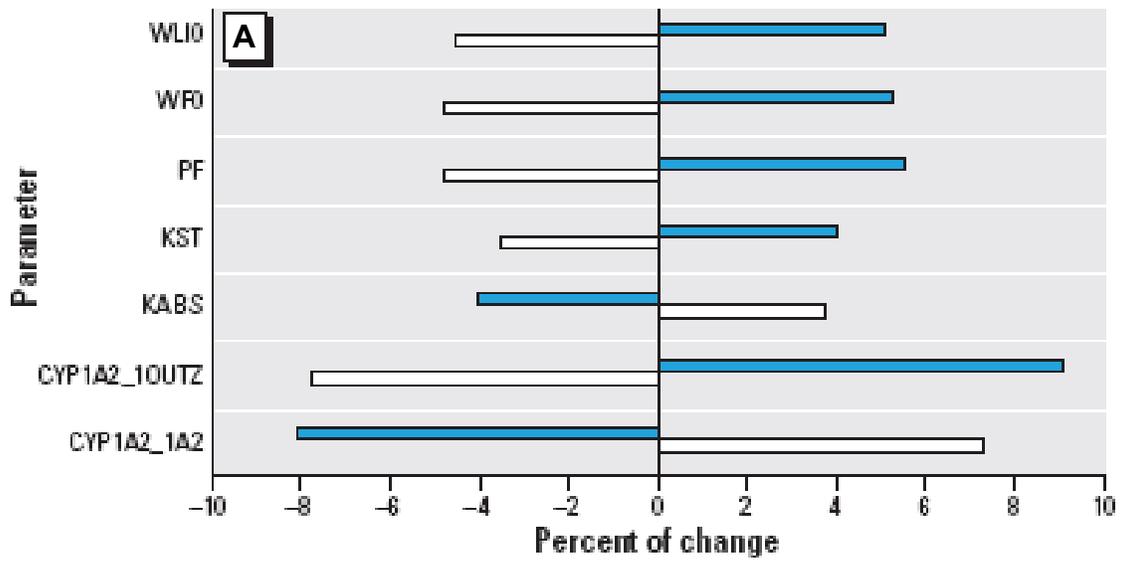
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Figure 3-18. Observed vs. Emond et al. (2005, [197317](#)) model simulated serum TCDD concentrations (pg/g lipid) over time (ln = natural log) in two Austrian women. Data from Geusau et al. (2002, [594259](#)).

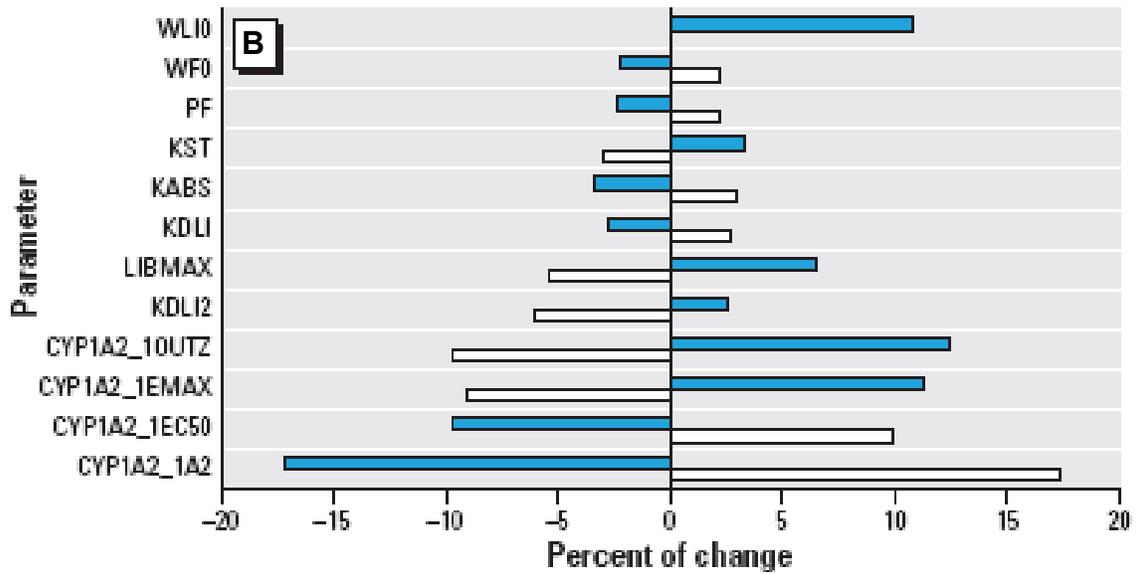


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 2 **Figure 3-19. Comparison of the dose dependency of TCDD elimination in the**
 3 **Emond model vs. observations of nine Ranch Hand veterans and two highly**
 4 **exposed Austrian patients. Circles are observed data.**

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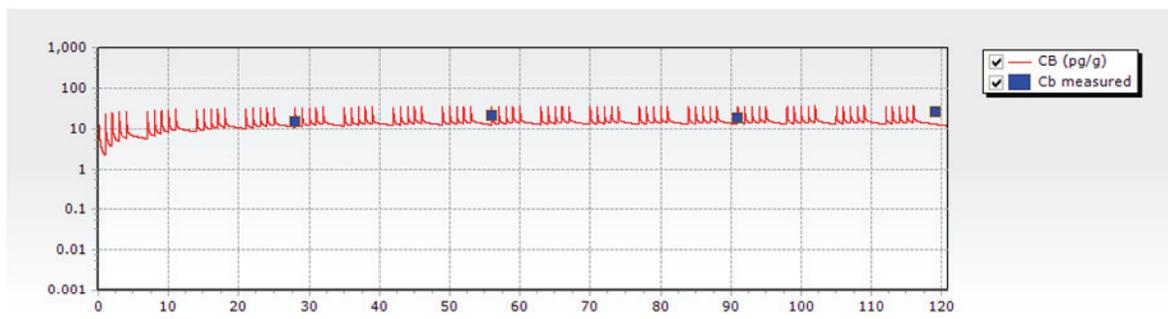
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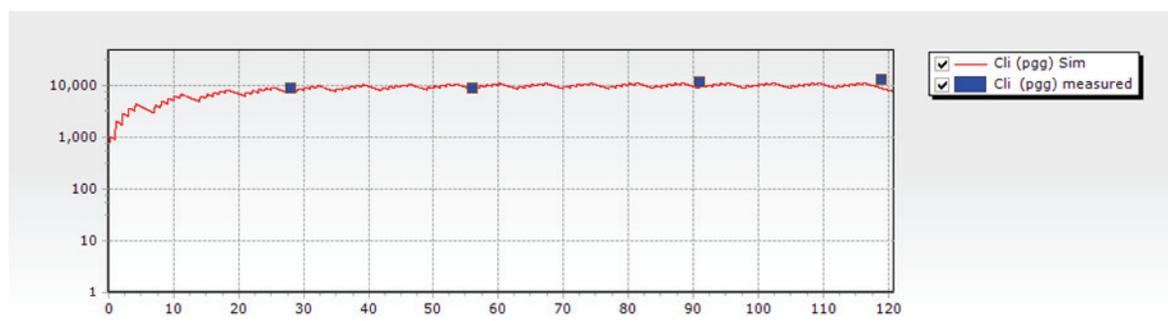
Figure 3-20. Sensitivity analysis was performed on the inducible elimination rate. The analysis was performed at 0.001 µg/kg (A) and at 10 µg/kg (B). The blue and white bars are results from -10% and +10% changes, respectively.

Source: Emond et al. (2006, [197316](#)).

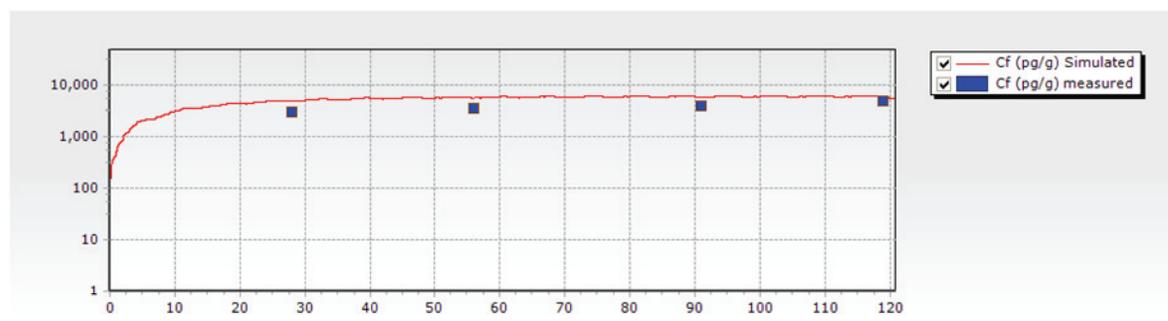
A



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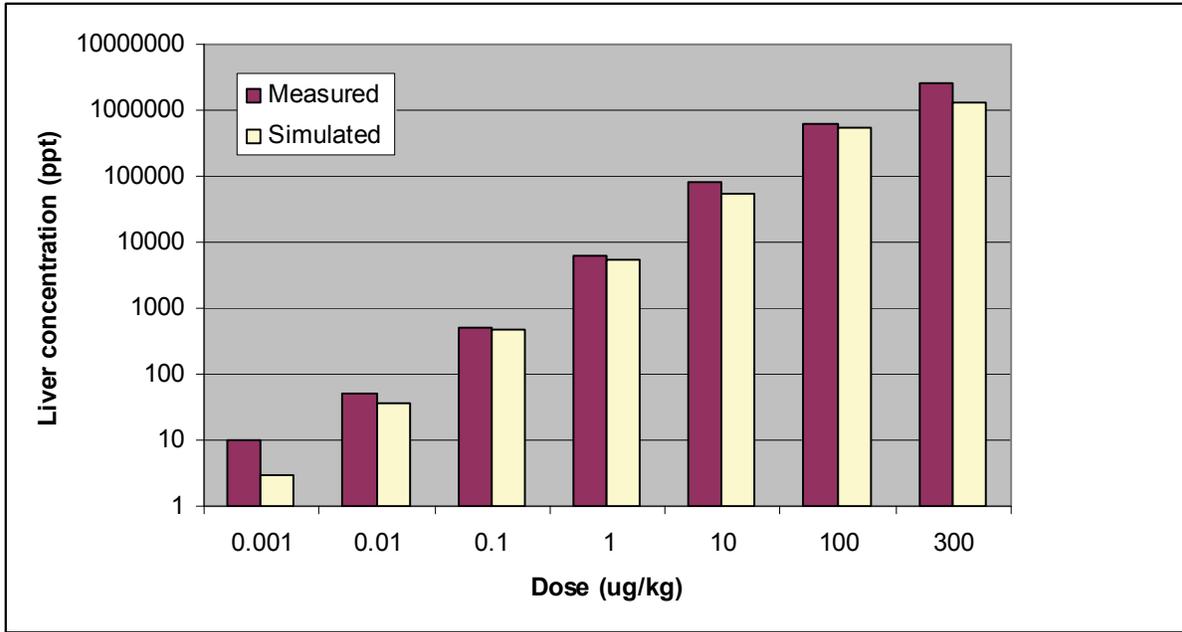


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2 **Figure 3-21. Experimental data (symbols) and model simulations (solid lines)**
3 **of (A) blood, (B) liver and (C) adipose tissue concentrations of TCDD after**
4 **oral exposure to 150 ng/kg-day, 5 days/week for 17 weeks in mice. Y-axis**
5 **represents concentration in pg/g and X-axis represents time in days.**

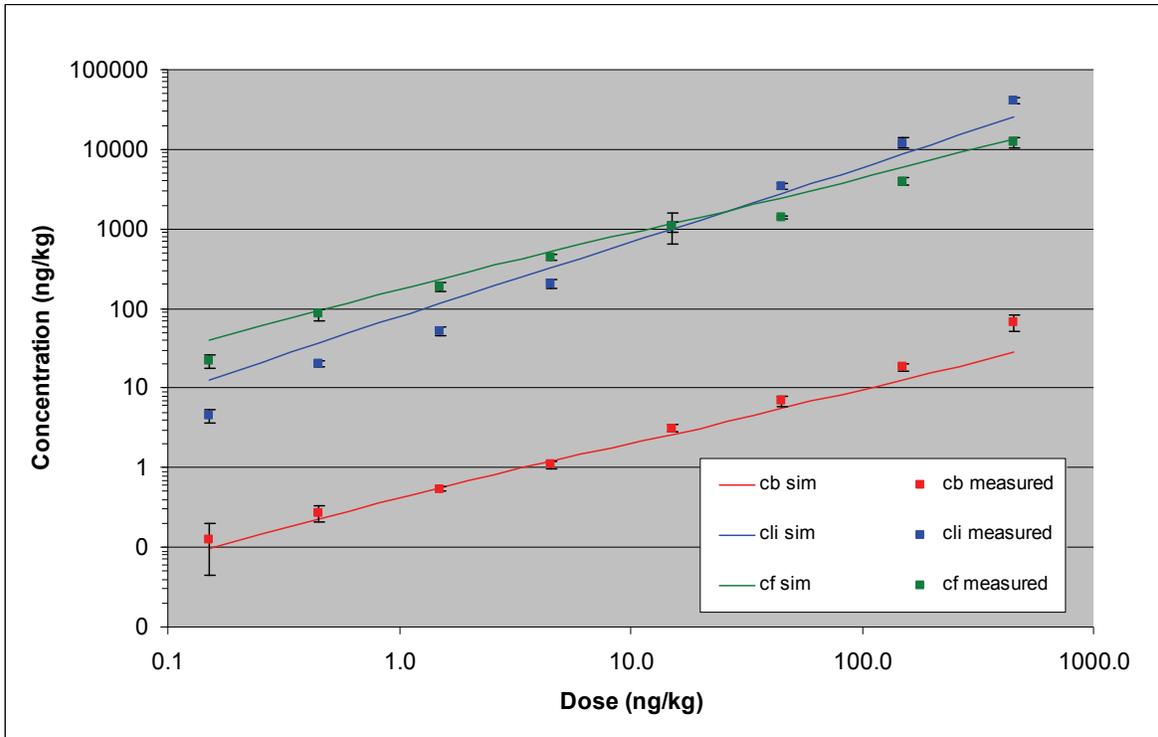
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7 Source: Experimental data were obtained from Diliberto et al. (2001, [197238](#)).



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Figure 3-22 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.

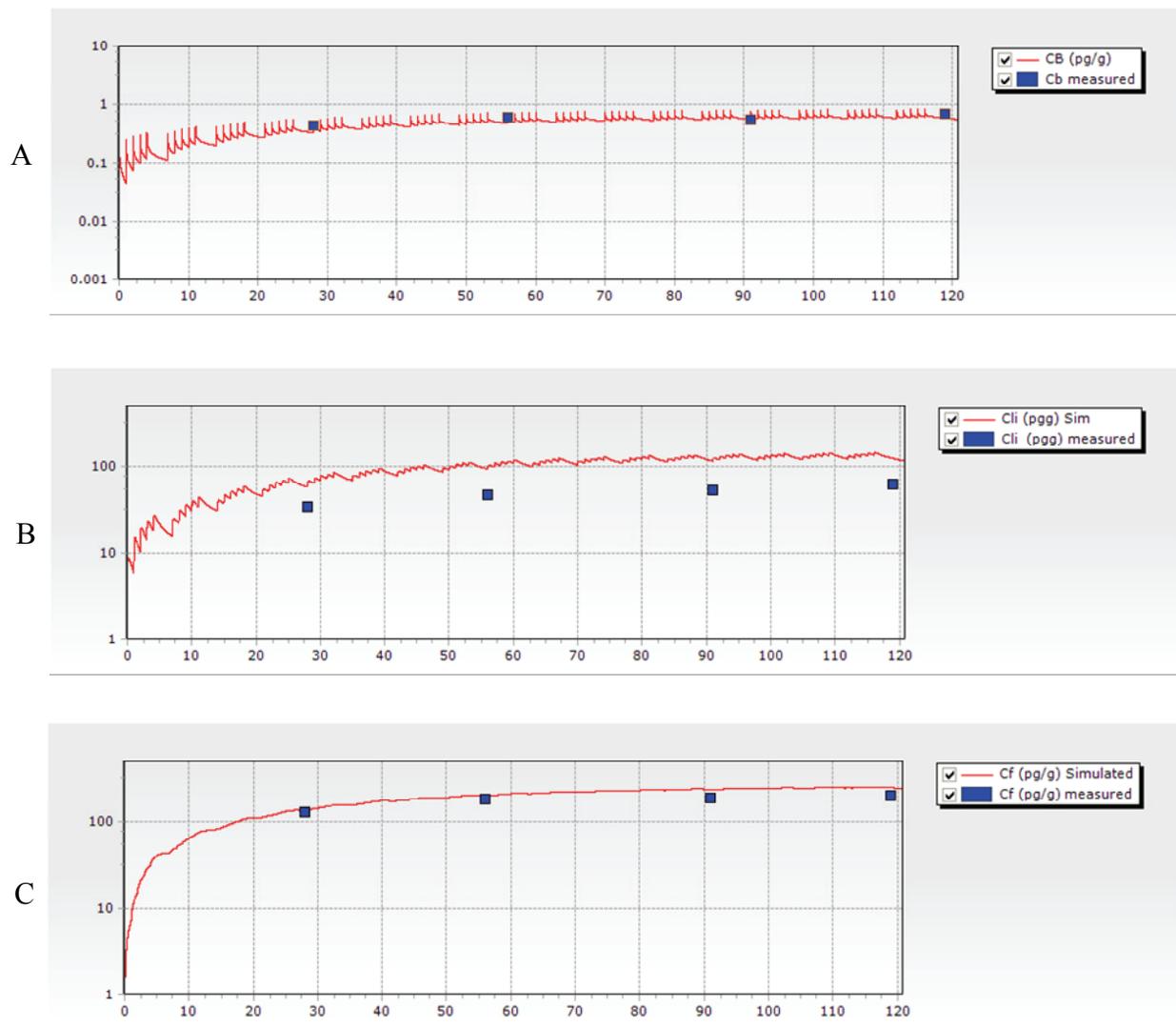
Source: Data obtained from Boverhoff et al. (2005, [594260](#)).



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Figure 3-23. 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.

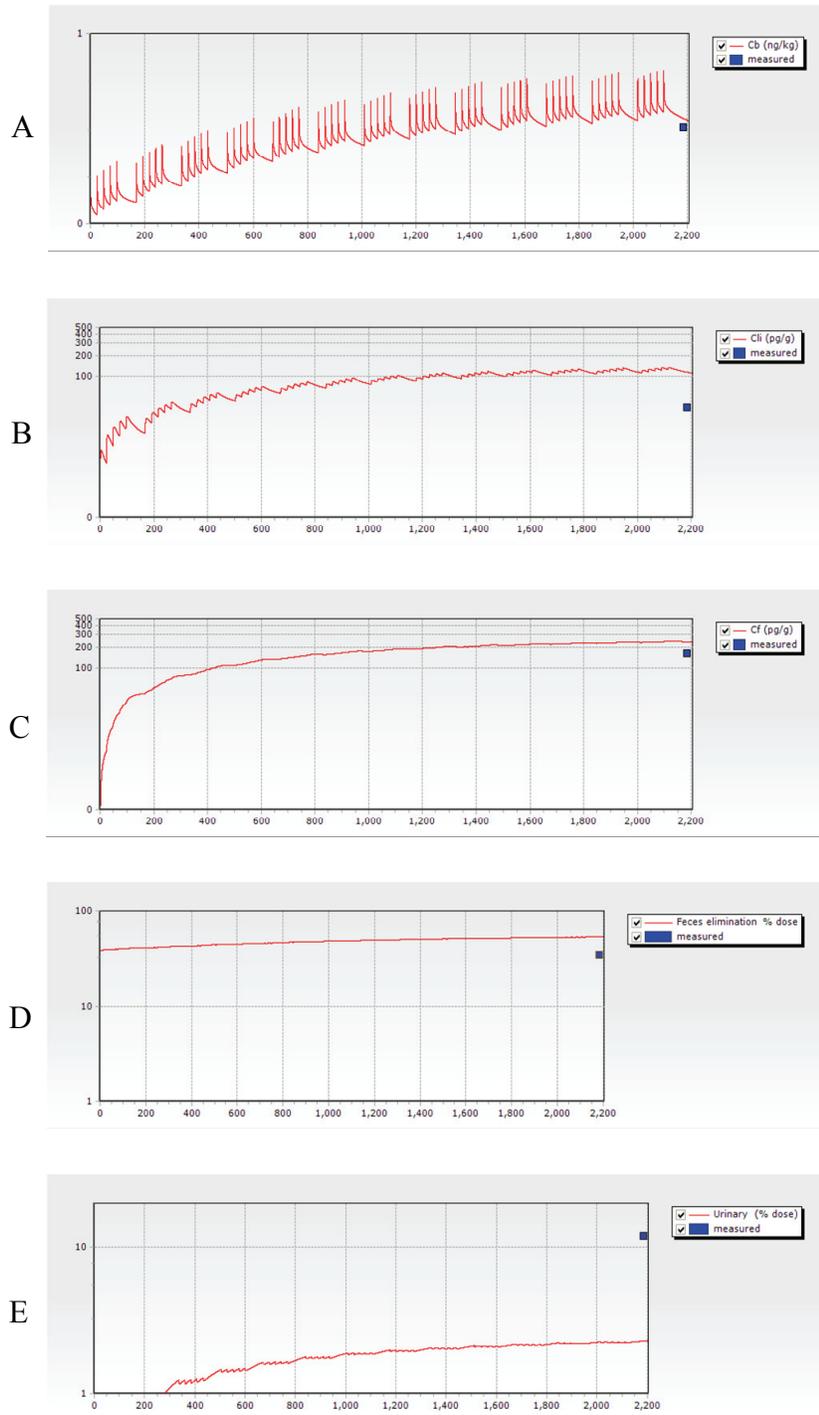
Source: Data obtained from Diliberto et al. (2001, [197238](#)).



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Figure 3-24. 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, [197238](#)).

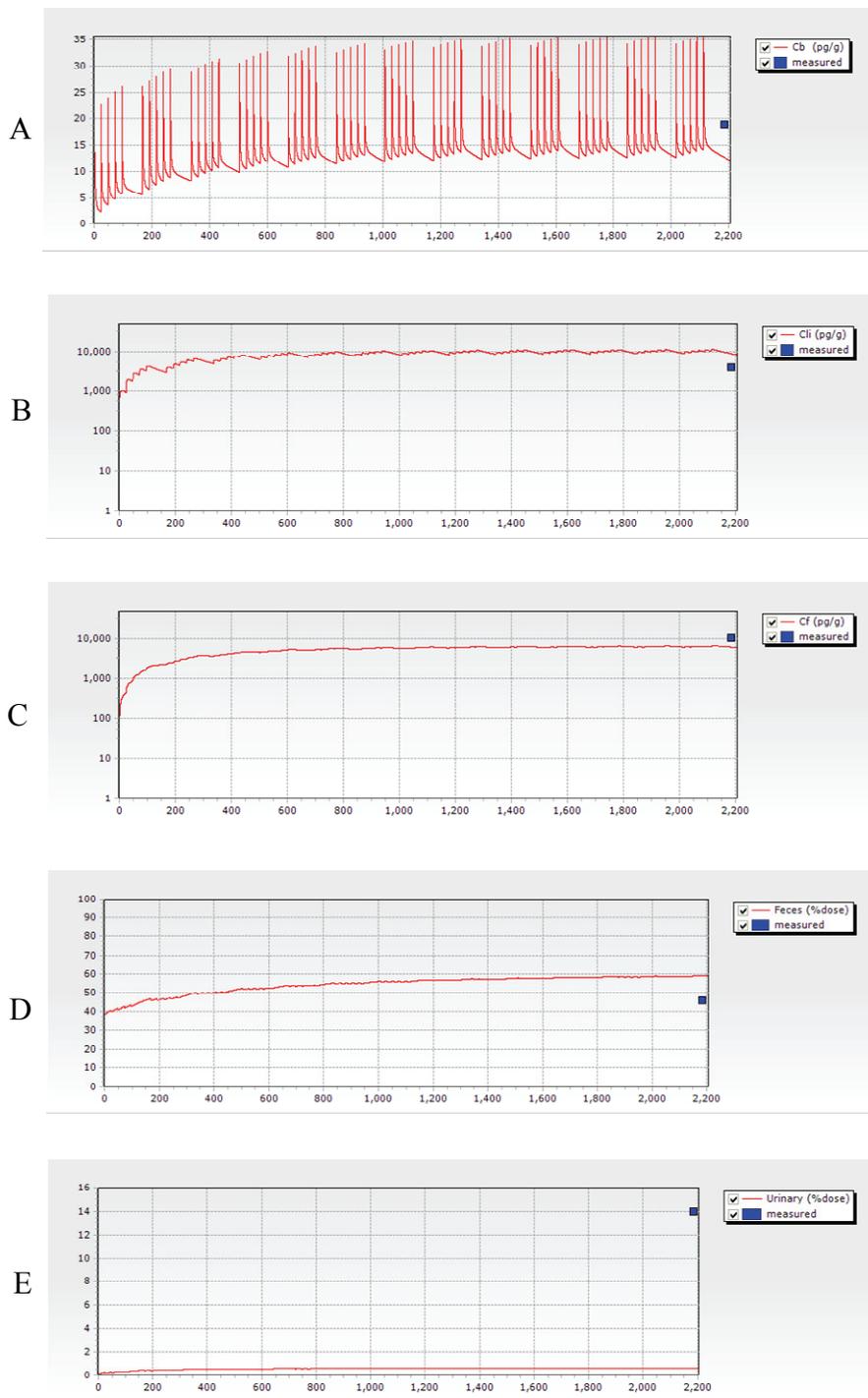


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Figure 3-25. Comparison of experimental data (symbols) and model predictions (solid lines) of (A) blood concentration, (B) liver concentration, (C) adipose tissue concentration (D) feces excretion (% dose) and (E) urinary elimination (% dose) of TCDD 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, [197238](#)).

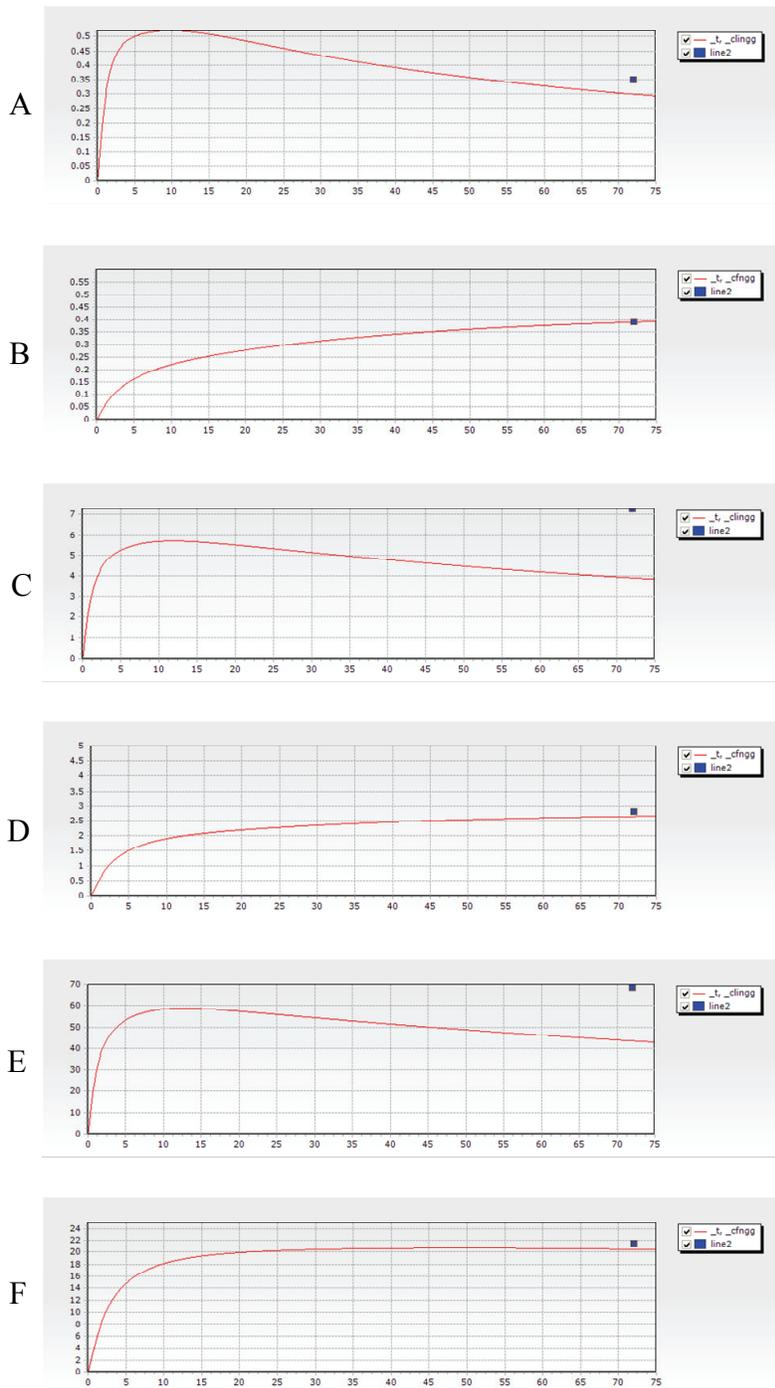
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 2 **Figure 3-26. Comparison of experimental data (symbols) and model**
 3 **predictions (solid lines) of (A) blood concentration, (B) liver concentration,**
 4 **(C) adipose tissue concentration (D) feces excretion (% dose) and (E) urinary**
 5 **elimination (% dose) of TCDD after oral exposure to 150 ng/kg-day,**
 6 **5 days/week for 13 weeks in mice. Y-axis represents concentration in pg/g and**
 7 **X-axis represents time in days.**

8 Source: Experimental data were obtained from Diliberto et al. (2001, [197238](#)).

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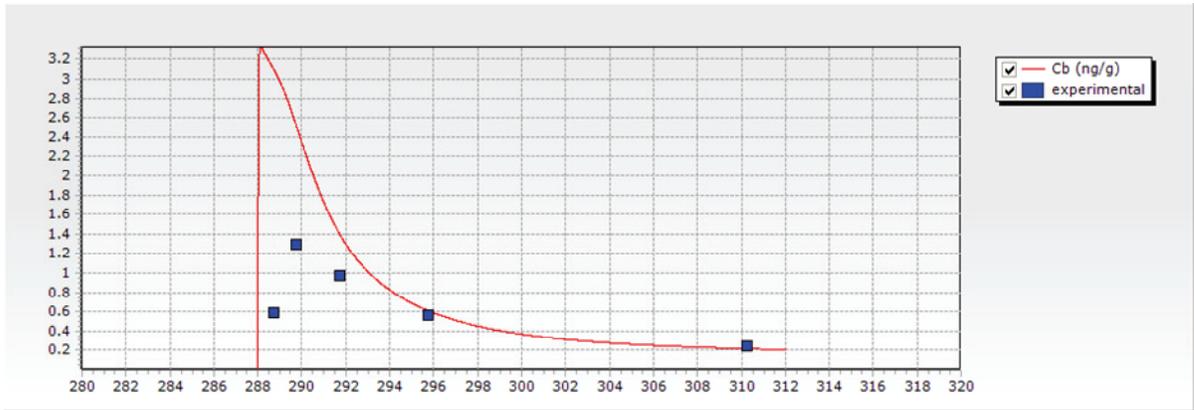


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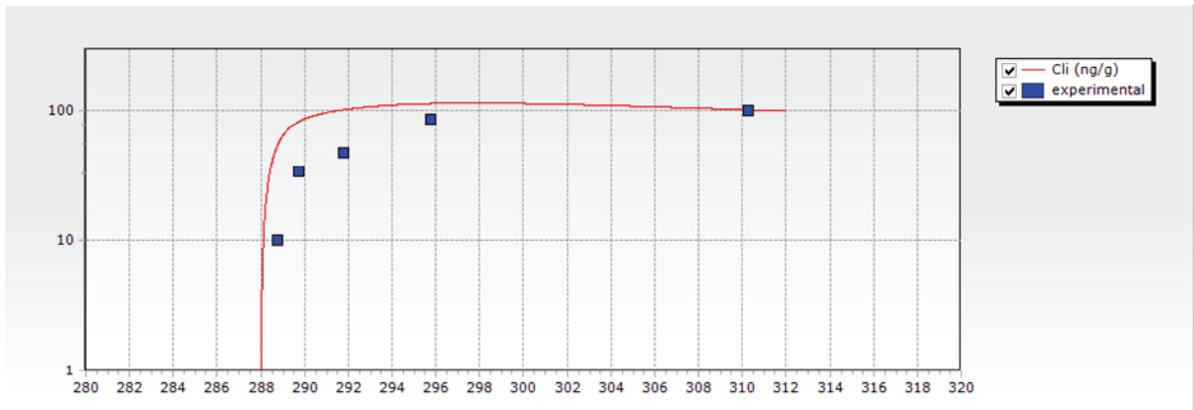
Figure 3-27. PBPK model simulations (solid lines) vs. experimental data (symbols) on the distribution of TCDD after a single acute oral exposure to A–B) 0.1, C–D) 1.0 and E–F) 10 µg of TCDD/kg of body weight in mice. Liver and adipose concentration for each dose was measured after 72 hours. Y-axis represents 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, [594258](#)).

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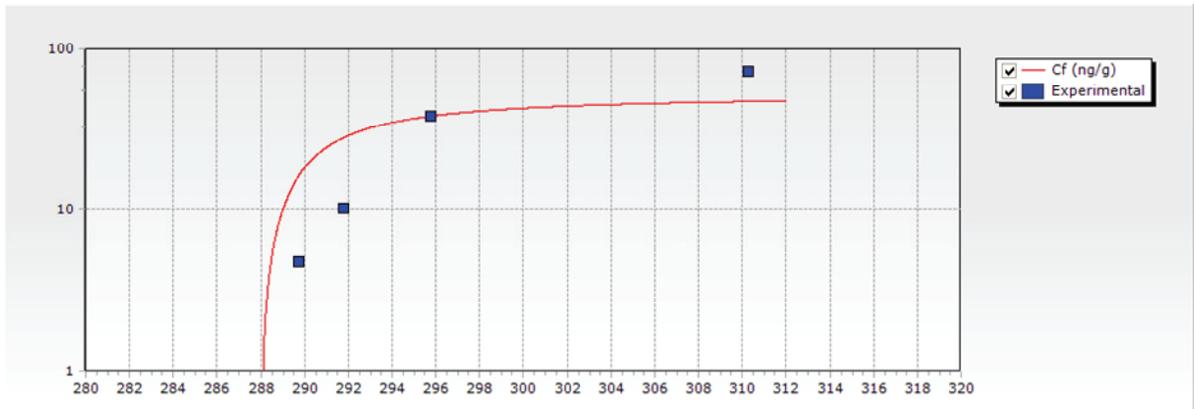
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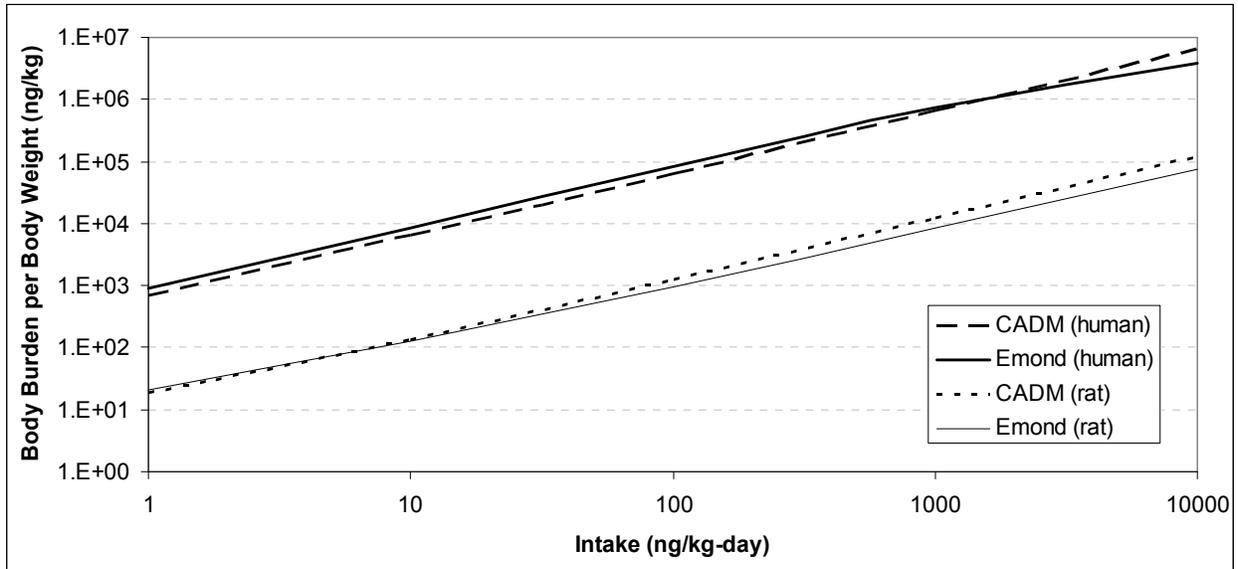
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Figure 3-28. PBPK model simulation (solid lines) vs. experimental data (symbols) on the distribution of TCDD after a single dose of 24 $\mu\text{g}/\text{kgBW}$ on GD 12 in mice. Concentrations expressed as ng TCDD/g tissue. (A) maternal blood, (B) 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, [155093](#)).



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Figure 3-29. Comparison of the near-steady-state body burden simulated with CADM and Emond models for a daily dose ranging from 1 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 age 20 to 30. The time-averaged concentration was used for each.

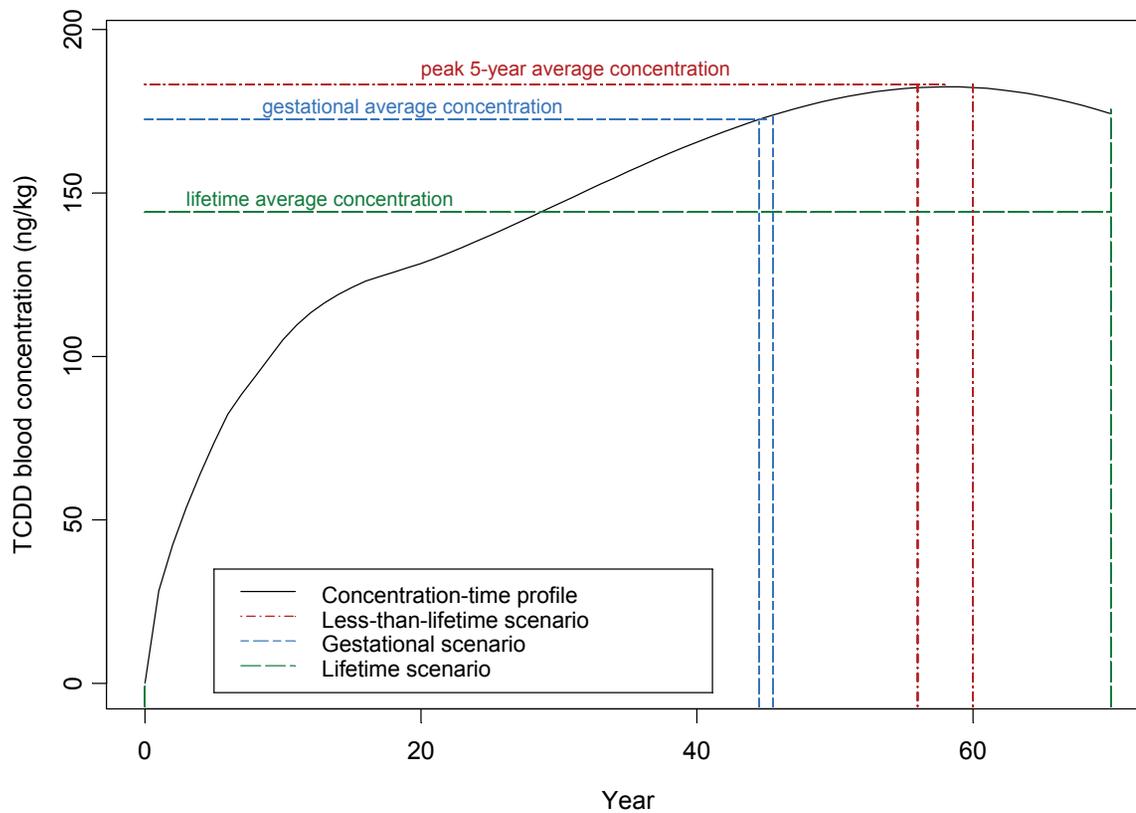


Figure 3-30. TCDD serum concentration-time profile for lifetime, less-than-lifetime and gestational exposure scenarios, with target concentrations shown for each; profiles generated with Emond human PBPK model.

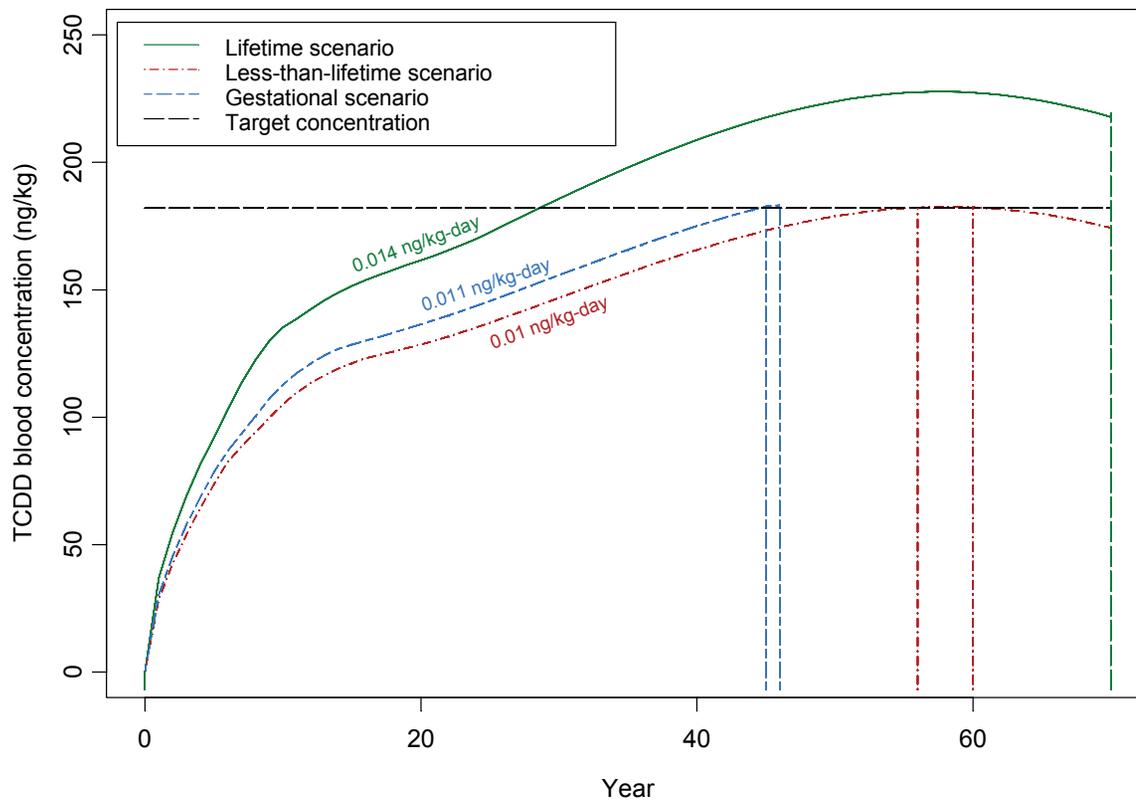


Figure 3-31. TCDD serum concentration-time profile for lifetime, less-than-lifetime and gestational exposure scenarios, showing continuous intake levels to fixed target concentration; profiles generated with Emond human PBPK model.

1 **4. CHRONIC ORAL REFERENCE DOSE**

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4 This section presents U.S. Environmental Protection Agency (EPA)’s response to the
5 National Academy of Sciences (NAS) recommendations that EPA more explicitly discuss the
6 modeling of noncancer endpoints and develop a reference dose (RfD) to address noncancer
7 effects associated with oral 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposures. Section 2
8 details the selection of the animal studies with the lowest TCDD doses associated with the
9 development of adverse noncancer effects and the selection of relevant epidemiologic studies of
10 adverse noncancer health effects. Section 3 discusses the kinetic modeling and estimation of
11 human equivalent daily oral doses that are used in TCDD RfD development in this section. This
12 section discusses the modeling of noncancer health effects data associated with TCDD exposure
13 and the derivation of an RfD. Specifically, Section 4.1 summarizes the NAS comments on
14 TCDD dose-response modeling and EPA’s response, including justification of selected
15 noncancer effects and statistical characterization of modeling results. Section 4.2 presents the
16 TCDD dose-response modeling undertaken for identification of candidate points of departure
17 (PODs) for derivation of an RfD. In Section 4.3, EPA derives an RfD for TCDD. Finally,
18 Section 4.4 describes the qualitative uncertainties in the RfD.

19
20 **4.1. NAS COMMENTS AND EPA’S RESPONSE ON IDENTIFYING NONCANCER**
21 **EFFECTS OBSERVED AT LOWEST DOSES**

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

25
26 With respect to noncancer end points, the committee notes that EPA does not use
27 a rigorous approach for evaluating evidence from studies... (NAS, 2006,
28 [198441](#)p. 47)

29
30 The Reassessment should describe clearly the following aspects:

- 31 1. The effects seen at the lowest body burdens that are the primary focus for any
32 risk assessment—the “critical effects.”
33 2. The modeling strategy used for each noncancer effect, paying particular
34 attention to the critical effects, and the selection of a point of comparison based
35 on the biological significance of the effect; if the ED₀₁ is retained, then the

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1 biological significance of the response should be defined and the precision of
2 the estimate given... (NAS, 2006, [198441](#) p. 187).
3

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

8 For all epidemiologic studies that were identified as suitable for further quantitative
9 dose-response analyses in Section 2.4.3, EPA has chosen to identify PODs (i.e., estimates of a
10 no-observed-adverse-effect level [NOAEL] or lowest-observed-adverse-effect level [LOAEL];
11 modeling of a benchmark dose lower confidence bound [BMDL] was not possible given the data
12 presented in these studies). Figure 4-1 shows EPA's process to select and identify candidate
13 PODs from these key epidemiologic studies. EPA first evaluated the dose-response information
14 in the study to determine whether it provided an estimate of TCDD dose and an observed
15 noncancer effect that was relevant for RfD derivation. If such data were available, then EPA
16 identified a NOAEL or LOAEL as a candidate POD. For each of these, EPA applied a human
17 kinetic model to estimate the continuous oral daily intake (ng/kg-day) associated with the POD
18 that could be used in the derivation of an RfD (see Section 4.2). If all of this information was
19 available, then the result was included as a candidate POD.

20 Figure 4-2 summarizes the strategy employed for identifying and selecting candidate
21 PODs from the key animal bioassays identified in Section 2.4.3 for use in noncancer
22 dose-response analysis of TCDD. For each noncancer endpoint, EPA first evaluated the
23 toxicologic relevance of each endpoint, rejecting those judged not to be relevant for RfD
24 derivation. Next, initial PODs (NOAELs, LOAELs, and BMDLs) based on the first-order body
25 burden metric (see Section 3.3.4.2) and expressed as human-equivalent doses (HEDs) were
26 determined for all relevant endpoints (summarized in Table 4-3). Because there were very few
27 NOAELs and BMDL modeling was largely unsuccessful due to data limitations, the next stage
28 of evaluation was carried out using LOAELs only. Within each study, endpoints not observed at
29 the LOAEL (i.e., reported at higher doses) with BMDLs greater than the LOAEL were
30 eliminated from further analysis, as they would not be considered as candidates for the final POD
31 on either a BMDL or NOAEL/LOAEL basis (i.e., the POD would be higher than the PODs of

1 other relevant endpoints). In addition, all endpoints with HED estimates based on LOAELs
2 (LOAEL_{HEDS}) beyond a 100-fold range of the lowest identified LOAEL_{HED} were eliminated
3 from further consideration, as they would not be potential POD candidates either (i.e., the POD
4 would be higher than the PODs of other relevant endpoints). For the remaining endpoints, EPA
5 then determined final potential PODs (NOAELs, LOAELs and BMDLs) based on TCDD blood
6 concentrations obtained from the Emond rodent physiologically based pharmacokinetic (PBPK)
7 models. HEDs were then estimated for each of these PODs using the Emond human PBPK
8 model. From these HEDs, a POD_{HED} was selected¹⁹ for each study as the basis for the candidate
9 RfD, to which appropriate uncertainty factors (UFs) were applied following EPA guidelines.
10 The resulting candidate RfDs were then considered in the final selection process for the RfD.
11 Other endpoints occurring at slightly higher doses representing additional effects associated with
12 TCDD exposure (beyond the 100-fold LOAEL range) were evaluated, modeled, and included in
13 the final candidate RfD array²⁰ to examine endpoints not evaluated by studies with lower PODs.
14 In addition, BMD modeling based on administered dose was performed on all endpoints for
15 comparison purposes. The final array of selected endpoints is shown in Table 4-4 (summary of
16 BMD analysis) and Table 4-5 (candidate RfDs).

17 The NAS recommended that EPA better justify the selection of response levels for
18 endpoints used to develop risk estimates. The NAS commented on EPA's decision to estimate
19 an ED₀₁ (effective dose eliciting a 1% response) for noncancer bioassay/data set combinations as
20 a comparative tool across studies, suggesting that EPA identify and evaluate the levels of change
21 associated with adverse effects to define the benchmark response (BMR) level for continuous
22 noncancer endpoints.

23

24 The committee notes that the choice of the 1% response level as the POD
25 substantially affects ... the noncancer analyses.... The committee recommends
26 that the Reassessment use levels of change that represent clinical adverse effects
27 to define the BMR level for noncancer continuous end points as the basis for an
28 appropriate POD in the assessment of noncancer effects (NAS, 2006, [198441](#),
29 p. 72).

30

¹⁹In the standard order of consideration: BMDL, NOAEL, and LOAEL.

²⁰However, studies with a lowest dose tested greater than 30 ng/kg-day were not included in the expanded evaluation.

1 The committee concludes that EPA did not adequately justify the use of the
2 1% response level (the ED₀₁) as the POD for analyzing epidemiological or animal
3 bioassay data for ... noncancer effects (NAS, 2006, [198441](#) p. 18).
4

5 In the 2003 Reassessment (U.S. EPA, 2003, [537122](#)), EPA was not attempting to derive
6 an RfD when it conducted TCDD dose-response modeling. The 2003 Reassessment developed
7 ED₀₁ estimates for noncancer effects in an attempt to compare disparate endpoints on a
8 consistent response scale. Importantly, the 2003 Reassessment defined the ED₀₁ as 1% of the
9 maximal response for a given endpoint, not as a 1% change from control. Because RfD
10 derivation is one goal of this document, the noncancer modeling effort undertaken here differs
11 substantially from the modeling in the 2003 Reassessment.

12 The NAS committee was concerned with the statistical power to determine the shape of
13 the dose-response curve at doses far below observed dose-response information. EPA agrees
14 that the shape of the dose-response curve in the low-dose region cannot be determined
15 confidently when based on higher-dose information. An observed response above background
16 near (or below) the BMR level is needed for discrimination of the shape of the curve and for
17 accurate estimation of an ED_x or BMDL. Although many of the ED₀₁s presented in the 2003
18 Reassessment were near the lowest dose tested, responses at the lowest doses were often high
19 and much greater than a 1% response (i.e., 1% of the maximum response). The lack of an
20 observed response near the BMR level is often a problem in interpretation of BMD modeling
21 results.

22 In this document, EPA has used a 10% BMR for dichotomous data for all endpoints;
23 there were no developmental studies that accounted for litter effects, for which a 5% BMR would
24 be used (U.S. EPA, 2000, [052150](#)). For continuous endpoints in this document, EPA has used a
25 BMR of 1 standard deviation from the control mean whenever a specific toxicologically-relevant
26 BMR could not be defined. For the vast majority of continuous endpoints, EPA could not
27 establish unambiguous levels of change representative of adversity, which EPA defines as “a
28 biochemical change, functional impairment, or pathologic lesion that affects the performance of
29 the whole organism, or reduces an organism's ability to respond to an additional environmental
30 challenge” (U.S. EPA, 2009, [192196](#)). For body and organ weight change, EPA has previously
31 established a BMR of 10% change, which also is used in this document.

1 The NAS commented on EPA’s development of ED₀₁ estimates for numerous study/data
2 set combinations in the 2003 Reassessment, suggesting that EPA had not appropriately
3 characterized the statistical confidence around such model predictions in the low-response region
4 of the model.

5
6 It is critical that the model used for determining a POD fits the data well,
7 especially at the lower end of the observed responses. Whenever feasible,
8 mechanistic and statistical information should be used to estimate the shape of the
9 dose-response curve at lower doses. At a minimum, EPA should use rigorous
10 statistical methods to assess model fit and to control and reduce the uncertainty of
11 the POD caused by a poorly fitted model. The overall quality of the study design
12 is also a critical element in deciding which data sets to use for quantitative
13 modeling (NAS, 2006, [198441](#), p. 18).

14
15 EPA should ... assess goodness-of-fit of dose-response models for data sets and
16 provide both upper and lower bounds on central estimates for all statistical
17 estimates. When quantitation is not possible, EPA should clearly state it and
18 explain what would be required to achieve quantitation (NAS, 2006, [198441](#),
19 p. 10).

20
21 The NAS also commented that EPA report information describing the adequacy of
22 dose-response model fits, particularly in the low response region. For those cases where
23 biostatistical modeling was not possible, NAS recommended that EPA identify the reasons.

24
25 The Reassessment should also explicitly address the importance of statistical
26 assessment of model fit at the lower end and the difficulties in such assessments,
27 particularly when using summary data from the literature instead of the raw data,
28 although estimates of the impacts of different choices of models would provide
29 valuable information about the role of this uncertainty in driving the risk estimates
30 (NAS, 2006, [198441](#), p. 73).

31
32 To address this concern, in this document EPA has reported the standard suite of
33 goodness-of-fit measures from the benchmark dose modeling software (BMDS 2.1). These
34 include chi-square *p*-values, Akaike’s Information Criterion (AIC), scaled residuals at each dose
35 level and plots of the fitted models. In some cases, when restricted parameters hit a bound, EPA
36 used likelihood ratio tests to evaluate whether the improvement in fit afforded by estimating
37 additional parameters could be justified. Goodness-of-fit measures are reported for all key data

1 sets in Appendix E. (See Section 4.2.4.2 for a more complete description of the benchmark dose
2 modeling criteria for model evaluation.)

4 4.2. NONCANCER DOSE-RESPONSE ASSESSMENT OF TCDD

5 This section describes EPA’s current effort to conduct an evaluation of TCDD
6 dose-response for the noncancer endpoints from studies that met the study inclusion criteria.
7 Discussions include benchmark dose modeling procedures, kinetic modeling, and POD
8 candidates for derivation of the RfD. Section 4.2.1 discusses the types of endpoints that are
9 considered relevant by EPA’s Integrated Risk Information System and lists the study/endpoint
10 combinations that were not considered for the TCDD RfD derivation, with supporting text in
11 Appendix G. Section 4.2.2 describes how EPA has used physiologically-based pharmacokinetic
12 (PBPK) modeling to estimate effective internal exposures as an alternative to using administered
13 doses or body burdens based on first-order kinetics. Section 4.2.3 details the dose-response
14 analysis of the epidemiologic data, with supporting information on kinetic modeling in
15 Appendix D. Section 4.2.4 details the dose-response analysis for the animal bioassay data;
16 Appendix E provides the BMDS input tables (see Section E.1) and output for all modeling,
17 including blood concentrations (see Section E.2) and administered dose (see Section E.3).

19 4.2.1. Determination of Toxicologically Relevant Endpoints

20 The NAS committee commented on the low dose model predictions and the need to
21 discuss the biological significance of the noncancer health effects modeled in the 2003
22 Reassessment. In selecting POD candidates from the animal bioassays for derivation of the
23 candidate RfDs, EPA had to consider the toxicological relevance of the identified endpoint(s)
24 from any given study. Some endpoints/effects may be sensitive, but lack general toxicological
25 significance due to not being clearly adverse (defined in EPA’s Integrated Risk Information
26 System glossary as “a biochemical change, functional impairment, or pathologic lesion that
27 affects the performance of the whole organism, or reduces an organism’s ability to respond to an
28 additional environmental challenge” (U.S. EPA, 2009, [192196](#))), being an adaptive response or
29 not being clearly linked to downstream functional or pathological alterations. For example, CYP
30 induction alone is not considered a significant toxicological effect given that CYPs are induced
31 as part of the hepatic metabolism of xenobiotic agents. Additionally, the role of CYP induction

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1 in hepatotoxicity and carcinogenicity of TCDD is unknown, thus, CYP induction is not
2 considered a relevant POD without obvious pathological significance. Another example is when
3 all oxidative stress markers are significantly affected, but no other indicators of brain pathology
4 are assessed. In this case, it is impracticable to link the markers of oxidative stress to a
5 toxicological outcome in the brain; thus, this endpoint is not considered a relevant POD
6 candidate. It is standard EPA practice for RfD derivation to base a reference value on endpoints
7 that are adverse or are immediate precursors to an adverse effect.

8 Studies meeting the study selection criteria with endpoints that were not considered for
9 derivation of a candidate RfD (because they were not considered to be toxicologically relevant
10 noncancer effects) are: Kitchin and Woods (1979, [198750](#)), Hassoun et al. (1998, [136626](#); 2000,
11 [197431](#); 2002, [543725](#); 2003, [198726](#)), Burleson et al. (1996, [196998](#)), Kuchiiwa et al. (2002,
12 [198355](#)), Mally and Chipman (2002, [198098](#)), Vanden Heuvel et al. (1994, [197551](#)), Devito
13 et al. (1994, [197278](#)), Lucier et al. (1986, [198398](#)), Sugita-Konishi et al. (2003, [198375](#)), and
14 Sewall et al. (1993, [197889](#)). Appendix G identifies the endpoints from these studies that were
15 not considered to be toxicologically relevant for derivation of an RfD (e.g., cytochrome P450
16 induction, oxidative stress measures, gap junction disruption, mRNA induction, brain serotonin
17 levels) and provides the rationales for the toxicological relevance decisions on the endpoints.
18 Note that for many of these studies, other endpoints were examined that are toxicologically
19 relevant and were considered in the RfD derivation process.

21 **4.2.2. Use of Toxicokinetic Modeling for TCDD Dose-Response Assessment**

22 Given that TCDD accumulates in fat with continuous exposure and is eliminated slowly
23 from the body, but at very different rates across species, EPA has determined that the standard
24 UF approach or allometric scaling of body weight for interspecies extrapolation is not
25 appropriate. Therefore, EPA has decided to use toxicokinetic modeling to estimate an effective
26 internal dose for equivalence across species. The toxicokinetic models chosen by EPA are the
27 rodent and human PBPK models described by Emond et al. (2004, [197315](#); 2006, [197316](#)) and
28 modified by EPA for this assessment as described in Section 3.3.4 (hereafter referred to as the
29 “Emond [rodent or human] PBPK model”). Both the rodent and human models have a
30 gestational component, which allow for more relevant exposure comparisons between general
31 adult exposures and the numerous gestational exposure studies. Ideally, a relevant tissue

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1 concentration for each effect would be estimated. However, no models exist for estimation of all
2 relevant tissue concentrations. As virtually all TCDD is found in the adipose fraction of tissues,
3 or bound to specific proteins, a preferred approach to developing a dose metric would be to
4 account for the fat fraction of each tissue and protein binding; however, EPA has decided that the
5 modeling of such estimates is too uncertain and EPA has not found sufficient data to implement
6 this approach. Therefore, EPA has decided to use the concentration of TCDD in blood as a
7 surrogate for tissue concentrations, assuming that tissue concentrations are proportional to blood
8 concentrations. Furthermore, because the RfD is necessarily expressed in terms of average daily
9 exposure, the blood concentrations are expressed as averages over the relevant period of
10 exposure for each endpoint. For the animal bioassay studies, the relevant period of exposure is
11 the duration of dosing, starting at the age of the animals at the beginning of the study. For
12 humans, the relevant period of exposure is generally lifetime, which is defined as 70 years by
13 convention. However, EPA varied the averaging time for the equivalent human blood
14 concentrations to correspond to the test-animal exposure duration in the following manner.

- 15
- 16 • For correspondence with animal chronic exposures,²¹ the human-equivalent
17 TCDD blood concentration is assumed to be the 70-year average.
 - 18 • For correspondence with animal gestational exposures, the human-equivalent
19 TCDD blood concentration is assumed to be the average over 45 years for a
20 female, beginning at birth, plus 9 months of gestational exposure. The choice of
21 45 years to beginning of pregnancy is health protective of the population in that
22 the TCDD daily oral intake achieving the target blood concentration is smaller
23 than for shorter averaging times.²²
 - 24 • For correspondence with any other animal exposure duration, the
25 human-equivalent TCDD blood concentration is assumed to be the average over
26 the equivalent human exposure duration calculated backward from the peak
27 exposure plateau at or near the end of the 70-year scenario. The average is
28 determined from the terminal end of the human exposure period because the daily
29 oral intake achieving the target blood concentration is smaller than for the same
30 exposure period beginning at birth and is health protective for effects occurring
31 after shorter-term exposure. The determination of equivalent exposure durations
32 across species is problematic and somewhat arbitrary, so EPA uses the average
33 peak blood concentration as the human equivalent for all less-than-chronic animal

²¹Assumed to be $\geq 75\%$ of nominal lifetime, or about 550 days in rodents.

²²See Section 3.3.4.2 for a discussion of this issue, including a comparison of the 45-year old pregnancy scenario to one beginning at age 25 in Table 3-15.

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1 exposures (other than gestational).²³ For the first-order kinetics model, the
2 average peak exposure is close to the theoretical steady-state asymptote (see
3 Section 3.3.4.2). However, for the Emond human PBPK model used by EPA in
4 this assessment, the timing of the peak exposure is dose-dependent and tends to
5 decline after 60 years in some cases. Therefore, the 5-year average TCDD blood
6 concentration that includes the peak (“5-year peak”) is used as the relevant
7 dose-metric for the PBPK model applications.

9 **4.2.3. Noncancer Dose-Response Assessment of Epidemiological Data**

10 The following four epidemiologic studies describing noncancer endpoints were identified
11 in Section 2.4.3 as studies to be evaluated for development of PODs for derivation of candidate
12 RfDs: Baccarelli et al. (2008, [197059](#)), Mocarelli et al. (2008, [199595](#)), Alaluusua et al. (2004,
13 [197142](#)) and Eskenazi et al. (2002, [197168](#)). Each of these studies described effects observed in
14 the Seveso cohort (see detailed study summaries in Section 2.4.1 and Table 2-5). Each study
15 modeled individual-level human exposure measures and provided information from which EPA
16 could determine an exposure window over which kinetic models could be used to quantify
17 TCDD exposures for dose-response assessment. EPA used kinetic information to estimate
18 group-mean daily TCDD intake rates for the exposure groups presented in these studies (see
19 Appendix D for details). EPA focused on identifying NOAELs and LOAELs for these studies;
20 EPA did not conduct Benchmark Dose modeling because the covariates identified by the study
21 authors could not be incorporated by modeling the grouped response data. EPA’s development
22 of PODs for these studies is described in this section and shown in Table 4-1.

24 **4.2.3.1. Baccarelli et al. (2008, [197059](#))**

25 For Baccarelli et al. (2008, [197059](#)), EPA was able to define a LOAEL as the group mean
26 of 39 ppt TCDD in neonatal plasma for thyroid stimulating hormone (TSH) values above
27 5 µU/mL. (See Section 2.4.1.2.1.5.7 for study details.) Baccarelli et al. (2008, [197059](#)) did not
28 estimate the equivalent oral intake associated with TCDD serum concentrations and gave only
29 neonatal serum TCDD concentrations for the groups above and below the 5 µU/mL standard.
30 The study authors, however, developed a regression model relating the level of TSH in 3-day-old

²³By comparison to a half-lifetime equivalent (1 year in rodents, 35 years in humans), the ratio of body burden (1st-order kinetic model) 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).

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1 neonates to TCDD concentrations in maternal plasma at birth (given as lipid-adjusted serum
2 concentrations, LASC). The authors extrapolated maternal plasma concentrations from previous
3 measurements using a simple first-order pharmacokinetic model. Because there is limited
4 information regarding the relationship between maternal and neonatal serum TCDD levels, EPA
5 determined that there was too much uncertainty in estimating maternal intake from neonatal
6 TCDD serum concentrations, directly. Therefore, EPA determined the maternal intake at the
7 LOAEL from the maternal serum-TCDD/TSH regression model by finding the maternal TCDD
8 LASC at which neonatal TSH exceeded 5 $\mu\text{U}/\text{mL}$. EPA then used the Emond PBPK model
9 under the human gestational scenario (see Section 4.2.2) to estimate the continuous daily oral
10 TCDD intake that would result in a TCDD LASC corresponding to a neonatal TSH of 5 $\mu\text{U}/\text{mL}$
11 at the end of gestation; EPA established the resulting maternal intake (0.024 ng/kg-day) as the
12 LOAEL, shown in Table 4-1 as a candidate POD for derivation of candidate RfDs (PBPK
13 modeling details are shown in Appendix D).

14

15 **4.2.3.2. Mocarrelli et al. (2008, [199595](#))**

16 Mocarrelli et al. (2008, [199595](#)) reported decreased sperm concentrations (20%) and
17 decreased motile sperm counts (11%) in men who were 1–9 years old in 1976 at the time of the
18 accident (initial TCDD exposure event) (see Section 2.4.1.2.1.5.8 for study details). Men who
19 were 10–17 years old in 1976 were not adversely affected. Serum (LASC) TCDD levels were
20 measured within one year of the initial exposure. Serum TCDD levels and corresponding
21 responses were reported by quartile, with a reference group of less-exposed individuals assigned
22 a TCDD LASC value of 15 ppt (which was the mean of individuals outside the contaminated
23 area). The lowest exposed group mean was 68 ppt (1st quartile). Because effects were detected
24 only among boys under the age of 10, EPA assumes there is a maximum 10-year critical
25 exposure window for elicitation of these effects. However, for the exposure profile, with a high
26 initial pulse followed by an extended period of elimination with only background exposure, the
27 estimation of an average exposure resulting in the effect is problematic. Therefore, EPA
28 implemented a procedure for the estimation of the continuous daily TCDD intake associated with
29 the LOAEL in the Mocarrelli et al. (2008, [199595](#)) study using the following 5-step process:

30

- 1 1. Using the Emond human PBPK model, the initial (peak) blood TCDD concentrations
2 associated with the accident were back-calculated based on the time that had elapsed
3 between the explosion and the serum collection. As serum measurements were taken
4 within 1 year after the event, a lag time of 0.5 years was assumed.
- 5 2. The oral exposure associated with the peak blood TCDD concentration (peak exposure)
6 was calculated using the Emond PBPK model.
- 7 3. Starting with the peak exposure and accounting for background TCDD intake, the
8 average daily blood TCDD concentration experienced by a representative individual in
9 the susceptible population (boys under 10 years old) was estimated using the Emond
10 PBPK model. Assuming a uniform distribution of subject ages at the time of the event,
11 the average age of the exposed male children would be 5 years. Consequently, a critical
12 exposure window for the cohort was estimated to be, on average, 5 years (i.e., a boy aged
13 5 years would remain in this exposure window for 5 more years until he was 10 years of
14 age).
- 15 4. Using the Emond PBPK model, the average daily TCDD intake rate needed to attain the
16 5-year average blood TCDD concentration in a boy 10 years old was calculated.
- 17 5. The LOAEL POD was calculated as the average of the peak exposure (0.032 ng/kg-day)
18 and the 5-year average exposure (0.0080 ng/kg-day), resulting in LOAEL of
19 0.020 ng/kg-day, shown in Table 4-1 as a candidate POD for derivation of a candidate
20 RfD. However, neither of the extremes was used because (1) the peak exposure does not
21 account for the continuing internal exposure from TCDD given its slow elimination, and
22 (2) the 5-year average does not reflect the influence of the much higher peak exposure,
23 which may be a significant factor in TCDD toxicity (Kim et al., 2003, [199146](#)).

24
25 The PBPK modeling details are shown in Appendix D.

26 27 **4.2.3.3. *Alaluusua et al. (2004, [197142](#))***

28 For Alaluusua et al. (2004, [197142](#)), the approach for estimation of daily oral TCDD
29 intake is virtually identical to the approach used for the Mocarelli et al. (2008, [199595](#)) data.
30 (See Section 2.4.1.2.1.5.5 for study details.) Alaluusua et al. (2004, [197142](#)) reported dental
31 effects in male and female adults who were less than 5 years of age at the time of the initial
32 exposure (1976). For the 75 boys and girls who were less than 5 years old at the time of the
33 accident, 25 (33%) were subsequently diagnosed with some form of dental enamel defect. For
34 the 38 individuals who were older than 5, only 2 (5.3%) suffered dental enamel defects at a later
35 date. A window of susceptibility of approximately 5 years is established. Serum measurements
36 for this cohort were taken within a year of the accident. Serum TCDD levels and corresponding
37 responses were reported by tertile, with a reference group of less-exposed individuals assigned a

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1 TCDD LASC value of 15 ppt (ng/kg); the tertile group means were 130, 383, and 1,830 ppt.
2 The incidence of dental effects for the reference group was 26% (10/39). The incidence of
3 dental effects in the 1st, 2nd and 3rd tertile exposure groups was 10% (1/10), 45% (5/11) and
4 60% (9/15), respectively. EPA judged that the NOAEL and LOAEL were 130 and 383 ppt
5 TCDD in serum. Following the same procedure used for the Mocarelli et al. (2008, [199595](#))
6 study (see Section 4.2.3.2), EPA estimated the continuous daily human oral TCDD intake
7 associated with each of the tertiles for both peak and average exposure across the critical
8 exposure window, assuming that the average age of the susceptible cohort at the time of the
9 accident was 2.5 years. Separate estimates for boys and girls were developed based on both the
10 peak intake and average intake across the critical exposure window (PBPK modeling details are
11 shown in Appendix D). The estimated averaged daily oral intakes for the tertiles, averaged for
12 boys and girls, are 0.20, 1.7, and 30 ng/kg-day for the peak exposure and 0.033, 0.15 and
13 1.5 ng/kg-day for the critical exposure window average. A study NOAEL at the second tertile of
14 0.12 ng/kg-day was identified as a candidate POD for derivation of a candidate RfD in Table 4-1.
15

16 **4.2.3.4. *Eskenazi et al. (2002, [197168](#))***

17 The approach used to estimate daily TCDD intake in Eskenazi et al. (2002, [197168](#))
18 combines the approaches EPA used for Baccarelli et al. (2008, [197059](#)), Mocarelli et al. (2008,
19 [199595](#)) and Alaluusua et al. (2004, [197142](#)). Eskenazi et al. (2002, [197168](#)) reported menstrual
20 effects in female adults who were premenarcheal in 1976 at the time of the initial exposure (see
21 Section 2.4.1.2.1.4.1 for study details). In Rigon et al. (2009), the median age at menarche was
22 shown to be 12.4 in Italian females with intergenerational decreases in age at menarche. Thus,
23 EPA established a window of susceptibility of approximately 13 years for this analysis. The
24 average age of the premenarcheal girls at the time of the initial exposure in 1976 was 6.8 years,
25 establishing an average critical-window exposure duration of 6.2 years for this cohort. Serum
26 samples were collected within a year of the accident from this cohort. However, serum TCDD
27 levels and corresponding responses were not reported by percentile and no internal reference
28 group was identified. As for Baccarelli et al. (2008, [197059](#)), Eskenazi et al. (2002, [197168](#))
29 developed a regression model relating menstrual cycle length to plasma TCDD concentrations
30 (LASC) measured in 1976. The model estimated that menstrual cycle length was increased
31 0.93 days for each 10-fold increase in TCDD LASC, with a 95% confidence interval of -0.01 to

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1 1.86 days. EPA judged a 1-day increase in menstrual cycle length to be adverse; a normal
2 menstrual cycle length is 28 days. EPA then determined the 1976 TCDD serum level
3 corresponding to a 29-day menstrual cycle length in the exposed cohort from the regression
4 model developed by Eskenazi et al. (2002, [197168](#)). Using this serum level, the peak initial
5 exposure and average exposure over the 6.2 year window were calculated using the Emond
6 human PBPK model, in the same manner as for Mocarelli et al. (2008, [199595](#)) and Alaluusua
7 et al. (2004, [197142](#)). The resulting peak TCDD intake is 3.2 ng/kg-day. The average exposure
8 experienced by this cohort over the critical exposure window is estimated to be 0.12 ng/kg-day.
9 The average of these two estimates is 1.64 ng/kg-day, which is designated as a LOAEL and
10 shown in Table 4-1. Because the LOAEL is almost 2 orders of magnitude higher than the
11 LOAELs for Baccarelli et al. (2008, [197059](#)) and Mocarelli et al. (2008, [199595](#)), it was not
12 considered further as a candidate POD for derivation of the RfD (PBPK modeling details are
13 shown in Appendix D).

14

15 **4.2.4. Noncancer Dose-Response Assessment of Animal Bioassay Data**

16 EPA followed the strategy illustrated in Figure 4-2 to evaluate the animal bioassay data
17 for TCDD dose-response. For the administered average daily doses (ng/kg-day) in each animal
18 bioassay, EPA identified NOAELs and/or LOAELs based on the original data presented by the
19 study author. Section 2.4.2 identifies these values in the study summaries and in Table 2-7.
20 These became candidate PODs for consideration in the derivation of an RfD for TCDD. The
21 candidate RfD values associated with these candidate PODs are presented in Table 4-5.
22 Additional PODs were identified using BMD modeling. All PODs were converted to HEDs
23 using the Emond PBPK models. The remainder of this Section describes the steps in this process
24 and concludes with the POD candidates from the animal bioassay data that were considered for
25 derivation of the RfD.

26

27 **4.2.4.1. Use of Kinetic Modeling for Animal Bioassay Data**

28 Blood concentrations corresponding to the administered doses in each mouse or rat
29 bioassay qualifying as a final RfD POD candidate were estimated using the appropriate Emond
30 rodent PBPK model. In each case, the simulation was performed using the exposure and
31 observation durations, body weights, and average daily doses from the original studies. For all

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1 multiple exposure protocols, the time-weighted average blood TCDD concentrations over the
2 exposure period were used as the relevant dose metric. For single (gestational and
3 nongestational) exposures, the initial peak blood TCDD concentrations were considered to be the
4 most relevant exposure metric. Gestational exposures were modeled using the species-specific
5 gestational component of the Emond rodent PBPK model. Bioassays employing exposure
6 protocols spanning gestational and postpartum life stages were modeled by sequential
7 application of the gestational and nongestational models.

8 The Emond PBPK models do not contain a lactation component, so exposure during
9 lactation was not modeled explicitly. Only one bioassay (Shi et al., 2007, [198147](#)) considered as
10 a POD candidate for RfD derivation included exposure during lactation. In Shi et al. (2007,
11 [198147](#)) pregnant animals were exposed weekly to TCDD throughout gestation and lactation.
12 Exposure was continued in the offspring following weaning for 10 months. For assessment of
13 maternal effects, the Emond gestational model was used, terminating at parturition. For
14 assessment of long-term exposure in the offspring, the Emond nongestational model was used,
15 ignoring prior gestational and lactational exposure, with the assumption that the total exposure
16 during these periods was small relative to exposure in the following 10 months. The assumption
17 is conservative in that effects observed in the offspring would be attributed entirely to adult
18 exposure, which is somewhat less than the actual total exposure.

19 The model code, input files and PBPK modeling results for each bioassay are reported in
20 Appendix C. These predicted TCDD blood concentrations were used for benchmark dose
21 modeling of bioassay response data and determination of NOAELs and LOAELs. BMD
22 modeling was performed, as described in Section 3.5.2.2.1, by substituting the modeled blood
23 concentrations for the administered doses and calculating the corresponding BMDL. For each of
24 these LOAEL, NOAEL, or BMDL blood-concentration equivalents, corresponding HEDs were
25 calculated using the Emond human PBPK model for the appropriate gestational or nongestational
26 scenario as described previously (see Section 4.2.2).

28 **4.2.4.2. Benchmark Dose Modeling of the Animal Bioassay Data**

29 Benchmark dose modeling was performed using BMDS 2.1, Build 06/16/09 to estimate
30 BMDs and BMDLs for each study/endpoint combination. The input data tables for these
31 noncancer studies are shown in Appendix E, Section E1, including both administered doses

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1 (ng/kg-day) and blood concentrations (ng/kg) and either incidence data for the dichotomous
2 endpoints or mean and standard deviations for the continuous endpoints. (See Section 4.2.4.1
3 and Sections 3.3.4 and 3.3.5 for a description of the development of TCDD blood concentrations
4 using kinetic modeling.)

5 Evaluation of BMD modeling performance, goodness-of-fit, dose-response data, and
6 resulting BMD and BMDL estimates included statistical criteria as well as expert judgment of
7 their statistical and toxicological properties. For the continuous endpoints, all available models
8 were run separately using both the assumption of constant variance and the assumption of
9 modeled variance. Saturated (0 degrees of freedom) model fits were rejected from consideration.
10 Parameters in models with power or slope parameters were constrained to prevent supralinear
11 fits, which EPA considers not to be biologically plausible and which often have undesirable
12 statistical properties (i.e., the BMDL diverges towards zero). However, if the constrained
13 parameters were estimated at their lower bounds, the unrestricted model was fit to the data,
14 primarily for elucidation of the degree of supralinearity present in the data. Depending on the
15 latter and the magnitude of the BMDL relative to the BMD, unrestricted model fits were
16 occasionally deemed acceptable. Table 4-2 shows each model and any restrictions imposed.

17 For the quantal/dichotomous endpoints, all primary BMDS dichotomous models were
18 run. The alternative dichotomous models were fit to several data sets, but the results were very
19 sensitive to the assumed independent background response and the fits were not accepted. The
20 confidence level was set to 95% and all initial parameter values were set to their defaults in
21 BMDS. For the continuous endpoints, one standard deviation was chosen as the default for the
22 BMR when a specific toxicologically-relevant BMR could not be defined. For the dichotomous
23 endpoints, a BMR of 10% extra risk was used for all endpoints.²⁴

24 The model output tables in Appendix E show all of the models that were run, both
25 restricted and unrestricted, goodness-of-fit statistics, BMD and BMDL estimates, and whether
26 bounds were hit for constrained parameters. After all models were run, the one giving the best
27 fit was selected using the selection criteria in the current BMDS draft guidance (U.S. EPA, 2000,
28 [052150](#)) where possible. Acceptable model fits were those with chi-square goodness-of-fit
29 *p*-values greater than 0.1. For continuous endpoints, a preference was held for models with an
30 asymptote term (plateau for high-dose response) because continuous measures do not continue to

²⁴There were no developmental studies that accounted for litter effects, for which a 5% BMR would be used.

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1 rise (or fall) with dose forever; this phenomenon is particularly evident for TCDD. Unbounded
2 models, such as the power model, must account for the plateauing effect entirely in the shape
3 parameter, generally resulting in an abnormally supralinear fit. Also, for the continuous
4 endpoints, the p -value for the homogenous variance test (Test 2) was used to determine whether
5 constant variance ($p > 0.1$) or modeled (nonconstant) variance ($p < 0.1$) should be used. As
6 BMDS offers only one variance model, model fits for nonconstant variance models were not
7 necessarily rejected if the variance model did not fit well (Test 3 p -value < 0.05). Within the
8 group of models with acceptable fits, the selected model was generally the one with the lowest
9 BMDL, unless the AIC was much higher (ca. +2) than another model. However, particularly for
10 continuous models, the fit of the model to the control mean and standard deviation and in the
11 lower response range was assessed. Models with higher BMDLs or AICs but much better fit to
12 the lower response data were often chosen over the nominally best-fitting model.

13 For many data sets, no models satisfied the acceptance criteria and no clear BMD/BMDL
14 selection could be made. In these cases, model fits were examined on an individual basis to
15 determine the reasons for the poor fits. On occasion, high doses were dropped and the models
16 were refit. Also, if a poor fit to the control mean was evident, the model was refit to the data
17 after fixing the control mean by specifying the relevant parameter in BMDS. However, these
18 techniques rarely resulted in better fits. If the fit was still not acceptable, the NOAEL/LOAEL
19 approach was applied to the study/data set combination. Most of the problems with BMD
20 modeling were a consequence of lack of response data near the BMR; many of the TCDD data
21 sets failed to show a response near the BMR, whether it was a 10% dichotomous relative change
22 or a continuous 1 standard deviation change. Responses at the lowest doses were generally much
23 higher than the BMR, resulting in a lack of anchoring at the critical response levels of interest
24 causing numerical problems in the estimation of BMDLs.

25

26 **4.2.4.3. *POD Candidates from Animal Bioassays Based on HED and BMD Modeling Results***

27 Table 4-3 summarizes the PODs that EPA estimated for each key animal study included
28 for TCDD noncancer dose-response modeling. After estimating the blood TCDD concentration
29 associated with a particular toxicity measure (NOAEL, LOAEL, or BMDL) obtained from a
30 rodent bioassay, EPA estimated a corresponding HED using the Emond human PBPK model
31 (described in Section 3). Table 4-3 summarizes the NOAEL, LOAEL, or BMDL (ng/kg) based

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1 on the administered animal doses for each key bioassay/data set combination. Table 4-3 also
2 summarizes the continuous daily HED corresponding to these administered doses as 1st order
3 body burdens and as blood concentrations. The doses in Table 4-3 are defined as follows, all in
4 units of ng/kg-day:
5

- 6 • Administered Dose NOAEL: Average daily dose defining the NOAEL for the test species
7 in the animal bioassay
- 8 • Administered Dose LOAEL: Average daily dose defining the LOAEL for the test species
9 in the animal bioassay
- 10 • Administered Dose BMDL: BMDL for the test species based on modeling of the
11 administered doses from the animal bioassay
- 12 • First-Order Body Burden HED NOAEL: Average daily dose defining the NOAEL for
13 humans derived from the animal bioassay using the first-order kinetics body-burden
14 model
- 15 • First-Order Body Burden HED LOAEL: Average daily dose defining the LOAEL for
16 humans derived from the animal bioassay using the first-order kinetics body-burden
17 model
- 18 • First-Order Body Burden HED BMDL: Human-equivalent BMDL from BMD modeling
19 of the animal bioassay data using first-order body burdens
- 20 • Blood Concentration HED NOAEL: Average daily dose defining the NOAEL for
21 humans derived from the animal bioassay using the Emond human PBPK model
- 22 • Blood Concentration HED LOAEL: Average daily dose defining the LOAEL for humans
23 derived from the animal bioassay using the Emond human PBPK model
- 24 • Blood Concentration HED BMDL: Human-equivalent BMDL from BMD modeling of
25 the animal bioassay data using the Emond human PBPK model

26
27 An evaluation of key BMD analyses is presented in Table 4-4. Tables showing the best
28 model fit for each study/endpoint combination and the associated BMD/BMDL are shown in
29 Appendix E. As described above in Section 4.3.4.2, the BMD modeling was largely
30 unsuccessful, primarily because of a lack of response data near the BMR, poor modeled
31 representation of control values, or nonmonotonic responses yielding poor fits. The comments
32 column in Table 4-4 lists reasons for poor results.
33

1 **4.3. RfD DERIVATION**

2 Table 4-5 lists all the studies and endpoints considered for derivation of the RfD. These
3 studies were chosen from the entire list of candidate study/data set combinations (see
4 Section 2.4.3) based on the toxicologic relevance of the endpoints and covering a range of the
5 most conservative RfD candidates that includes three of the four human studies.²⁵ Figure 4-3
6 (exposure-response array) shows all of the endpoints listed in Table 4-5 graphically in terms of
7 PODs in human-equivalent intake units (ng/kg-day). The human study endpoints are shown at
8 the far left of the figure and the rodent endpoints are arranged by category to the right. (Note the
9 two studies in guinea pigs were estimated using first-order body burden kinetics which are not
10 directly comparable to the PODs based on the mouse, rat and human studies that were generated
11 from the Emond PBPK model. There are no published models for TCDD disposition in guinea
12 pigs and EPA did not develop one for this assessment.) Figure 4-4 demonstrates the same
13 endpoints, arrayed by RfD value, showing the POD, applicable UFs and candidate RfD.

14 Table 4-5 illustrates the study, species, strain and sex, study protocol, and toxicologic
15 endpoints observed at the lowest TCDD doses. The table also identifies the human-equivalent
16 BMDLs (when applicable), NOAELs and LOAELs, as well as the composite UF that applies to
17 the specific endpoint, and finally, the corresponding candidate RfD.²⁶ The NOAELs, LOAELs,
18 and BMDLs are presented as HEDs, based on the assumption that blood concentration is the
19 toxicokinetically-equivalent TCDD dose metric across species and serves as a surrogate for
20 tissue concentration.²⁷ For rats and mice, these estimates relied on the two Emond PBPK
21 models—one for the relevant rodent species and one for the human—as described previously
22 (see Section 3.3.4.3). The two guinea pig studies that are included in Table 4-5 are given in
23 HED units based on the first-order body burden model described in Section 3.3.4.2; there is
24 currently no TCDD PBPK model for the guinea pig. The values listed for guinea pigs are not
25 directly comparable to those for rats and mice but are probably biased low, as first-order body
26 burden HED estimates for rats and mice are generally 2- to 5-fold lower than the corresponding
27 PBPK model estimates. The LOAELs for the human studies also rely on the Emond PBPK
28 model, as described in Sections 4.2.2 and 4.2.3.

²⁵The RfD derived from the study of Eskenazi et al. (2002, [197168](#)) was outside the RfD range presented in Table 4-5.

²⁶Extra significant digits are retained for comparison prior to rounding to one significant digit for the final RfD.

²⁷The procedures for estimating HEDs based on TCDD blood concentration are described in the preceding section.

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1 As is evident from the Table 4-5, very few NOAELs and even fewer BMDLs have been
2 established for low-dose TCDD studies. BMD modeling was unsuccessful for all of the
3 endpoints without a NOAEL, primarily because of the lack of dose-response data near the BMR
4 (see discussion in Section 4.2). Therefore, the RfD assessment rests largely on evaluation of
5 LOAELs to determine the POD.

6 The rows in Table 4-5 are arranged in order of increasing candidate RfD magnitude.
7 Endpoints projected to occur at higher exposure levels are still considered for qualitative support
8 of the effects shown in Table 4-5.

10 **4.3.1. Toxicological Endpoints**

11 As can be seen in Table 4-5, a wide array of toxicological endpoints has been observed
12 following TCDD exposure, ranging from subtle developmental effects to overt chronic liver
13 toxicity. Developmental effects in rodents include dental defects, delayed puberty in males, and
14 several neurobehavioral effects. Reproductive effects reported in rodents include altered
15 hormone levels in females and decreased sperm production in males. Immunotoxicity endpoints
16 such as decreased response to SRBC challenge in mice and decreased delayed-type
17 hypersensitivity response in guinea pigs are also observed. Longer durations of TCDD exposure
18 in rodents elicit results such as organ and body weight changes, renal toxicity, and liver and lung
19 lesions. Adverse effects in human studies are also observed, which include male reproductive
20 effects, increased TSH in neonates, and dental defects in children. Analogous results have been
21 observed in animal bioassays for each of these human endpoints.

22 All but two of the study/endpoint combinations from animal bioassays listed in Table 4-5
23 are on TCDD-induced toxicity observed in mice and rats; the other two study/endpoint
24 combinations are effects in guinea pigs. Although the effects of TCDD have been investigated in
25 several other species (i.e., hamsters, monkeys, and mink), those studies were not included for
26 final POD consideration because the effect levels were greater than those in Table 4-5, or
27 because the effects could not be attributed solely to TCDD exposure (i.e., confounding by
28 dioxin-like compounds [DLCs]).

29 Three human studies were also included for final POD consideration in the derivation of
30 an RfD and are presented in Table 4-5 as candidate RfDs. All three human study/endpoint
31 combinations are from studies on the Seveso cohort. The developmental effects observed in

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1 these studies were associated with TCDD exposures either in utero or in early childhood between
2 1 and 10 years of age. Baccarelli et al. (2008, [197059](#)) reported increased levels of TSH in
3 newborns exposed to TCDD in utero, indicating a possible dysregulation of thyroid hormone
4 metabolism. Mocarelli et al. (2008, [199595](#)) reported decreased sperm concentrations and
5 decreased motile sperm counts in men who were 1–9 years old in 1976 at the time of the Seveso
6 accident (initial TCDD exposure event). Alaluusua et al. (2004, [197142](#)) reported dental effects
7 in adults who were less than 9.5 years of age at the time of the initial exposure (1976).

9 **4.3.2. Exposure Protocols of Candidate PODs**

10 The studies in Table 4-5 represent a wide variety of exposure protocols, involving
11 different methods of administration and exposure patterns across virtually all exposure durations
12 and life stages. Both dietary and gavage administration have been used in rodent studies, with
13 gavage being the predominant method. Gavage dosing protocols vary quite widely and include
14 single gestational exposures, multiple daily exposures (for up to 2 weeks, intermittent schedules
15 that include 5 days/week, once weekly, or once every 2 weeks), and loading/maintenance dose
16 protocols, in which a relatively high dose is initially administered followed by lower weekly
17 doses. The intermittent dosing schedules require dose-averaging over time periods as long as
18 2 weeks, which introduces uncertainty in the effective exposures. In other words, the high unit
19 dose may be more of a factor in eliciting the effect than the average TCDD tissue levels over
20 time. Although the loading/maintenance dose protocols are designed to maintain a constant
21 internal exposure, these protocols are somewhat inconsistent with the constant daily TCDD
22 dietary exposures associated with human ingestion patterns.

23 The epidemiologic studies conducted in the Seveso cohort represent exposures over
24 different life stages including gestation, childhood, and young adulthood. The Seveso exposure
25 profile is essentially a high initial pulse TCDD exposure followed by a 20–30 year period of
26 elimination. Effects are realized, or measured, 10–20 years following the initial exposure; the
27 critical exposure window for susceptibility varies with effect and is often unknown. Therefore,
28 the effective exposure profiles for the Seveso cohort studies vary considerably. For the
29 Mocarelli et al. (2008, [199595](#)) and Alaluusua et al. (2004, [197142](#)) studies where early
30 childhood exposures proximate to the initial event are associated with the outcomes, there is
31 some uncertainty as to the magnitude of the effective doses. Although the effects are associated

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1 with TCDD exposure in the first 10 years of life, it is not clear to what extent the initial peak
2 exposure is primarily responsible for the effects. It is also not clear if averaging exposure over
3 the critical window is appropriate given the large difference between initial TCDD body burden
4 and body burden at the end of the critical exposure window. The LOAELs for both Mocarelli
5 et al. (2008, [199595](#)) and Alaluusua et al. (2004, [197142](#)) are calculated as the average of the
6 peak exposure and average exposure across the critical exposure window (see Section 4.2 for
7 details).

8 For the gestational exposure study (Baccarelli et al., 2008, [197059](#)), the critical exposure
9 window is strictly defined and relatively short (9 months) and occurs long after the initial
10 exposure (15–20 years). In addition, the maternal serum TCDD concentrations were measured
11 10–15 years after the initial exposure and are proximate to the actual pregnancies; consequently,
12 there is less uncertainty in the kinetic extrapolation between time of measurement and time of
13 birth (i.e., the critical exposure window). The narrow critical exposure window at a much later
14 time than the initial exposure (where the TCDD elimination curve is flattening) is assumed to
15 lead to a relatively steady-state exposure over the critical time period with much less uncertainty
16 in the magnitude of the effective dose. With the exception of Eskenazi et al. (2002, [197168](#)) (see
17 Section 4.2), the effective doses for other effects reported for the Seveso cohort (see
18 Section 2.4.1.1.1.4) have not been quantified and are not represented in Table 4-5 because no
19 critical exposure windows can be identified or individual exposure estimates were not reported.

21 **4.3.3. Uncertainty Factors (UFs)**

22 The UF column in Table 4-5 shows the composite (total) UF that would be applied to the
23 POD for each endpoint. For the animal bioassays, a UF of 3 for the toxicodynamic component
24 of the interspecies extrapolation factor (UF_A) was applied to all PODs. For both animal and
25 human studies, when a NOAEL was used as the POD, a factor of 10 was applied for human
26 interindividual variability (UF_H). For all of the animal bioassay endpoints lacking a NOAEL, a
27 UF of 10 for the LOAEL-to-NOAEL UF (UF_L) was included. For the human LOAELs, a UF_L of
28 3 was applied because sensitive populations were identified. A subchronic-to-chronic UF (UF_S)
29 of 1 and a database factor (UF_D) of 1 are applied to all endpoints. A rationale for each UF is
30 provided for the derivation of the RfD below.

4.3.4. Choice of Human Studies for RfD Derivation

For selection of the POD, the human studies are given the highest consideration, as quality human data are always preferred by the EPA to animal data of comparable quality. The human studies included in Table 4-5 (Alaluusua et al., 2004, [197142](#); Baccarelli et al., 2008, [197059](#); Mocarelli et al., 2008, [199595](#)) 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, the chemical of concern, with apparently minimal DLC exposures beyond those associated with background intake,²⁸ making these studies highly appropriate for use in RfD derivation for TCDD. In addition, health effects associated with TCDD exposures were observed in humans, the species of concern whose health protection is represented by the RfD, 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 population subgroups. Their inclusion among the RfDs derived also may characterize noncancer health effects associated with TCDD exposures in potentially vulnerable populations, thus accounting for some part of the intraspecies uncertainty in the RfD. 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 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. To a large extent, this is expected because a 10-fold interspecies uncertainty factor is generally used to extrapolate from test-animal species to humans, intended to provide a conservative estimate of an RfD that would be derived directly from human data. Two of the rat bioassays among this group of studies—Bell et al. (2007, [197041](#); RfD = 1.4E-9 mg/kg day based on delay in the onset of puberty) and NTP (2006, [197605](#); RfD = 4.6E-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

²⁸As an example, note the lack of statistically significant effects reported by Baccarelli et al. (2008, [197059](#); Figure 2 C and D) in regression models based on either maternal plasma levels of noncoplaner PCBs or total TEQ on neonatal TSH levels.

1 more animals per dose group (see Table 4-6 for a discussion of these studies' strengths and
2 weaknesses); both also are consistent with and, in part, have helped to define the current state of
3 practice in the field. Bell et al. (2007, [197041](#)) evaluated several reproductive and
4 developmental endpoints, initiating TCDD exposures well before mating and continuing through
5 gestation. NTP (2006, [197605](#)) is the most comprehensive evaluation of TCDD chronic toxicity
6 in rodents to date, evaluating dozens of endpoints at several time points in all major tissues.
7 Thus, proximity of the RfDs derived from these two high quality, recent studies provide
8 additional support for the use of the human data for RfD derivation.

9 There are several animal bioassay candidate RfDs at the lower end of the RfD range in
10 Table 4-5 that are more than 10-fold below the human-based RfDs. Two of these studies report
11 effects that are analogous to the endpoints reported in the three human studies and support the
12 RfDs based on human data. Specifically, decreased sperm production in Latchoumydandane and
13 Mathur (2002, [197498](#)) is consistent with the decreased sperm counts and other sperm effects in
14 Baccarelli et al. (2008, [197059](#)), and missing molars in Keller et al. (2007, [198526](#); 2008,
15 [198531](#); 2008, [198033](#)) are similar to the dental defects seen in Alaluusua et al. (2004, [197142](#)).
16 Thus, because these endpoints have been associated with TCDD exposures in humans, these
17 animal studies would not be selected for RfD derivation in preference to human data showing the
18 same effects.

19 Another characteristic of the remaining studies in the lower end of the candidate RfD
20 distribution is that they are dominated by mouse studies (comprising 6 of the 8 lowest
21 rodent-based RfDs). EPA considers the candidate RfD estimates based on mouse data to be
22 much more uncertain than either the rat or human candidate RfD estimates. The EPA considers
23 the Emond mouse PBPK model to be the most uncertain of toxicokinetic models used to estimate
24 the PODs because of the lack of key mouse-specific data, particularly for the gestational
25 component (see Section 3.3.4.3.2.5). The LOAEL_{HEDS} identified in mouse bioassays are low
26 primarily because of the large toxicokinetic interspecies extrapolation factors used for mice, for
27 which there is more potential for error. The ratio of administered dose to HED (D_a :HED) ranges
28 from 65 to 1,227 depending on the duration of exposure. The D_a :HED for mice is, on average,
29 about four times larger than that used for rats. In addition, each one of the mouse studies has
30 other qualitative limitations and uncertainties (discussed above and in Table 4-6) that make them
31 less desirable candidates as the basis for the RfD than the human studies.

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1 **4.3.4.1. Identification of POD from Baccarelli et al. (2008, [197059](#))**

2 Baccarelli et al. (2008, [197059](#)) reported increased levels of TSH in newborns exposed to
3 TCDD in utero, indicating a possible dysregulation of thyroid hormone metabolism. The study
4 authors related TCDD concentrations in neonatal blood to TSH levels, reporting group mean
5 TCDD concentrations associated with TSH levels above or below 5 μ -Units TSH per mL of
6 serum (5 μ U/mL).

7 The World Health Organization (WHO, 1994) established the 5 μ U/mL standard as an
8 indicator of potential iodine deficiency and potential thyroid problems in neonates. Increased
9 TSH levels are indicative of decreased thyroid hormone (T4 and/or T3) levels. The 5 μ U/mL
10 “cutoff” for TSH measurements in neonates was recommended by WHO (1994) for use in
11 population surveillance programs as an indicator of iodine deficiency disease (IDD). In
12 explaining this recommendation, WHO (1994) stated that:

13
14 “While further study of iodine replete populations is needed, a cutoff of 5 μ U/ml whole
15 blood... may be appropriate for epidemiological studies of IDD [iodine deficiency
16 disease.] Populations with a substantial number of newborns with TSH levels above the
17 cutoff could indicate a significant IDD problem.”
18

19 For TCDD, the toxicological concern is not likely to be iodine uptake inhibition, but
20 rather increased metabolism and clearance of T4, as evidenced in a number of animal studies
21 (e.g., Seo et al., 1995, [197869](#)). Clinically, a TSH level of >4 μ U/mL in a pregnant woman is
22 followed up by an assessment of free T4, and treatment with L-thyroxine is prescribed if
23 T4 levels are low (Glinioer and Delange, 2000). This is to ensure a sufficient supply of T4 for the
24 fetus, which relies on maternal T4 exclusively during the 1st half of pregnancy (Chan et al., 2005;
25 (Calvo et al., 2002, [051690](#); Morreale et al., 2000, [019231](#)).

26 Adequate levels of thyroid hormone also are essential in the newborn and young infant as
27 this is a period of active brain development (Glinioer and Delange, 2000; Zoeller and Rovet,
28 2004). Smaller reserves, higher demand, and shorter half-life of thyroid hormones in newborns
29 and young infants also could make this population more susceptible to the impact of insufficient
30 levels of T4 (Savin et al., 2003(Greer et al., 2002, [051202](#); Van Den et al., 1999, [016478](#)).

31 Thyroid hormone disruption during pregnancy and in the neonatal period can lead to
32 neurological deficiencies. However, the exact relationship between TSH increases and adverse

1 neurodevelopmental outcome is not well defined. A TSH level above 20 $\mu\text{U/L}$ in a newborn
2 infant is cause for immediate intervention to prevent mental retardation, often caused by a
3 malformed or ectopic thyroid gland in the newborn (Glinioer and Delange, 2000; Rovet, 2002;
4 WHO, 2007). Recent epidemiological data indicate concern for even lower level thyroid
5 hormone perturbations during pregnancy. For example, Haddow et al. (1999, [002176](#)) reported
6 that women with subclinical hypothyroidism, with a mean TSH of 13.2 $\mu\text{U/L}$ had children with
7 IQ deficits of up to 4 IQ points on the Wechsler IQ scale. Neonatal TSH within the first
8 72 hours of birth (as was evaluated by Baccarelli et al., 2008, [197059](#)) is a sensitive indicator of
9 both neonatal and maternal thyroid status (DeLange et al., 1983). Animal models have recently
10 indicated that very modest perturbations in thyroid status for even a relatively short period of
11 time can lead to altered brain development (e.g., Auso et al., 2004; Lavado-Autric et al., 2003;
12 Sharlin et al., 2008, 2010; Royland et al., 2008).

13 Baccarelli et al. (2008, [197059](#)) discount iodine status in the population as a confounder,
14 as exposed and referent populations all lived in a relatively small geographical area. It is
15 unlikely that there was iodine deficiency in one population and not in the other population based
16 on iodine levels in the soil.

17 Baccarelli et al. (2008, [197059](#)) also showed, in graphical form, how the TSH distribution
18 in each of three categorical exposure groups (reference, zone A, and zone B—representing
19 increasing TCDD exposure) shifted to higher TSH values with increasing exposure. The
20 individuals comprising the above 5 $\mu\text{U/mL}$ group were from all three categorical exposure
21 groups, not just from the highest exposure group. Therefore, EPA was able to designate a
22 LOAEL independently of the nominal categorical exposure groups; the LOAEL is designated as
23 the group mean of 39 ppt TCDD in neonatal plasma as a LOAEL for TSH values above
24 5 $\mu\text{U/mL}$. Using the Emond human PBPK model, the daily oral intake at the LOAEL is
25 estimated to be 0.024 ng/kg-day (see Section 4.2.3.1). A NOAEL is not defined because it is not
26 clear what maternal intake should be assigned to the group below 5 $\mu\text{U/mL}$.

27

28 **4.3.4.2. Identification of POD from Mocarelli et al. (2008, [199595](#))**

29 Mocarelli et al. (2008, [199595](#)) reported decreased sperm concentrations (20%) and
30 decreased motile sperm counts (11%) in men who were 1–9 years old in 1976 at the time of the
31 Seveso accident (initial TCDD exposure event). The sperm concentrations and motile sperm

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1 counts in men who were 10–17 years old in 1976 were not affected. Serum (LASC) TCDD
2 levels were measured within one year of the initial exposure. Serum TCDD levels and
3 corresponding responses were reported by quartile, with a reference group of less-exposed
4 individuals assigned a TCDD LASC value of 15 ppt (which was the mean of the TCDD LASC
5 reported in individuals outside the contaminated area). The lowest exposed group mean was
6 68 ppt (1st-quartile). Mean sperm concentrations and motile sperm counts were reduced about
7 20% from the reference group. Further decrease in these values in the groups exposed to more
8 than 68 ppt was slight and reached a maximum of about 33%.

9 Although a decrease in sperm concentration of 20% likely would not have clinical
10 significance for an individual EPA’s concern with the reported decreases in sperm concentration
11 and total number of motile sperm (relative to the comparison group) is that such decreases
12 associated with TCDD exposures could lead to shifts in the distributions of these measures in the
13 general population. Such shifts could result in decreased fertility in men at the low end of these
14 population distributions. While there is no clear cut-off indicating male fertility problems for
15 either of these measured effects. A sperm concentration of 20 million/ml is typically used as a
16 cut-off by clinicians to indicate follow-up for potential reproductive impact in affected
17 individuals. Low sperm counts are typically accompanied by poor sperm quality (morphology
18 and motility). For fertile men, between 50% and 60% of sperm are motile (Swan et al., 2003;
19 Slama et al., 2002; Wijchman et al., 2001). Any impacts on these reported levels could become
20 functionally significant.

21 For the 22–31 year-old men exposed to TCDD as a consequence of the Seveso accident,
22 the mean total sperm concentration was reported by Mocarelli et al. (2008, [199595](#)) to be
23 53.6 million/ml, with a value of 21.8 million/ml at one standard deviation below the mean. In
24 the comparison group that consisted of men not exposed to TCDD by the Seveso explosion and
25 of the same age as the exposed men, the mean total sperm concentration was 72.5 million/ml
26 (31.7 million/ml at one standard deviation below the mean). In the group exposed due to the
27 Seveso accident, individuals one standard deviation below the mean are just above the cut-off
28 used by clinicians, indicating a that a number of individuals in the exposed group likely had
29 sperm concentrations less than 20 million/ml; EPA could not obtain the individual data to
30 determine the exact number of men in this category. EPA judged that the impact on sperm

1 concentration and quality reported by Mocarelli et al. (2008, [199595](#)) is biologically significant
2 given the potential for functional impairment.

3 EPA has designated the lowest exposure group (68 ppt) as a LOAEL, which translates to
4 a continuous daily oral intake of 0.020 ng/kg-day (see Section 4.2.3.2). The reference group is
5 not designated as a NOAEL because there is no clear zero-exposure measurement for any of
6 these endpoints, particularly considering the contribution of background exposure to DLCs,
7 which further complicates the interpretation of the reference group response as a true “control”
8 response (see discussion in Section 4.4). However, males less than 10 years old can be
9 designated as a sensitive population by comparison to older males who were not affected.

11 **4.3.4.3. Identification of POD from Alaluusua et al. (2004, [197142](#))**

12 Alaluusua et al. (2004, [197142](#)) reported dental effects in male and female adults who
13 were less than 9.5 years of age, but not older, at the time of the initial exposure (1976) in Seveso.
14 EPA used the same approach to estimate daily TCDD intake as was used for the Mocarelli et al.
15 (2008, [199595](#)) data; a window of susceptibility of about 5 years was established. Serum
16 measurements for this cohort were taken within a year of the accident. Serum TCDD levels and
17 corresponding responses were reported by tertile, with a reference group of less-exposed
18 individuals assigned a TCDD LASC value of 15 ppt (ng/kg); the tertile group means were 130,
19 383, and 1,830 ppt. Both a NOAEL and LOAEL can be defined for this study. The NOAEL is
20 0.12 ng/kg-day, corresponding to the TCDD LASC of 130 ppt at the first tertile. The LOAEL is
21 0.93 ng/kg-day at the second tertile. The children in this cohort less than 5 years old can be
22 designated as a sensitive population by comparison to older individuals who were not affected
23 relative to the reference group.

25 **4.3.5. Derivation of the RfD**

26 The two human studies, Baccarelli et al. (2008, [197059](#)) and Mocarelli et al. (2008,
27 [199595](#)), have similar LOAELs of 0.024 and 0.020 ng/kg-day, respectively. Together, these
28 two studies constitute the best foundation for establishing a POD for the RfD, and are designated
29 as coprincipal studies. Therefore, increased TSH in neonates in Baccarelli et al. (2008, [197059](#))
30 and male reproductive effects (decreased sperm count and motility) in Mocarelli et al. (2008,
31 [199595](#)) are designated as cocritical effects. Although the exposure estimate used in

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1 determination of the LOAEL for Mocarelli et al. (2008, [199595](#)) is more uncertain than the
2 Baccarelli et al. (2008, [197059](#)) exposure estimate, the slightly lower LOAEL of
3 0.020 ng/kg-day from Mocarelli et al. is designated as the POD. A composite UF of 30 is
4 applied to account for lack of a NOAEL ($UF_L = 10$) and human interindividual variability
5 ($UF_H = 3$); the resulting RfD in standard units is 7×10^{-10} mg/kg-day. Table 4-7 presents the
6 details of the RfD derivation.

8 **4.4. UNCERTAINTY IN THE RfD**

9 Exposure assessment is a key limitation of the epidemiologic studies (of the Seveso
10 cohort) used to derive the RfD. The Seveso cohort exposure profile consists of an initial high
11 dose followed by a drop in body burden to background levels over a period of about 20 years, at
12 which time the effects were observed. This exposure scenario is a mismatch with the constant
13 daily intake scenario addressed by the RfD methodology. The determination of an effective
14 average daily dose from the Seveso exposure scenario requires an understanding of the critical
15 time-window of susceptibility and the influence of the peak exposure on the occurrence of the
16 observed effects, particularly when the peak exposure is high relative to the average exposure
17 over the critical exposure window. For one of the principal studies (Mocarelli et al., 2008,
18 [199595](#)), a maximum susceptibility exposure window can be identified based on the age of the
19 population at risk. However, the influence of the peak exposure on the effects observed 20 years
20 later is unknown and the biological significance of averaging the exposure over several years,
21 with internal exposure measures spanning a 4.5-fold range, is unknown. EPA, in this
22 assessment, has averaged intermittent exposures for rodent bioassays over weekly dosing
23 intervals, but the peak and average body burdens varied by less than 50%. EPA has not
24 developed guidance for larger-interval averaging. Furthermore, because there is an assumption
25 of a threshold level of exposure below which the effects are not expected to occur, averaging
26 over large intervals could include below-threshold exposures. The process used by EPA to
27 estimate the LOAEL exposure for the Mocarelli study is a compromise between the extremes; as
28 such, there is some uncertainty in the estimate, perhaps in the range of 3- to 10-fold in either
29 direction. This uncertainty also holds for the LOAEL determined for the dental effects reported
30 in Alaluusua et al. (2004, [197142](#)) and the increased menstrual cycle length reported in Eskenazi
31 et al. (2002, [197168](#) see Section 4.2.3.4); in both of those studies, the uncertainty is greater, as

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1 the difference between peak and average internal exposures is an order of magnitude or more.
2 The LOAEL for increased TSH in neonates (Baccarelli et al., 2008, [197059](#)), however, is less
3 uncertain because the critical exposure window is much narrower (9 months) and the
4 developmental exposures occurred 10 to 15 years after the initial exposure, when internal TCDD
5 concentrations for the pregnant women likely were leveling off; that is, exposure over the critical
6 window was more constant and estimation of the relevant exposures was less uncertain.
7 However, there is some uncertainty in the magnitude of the exposures because they were
8 estimated from measurements in sera taken several years prior to pregnancy.

9 Another source of uncertainty using human epidemiologic data is the lack of completely
10 unexposed populations. The available TCDD epidemiologic data were obtained by comparing
11 populations that experienced elevated TCDD exposures to populations that experienced lower
12 exposures, rather than to a population with no TCDD exposure. An additional complicating
13 factor is coexposure to DLCs, which can behave in the same way as TCDD. Although the
14 accidental exposure to the Seveso women's cohort was primarily to TCDD, background
15 exposure was largely to DLCs.²⁹ Eskenazi et al. (2004, [197160](#)) reported that TCDD comprised
16 only 20% of the total toxicity equivalence (TEQ) in the serum of the reference group that was
17 not exposed as a result of the factory explosion, which implies that the effective background
18 TEQ exposure was approximately 5-fold higher.

19 The higher background exposure could be significant at the lower TCDD exposure levels,
20 with the effect diminishing as TCDD exposure increased. For dose-response modeling, the
21 effect of a higher background dose (i.e., total TEQ), if included, would be to shift the response
22 curve to the right (responses associated with higher exposures) but, primarily, would reduce the
23 spread of the exposures, which would tend to alter the shape of the dose response towards
24 sublinear. Both the right shift and the more sublinear shape would result in higher ED_x
25 estimates, such as BMDs and BMDLs, from fitting dose-response models. However, for
26 determination of a LOAEL, which is the case for all the human studies in Table 4-5, the impact
27 may be minimal, as the LOAEL depends only on establishing that an effect of sufficient

²⁹Moccarelli (2001, [197002](#)) reported the release from the Seveso plant to contain a mixture of TCDD, ethylene glycol and sodium hydroxide. As 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, 2009, [192196](#)). It is unlikely that sodium hydroxide, being primarily a caustic agent, would cause these effects.

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1 magnitude was observed at some TCDD exposure level. In this case, the effect of the increased
2 effective background exposure would be to inflate the “control” (zero-TEQ) response, providing
3 the threshold for the response had been exceeded. The potential impact of an inflated control
4 response would be to mask a significant effect of the added TCDD exposure, when the latter
5 effect is determined by comparison to the reference group response. To compensate for this,
6 EPA has been somewhat conservative in interpreting the magnitude of responses defining
7 LOAELs for the Seveso cohort studies. The actual magnitude of the impact of the DLC
8 background exposure is impossible to assess without knowing the true (TEQ-free) background
9 response.

10 A primary strength of the TCDD database is that analogous effects have been observed in
11 animal bioassays for most of the human endpoints, increasing the overall confidence in the
12 relevance to humans of the effects reported in rodents and the association of TCDD exposure
13 with the effects reported in humans. Table 4-5 shows that low dose TCDD exposures are
14 associated with a wide array of toxicological endpoints in rodents including developmental
15 effects, reproductive effects, immunotoxicity and chronic toxicity. Effects reported in human
16 studies are similar, including male reproductive effects, increased TSH in neonates and dental
17 defects in children; other human health effects such as female reproductive effects and chloracne
18 have been observed at higher exposures (see Section 2.4.1). Other effects reported in rodent
19 studies such as liver toxicity and overt immunological endpoints have not been reported in
20 human studies. However, with respect to immunological effects, Baccarelli et al. (2002, [197062](#);
21 2004, [197045](#)) evaluated immunoglobulin and complement levels in the sera of TCDD-exposed
22 individuals from the Seveso cohort and found slightly reduced immunoglobulin in the highest
23 exposure groups but no effect on other immunoglobulins or on C3 or C4 complement levels.
24 The latter finding indicates that at least one immunological measure in humans is not a sensitive
25 endpoint, as it is for mice, with large reductions in serum complement at low exposure levels
26 (White et al., 1986, [197531](#)).

27 Although there is a substantial amount of qualitative concordance of effects between
28 rodents and humans, quantitative concordance is not evident in Table 4-5. The differential
29 sensitivity of mice and humans for the serum complement endpoint is one example. Other
30 examples of differential sensitivity are developmental dental effects and thyroid hormonal
31 dysregulation. Developmental dental defects are relatively sensitive effects in rodents, appearing

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1 at exposure levels in mice (Keller et al., 2007, [198526](#); Keller et al., 2008, [198531](#); Keller et al.,
2 2008, [198033](#)) more than an order of magnitude lower than effect levels in humans (Alaluusua et
3 al., 2004, [197142](#)). In contrast, thyroid hormone effects are seen in rats (Crofton et al., 2005,
4 [197381](#)) at 30-fold higher exposures than for humans (Baccarelli et al., 2008, [197059](#)). Male
5 reproductive effects (sperm production) occur in rats (Latchoumycandane and Mathur, 2002,
6 [197498](#)) and humans (Mocaelli et al., 2008, [199595](#)) at about the same dose. To what extent
7 these differential sensitivities depend on specifics of the comparison, such as species (mouse vs.
8 rat), life-stage (e.g., fetal vs. adult), endpoint measure (e.g., thyroxine [T4] vs. TSH) or
9 magnitude of the lowest dose tested, cannot be determined, so strong conclusions about
10 quantitative concordance cannot be made.

11 A number of qualitative strengths and limitations/uncertainties are associated with the top
12 animal bioassays listed in Table 4-5, as articulated in Table 4-6. Considering the issue of lowest
13 tested dose, the general lack of NOAELs and acceptable BMDLs is a primary weakness of the
14 rodent bioassay database. None of the 6 most sensitive rodent studies in Table 4-5, spanning a
15 30-fold range of LOAELs, had defined NOAELs or BMDLs. NOAELs or BMDLs were
16 established for only 4 of the next 10 rodent studies. In addition, many of these LOAELs are
17 characterized by relatively high responses with respect to the control population, so it is not
18 certain that a 10-fold lower dose (based on the application of UF_L of 10) would be approximately
19 equivalent to a NOAEL. A major reason for the failure of BMD modeling was that the responses
20 were not “anchored” at the low end (i.e., first response levels were far from the BMR [see
21 Table 4-4]). Another major problem with the animal bioassay data was nonmonotone and flat
22 response profiles. The small dose-group sizes and large dose intervals probably contributed to
23 many of these response characteristics that prevented successful BMD modeling. Larger study
24 sizes with narrower dose intervals at lower doses are still needed to clarify rodent response to
25 TCDD.

26 Lower TCDD doses have been tested in rodents but almost entirely for investigation of
27 specialized biochemical endpoints³⁰ that EPA does not consider to be adverse health effects (see
28 Appendix G). There is, however, a fundamental limit to the lowest dose of TCDD that can be
29 tested meaningfully, as TCDD is present in feed stock and accumulates in unexposed animals
30 prior to the start of any study. This issue is illustrated by the presence of TCDD in tissues of

³⁰Enzyme induction, oxidative stress indicators, mRNA levels, etc.

1 unexposed control animals, often at significant levels relative to the lowest tested dose in low
2 dose studies (Bell et al., 2007, [197041](#); Ohsako et al., 2001, [198497](#)) (Vanden Heuvel et al.,
3 1994, [594318](#), see Text Box 4-1). Some DLCs also have been measured in animal feeds and are
4 anticipated to accumulate in unexposed test animals further complicating the interpretation of
5 low dose studies.

6

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, [197551](#)), 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. The equivalent 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 ng/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, [197041](#)) 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, [198497](#)) 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, [197041](#)) 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, [197041](#)), as the lowest TCDD exposure level was 2.4 ng/kg-day (28-day dietary exposure).

NTP (2006, [543749](#)) reported TCDD concentrations in the liver and fat of untreated female S-D 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, 2006, [197605](#))), 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, [197041](#)). As for the latter study, background intake for the NTP (2006, [197605](#)) 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, [197041](#)), control animals were gavaged with corn oil vehicle. TCDD concentrations in corn oil were not reported in any of the studies.

7

Table 4-1. POD candidates for epidemiologic studies of TCDD

Study	POD (ng/kg-day)	Critical effects
Alaluusua et al. (2004, 197142)	1.2E-01 ^a (NOAEL)	Dental effects in adults exposed to TCDD in childhood
Baccarelli et al. (2008, 197059)	2.4E-02 ^b (LOAEL)	Elevated TSH in neonates
Eskenazi et al. (2002, 197168)	1.64E+00 ^c (LOAEL)	Increased length of menstrual cycle in women exposed to TCDD in childhood
Mocarelli et al. (2008, 199595)	2.0E-02 ^d (LOAEL)	Decreased sperm count and motility in men exposed to TCDD in childhood

^aMean of peak exposure (0.15 ng/kg-day) and average exposure over 10-year critical window (0.0093 ng/kg-day).

^bMaternal exposure corresponding to neonatal TSH concentration exceeding 5 μU/mL.

^cMean of peak exposure (3.2 ng/kg-day) and average exposure over 10-year critical window (0.12 ng/kg-day).

^dMean of peak exposure (0.035 ng/kg-day) and average exposure over 10-year critical window (0.0078 ng/kg-day).

Table 4-2. Models run for each study/endpoint combination in the animal bioassay benchmark dose 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, 2 nd degree polynomial
Probit	None
Weibull	Power ≥ 1

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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			1 st -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, 197169)	Saccharin preference ratio, female	–	2.50E+01	5.10E+01	–	2.49E-02	5.08E-02	–	1.71E-01	3.20E-01
Bell et al. (2007, 197041)	Balano-preputial separation in male pups	–	2.40E+00	2.87E+00	–	1.26E-02	1.50E-02	–	8.83E-02	4.33E-02
Cantoni et al. (1981, 197092)	Urinary coproporphyrins	–	1.43E+00	1.25E-01	–	1.24E-02	1.09E-03	–	6.51E-02	1.60E-03
Chu et al. (2001, 521829)	Tissue weight changes	2.50E+02	1.00E+03	–	7.55E-01	3.02E+00	–	–	–	–
Chu et al., 2007	Liver lesions	2.50E+00	2.50E+01	–	7.55E-03	7.55E-02	–	3.56E-02	5.76E-01	–
Crofton et al. (2005, 197381)	Serum T4	3.00E+01	1.00E+02	3.01E+01	1.92E-02	6.40E-02	1.92E-02	1.72E-01	7.61E-01	1.40E-01
Croutch et al. (2005, 197382)	Decreased body weight	5.43E+01	2.17E+02	–	2.22E-01	8.89E-01	–	–	–	–
DeCaprio et al. (1986, 197403)	Decreased body weight	6.10E-01	4.90E+00	–	4.11E-03	3.30E-02	–	–	–	–
Fattore et al. (2000, 197446)	Decreased hepatic retinol	–	2.00E+01	–	–	1.23E-01	–	–	8.01E-01	–
Fox et al. (1993, 197344)	Increased liver weight	5.70E-01	3.27E+02	–	1.42E-03	8.12E-01	–	–	–	–
Franc et al. (2001, 197353)	Organ weight changes	1.00E+01	3.00E+01	1.59E+00	6.62E-02	1.99E-01	1.05E-02	4.60E-01	1.45E+00	3.37E-02
Franczak et al. (2006, 197354)	Abnormal estrous cycle	–	7.14E+00	–	–	5.95E-02	–	–	3.25E-01	–
Hojo et al. (2002, 198785)	DRL response per min	–	2.00E+01	2.70E-01	–	5.26E-03	7.11E-05	–	5.50E-02	7.37E-05
Hutt et al. (2008, 198268)	Embryotoxicity	–	7.14E+00	–	–	4.67E-02	–	–	2.57E-01	–

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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)

Study	Endpoint	Administered dose ^a			1 st -order body burden HED ^b			Blood concentration HED ^c		
		NOAEL	LOAEL	BMDL ^d	NOAEL	LOAEL	BMDL ^d	NOAEL	LOAEL	BMDL ^d
Ikeda et al. (2005, 197834)	Sex ratio	–	1.65E+01	–	–	1.05E–01	–	–	2.75E+00	–
Ishihara et al. (2007, 197677)	Sex ratio	1.00E–01	1.00E+02	–	3.18E–04	3.18E–01	–	–	–	–
Kattainen et al. (2001, 198952)	3 rd molar length	–	3.00E+01	2.14E+00	–	7.89E–03	5.64E–04	–	8.99E–02	1.71E–03
Keller et al. (2007, 198526 ; 2008, 198531 ; 2008, 198033)	Missing mandibular molars	–	1.00E+01	1.88E+01	–	2.58E–03	4.85E–03	–	9.81E–03	1.70E–02
Kociba et al. (1976, 198594)	Liver and hematologic effects and body weight changes	7.14E+00	7.14E+01	–	4.53E–02	4.53E–01	–	2.68E–01	3.10E+00	–
Kociba et al. (1978, 001818)	Liver and lung lesions, increased urinary porphyrins	1.00E+00	1.00E+01	7.30E–01	1.07E–02	1.07E–01	7.84E–03	6.46E–02	6.46E–01	2.00E–02
Latchoumycandane and Mathur (2002, 197498)	Sperm production	–	1.00E+00	1.56E–02	–	3.87E–03	6.03E–05	–	1.67E–02	3.83E–05
Li et al. (1997, 199060)	Increased serum FSH	3.00E+00	1.00E+01	3.60E+03	7.89E–04	2.63E–03	9.47E–01	2.97E–03	1.72E–02	2.38E+01
Li et al. (2006, 199059)	Hormone levels (serum estradiol)	–	2.00E+00	1.08E+02	–	9.85E–04	5.33E–02	–	1.57E–03	3.46E–01
Markowski et al. (2001, 197442)	FR2 revolutions	–	2.00E+01	7.34E+00	–	6.25E–03	2.29E–03	–	5.14E–02	1.18E–02
Maronpot et al. (1993, 198386)	Increased relative liver weight	1.07E+01	3.50E+01	–	8.97E–02	2.93E–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)

Study	Endpoint	Administered dose ^a			1 st -order body burden HED ^b			Blood concentration HED ^c		
		NOAEL	LOAEL	BMDL ^d	NOAEL	LOAEL	BMDL ^d	NOAEL	LOAEL	BMDL ^d
Miettinen et al. (2006, 198266)	Cariogenic lesions in pups	–	3.00E+01	1.05E+01	–	7.89E–03	2.77E–03	–	8.93E–02	9.32E–03
Murray et al. (1979, 197983)	Fertility index in f2 generation	1.00E+00	1.00E+01	1.63E+00	9.43E–03	9.43E–02	1.54E–02	2.96E–02	3.88E–01	4.05E–02
NTP (1982, 200870)	Liver lesions	–	1.39E+00	4.68E+00	–	6.47E–03	2.18E–02	–	2.21E–02	5.20E–02
NTP (2006, 197605)	Liver and lung lesions	–	2.14E+00	5.04E–01	–	2.34E–02	5.50E–03	–	1.39E–01	7.38E–03
Nohara et al. (2000, 200027)	Decreased spleen cellularity	8.00E+02	–	–	2.10E–01	–	–	5.34E+00	–	–
Ohsako et al. (2001, 198497)	Anogenital distance in pups	1.25E+01	5.00E+01	9.75E+00	3.29E–03	1.32E–02	2.57E–03	2.75E–02	1.78E–01	1.84E–02
Seo et al. (1995, 197869)	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, 198145)	Serum T4	1.07E+01	3.50E+01	5.16E+00	8.97E–02	2.93E–01	4.33E–02	5.15E–01	1.76E+00	1.84E–01
Shi et al. (2007, 198147)	Serum estradiol in female pups	1.43E–01	7.14E–01	2.24E–01	1.23E–03	6.13E–03	1.92E–03	4.71E–03	2.75E–02	4.95E–03
Simanainen et al. (2002, 201369)	Decreased serum T4	1.00E+02	3.00E+02	–	2.63E–02	7.89E–02	–	–	–	–
Simanainen et al. (2003, 198582)	Decreased thymus weight and change in EROD activity	1.00E+02	3.00E+02	–	2.63E–02	7.89E–02	–	–	–	–
Simanainen et al. (2004, 198948)	Decreased daily sperm production	1.00E+02	3.00E+02	–	2.63E–02	7.89E–02	–	–	–	–
Smialowicz et al. (2004, 198948)	Decreased antibody response to SRBCs	3.00E+02	1.00E+03	–	7.73E–02	2.58E–01	–	–	–	–
Smialowicz et al. (2008, 198341)	PFC per 10 ⁶ cells	–	1.07E+00	4.09E–01	–	5.00E–03	1.91E–03	–	6.38E–03	2.00E–03

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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)

Study	Endpoint	Administered dose ^a			1 st -order body burden HED ^b			Blood concentration HED ^c		
		NOAEL	LOAEL	BMDL ^d	NOAEL	LOAEL	BMDL ^d	NOAEL	LOAEL	BMDL ^d
Toth et al. (1979, 197109)	Skin lesions	–	1.00E+00	2.15E+02	–	3.70E-03	7.94E-01	–	1.00E-02	2.18E-01
VanBirgelen et al. (1995, 198052)	Decreased liver retinyl palmitate	–	1.40E+01	9.89E+02	–	8.63E-02	6.09E+00	–	5.25E-01	5.00E+00
Vos et al. (1973, 198367)	Decreased delayed-type hypersensitivity response to tuberculin	1.14E+00	5.71E+00	–	6.43E-03	3.22E-02	–	–	–	–
White et al. (1986, 197531)	Decreased serum complement	–	1.00E+01	3.59E+01	–	2.23E-02	7.98E-02	–	2.83E-02	4.65E-02
Yang et al. (2000, 198590)	Increased endometrial implant survival	1.79E+01	–	–	6.74E-01	–	–	–	–	–

^aAverage administered daily dose over the experimental exposure period.

^bHED based on 1st-order body burden model described in Section 3.2.4.4.

^cHED based on Emond rodent and human PBPK models described in Section 3.3.6.

^dBMR = 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.

– = value not established or not modeled.

Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg)^a

Study	NOAEL/ LOAEL	Endpoint	Control response	First response ^b	Max response ^c	Model fit detail	BMD/ BMDL	Comments
Amin et al. (2000, 197169) (rat)	3.38E+00	Saccharin consumed, female, (0.25%) (<i>n</i> = 10)	—	22% ↓ (0.3 SD)	66% ↓	Continuous linear, nonconstant variance (<i>p</i> = 0.55)	9.15E+00 6.09E+00	BMDL > LOAEL; restricted power model, constrained parameter hit lower bound
						Continuous power, nonconstant variance, unrestricted (<i>p</i> = NA)	8.37E+00 3.42E+00	Saturated model; supralinear fit (power = 0.74)
		Saccharin consumed, female (0.50%) (<i>n</i> = 10)	—	49% ↓ (0.7 SD)	80% ↓	Continuous linear, nonconstant variance (<i>p</i> = 0.06)	1.02E+01 6.57E+00	Restricted power model, constrained parameter hit lower bound
						Continuous power, nonconstant variance, unrestricted (<i>p</i> = NA)	6.57E+00 1.15E+00	Saturated model; supralinear fit (power = 0.40)
		Saccharin preference ratio, female (0.25%) (<i>n</i> = 10)	—	29% ↓ (1.8 SD)	33% ↓	Continuous linear, nonconstant variance (<i>p</i> = 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%) (<i>n</i> = 10)	—	39% ↓ (1.1 SD)	54% ↓	Continuous linear, constant variance (<i>p</i> = 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 (<i>p</i> = NA)	2.60E+00 1.06E-14	Saturated model; supralinear fit (power = 0.28)

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg^a) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
Bell et al. (2007, 197041) (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, 197092) (rat)	– 1.85E+00	Urinary uroporphyrins (n = 4)	–	2.4-fold ↑ (5.7 SD)	87-fold ↑	Continuous exponential (M2), nonconstant variance (p = 0.0003)	3.76E+00 2.76E+00	No response near BMR; poor fits for all nonconstant variance models; constant variance poor representation of control SD; BMDL > LOAEL
		Urinary coproporphyrins (n = 4)	–	2.4-fold ↑ (3.1 SD)	4.0-fold ↑	Continuous exponential (M4), nonconstant variance (p = 0.49)	5.34E–01 1.80E–01	No response near BMR
			–			Continuous power, nonconstant 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, 197381) (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

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg^a) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments	
Franc et al. (2001, 197353) (rat)	6.58E+00 1.45E+01	S-D Rats, Relative Liver Weight	—	8.1% ↑ (0.58 SD)	55% ↑	Continuous power, constant variance (<i>p</i> = 0.84)	9.47E+00 4.59E+00	Acceptable fit	
		L-E Rats, Relative Liver Weight	—	6.3% ↑ (0.63 SD)	22% ↑	Continuous Hill, nonconstant variance, restricted (<i>p</i> = 0.83)	7.72E+00 1.22E+00	Constrained parameter hit lower bound; otherwise acceptable fit; selected	
							Continuous Hill, nonconstant variance, unrestricted (<i>p</i> = 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), nonconstant variance (<i>p</i> = 0.72)	1.88E+00 9.22E-01	Poor fit for responses in controls and lowest exposure group	
							Continuous polynomial, nonconstant variance (<i>p</i> = 0.40)	4.78E+00 3.89E+00	Acceptable fit
		L-E Rats, Relative Thymus Weight	—	7.7% ↓ (0.15 SD)	66% ↓	Continuous exponential (M4), constant variance (<i>p</i> = 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 (<i>p</i> = 0.70)	5.09E+00 3.13E+00		

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg^a) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
Hojo et al. (2002, 198785) (rat)	– 1.62E+00	DRL reinforce per min (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 min (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, 198952) (rat)	– 2.23E+00	3 rd molar length in pups (n = 4–8)	–	15% ↓ (4.2 SD)	27% ↓	Continuous Hill, nonconstant variance, restricted (p = 0.02)	3.13E–01 1.68E–01	No response data near BMR; Constrained parameter lower bound hit
						Continuous Hill, nonconstant variance, unrestricted (p < 0.001)	1.21E–02 –	BMDL could not be calculated
		3 rd 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. (2007, 198526 ; 2008, 198531 ; 2008, 198033) (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

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg^a) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
Kociba et al. (1978, 001818) (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), nonconstant 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
Latchoumy-candane and Mathur (2002, 197498) (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, 199060) (rat)	2.66E-01 7.99E-01	FSH in female rats (n = 10)	—	3.6-fold ↑ (2.0 SD)	19-fold ↑	Continuous power, nonconstant variance, restricted (p < 0.01)	2.00E+02 1.36E+02	Power hit lower bound
						Continuous power, nonconstant variance, unrestricted (p = 0.003)	1.96E-01 2.48E-02	supralinear fit (power = 0.31)

Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg^a) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
Li et al. (2006, 199059) (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 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, nonconstant 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)
Markowski et al. (2001, 197442) (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

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg^a) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
Miettinen et al. (2006, 198266) (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, 197983) (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 (1982, 200870) (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
NTP (2006, 197605) (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

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg^a) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
NTP (2006, 197605) (rat) (continued)	– 2.56E+00 (continued)	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
		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
Ohsako et al. (2001, 198497) (rat)	1.04E+00 3.47E+00	Ano-genital 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)

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg^a) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
Sewall et al. (1995, 198145) (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, 198147) (rat)	3.42E-01 1.07E+00	Serum estradiol in female pups (n = 10)	—	38% ↓ (0.4 SD)	62% ↓	Continuous exponential (M4), nonconstant variance (p = 0.69)	8.07E-01 3.54E-01	Adequate fit; selected
Smialowicz et al. (2008, 198341) (mouse)	— 4.38E-01	PFC per spleen (n = 15)	—	24% ↓ (0.5 SD)	89% ↓	Continuous power, unrestricted, nonconstant 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 nonconstant variance models
Toth et al. (1979, 197109) (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)

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg^a) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
Toth et al. (1979, 197109) (mouse) (continued)	– 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. (1995, 198052) (rat)	– 7.20E+00	Hepatitis retinol (n = 8)	–	44% ↓ (0.74 SD)	96% ↓	Continuous exponential (M4), nonconstant variance (p < 0.01)	2.49E+01 3.36E+00	Poor fit
						Continuous power, nonconstant variance, unrestricted (p = 0.01)	3.80E–01 1.39E–02	Poor fit; supralinear fit (power = 0.14)
		Hepatitis retinyl palmitate (n = 8)	–	80% ↓ (1.4 SD)	99% ↓	Continuous exponential (M4), nonconstant variance (p < 0.01)	1.42E+02 3.65E+01	Poor fit; no response near BMR
						Continuous power, nonconstant variance, unrestricted (p = 0.24)	5.26E–02 5.89E–05	Supralinear fit (power = 0.06)

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Table 4-4. TCDD BMDL analysis (NOAEL, LOAEL, BMD, and BMDL values given as animal whole blood concentrations in ng/kg^a) (continued)

Study	NOAEL/ LOAEL	Endpoint	Control response	First response	Max response	Model fit detail	BMD/ BMDL	Comments
White et al. (1986, 197531) (mouse)	– 1.09E+00	Total hemolytic complement activity (CH50) (<i>n</i> = 8)	–	41% ↓ (2.6 SD)	81% ↓	Continuous Hill, nonconstant variance, restricted (<i>p</i> = 0.002)	8.63E+00 1.50E+00	Poor fit; no response near BMR; constrained parameter bound hit; BMDL > LOAEL
						Continuous Hill, nonconstant variance, unrestricted (<i>p</i> = 0.07)	1.48E–01 4.35E–03	

^aAnimal whole blood concentrations were used to determine the HEDs in Table 4-5.

^bMagnitude 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.

^cMagnitude of response maximally differing from control value (in the adverse direction).

S-D = Sprague-Dawley.

SD = standard deviation.

Table 4-5. Candidate points of departure for the TCDD RfD 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, 199059)	Mouse, NIH (F)	Gavage GD 1–3; n = 10	Hormone levels in pregnant dams (decreased progesterone, increased estradiol)	–	1.6E–03	300	5.2E–12
Smialowicz et al. (2008, 198341)	Mouse, B6C3F1 (F)	90-day gavage; n = 8–15	Decreased SRBC response	–	6.4E–03	300	2.1E–11
Keller et al. (2007, 198526 ; 2008, 198531 ; 2008, 198033) ^b	Mouse, CBA/J and C3H/HeJ	Gavage GD 13; n = 23–36 (pups)	Missing molars, mandibular shape changes in pups	–	9.8E–03	300	3.3E–11
Toth et al. (1979, 197109)	Mouse, Swiss/H/Riop (M)	1-year gavage; n = 38–44	Dermal amyloidosis, skin lesions	–	1.0E–02	300	3.3E–11
Latchoumy-candane and Mathur (2002, 197498)	Rat, Wistar (M)	45-day oral pipetting; n = 6	Decreased sperm production	–	1.7E–02	300	5.6E–11
NTP (1982, 200870)	Mouse, B6C3F1 (M)	2-year gavage; n = 50	Liver lesions	–	2.2E–02	300	7.4E–11
White et al. (1986, 197531)	Mouse, B6C3F1 (F)	14-day gavage; n = 6–8	Decreased serum complement	–	2.8E–02	300	9.4E–11
Li et al. (1997, 199060)	Rat, S-D (F, 22 day-old)	Single gavage; n = 10	Increased serum FSH	3.0E–03 (N)	1.7E–02	30 ^c	9.9E–11
DeCaprio et al. (1986, 197403)	Guinea pig, Hartley	90-day dietary; n = 10	Decreased body weight, organ weight changes (liver, kidney, thymus, brain)	4.1E–03 ^d (N)	3.3E–02 ^d	30 ^c	1.4E–10
Shi et al. (2007, 198147)	Rat, S-D (F)	11-month gavage; n = 10	Decreased serum estradiol	4.7E–03 (N) 5.0E–03 (B)	2.8E–02	30 ^c	1.6E–10
Markowski et al. (2001, 197442)	Rat, Holtzman	Gavage GD 18; n = 4–7	Neurobehavioral effects in pups (running, lever press, wheel spinning)	–	5.1E–02	300	1.7E–10

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Table 4-5. Candidate points of departure for the TCDD RfD 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, 198785)	Rat, S-D	Gavage GD 8; <i>n</i> = 12	Food-reinforced operant behavior in pups	–	5.5E-02	300	1.8E-10
Vos et al. (1973, 198367)	Guinea pig, Hartley (F)	8-week gavage; <i>n</i> = 10	Decreased delayed-type hypersensitivity response to tuberculin	6.4E-03 ^d (N)	3.2E-02 ^d	30 ^c	2.1E-10
Cantoni et al. (1981, 197092)	Rat, CD-COBS (F)	45-week gavage; <i>n</i> = 4	Increased urinary porphyrins	–	6.5E-02	300	2.2E-10
Miettinen et al. (2006, 198266)	Rat, Line C	Gavage GD 15; <i>n</i> = 3-10	Cariogenic lesions in pups	–	8.9E-02	300	3.0E-10
Kattainen et al. (2001, 198952)	Rat, Line C	Gavage GD 15; <i>n</i> = 4-8	Inhibited molar development in pups	–	9.0E-02	300	3.0E-10
NTP (2006, 197605)	Rat, S-D (F)	2-year gavage; <i>n</i> = 53	Liver and lung lesions	–	1.4E-01	300	4.6E-10
Amin et al. (2000, 197169)	Rat, S-D	Gavage GD 10-16; <i>n</i> = 10	Reduced saccharin consumption and preference	–	1.7E-01	300	5.7E-10
Mocarelli et al. (2008, 199595)	Human (M)	Childhood exposure; <i>n</i> = 157	Decreased sperm concentration and sperm motility, as adults	–	2.0E-02^e	30^f	6.7E-10
Baccarelli et al. (2008, 197059)	Human infants	Gestational exposure; <i>n</i> = 51	Increased TSH in newborn infants	–	2.4E-02^g	30^f	8.2E-10
Hutt et al. (2008, 198268)	Rat, S-D (F)	13-week dietary; <i>n</i> = 3	Embryotoxicity	–	2.6E+00	300	8.6E-10
Ohsako et al. (2001, 198497)	Rat, Holtzman	Gavage GD 15; <i>n</i> = 5	Decreased ano-genital distance in male pups	2.8E-02 (N)	1.8E-01	30 ^c	9.2E-10
Murray et al. (1979, 197983)	Rat, S-D	3-generation dietary	Reduced fertility and neonatal survival (f 0 and f 1)	3.0E-02 (N)	3.9E-01	30 ^c	9.9E-10

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Table 4-5. Candidate points of departure for the TCDD RfD 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)
Franc et al. (2001, 197353)	Rat, Long-Evans (F)	22-week gavage; n = 8	Increased Relative Liver Weight; decreased relative thymus weight	4.6E-01 (N) 3.4E-02 (B)	1.45E+00	30 ^c	1.1E-09
Chu et al., 2007	Rat, S-D (F)	28-day gavage, n = 5	Liver lesions	3.6E-02 (N)	5.8E-01	30 ^c	1.2E-09
Bell et al. (2007, 197041)	Rat, CRL:WI (Han) (M)	17-week dietary; n = 30	Delay in onset of puberty	4.3E-02 (B)	8.8E-02	30 ^c	1.4E-09
Van Birgelen et al. (1995, 198052)	Rat, S-D (F)	13-week dietary; n = 8	Decreased liver retinyl palmitate	–	5.3E-01	300	1.8E-09
Kociba et al. (1978, 001818)	Rat, S-D (F)	2-year dietary; n = 50	Liver and lung lesions, increased urinary porphyrins	6.5E-02 (N)	6.5E-01	30 ^c	2.2E-09
Fattore et al., (2000, 197446)	Rat, S-D	13-week dietary; n = 6	Decreased hepatic retinol	–	8.0E-01	300	2.7E-09
Seo et al. (1995, 197869)	Rat, S-D	Gavage GD 10–16; n = 10	Decreased serum T4 and thymus weight	1.7E-01 (N)	9.1E-01	30 ^c	5.6E-09
Crofton et al. (2005, 197381)	Rat, Long-Evans (F)	4-day gavage; n = 4–14	Decreased serum T4	1.7E-01 (N)	7.6E-01	30 ^c	5.7E-09
Sewall et al. (1995, 198145)	Rat, S-D (F)	30-week gavage; n = 9	Decreased serum T4	5.2E-01 (N) 1.8E-01 (B)	1.8E+00	30 ^c	6.1E-09
Alaluusua et al. (2004, 197142)	Human	Childhood exposure; n = 48	Dental defects	1.2E-01 ^h (N)	9.3E-01 ⁱ	3 ^j	3.9E-08

^aExcept where indicated, UF_A = 3 (for dynamics), UF_H = 10, UF_L = 10.

^bResults from 3 separate studies with identical designs combined.

^cUF_L = 1 (NOAEL or BMDL).

^dHED determined from 1st-order body burden model; no PBPK model available for guinea pigs.

^eMean of peak exposure (0.0319 ng/kg-day) and average exposure over 10-year critical window (0.00802 ng/kg-day).

^fUF_H = 3, UF_L = 10.

^gMaternal exposure corresponding to neonatal TSH concentration exceeding 5 µU/mL.

Table 4-5. Candidate points of departure for the TCDD RfD using blood-concentration-based human equivalent doses (continued)

^hMean of peak exposure (0.200 ng/kg-day) and average exposure over 10-year critical window (0.0335 ng/kg-day).

ⁱMean of peak exposure (1.71 ng/kg-day) and average exposure over 10-year critical window (0.153 ng/kg-day).

^jUF_H = 3.

S-D = Sprague-Dawley.

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Table 4-6. Qualitative analysis of the strengths and limitations/uncertainties associated with animal bioassays possessing candidate points-of-departure for the TCDD RfD

Study	Strengths	Limitations	Remarks
Bell et al. (2007, 197041)	<ul style="list-style-type: none"> Large sample size of both rat dams and offspring/dose employed Several developmental effects tested 	<ul style="list-style-type: none"> 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, 197092)	<ul style="list-style-type: none"> Experiments were designed to test qualitative and quantitative composition and the course of urinary excretion in TCDD-induced porphyria 	<ul style="list-style-type: none"> 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, 197403)	<ul style="list-style-type: none"> Subchronic oral dosing duration up to 90 days. Male and female guinea pigs tested 	<ul style="list-style-type: none"> 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, 197353)	<ul style="list-style-type: none"> 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 	<ul style="list-style-type: none"> 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, 198785)	<ul style="list-style-type: none"> 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 	<ul style="list-style-type: none"> 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

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Table 4-6. Qualitative analysis of the strengths and limitations/uncertainties associated with animal bioassays possessing candidate points-of-departure for the TCDD RfD (continued)

Study	Strengths	Limitations	Remarks
Keller et al. (2007, 198526 ; 2008, 198531 ; 2008, 198033)	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • Unknown sample size of mouse dams/dose/strain employed • All inbred strains possessed sensitive <i>b</i> allele at the <i>Ahr</i> 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, b) 	Endpoint similar to effects observed at higher exposure levels in humans; HED highly uncertain using mouse PBPK model
Latchoumy-candane and Mathur (2002, 197498)	<ul style="list-style-type: none"> • Compared to epididymal sperm counts, the testicular spermatid head count provides better quantitation of acute changes in sperm production and can indicate pathology 	<ul style="list-style-type: none"> • 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, 199059)	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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, 197442)	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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 2 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

Table 4-6. Qualitative analysis of the strengths and limitations/uncertainties associated with animal bioassays possessing candidate points-of-departure for the TCDD RfD (continued)

Study	Strengths	Limitations	Remarks
NTP (1982, 200870)	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • Elevated background levels of hepatocellular tumors in untreated male mice • Gavage exposure was only 2 days/week • Only 2 treatment levels 	Comprehensive chronic toxicity evaluations of TCDD in rodents; HED highly uncertain using mouse PBPK model
NTP (2006, 197605)	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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, 198147)	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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, 198341)	<ul style="list-style-type: none"> • Sheep red blood cell (SRBC) plaque forming cell assay is highly sensitive and reproducible across laboratories when examining TCDD 	<ul style="list-style-type: none"> • 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, 197109)	<ul style="list-style-type: none"> • Large sample size of mice/dose employed • Chronic exposure duration 	<ul style="list-style-type: none"> • 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

Table 4-6. Qualitative analysis of the strengths and limitations/uncertainties associated with animal bioassays possessing candidate points-of-departure for the TCDD RfD (continued)

Study	Strengths	Limitations	Remarks
Vos et al. (1973, 198367)	<ul style="list-style-type: none"> • Three different animal species tested (guinea pigs, mice, and rats) • Effects of TCDD tested on both cell-mediated and humoral immunity 	<ul style="list-style-type: none"> • 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 and humoral immunity in each animal species; 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
White et al. (1986, 197531)	<ul style="list-style-type: none"> • Total hemolytic complement (CH50) is representative functional assay of the complete complement sequence 	<ul style="list-style-type: none"> • 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

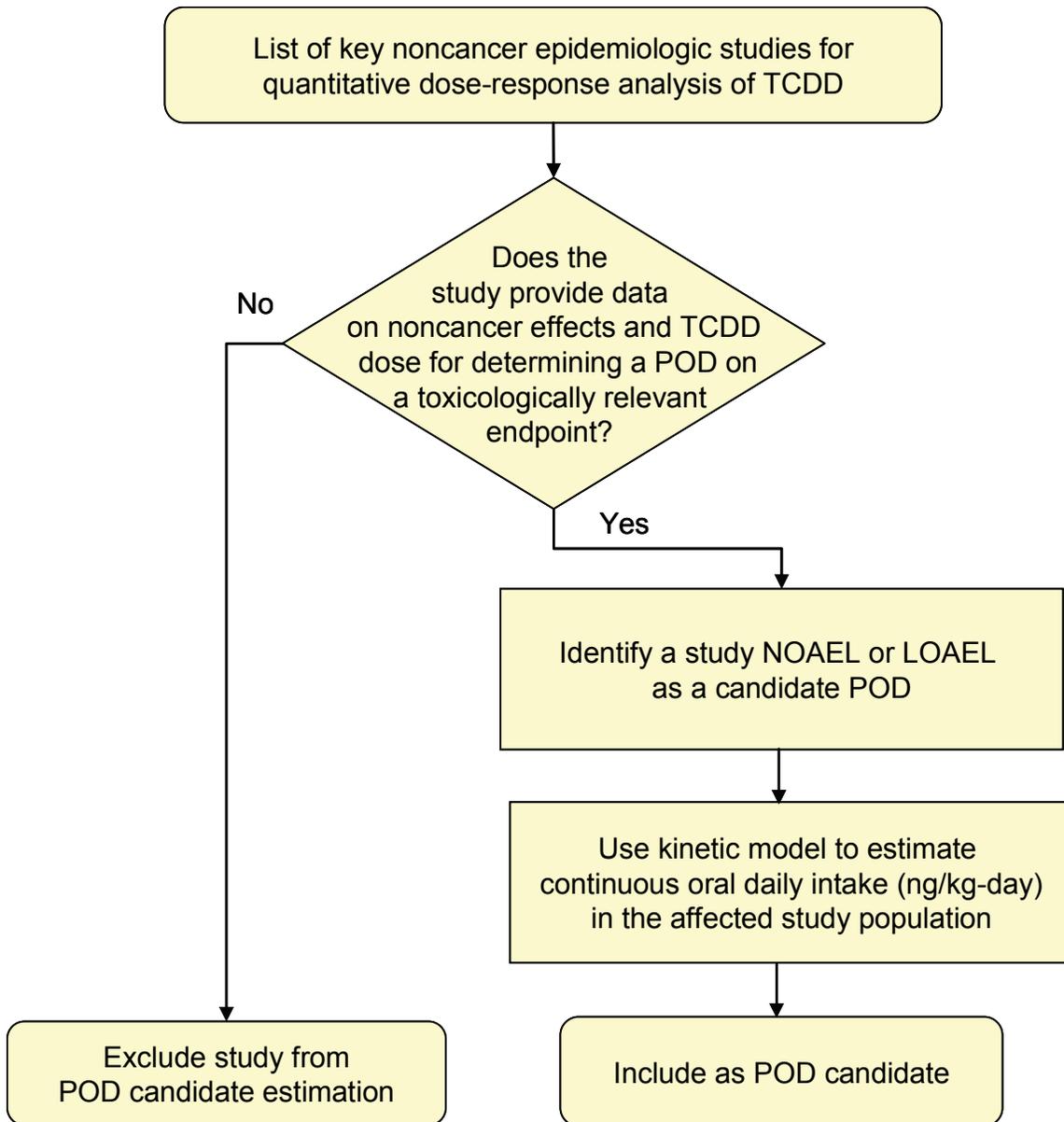
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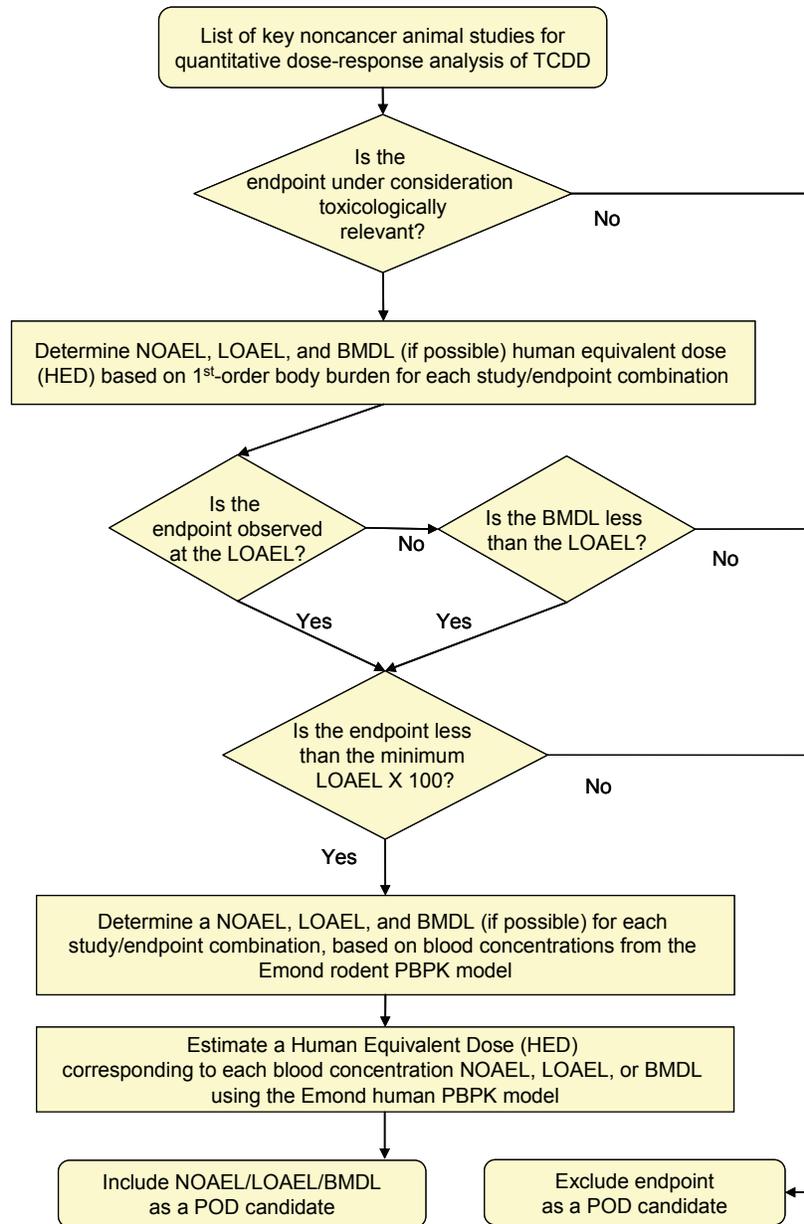
Table 4-7. Basis and derivation of the TCDD reference dose

Principal study detail		
Study	POD (ng/kg-day)	Critical effects
Mocarelli et al. (2008, 199595)	0.020 (LOAEL)	Decreased sperm count (20%) and motility (11%) in men exposed to TCDD during childhood
Baccarelli et al. (2008, 197059)	0.024 (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, 197059); 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 populations. 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.
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 developmental effects are the most sensitive, occurring at doses lower than other effects noted in chronic studies. Considering that exposure in the principal studies encompasses the critical window of susceptibility associated with development, an UF to account for exposure duration is not warranted.
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 reference dose.

3



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



1
 2 **Figure 4-2. EPA’s process to select and identify candidate PODs from key**
 3 **animal bioassays for use in noncancer dose-response analysis of TCDD.** For
 4 each noncancer endpoint reported in the studies that qualified for TCDD
 5 dose-response assessment using the study inclusion criteria, EPA evaluated the
 6 endpoint and eliminated it if it was not toxicologically relevant for RfD derivation.
 7 Then, relevant endpoints not observed at the LOAEL (i.e., reported at higher
 8 doses) with BMDLs greater than the LOAEL were eliminated from further
 9 analysis. Endpoints with LOAELS greater than the minimum LOAEL times 100
 10 also were eliminated from further analysis. Using kinetic modeling, EPA
 11 developed human equivalent doses for each remaining NOAEL/LOAEL/BMDL
 12 associated with selected endpoints and included these as candidate PODs.

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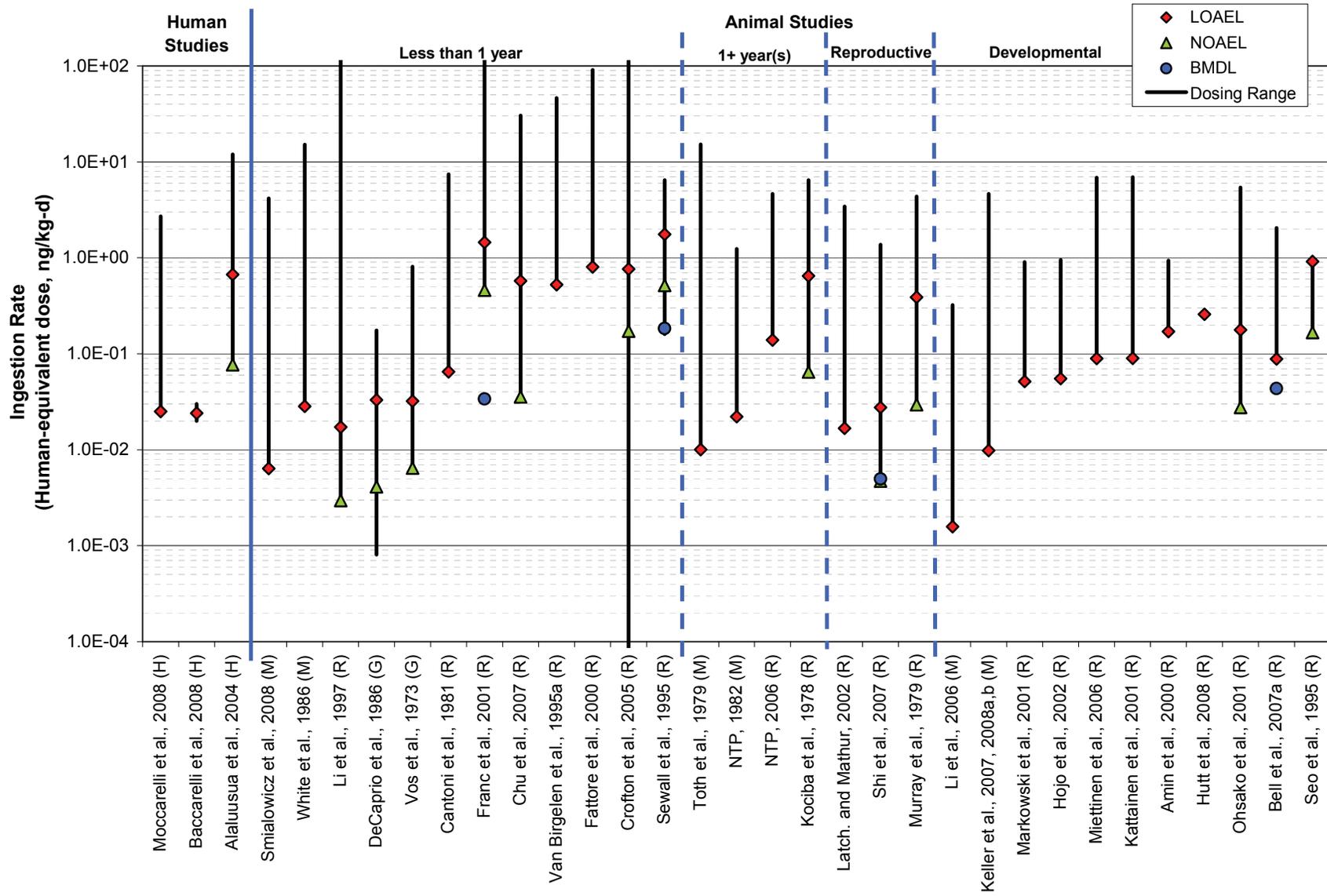


Figure 4-3. Exposure-response array for ingestion exposures to TCDD.

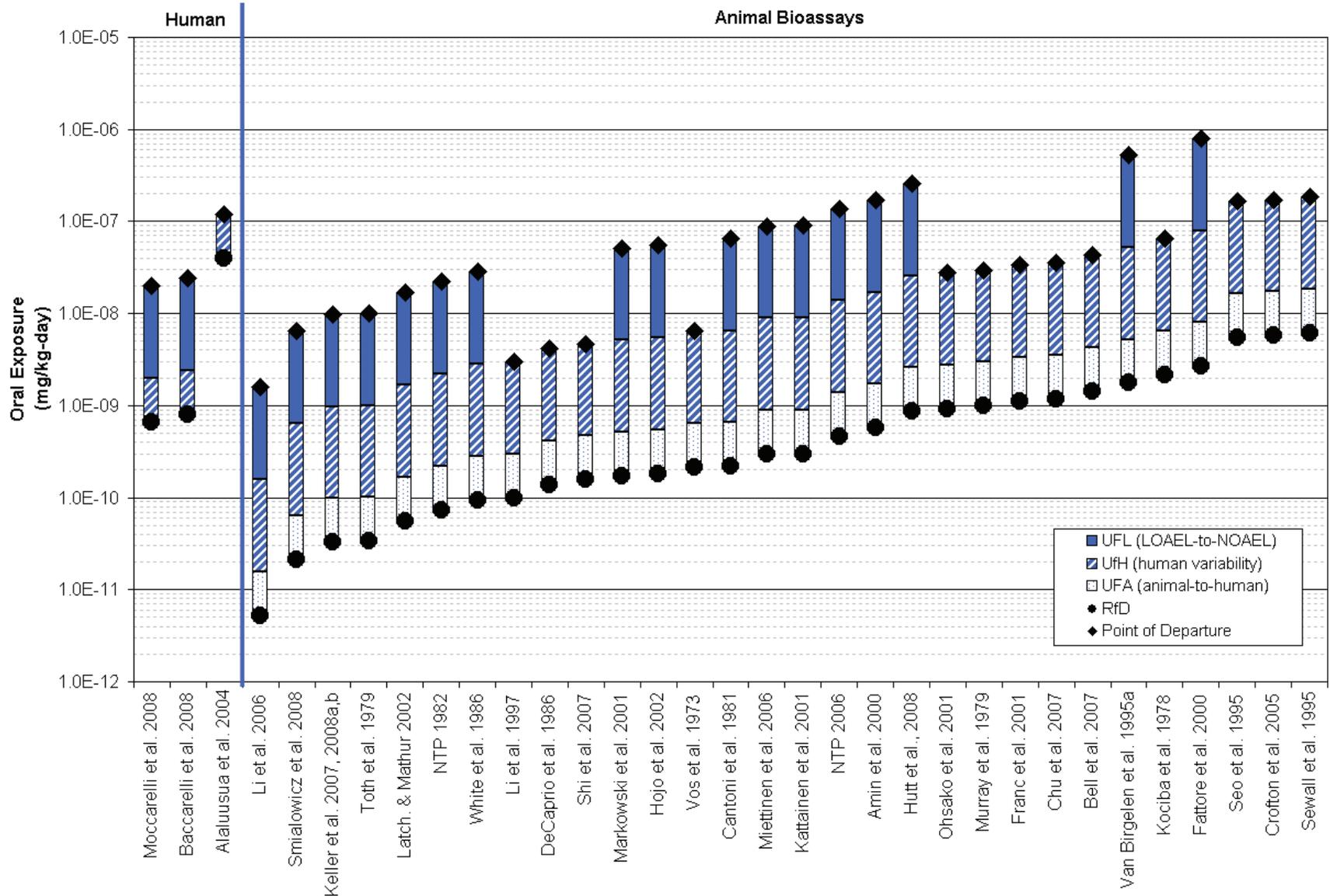


Figure 4-4. Candidate RfD array.

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4 **5. CANCER ASSESSMENT**

5 **5.1. QUALITATIVE WEIGHT-OF-EVIDENCE CARCINOGEN CLASSIFICATION**
6 **FOR 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD)**

7 **5.1.1. Summary of National Academy of Sciences (NAS) Comments on the Qualitative**
8 **Weight-of-Evidence Carcinogen Classification for 2,3,7,8-Tetrachlorodibenzo-**
9 ***p*-Dioxin (TCDD)**

10 In its charge, the National Academy of Sciences (NAS) was requested to comment
11 specifically on U.S. Environmental Protection Agency (EPA)’s conclusion that
12 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is best characterized as “carcinogenic to humans.”
13 While indicating that distinction between the categories of “carcinogenic to humans” and “likely
14 to be carcinogenic to humans” is “...based more on semantics than on science...” (NAS, 2006,
15 [198441](#), p. 141) and recommending that EPA “...spend its energies and resources on more
16 carefully delineating the assumptions used in quantitative risk estimates for TCDD...” (NAS,
17 2006, [198441](#), p. 141) rather than on the qualitative cancer descriptor for TCDD, the NAS
18 provided the following comments:

19 ...the classification of dioxin as “carcinogenic to humans” versus “likely to be
20 carcinogenic to humans” depends greatly on the definition and interpretation of
21 the specific criteria used for classification, with the explicit recognition that the
22 true weight of evidence lies on a continuum with no bright line that easily
23 distinguishes between these two categories. The committee agreed that, although
24 the weight of epidemiological evidence that dioxin is a human carcinogen is not
25 strong, the human data available from occupational cohorts are consistent with a
26 modest positive association between relatively high body burdens of dioxin and
27 increased mortality from all cancers. Positive animal studies and mechanistic data
28 provide additional support for classification of dioxin as a human carcinogen.
29 However, the committee was split on whether the weight of evidence met all the
30 necessary criteria described in the cancer guidelines for classification of dioxin as
31 “carcinogenic to humans.” EPA should summarize its rationale for concluding
32 that dioxin satisfies the criteria set out in the most recent cancer guidelines for
33 designation as either “carcinogenic to humans” or “likely to be carcinogenic to
34 humans (NAS, 2006, [198441](#), p. 140).

35
36 If EPA continues to designate dioxin as “carcinogenic to humans,” it should
37 explain whether this conclusion reflects a finding that there is a strong association
38 between dioxin exposure and human cancer or between dioxin exposure and a key
39 precursor event of dioxin’s mode of action (presumably AhR binding). If EPA’s
40 finding reflects the latter association, EPA should explain why that end point

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1 (e.g., AhR binding) represents a “key precursor event (NAS, 2006, [198441](#), p.
2 141).

3 4 **5.1.2. EPA’s Response to the NAS Comments on the Qualitative Weight-of-Evidence** 5 **Carcinogen Classification for TCDD**

6 A cancer descriptor is used to express the conclusion of the weight of evidence regarding
7 the carcinogenic hazard potential of a compound. EPA agrees with the NAS committee that
8 cancer descriptors represent points along a continuum of evidence. Relatedly, EPA
9 acknowledges that there are gradations and borderline situations that cannot be communicated
10 through a descriptor and are best clarified by a full weight of evidence narrative.

11 The 2003 Reassessment contains a detailed discussion of TCDD carcinogenicity in both
12 humans (Part II, Chapter 7a; 8) and animals (Part II, Chapter 6; 8) as well as an overall summary
13 of TCDD carcinogenicity (Part III, Chapter 2.2.1). Since the release of the 2003 Reassessment,
14 the database pertaining to TCDD carcinogenicity has been strengthened and expanded by
15 numerous publications (U.S. EPA, 2008, [519261](#)), including a new chronic bioassay in female
16 rats (NTP, 2006, [543749](#)) and several new follow-up epidemiological investigations (see
17 Section 2.4.1 and references therein). Many of these studies have been published subsequent to
18 the NAS review. These new data are summarized and evaluated in Section 2.4 of this document.

19 As noted by the NAS, the 2003 Reassessment was released prior to EPA’s publication of
20 the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (“2005 Cancer Guidelines”; U.S. EPA,
21 2005, [086237](#)). Using EPA’s guidance at the time of its release (U.S. EPA, 1996, [198087](#)), the
22 2003 Reassessment determined that the available evidence was sufficient to classify TCDD as a
23 “human carcinogen.” The 1996 guidance suggested “human carcinogen” to be an appropriate
24 descriptor of carcinogenic potential when there is an absence of conclusive epidemiologic
25 evidence to clearly establish a cause-and-effect relationship between human exposure and
26 cancer, but there are compelling carcinogenicity data in animals and mechanistic information in
27 animals and humans demonstrating similar modes of carcinogenic action.

28 The 2005 Cancer Guidelines (U.S. EPA, 2005, [086237](#)) are intended to promote greater
29 use of the increasing scientific understanding of the mechanisms that underlie the carcinogenic
30 process. The 2005 Cancer Guidelines expand upon earlier guidance applied in the 2003
31 Reassessment and encourage the use of chemical- and site-specific data versus default options,
32 the consideration of mode of action information and understanding of biological changes, fuller

1 characterization of carcinogenic potential, and consideration of differences in susceptibility. The
2 2005 Cancer Guidelines also emphasize the importance of weighing all of the available evidence
3 in reaching conclusions about the human carcinogenic potential of an agent. As noted above,
4 additional information on TCDD carcinogenicity has been published since the release of the
5 2003 Reassessment. This information has expanded the TCDD database and provided additional
6 support for conclusions made in the 2003 Reassessment regarding the carcinogenic potential of
7 TCDD.

8 Under the 2005 Cancer Guidelines (U.S. EPA, 2005, [086237](#)), TCDD is characterized as
9 *carcinogenic to humans*, based on the available data as of 2009. The 2005 Cancer Guidelines
10 indicate that this descriptor is appropriate when there is convincing epidemiologic evidence of a
11 causal association between human exposure and cancer or when all of the following conditions
12 are met (a) there is strong evidence of an association between human exposure and either cancer
13 or the key precursor events of the agent’s mode of action, but not enough for a causal
14 association, and (b) there is extensive evidence of carcinogenicity in animals, and (c) the mode(s)
15 of carcinogenic action and associated key precursor events have been identified in animals, and
16 (d) there is strong evidence that the key precursor events that precede the cancer response in
17 animals are anticipated to occur in humans and progress to tumors, based on available biological
18 information.

19 As noted above, the NAS commented that EPA should “...explain whether this
20 conclusion reflects a finding that there is a strong association between dioxin exposure and
21 human cancer or between dioxin exposure and a key precursor event of dioxin’s mode of action
22 (presumably AhR binding)” (NAS, 2006, [198441](#)). When evaluating the carcinogenic potential
23 of a compound, EPA employs a weight of evidence approach in which all available information
24 is evaluated and considered in reaching a conclusion. The following sections provide a summary
25 of EPA’s weight of evidence evaluation for TCDD.

26

27 **5.1.2.1. Summary Evaluation of Epidemiologic Evidence of TCDD and Cancer**

28 The available occupational epidemiologic studies provide convincing evidence of an
29 association between TCDD exposure and all cancer mortality. Among the strongest of these are
30 the studies of over 5,000 U.S. chemical manufacturing workers (the National Institute for
31 Occupational Safety and Health [NIOSH] cohort) (Aylward et al., 1997, [594365](#); Cheng et al.,

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1 2006, [523122](#); Collins et al., 2009, [197627](#); Fingerhut et al., 1991, [197301](#); Steenland et al.,
2 1999, [197437](#); Steenland et al., 2001, [198589](#)); a study of nearly 2,500 German workers involved
3 in the production of phenoxy herbicides and chlorophenols (the Hamburg cohort) (Becher et al.,
4 1996, [197121](#); Becher et al., 1998, [197173](#); Flesch-Janys et al., 1995, [197261](#); Flesch-Janys et
5 al., 1998, [197339](#); Manz et al., 1991, [199061](#); Nagel et al., 1994, [594369](#)); a study of more than
6 2,000 Dutch workers in two plants involved in the synthesis and formulation of phenoxy
7 herbicides and chlorophenols (the Dutch cohort) (Bueno et al., 1993, [196993](#); Hooiveld et al.,
8 1998, [197829](#)); a smaller study of roughly 250 workers involved in a chemical accident cleanup
9 (the BASF cohort) ed in a chemical accident cleanup (the BASF cohort) (Ott and Zober, 1996,
10 [198101](#); Thiess et al., 1982, [064999](#); Zober et al., 1990, [197604](#)); and an international study of
11 more than 18,000 workers exposed to phenoxy herbicides and chlorophenols (Kogevinas et al.,
12 1997, [198598](#); Saracci et al., 1991, [199190](#)) including newer studies of smaller subsets of these
13 workers (McBride, 2009, [198490](#); McBride et al., 2009, [197296](#); t' Mannetje et al., 2005,
14 [197593](#)). The findings from these studies have been thoroughly described either in the 2003
15 Reassessment or in Section 2.4.1 of this document.

16 As noted in Section 2.4, there are considerable challenges inherent in addressing potential
17 sources of confounding from smoking and co-exposure to other carcinogens, (which could
18 produce inflated or spurious associations), the healthy worker effect, (which could result in
19 attenuated effects through comparison with a referent background with an inappropriately high
20 background risk), and quantifying exposure to the populations included in many of these
21 retrospective studies. The more recent studies of these cohorts have made significant advances
22 in reducing the potential for bias from the healthy worker effect through use of internal cohort
23 analyses and/or controlling for potential confounders through statistical adjustment, restriction,
24 and use of internal comparisons. Although some exposure assessment uncertainties remain,
25 some of these studies have also collected individual-level TCDD exposure estimates that allow
26 quantification of effective dose necessary for dose-response modeling. Overall, the occupational
27 data provide consistent support for an association between exposure to TCDD and increased
28 cancer mortality.

29 Additional epidemiologic evidence supporting an association between TCDD exposure
30 and cancer comes from studies investigating the morbidity and mortality of residents exposed to
31 TCDD following an accidental release from a chemical plant near Seveso, Italy (the Seveso

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1 cohort) (Bertazzi et al., 1989, [197013](#); Bertazzi et al., 1993, [192445](#); Bertazzi et al., 1997,
2 [197097](#); Bertazzi et al., 2001, [197005](#); Consonni et al., 2008, [524825](#); Pesatori et al., 1998,
3 [523076](#); Pesatori et al., 2003, [197001](#); Warner et al., 2002, [197489](#)). Pesatori et al. (2003,
4 [197001](#)) and Consonni et al. (2008, [524825](#)) were not available at the time the 2003
5 Reassessment was released. Among individuals with relatively high exposure at Seveso
6 (Zones A and B combined), all-cancer mortality in the 20-year post-accident period and all-
7 cancer incidence in the 15-year post-accident period failed to exhibit significant departures from
8 the expected [197001](#)). However, an increased risk of all-cancer mortality was noted among men
9 15–20 years after first exposure; not only is the association similar in magnitude to other studies
10 (relative risk [RR] = 1.3; 95% confidence interval [CI] = 1.0–1.7) but also emphasizes the
11 importance of consideration of latency (Bertazzi et al., 2001, [197005](#)). Furthermore, associations
12 between TCDD and some specific cancer sites were detected in this cohort, including increased
13 incidence (based on 15 years of follow-up) and mortality (based on 20 years follow-up) from
14 lymphatic and hematopoietic neoplasms in both males and females from Zones A and B
15 (Consonni et al., 2008, [524825](#)). This excess was primarily due to non-Hodgkin’s lymphoma.
16 Additionally, there was an increase in lung and rectal cancer mortality in men (Bertazzi et al.,
17 2001, [197005](#)) and limited evidence of increased liver cancer incidence in women based on the
18 15-year follow-up study (Bertazzi et al., 1993, [192445](#)). In a separate analysis of 981 women in
19 Zone A, breast cancer incidence ($n = 15$) was associated (a 2-fold increase for a 10-fold increase
20 in serum TCDD) with TCDD measurements first collected in 1976 and 1977 (Warner et al.,
21 2002, [197489](#)). The authors also reported a 2–3-fold increase in all cancer incidence ($n = 21$) for
22 the two upper quartiles of TCDD exposure.

23 Overall, the newer studies of the Seveso cohort have reported significant increases in
24 cancer incidence and elevations in cancer mortality that were not evident in earlier studies of this
25 cohort. While these studies demonstrate an association between TCDD exposure and different
26 types of cancer, one of the main limitations is the small number of cancer cases to assess
27 site-specific associations with TCDD exposure. Ongoing studies in that cohort should help
28 further elucidate potential risk for specific cancer types (and other endpoints) associated with
29 TCDD exposures among this population.

30

1 **5.1.2.1.1. Evidence for causality.**

2 The evidence for causality for cancer from the human studies is briefly summarized in the
3 paragraphs that follow and is based on recommendations from the 2005 Cancer Guidelines. It
4 should be noted that there are methodological limitations of the epidemiologic studies that may
5 temper some of the conclusions regarding causality. These limitations include limited statistical
6 power, exposure assessment uncertainty, and lack of control of confounders (e.g., dioxin-like
7 compounds and smoking) in some studies. There also is additional uncertainty in the evidence
8 for causality due to the lack of organ specificity in TCDD associated cancers, as the most
9 consistent results occurred for all-cancer mortality; however, this would be consistent with a
10 hypothesized carcinogenic mode of action of TCDD as a promoter. Despite these uncertainties,
11 many of the more recent studies have greatly improved exposure assessments compared to
12 earlier studies of the same cohorts and have addressed the potential for confounding and other
13 types of biases.

14 **Temporality**—exposure must precede the effect for causal inference. Given the long
15 induction period for many types of cancers, exposure should precede the effect with a sufficient
16 latency (i.e., typically 15–20 years for environmental carcinogens). In all the occupational
17 studies reviewed (with the exception of (McBride, 2009, [198490](#))), TCDD exposure has
18 preceded the effect with sufficient latency to be considered causally associated. In the studies of
19 the Seveso cohort, the follow-up exposure period has now reached 20 years, a latency sufficient
20 to address some carcinogenic endpoints. Since most of the studies are based on occupational
21 exposures or accidental releases into the environment, temporality is more readily established
22 due to the obvious determination of the specific exposure windows prior to disease onset.

23 **Strength of Association**—refers to the magnitude of measures of association such as the
24 ratio of incidence or mortality (e.g., standardized mortality ratio [SMRs], standardized incidence
25 ratios, RRs, or odds ratios) in addition to statistical significance considerations. Effect estimates
26 that are large in magnitude are less likely to be due to chance, bias, or confounding. Reports of
27 modest risk, however, do not preclude a causal association and may reflect an agent of lower
28 potency, lower levels of exposure or attenuation due to nondifferential exposure
29 misclassification. The four occupational cohorts with the highest exposures (NIOSH, Hamburg,
30 Dutch, and BASF) consistently showed statistically significant, although moderate, elevations in
31 cancer mortality. When the data were combined, the SMR for all four subcohorts was 1.4

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1 [95% CI = 1.2–1.6] (IARC, 1997, [537123](#)). Based on findings from the International Agency for
2 Research on Cancer (IARC) Working Group, increases in all cancer (combined) mortality of the
3 magnitude reported for TCDD have rarely been found in occupational cohort studies (IARC,
4 1997, [537123](#)). Although these estimates are higher than the all-cancer mortality results among
5 Seveso men (RR = 1.1; 95% CI = 1.0–1.3), they are comparable to the risk estimated in this
6 population (RR = 1.3; 95% CI = 1.0–1.7) 15–20 years after first exposure.³¹ These consistent
7 results comparable in magnitude from the occupational cohorts and Seveso population are not
8 likely due to chance.

9 The occupational cohort studies also show an increased risk for lung cancer in the
10 previously mentioned four subcohorts. The relative risk for lung cancer in the combined highly
11 exposed subcohorts was estimated to be 1.4 (95% CI = 1.1–1.7) (IARC, 1997). This is
12 consistent with the lung cancer mortality findings for the highest exposed group of men in
13 Seveso (RR = 1.3; 95% CI = 1.0–1.7). Additionally, there was an increase in rectal cancer
14 mortality in the Seveso cohort (RR = 2.4; 95% CI = 1.2–4.6) (Bertazzi et al., 2001, [197005](#)) with
15 a corresponding increase in incidence. Consistent relative risks of more than two were also
16 detected for rectal cancer in the Hamburg and New Zealand cohorts, but increased risks were not
17 found in the other cohorts. Although there was limited evidence of increased incidence or
18 mortality from hepatobiliary cancers across the cohorts, liver cancer incidence was elevated in the
19 15-year post accident period among women in the Seveso cohort (RR = 2.4; 95% CI = 1.1–5.1,
20 (Warner et al., 2002, [197489](#))). An association in this population was also detected for between
21 breast cancer incidence (RR = 2.1; 95% CI = 1.0–4.6) and serum TCDD levels (per a 10-fold
22 increase in serum TCDD). Although findings were based on small numbers, three- and four-fold
23 increased risks of soft tissue sarcoma were detected among the NIOSH (Collins et al., 2009,
24 [197627](#)) and New Zealand cohorts (McBride, 2009, [198490](#)). No other cases of this very rare
25 cancer were detected in the exposed populations from the other cohorts.

³¹In addition to consideration of statistical significance to address the possibility of random variability (i.e., chance), many other factors are important to consider when assessing causality using a weight of evidence determination. As noted in the EPA's Cancer Guidelines, a number of factors besides statistical significance are relevant for assessing evidence of adverse health effects based on human data. These include strength of association, temporality, biological gradient (i.e., dose-response concordance), biological plausibility, etc.). In analyzing the body of information in the literature, the consistency of the magnitude of reported risk estimates (across different studies) is considered when addressing causality; rather than relying solely on statistical significance.

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1 Elevated risk of lymphohemopoietic cancer mortality was noted among the Seveso cohort
2 (RR = 1.7; 95% CI = 1.2, 2.5) (Consonni et al., 2008, [524825](#)). Increased SMRs for
3 lymphohemopoietic cancer comparable in magnitude (range: 1.6–2.2) were also detected among
4 the Hamburg and New Zealand occupational cohorts, but limited evidence (range: 1.0 to 1.2) of
5 increased mortality was found in the BASF, NIOSH and Ranch Hands employees (Akhtar et al.,
6 2004, [197141](#); Ott and Zober, 1996, [198101](#); Steenland et al., 1999, [197437](#)). Most of the
7 lymphohemopoietic cancer mortality risk was reportedly due to non-Hodgkin’s lymphoma in
8 most of the cohorts. Relative risks for non-Hodgkin’s lymphoma among TCDD exposed
9 populations from the NIOSH, Hamburg, New Zealand, Dutch, and Seveso cohorts ranged from
10 1.2 to 3.8. Although statistical power was limited in most of these studies, relative risks
11 exceeded 3.0 for non-Hodgkin’s lymphoma in three of these cohorts (Consonni et al., 2008,
12 [524825](#); Flesch-Janys et al., 1998, [197339](#); Hooiveld et al., 1998, [197829](#)).

13 **Consistency**—the observation of the same site-specific effect across several independent
14 study populations strengthens an inference of causality. Despite differences across occupational
15 cohorts, most studies have consistently reported increases in all-cancer mortality with TCDD
16 exposure. Several of these studies have also reported increases in lung cancer related to TCDD
17 exposure. As noted above, there is also suggestive evidence of an increased risk in all-cancer
18 and lung cancer mortality among the Seveso cohort consistent in magnitude to the occupational
19 cohorts. Elevated risk of lymphohemopoietic cancer mortality consistent in magnitude
20 (range: 1.6–2.2) was also detected among the Seveso, Hamburg and New Zealand cohorts. An
21 increased risk for non-Hodgkin’s lymphoma was found in two of the occupational cohorts as
22 well as in the Seveso cohort, although the relative risks largely did not achieve statistical
23 significance. Among those studies detecting an association, consistent two-fold relative risks
24 were found for rectal cancer (Bertazzi et al., 2001, [197005](#); Flesch-Janys et al., 1998, [197339](#);
25 McBride, 2009, [198490](#)) and relative risks in excess of three were detected for soft tissue
26 sarcoma (Collins et al., 2009, [197627](#); McBride, 2009, [198490](#)).

27 **Biological Gradient**—refers to the presence of a dose-response and/or duration-response
28 between a health outcome and exposure of interest. Several of the occupational cohort studies
29 (Flesch-Janys et al., 1998, [197339](#); Manz et al., 1991, [199061](#); Michalek and Pavuk, 2008,
30 [199573](#); Ott and Zober, 1996, [198101](#); Steenland et al., 1999, [197437](#)) found evidence of a
31 dose-response relationship for all cancers and various TCDD exposure measures. The SMR

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1 analyses based on internal comparisons within the occupational cohorts show a biological
2 gradient by comparing highly TCDD exposed workers to low or unexposed workers. A
3 biological gradient was also demonstrated in the Seveso cohort by comparing highly exposed
4 individuals (Zones A and B) to individuals in lower exposure zones (Zones C and R). Warner et
5 al. (2002, [197489](#)) also reported evidence of a dose-response trend for breast cancer and
6 increasing TCDD exposures.

7 **Biological Plausibility**—refers to the observed effect having some biological link to the
8 exposure. Most evidence suggests that toxic effects of TCDD are mediated by interaction with
9 the aryl hydrocarbon receptor (AhR). AhR is a highly conserved protein among mammals,
10 including humans (Fujii-Kuriyama et al., 1995, [543727](#); Harper et al., 2002, [198124](#); Nebert et
11 al., 1991, [543728](#)). Several hypothesized modes of action have been presented for TCDD-
12 induced tumors in rodents, all involving AhR activation. The available evidence does not
13 preclude the relevance of these hypothesized modes of action to humans.

14 **Specificity**—as originally intended, refers to increased inference of causation if a single
15 site effect, as opposed to multiple effects, is observed and associated with exposure. Based on
16 current biological understanding, this is now considered one of the weaker guidelines for
17 causality. As stated in the 2005 Cancer Guidelines, given the current understanding that many
18 agents cause cancer at multiple sites, and cancers have multiple causes, the absence of specificity
19 does not detract from evidence for a causal effect. Given that the most consistent findings
20 associating TCDD and cancer are for all-cause cancer mortality, epidemiological evidence
21 suggests that TCDD lacks specificity for particular tumor sites. A key event in TCDD's mode of
22 action is binding to and activating AhR; however, downstream events leading to tumor formation
23 are uncertain and may likely be tissue specific. Given that the AhR is highly conserved among
24 species and is expressed in various human tissues, the lack of tumor site specificity does not
25 preclude a determination of causality.

26 In summary, EPA finds the available epidemiological information provides strong
27 evidence of an association between TCDD exposure and human cancer that cannot be reasonably
28 attributed to chance or confounding and other types of bias, and with a demonstration of
29 temporality, strength of association, consistency, biological plausibility, and a biological
30 gradient. Additional evidence from animal studies and from mechanistic studies (described
31 below) provides additional support for the classification of TCDD as carcinogenic to humans.

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1 **5.1.2.2. Summary of Evidence for TCDD Carcinogenicity in Experimental Animals**

2 An extensive database on the carcinogenicity of TCDD in experimental animals is
3 described in detail in Part II, Chapter 6 of the 2003 Reassessment. There is substantial evidence
4 that TCDD is carcinogenic in experimental animals based on long-term bioassays conducted in
5 both sexes of rats and mice (Kociba et al., 1978, [001818](#); NTP, 1982, [594255](#); NTP, 2006,
6 [543749](#)) and in male hamsters (Rao et al., 1988, [199032](#)). Additionally, National Toxicology
7 Program (NTP, 2006, [543749](#)) has completed a new chronic bioassay in female Sprague Dawley
8 rats. These studies are summarized in Section 2.4.2 of this document. All studies have produced
9 positive results, with TCDD increasing the incidence of tumors at sites distant from the site of
10 treatment and at doses well below the maximum tolerated dose. In both sexes of rodents, when
11 administered by different routes and at low doses, TCDD caused tumors at multiple sites; tumors
12 were observed in liver, lung, lymphatic system, soft tissue, nasal turbinates, hard palate, thyroid,
13 adrenal, pancreas, and tongue. The most consistent and best characterized carcinogenic
14 responses to TCDD are in the rodent liver, lung, and thyroid (discussed below in
15 Section 5.1.2.3).

16 17 **5.1.2.3. TCDD Mode of Action**

18 The 2005 Cancer Guidelines defines the term “mode of action” as “a sequence of key
19 events and processes, starting with interaction of an agent with a cell, proceeding through
20 operational and anatomical changes, and resulting in cancer formation.” A “key event” is an
21 empirically observable precursor step that is itself a necessary element of the mode of action or is
22 a biologically based marker for such an element. Mode of action is contrasted with “mechanism
23 of action,” which implies a more detailed understanding and description of events, often at the
24 molecular level. In the case of TCDD, the terms ‘mechanism of action’ and ‘mode of action’ are
25 often used interchangeably in the scientific literature in reference to TCDD’s interaction with the
26 AhR. A thorough discussion of TCDD’s interaction with the AhR can be found in the 2003
27 Reassessment (Part II, Chapter 2; Part III, Chapter 3), and is summarized below (see
28 Section 5.1.2.3.1).

29 Most evidence suggests that the majority of toxic effects of TCDD are mediated by
30 interaction with the AhR. EPA considers interaction with the AhR to be a necessary, but not
31 sufficient, event in TCDD carcinogenesis. The sequence of key events following binding of

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1 TCDD to the AhR and that ultimately leads to the development of cancer is unknown.
2 Therefore, in the strictest sense, TCDD’s interaction with the AhR does not constitute a “mode
3 of action” as defined by the 2005 Cancer Guidelines because information about the progression
4 of necessary events is lacking. However, AhR binding and activation by TCDD is considered to
5 be a key event in TCDD carcinogenesis.

6

7 **5.1.2.3.1. *The aryl hydrocarbon receptor (AhR).***

8 While substantial evidence suggests that most toxic effects of TCDD are mediated by
9 interaction with the AhR, less is known about the complex responses that result in tumor
10 formation. Nonetheless, a picture is emerging wherein TCDD is considered a
11 “receptor-mediated carcinogen” in laboratory animals (see Figure 5-1), acting in a manner
12 similar to peroxisome proliferators, phorbol esters, or estrogen (Woods et al., 2007, [543735](#)).

13 TCDD activates the AhR, a member of the basic helix-loop-helix, Per-Arnt-Sim
14 (bHLH-PAS) family of transcription factors. AhR is present in most cell types and in the
15 inactivated state is cytosolic and exists in a complex with chaperone proteins, such as heat shock
16 protein 90 (Hsp90). Binding of TCDD to AhR leads to nuclear translocation and
17 heterodimerization with its partner protein Arnt, another bHLH-PAS family member. The
18 AhR:Arnt heterodimer binds to specific cognate DNA sequence elements known as
19 dioxin/xenobiotic response elements (DRE/XRE) present in the regulatory region of specific
20 genes. Binding of the AhR:Arnt heterodimer to these elements, and subsequent recruitment of
21 tissue specific transcriptional coactivator complexes, leads to increased transcription of specific
22 genes, known as “target genes.” There is a battery of genes affected in this manner and targets
23 include certain xenobiotic-metabolizing enzymes, such as cytochrome P450 (CYP)1A1,
24 CYP1A2, CYP2B1, and UDP-glucuronosyltransferase (UGT)1A6 (reviewed in Schwartz and
25 Appel, 2005, [543737](#)). In addition, genes affected by the TCDD/AhR-complex code for both
26 inhibitory and stimulatory growth factors; their gene products affect cellular growth,
27 differentiation and homeostasis and have been shown to contribute to carcinogenicity as well as
28 other forms of toxicity (reviewed in Popp et al., 2006, [197074](#)).

29 Detailed molecular biology research has been performed to identify the extent of the
30 genes regulated by AhR (Woods et al., 2007, [543735](#)); however a complex and still ill-defined
31 profile remains. The basic physiology of AhR signaling is still poorly understood, despite being

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1 highly conserved among vertebrate species (reviewed in Hahn, 2002, [099302](#)). In fact, it is now
2 known that the AhR recognizes a large number of chemical structures, including nonaromatic
3 and nonhalogenated compounds (Denison and Nagy, 2003, [197226](#)), which supports the
4 biological role of the AhR as a receptor that helps regulate the expression of genes necessary for
5 biotransformation of environmental chemicals (i.e., CYP1A1). However, the endogenous
6 physiological role of AhR is complicated, as evidenced by the numerous studies examining AhR
7 null (ArH -/-) mice, which demonstrate alterations in the liver, immune system, ovary, heart and
8 other organs (reviewed in Hahn, 2009, [477460](#)). The endogenous function of AhR remains
9 unknown.

10 Given that the AhR is expressed in most tissues (Dolwick et al., 1993, [543762](#)) with
11 tissue-specificity in terms of level of expression and the profile of target genes, there is
12 substantial complexity and difficulty associating TCDD-mediated transcription of specific target
13 genes and tissue-specific toxic responses, including cancer. It is important to note that the extent
14 of the response of individual TCDD target genes does not correlate with site-specific
15 tumorigenicity. For example, while TCDD is ineffective as a tumor promoter in ovariectomized
16 rats and does not stimulate liver cell proliferation in these animals, it is still capable of inducing
17 CYP1A2 in roughly the same magnitude as in the intact female rats (Lucier, 1991, [198691](#)).
18 Similarly, CYP1A1 induction by TCDD is very similar in male and female rats even though
19 males are almost completely resistant to TCDD carcinogenicity (Wyde et al., 2002, [197009](#)).

20 Some of AhR's effects on gene expression may be the result of interaction with other
21 transcription factors (such as the retinoblastoma protein (Ge and Elferink, 1998, [197702](#)), NF- κ B
22 (Tian et al., 1999, [198378](#)) or with the tyrosine kinase c-Src (Blankenship and Matsumura, 1997,
23 [543751](#)) rather than via direct interaction with DNA. By far the most extensive studies involving
24 cross-talk between AhR and another transcription factor are those involving the estrogen receptor
25 alpha (ER α). The anti-estrogenic properties of TCDD have been well-documented, beginning
26 with the observations that TCDD repressed estradiol function in rat uterus and liver. The
27 AhR-ER α cross-talk can be manifested at several levels including direct protein interaction,
28 association of the receptors with the other's response element and altered metabolism of estradiol
29 by AhR ligand (Takemoto et al., 2004, [543753](#)). The interactions between AhR/Arnt- and
30 estrogen receptor-dependent signaling pathways, which mediate anti-estrogenic effects of
31 dioxins and dioxin-like polychlorinated biphenyls (PCBs; Bock, 1994, [543755](#)), is probably

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1 causal for the well-documented gender-specificity of the carcinogenic effects of these agents
2 (e.g., hepatocarcinogenicity of TCDD in female as opposed to male rats) (Lucier, 1991, [198691](#)).
3 In addition, cross-talk between AhR/Arnt and other nuclear receptors, their coactivators, and
4 corepressors, has been described. In fact, cross-talk has been reported for AhR and numerous
5 signaling pathways involved in a broad range of physiological processes. The molecular
6 mechanisms by which the AhR interferes with these signaling networks are multifaceted and
7 occur at multiple levels of regulation (many beyond transcriptional control)
8 (Haarmann-Stemmann et al., 2009, [197874](#)). It remains unknown how any of these molecular
9 pathways involving AhR signaling are linked to TCDD-mediated carcinogenesis.

10 Pertinent to human risk assessment, there are wide inter- and intraspecies differences in
11 the toxicological responses to TCDD (Ema et al., 1994, [197313](#); Poland and Glover, 1990,
12 [543759](#); Poland et al., 1994, [198439](#)) some of which can be explained by polymorphisms in
13 AhR. For instance, there is a 10-fold difference in susceptibility to TCDD-induced toxicity
14 between the TCDD-sensitive C57BL/6 and the TCDD-resistant DBA/2 strains of mice (Poland
15 and Glover, 1980, [543761](#)) that can be explained by polymorphic variations in the ligand-binding
16 domain and in the C-terminal region of the AhR molecule of each strain (Dolwick et al., 1993,
17 [543762](#)). Depending on the system examined, the estimated affinity of binding of TCDD (and
18 related compounds) to the human AhR is about 10-fold lower than that observed to the AhR
19 from “responsive” rodent species and is comparable to that observed to the AhR from
20 “nonresponsive” mouse strains (Ramadoss and Perdew, 2004, [198824](#)). This reduced affinity is
21 due, in part, to a single amino acid substitution within the ligand binding domain of the human
22 and “nonresponsive” mouse AhRs (Ramadoss and Perdew, 2004, [198824](#)). Although the affinity
23 of binding of TCDD and related compounds to the human AhR is reduced compared with rodent
24 AhRs, the qualitative and quantitative rank-order potency of these chemicals is similar. The
25 considerable tissue and species variability in response to TCDD cannot be ascribed solely to
26 polymorphisms of the AhR gene (Geyer et al., 1997, [543768](#); Pohjanvirta and Tuomisto, 1994,
27 [543767](#)), further complicating this key event in TCDD-mediated carcinogenesis.

28 29 **5.1.2.3.1.1. Other AhR considerations.**

30 In addition to the potent agonist TCDD, there are many other exogenous ligands for the
31 AhR, including certain polycyclic aromatic hydrocarbons, polychlorinated dibenzofurans, and

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1 PCBs (Bock, 1994, [543755](#)). Several natural and endogenous compounds are also regulators of
2 AhR (Chiaro et al., 2008, [543771](#)). The classes of endogenous compounds that have been shown
3 to induce CYP1 and/or activate AhR include: (a) tryptophan metabolites, other indole-containing
4 molecules, and phenylethylamines (Gielen and Nebert, 1971, [543775](#)); (b) tetrapyrroles such as
5 bilirubin and biliverdin; (c) sterols such as 7-ketocholesterol and the horse steroid equilenin;
6 (d) fatty acid metabolites, including at least six different prostaglandins (Seidel et al., 2001,
7 [543776](#)) and lipoxin A4; and (e) the ubiquitous second messenger cAMP (reviewed in McMillan
8 and Bradfield (2007, [543777](#)) and Barouki et al. (2007, [543778](#))). Several of these endogenous
9 and exogenous compounds, including bilirubin, biliverdin, and β -naphthoflavone, that also bind to
10 the AhR are not carcinogenic in rodent models, therefore, some other key precursor event(s)
11 need to be identified. Further, the existence of multiple ligands with varying affinity and
12 responses suggests that “selective receptor modulators” (or SRMs) of the AhR exist. SRMs are
13 ligands for a receptor that, upon binding, elicit a conformational change in the receptor that
14 results in differential recruitment of coregulatory molecules to the target gene promoter region,
15 thereby imparting a different biological activity relative to the prototypical ligand. This
16 phenomenon has been most studied for nuclear receptors such as the ER α with the classic
17 example being tamoxifen, which has estrogen-like activity in the uterus but anti-estrogen-like
18 effects in the breast. Thus, the relative abilities of compounds to stimulate gene expression or
19 other effects vary in promoter- and cell type-specific manners. It is now apparent that SRMs
20 exist for the AhR as well (SAhRMs, Fretland et al., 2004, [197357](#)). For example,
21 6-methyl-1,3,8-trichlorodibenzofuran (6-MCDF), a SAhRM whose structure is similar to that of
22 TCDD, can induce CYP1A1 gene expression in liver but does not lead to the toxic responses
23 associated with TCDD (Fritz et al., 2009, [594372](#)). The existence of SAhRMs further
24 complicates the role of TCDD binding to AhR as a key event in TCDD-mediated
25 carcinogenicity, and suggests that additional information is necessary to elucidate the
26 carcinogenic mode of action of TCDD.

27 TCDD may have dose-dependent modes of action. It has been demonstrated that
28 AhR-deficient (AhR $^{-/-}$) mice show no signs of toxicity at doses of TCDD approximating the
29 lethal dose eliciting 50% response (LD₅₀) dose (200 μ g/kg) in AhR $^{+/+}$ mice (Fernandez-
30 Salguero et al., 1996, [197650](#)). However, a single high exposure of 2,000 μ g/kg to
31 AhR-deficient mice produced several minor lesions including scattered necrosis and vasculitis in

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1 the liver and lungs. These data suggest that a pathway leading to toxicity exists, albeit at very
2 high doses, that is independent of the AhR. However, these data also indicate that, at least in
3 mice, the major in vivo effects of TCDD are mediated through the AhR. The finding of
4 carcinogenicity in hamsters (Rao et al., 1988, [199032](#)) is of special interest since hamsters have
5 been found to be relatively resistant to the lethal effects of TCDD (Henck et al., 1981, [543779](#);
6 Olson et al., 1980, [197976](#)). To date, there have been no chronic bioassay studies of TCDD
7 carcinogenicity in AhR-deficient transgenic animals.

8 There are additional insights into the complexity of TCDD's mechanism of action
9 involving AhR. Some biochemical responses to TCDD treatment in isolated cells have been
10 reported in cells lacking Arnt, in cells expressing a mutated Arnt protein and in cells with highly
11 reduced levels of AhR (Kolluri et al., 1999, [548721](#); Puga et al., 1992, [543784](#)), implying either
12 a non nuclear role of the AhR in mediating these events or an AhR-independent process.

13 Additionally, recent studies have linked AhR activation in the absence of exogenous
14 ligand to a multitude of biological effects, ranging from control of mammary tumorigenesis to
15 regulation of autoimmunity (Hahn et al., 2009, [548725](#)). Finally, constitutively activated AhR in
16 rodents has been shown to induce stomach tumors (Andersson et al., 2002, [197101](#)). This
17 indicates that AhR activation alone (i.e., in the absence of ligand) is sufficient to induce tumors.

18 19 **5.1.2.3.2. TCDD as a tumor promoter.**

20 The role of TCDD as a tumor promoter is discussed in the 2003 Reassessment (Part II,
21 Chapter 6). The following is a brief summary of the information regarding TCDD as a tumor
22 promoter.

23 Numerous studies have examined the tumor promoting potential of TCDD. Using the
24 traditional two-stage initiation-promotion study design in the liver, studies have demonstrated
25 that TCDD is a dose- and duration-dependent liver tumor promoter (Dragan and Schrenk, 2000,
26 [197243](#); Maronpot et al., 1993, [198386](#); Pitot et al., 1980, [197885](#); Teeguarden et al., 1999,
27 [198274](#); Walker et al., 2000, [198733](#))(Walker et al., 1998). TCDD has also tested positive for
28 tumor promoting ability in the two-stage models of mouse skin tumorigenesis (Dragan and
29 Schrenk, 2000, [197243](#); IARC, 1997, [537123](#)), and in the lung (Anderson et al., 1991, [201761](#);
30 Beebe et al., 1995, [548754](#)). Overall, the data demonstrate that TCDD is a tumor promoter and
31 potentially harbors only weak initiating activity.

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1 TCDD is typically designated as a nongenotoxic and nonmutagenic carcinogen because it
2 does not damage DNA directly through the formation of DNA adducts, is negative in most
3 short-term assays for genotoxicity, and is a potent tumor promoter and a weak initiator or
4 noninitiator in multistage models for chemical carcinogenesis (Clark et al., 1991, [594378](#);
5 Flodstrom and Ahlborg, 1991, [548728](#); Graham et al., 1988, [594375](#); Lucier, 1991, [198691](#); Pitot
6 et al., 1980, [197885](#); Poland et al., 1982, [199756](#)). However, mechanisms have been proposed
7 that support the possibility that TCDD might be indirectly genotoxic, either through the
8 induction of oxidative stress or by altering the DNA-damaging potential of exogenous and
9 endogenous compounds, such as estrogens. In addition, there have been numerous reports
10 demonstrating TCDD-induced modifications of growth factor signaling pathways and cytokines
11 in experimental animals and cell culture systems. Some of the altered signaling pathways
12 include those for epidermal growth factor, transforming growth factor alpha, glucocorticoids,
13 estrogen, tumor necrosis factor-alpha, interleukin 1-beta, plasminogen inactivating factor-2, and
14 gastrin. Many of these pathways are involved in cell homeostasis, proliferation, and
15 differentiation and provide plausible mechanisms responsible for the carcinogenic actions of
16 TCDD. Unfortunately, information on the etiology of the different tumor types is lacking to
17 equivocally link tumor promotion or indirect genotoxic action of TCDD to a specific mechanism
18 or mode of TCDD carcinogenesis.

19

20 **5.1.2.3.3. Hypothesized modes of action of TCDD in rodents.**

21 TCDD has been shown to consistently induce multiple tumors in both sexes in several
22 rodent species. These tumors are observed in various tissues, including (but not limited to):
23 liver, lung, thyroid, lymphatic system, soft tissue, nasal turbinates, hard palate, adrenal, pancreas,
24 and tongue. While the mode of action of TCDD in producing cancer has not been elucidated for
25 any tumor type, the best characterized carcinogenic actions of TCDD are in rodent liver, lung,
26 and thyroid. The hypothesized mode of action for each of these three tumor types is briefly
27 discussed below and is described in Figure 5-2. The hypothesized sequence of events following
28 TCDD interaction with the AhR is markedly different for each of these three tumor types. No
29 detailed hypothesized mode of action information exists for any of the other reported tumor
30 types. Further, no single definitive mode of action of TCDD-mediated carcinogenicity has been
31 identified.

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1 **5.1.2.3.3.1. Liver tumors.**

2 The mode of action of TCDD in producing liver cancer in rodents has not been
3 elucidated. One hypothesized mode of carcinogenic action of TCDD in the liver is mediated
4 through hepatotoxicity. Generically speaking, TCDD activation of the AhR leads to a variety of
5 changes in gene expression, which then lead to hepatotoxicity, followed by compensatory
6 regenerative cellular proliferation and subsequent tumor development (see Figure 5-2). The
7 details of the mechanism of TCDD-induced hepatotoxicity have not been fully determined but
8 both CYP induction and oxidative stress have been postulated to be involved (Maronpot et al.,
9 1993, [198386](#); Viluksela et al., 2000, [198968](#)). The enhanced cell proliferation arising from
10 either altered gene expression or hepatotoxicity, or both, may lead to the promotion of
11 hepatocellular tumors (Whysner and Williams, 1996, [197556](#)). The sensitivity of female rat liver
12 to TCDD, which apparently does not extend to the mouse, depends on ovarian hormones (Lucier,
13 1991, [198691](#); Wyde et al., 2001, [198575](#)). This sensitivity has been ascribed to induction of
14 estradiol metabolizing enzymes (Graham et al., 1988, [594375](#)) and is hypothesized to lead either
15 to generation of reactive metabolites of endogenous estrogen or to active oxygen species of
16 estrogens. Oxidative DNA damage has been implicated in liver tumor promotion (Umemura et
17 al., 1999, [198001](#)).

18 A dose-response relationship exists for TCDD-mediated hepatotoxicity, and this parallels
19 the dose-response relationship for tumor formation (or formation of foci of cellular alteration as a
20 surrogate of tumor formation). However, the dose-response relationship for other
21 TCDD-induced responses such as enhanced gene expression is different from the dose-response
22 for tumor formation in terms of both efficacy and potency (see Popp et al. (2006, [197074](#)) for
23 review). It is important to note that differences in potency between events (i.e., gene expression
24 versus cell proliferation) does not necessary imply alternative mechanisms of action.

25
26 **5.1.2.3.3.2. Lung tumors.**

27 The mode of action of TCDD in producing lung cancer in rodents (predominantly
28 keratinizing squamous cell carcinoma, (Larsen, 2006, [548744](#))) has not been elucidated. One
29 hypothesized mechanism of the carcinogenic action of TCDD in the lung involves disruption of
30 retinoid homeostasis in the liver (see Figure 5-2). Retinoic acids and their corresponding nuclear
31 receptors, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs), work together

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1 to regulate cell growth, differentiation, and apoptosis. It is hypothesized that TCDD, through
2 activation of the AhR, can affect parts of the complex retinoid system and/or other signaling
3 systems regulated by, and/or cross-talking with, the retinoid system (reviewed in (Nilsson and
4 Håkansson, 2002, [548746](#))). These effects are then hypothesized to lead to lung tumor
5 development; however, the mechanisms underlying this hypothesis are not well-defined.
6 Pulmonary squamous proliferative lesions have been reported following oral exposure to TCDD
7 in rats (Tritscher et al., 2000, [197265](#)). In general, squamous metaplasia with some
8 inflammation is associated with significant forms of injury via inhalation of toxic compounds but
9 is also seen with vitamin A deficiency (Tritscher et al., 2000, [197265](#)) and gives some credence
10 to this hypothesis.

11 Another hypothesized mechanism for the carcinogenic action of TCDD in the lung is
12 through induction of metabolic enzymes. Through activation of AhR and subsequent induction
13 of metabolizing enzymes (such as CYP1A1), TCDD may enhance bioactivation of other
14 carcinogens in lung (Tritscher et al., 2000, [197265](#)). There have been few studies to support this
15 hypothesis; however, in a long-term continuous-application study of carcinogenesis using
16 airborne particulate extract (APE), squamous cell carcinoma occurred in 8 of 17 AhR^{+/+} mice
17 (47%) while no tumors were found in AhR^{-/-} mice (Matsumoto et al., 2007, [548748](#)). In
18 addition CYP1A1 was induced in AhR^{+/+} mice but not in AhR^{-/-} mice in this study. These
19 results suggest that AhR plays a significant role in APE-induced carcinogenesis in AhR^{+/+} mice
20 and CYP1A1 activation of carcinogenic polycyclic aromatic hydrocarbons (the primary
21 carcinogenic component of APE) is also of importance.

22

23 **5.1.2.3.3.3. Thyroid tumors.**

24 The mode of action of TCDD in producing thyroid cancer in rodents has not been
25 elucidated. It is hypothesized that TCDD increases the incidence of thyroid tumors through an
26 extrathyroidal mechanism (see Figure 5-2). The prevailing hypothesis for the induction of
27 thyroid tumors by TCDD involves the disruption of thyroid hormone homeostasis via induction
28 of Phase II enzymes UGTs in the liver (reviewed in Brouwer et al., 1998, [201801](#)) by an
29 AhR-dependent transcriptional mechanism (Bock et al., 1998, [548752](#); Nebert et al., 1990,
30 [548756](#)). This induction of hepatic UGT results in increased conjugation and elimination of
31 thyroxine (T4), leading to reduced serum T4 concentrations. T4 synthesis is controlled by the

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1 thyroid stimulating hormone (TSH) which is under negative and positive regulation from the
2 hypothalamus, pituitary, and thyroid via thyrotrophin-releasing hormone, TSH, T4, and
3 triiodothyronine. Consequently, the reduced serum T4 concentrations lead to a decrease in the
4 negative feedback inhibition on the pituitary gland. This would then lead to a rise in secreted
5 TSH and stimulation of the thyroid. The persistent induction of UGT by TCDD and the
6 subsequent prolonged stimulation of the thyroid could result in thyroid follicular cell hyperplasia
7 and hypertrophy of the thyroid, thereby increasing the risk of progression to neoplasia. Increases
8 in blood TSH levels are consistent with prolonged stimulation of the thyroid and may represent
9 an early stage in the induction of thyroid tumors identified in animal bioassays. Statistically
10 significant increases in neonatal blood TSH levels have been recently been reported in children
11 born to TCDD-exposed mothers in the Seveso cohort (Baccarelli et al., 2008, [197059](#), discussed
12 in Section 2.4.1.1.4.4). Support for this hypothesis comes from several studies showing that
13 TCDD decreases serum total thyroxine and free thyroxine concentrations in rats following both
14 single dose and repeated dose exposures (Bastomsky, 1977, [548760](#); Brouwer et al., 1998,
15 [201801](#); Pohjanvirta et al., 1989, [548766](#); Potter et al., 1983, [548769](#); Potter et al., 1986, [548771](#);
16 Sewall et al., 1995, [198145](#); Van Birgelen et al., 1995, [198052](#)). Further support comes from
17 studies of transgenic animals in which TCDD exposure resulted in a marked reduction of total
18 thyroxin and free T4 levels in the serum of AhR+/- mice but not AhR-/- mice (Nishimura et al.,
19 2005, [197860](#)). Additionally, gene expression of UGT1A6, CYP1A1, and CYP1A2 in the liver
20 was markedly induced by TCDD in AhR+/- but not AhR-/- mice (Nishimura et al., 2005,
21 [197860](#)).

22

23 **5.1.2.3.4. Summary of TCDD mode of action in rodents.**

24 Overall, there are inadequate data to support the conclusion that any of the particular
25 mode of action hypotheses described above is operant in TCDD-induced carcinogenesis.
26 However, the wealth of scientific evidence available indicates that most, if not all, of the
27 biological and toxic effects of TCDD are mediated by the AhR. Although the receptor may be
28 necessary for the occurrence of these events, it is not sufficient because other proteins and
29 conditions are known to affect the activity of the receptor and its ability to alter gene expression
30 or to induce other effects. Certain studies could be interpreted to indicate AhR-independent
31 mechanisms, although these studies have not clearly ruled out involvement of the AhR. The

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1 only consistent, but limited, evidence for TCDD-induced effects that do not involve the AhR
2 comes from studies using AhR-deficient transgenic animals. Here however, only minor effects
3 occurred following treatment with extremely high doses of TCDD. Thus, a toxic response to
4 TCDD has AhR interaction as a key event, but there are various species-, cell-, development-,
5 gender-, and disease-dependent differences in the cellular milieu that can affect the nature and
6 extent of the response observed.

7 The findings that many AhR-modulated effects are regulated with distinct specificity
8 supports the understanding that the molecular and cellular pathways leading to any particular
9 toxic event are extremely complex. Precise dissection of these events represents a considerable
10 challenge, especially in that a toxic response may depend on timely modulation of several genes
11 rather than of just one particular gene, and possibly modulation of these genes in several rather
12 than just one cell type or tissue.

13 While a defined mechanism at the molecular level or a defined mode of action for
14 TCDD-induced carcinogenicity is lacking, EPA concludes the following

15

- 16 • interaction with the AhR is a necessary early event in TCDD carcinogenicity in
17 experimental animals.
- 18 • through interaction with the AhR, TCDD modifies one or more of a number of cellular
19 processes, such as induction of enzymes, changes in growth factor and/or hormone
20 regulation, and/or alterations in cellular proliferation and differentiation.
- 21 • AhR activation is anticipated to occur in humans and may progress to tumors. AhR is
22 present in human cells and tissues, studies using human cells are consistent with the
23 hypothesis that the AhR mediates TCDD toxicity and no data exist to suggest that the
24 biological effects of AhR activation by TCDD are precluded in humans.
- 25 • non-AhR mediated carcinogenic effects of TCDD are possible.

26

27 **5.1.3. Summary of the Qualitative Weight of Evidence Classification for TCDD**

28 Under the 2005 Cancer Guidelines (U.S. EPA, 2005, [086237](#)), TCDD is characterized as
29 carcinogenic to humans, based on the available data as of 2009. This conclusion is based on

- 30 • Multiple occupational epidemiologic studies showing strong evidence of an association
31 between TCDD exposure and increased mortality from all cancers.
- 32 • Epidemiological studies showing an association between TCDD exposure and certain
33 cancers in individuals accidentally exposed to TCDD in Seveso, Italy.

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- 1 • Extensive evidence of carcinogenicity at multiple tumor sites in both sexes of multiple
2 species of experimental animals.
- 3 • General scientific consensus that the mode of TCDD’s carcinogenic action in animals
4 involves AhR-dependent key precursor events and proceeds through modification of one
5 or more of a number of cellular processes, such as induction of enzymes, changes in
6 growth factor and/or hormone regulation, and/or alterations in cellular proliferation and
7 differentiation.
- 8 • The human AhR and rodent AhR are similar in structure and function and human and
9 rodent tissue and organ cultures respond to TCDD in a similar manner and at similar
10 concentrations.
- 11 • General scientific consensus that AhR activation is anticipated to occur in humans and
12 may progress to cancers.
13

14 **5.2. QUANTITATIVE CANCER ASSESSMENT**

15 **5.2.1. Summary of NAS Comments on Cancer Dose-Response Modeling**

16 **5.2.1.1. *Choice of Response Level and Characterization of the Statistical Confidence Around*** 17 ***Low Dose Model Predictions***

18 The NAS commented on the low dose model predictions in the 2003 Reassessment,
19 including EPA’s development of ED₀₁ (effective dose eliciting x percent response) estimates for
20 numerous study/endpoint combinations. The committee also suggested that EPA had not
21 appropriately characterized the statistical confidence around such model predictions in the low-
22 response region of the model.

23 The committee concludes that EPA did not adequately justify the use of the 1%
24 response level (the ED₀₁) as the POD for analyzing epidemiological or animal
25 bioassay data for both cancer and noncancer effects. The committee recommends
26 that EPA more explicitly address the importance of the selection of the POD and
27 its impact on risk estimates by calculating risk estimates using alternative
28 assumptions (e.g., the ED₀₅) (NAS, 2006, [198441](#), p. 18)
29

30 It is critical that the model used for determining a POD fits the data well,
31 especially at the lower end of the observed responses. Whenever feasible,
32 mechanistic and statistical information should be used to estimate the shape of the
33 dose-response curve at lower doses. At a minimum, EPA should use rigorous
34 statistical methods to assess model fit, and to control and reduce the uncertainty of
35 the POD caused by a poorly fitted model. The overall quality of the study design
36 is also a critical element in deciding which data sets to use for quantitative
37 modeling (NAS, 2006, [198441](#), p. 18).
38

39 EPA should ... assess goodness-of-fit of dose-response models for data sets and
40 provide both upper and lower bounds on central estimates for all statistical

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1 estimates. When quantitation is not possible, EPA should clearly state it and
2 explain what would be required to achieve quantitation (NAS, 2006, [198441](#), p.
3 10).

4
5 The NAS also suggested that EPA report information describing the adequacy of dose-
6 response model fits, particularly in the low-response region. For those cases where biostatistical
7 modeling was not possible, the NAS recommended that EPA identify the reasons.

8
9 The Reassessment should also explicitly address the importance of statistical
10 assessment of model fit at the lower end and the difficulties in such assessments,
11 particularly when using summary data from the literature instead of the raw data,
12 although estimates of the impacts of different choices of models would provide
13 valuable information about the role of this uncertainty in driving the risk estimates
14 (NAS, 2006, [198441](#), p. 73).

16 **5.2.1.2. Model Forms for Predicting Cancer Risks Below the Point of Departure (POD)**

17 The NAS focused much of its review on EPA's derivation of a cancer slope factor.
18 Specifically, the NAS commented extensively on the selection of the appropriate point of
19 departure (POD) and the extrapolation of dose response modeling below the POD.

20 The NAS questioned EPA's choice of a linear, nonthreshold model for extrapolating risk
21 associated with exposure levels below the POD, concluding that the current scientific evidence
22 was sufficient to justify the use of nonlinear methods when extrapolating below the POD for
23 TCDD carcinogenicity. The committee further recommended that EPA include a nonlinear
24 model for low dose cancer risk estimates as a comparison to the results from the linear model.

25
26 The committee concludes that EPA's decision to rely solely on a default linear
27 model lacked adequate scientific support. The report recommends that EPA
28 provide risk estimates using both nonlinear and linear methods to extrapolate
29 below PODs (NAS, 2006, [198441](#), p. 5).

30 After reviewing EPA's 2003 Reassessment and additional scientific data
31 published since completion of the Reassessment, the committee unanimously
32 agreed that the current weight of scientific evidence on the carcinogenicity of
33 dioxin is adequate to justify the use of nonlinear methods consistent with a
34 receptor-mediated response to extrapolate below the POD. The committee points
35 out that data from NTP released after EPA generated the 2003 Reassessment
36 provide the most extensive information collected to date about TCDD
37 carcinogenicity in test animals, and the committee found the NTP results to be

1 compelling. The committee concludes that EPA should reevaluate how it models
2 the dose-response relationships for TCDD... (NAS, 2006, [198441](#), p. 16).

3
4 Because EPA's assumption of linearity at doses below the 1% excess risk level
5 for carcinogenic effects of TCDD, other dioxins, and DLCs is central to the
6 ultimate determination of regulatory values, it is important to critically address the
7 available scientific evidence on the most plausible shape of the dose-response
8 relationship at doses below the POD (LED₀₁). On the basis of a review of the
9 literature, including the detailed review prepared by EPA and presented in Part II
10 of EPA's Dioxin Risk Assessment and new literature available since the last EPA
11 review, the committee concludes that, although it is not possible to scientifically
12 prove the absence of linearity at low doses, the scientific evidence, based largely
13 on mode of action, is adequate to favor the use of a nonlinear model that would
14 include a threshold response over the use of the default linear assumption (NAS,
15 2006, [198441](#), p. 122).

16
17 On the whole, the committee concluded that the empirical evidence supports a
18 nonlinear dose-response below the ED₀₁, while acknowledging that the possibility
19 of a linear response cannot be completely ruled out. The Reassessment
20 emphasizes the lack of such nonlinear models, hence its adoption of the approach
21 of linear extrapolation below the POD level. Although this approach remains
22 consistent with the cancer guidelines (U.S. EPA, 2005, [086237](#); see also
23 Appendix B), EPA should acknowledge the qualitative evidence of nonlinear dose
24 response in a more balanced way, continue to fill in the quantitative data gaps,
25 and look for opportunities to incorporate mechanistic information as it becomes
26 available. The committee recommends adopting both linear and nonlinear
27 methods of risk characterization to account for the uncertainty of dose-response
28 relationship shape below ED₀₁ (NAS, 2006, [198441](#), p. 72).

29 **5.2.2. Overview of EPA Response to NAS Comments on Cancer Dose-Response Modeling**

30 EPA agrees with the NAS that the approaches to cancer dose-response modeling for
31 TCDD should be clearly communicated and justified. Furthermore, due to the abundance of new
32 information on TCDD carcinogenicity published since the 2003 Reassessment, EPA has
33 reevaluated the cancer dose-response modeling for TCDD presented in the 2003 Reassessment.
34 As detailed below in Section 5.2.3, EPA has conducted an updated cancer dose-response
35 assessment for TCDD that incorporates key NAS recommendations discussed in this document,
36 reflects the current state-of-the science in cancer dose-response modeling and integrates new
37 TCDD carcinogenic information. Detailed responses to the NAS comments summarized above
38 are found in Section 5.2.3.3.

1 The 2003 Reassessment presents an extensive dose-response assessment of TCDD and
2 provides a comprehensive summary of dose-response relationships. The analyses and
3 discussions synthesized a considerable breadth of data and model types, highlighting the
4 strengths and weaknesses of the then-available scientific information. Modeling included both
5 administered dose and steady state body burden dose metrics, taking into account variation in
6 half-lives of TCDD across species. These body burden calculations used a simple one-
7 compartment kinetic model based on the assumption of a first-order decrease in the levels of
8 administered dose as a function of time. An excess risk of 1% was chosen to model the cancer
9 data, but comparative results were also shown for 5% and 10% excess risk (see Table 8-2 of the
10 2003 Reassessment). Dose response was also explored thoroughly for a number of in vitro and
11 biochemical endpoints in addition to the in vivo data analyses, and ranges of these values were
12 presented (see Figures 8-1, 8-2 and 8-3 of the 2003 Reassessment). Thus, the 2003
13 Reassessment provides an initial evaluation of the carcinogenic database for TCDD and serves as
14 the foundation for the analyses presented below.

15

16 **5.2.3. Updated Cancer Dose-Response Modeling for Derivation of Oral Slope Factor**

17 The following sections describe the dose-response analysis of the cancer data from
18 epidemiologic cohort studies (see Section 2.4.1 and Table 2-4) and rodent bioassays (see
19 Section 2.4.2 and Table 2-6), concluding with the derivation of oral slope factors for TCDD
20 based on epidemiologic data (see Section 5.2.3.1) and rodent bioassay data (see Section 5.2.3.2).

21

22 **5.2.3.1. Dose-Response Modeling Based on Epidemiologic Cohort Data**

23 The 2003 Reassessment included dose-response analyses and the development of oral
24 slope factors from the following three occupational cohorts: the NIOSH cohort, the Hamburg
25 cohort, and the BASF cohort. In this document, EPA determined that specific studies from each
26 of these cohorts (Becher et al., 1998, [197173](#); Ott and Zober, 1996, [198408](#); Steenland et al.,
27 2001, [198589](#)) met the epidemiologic study inclusion criteria (see Section 2.3.1 and
28 Section 2.4.1). In Section 5.2.3.1.1, the oral slope factors derived from these studies in the 2003
29 Reassessment are reviewed. Another study that met the current epidemiologic study inclusion
30 criteria (Warner et al., 2002, [197489](#)) was also briefly discussed in the 2003 Reassessment, but
31 an oral slope factor was not derived from that study. In Section 5.2.3.1.2.2, EPA discusses its

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1 unsuccessful attempt to use the categorical results published by (Warner et al., 2002, [197489](#)) to
2 develop an oral cancer risk estimate.

3 Since the publication of the 2003 Reassessment, additional cancer epidemiologic studies
4 based on these cohorts have been published in the peer-reviewed literature. Of these, Collins et
5 al. (2009, [197627](#)) and Cheng et al. (2006, [523122](#)) met the epidemiologic study inclusion
6 criteria (see Section 2.3.1 and Section 2.4.1). In Section 5.2.3.1.2, EPA evaluates the suitability
7 of deriving an oral slope factor from the Cheng et al. (2006, [523122](#)) study and derives oral slope
8 factor estimates. Although the Collins et al. (2009, [197627](#)) study met the study inclusion
9 criteria, EPA could not derive an oral slope factor from that study. In Section 5.2.3.1.2.3, EPA
10 discusses why an oral cancer risk estimate was not developed using the positive results for the
11 soft-tissue sarcoma mortality published by Collins et al. (2009, [197627](#)).
12

13 **5.2.3.1.1. Evaluation of Epidemiologic Studies Used in the 2003 Reassessment for OSF** 14 **Derivation.**

15 In the 2003 Reassessment, EPA reported dose-response modeling results for three
16 epidemiologic studies of human occupational cohorts: the NIOSH cohort with data published by
17 Steenland et al. (2001, [198589](#)); the Hamburg cohort with data published by Becher et al. (1998,
18 [197173](#)); and the BASF cohort with data published by Ott and Zober (1996, [198408](#)). Each of
19 these studies is summarized in Section 2.4.1 of this document and in the 2003 Reassessment
20 (Part II, Chapter 8; Part III, Chapter 5). Furthermore, EPA has evaluated the suitability of these
21 studies for use in TCDD dose-response modeling and concluded that each of these studies meet
22 the inclusion criteria for epidemiology studies presented in Section 2.3.1.

23 Each of these studies reports all cancer mortality as an outcome. Steenland et al. (2001,
24 [198589](#)) and Becher et al. (1998, [197173](#)) analyzed cohorts of primarily male workers who
25 experienced occupational exposures to TCDD over long periods of time, while Ott and Zober
26 (1996, [198408](#)) studied a cohort of primarily male workers who were exposed to high TCDD
27 concentrations at a single point in time due to an industrial accident.

28 The authors of all three of these studies measured, and then back-extrapolated, TCDD
29 levels in a subset of workers to estimate exposures during employment and then the authors used
30 this information to estimate exposures in the remainder of the cohort. These measured TCDD
31 samples generally were collected decades after the last known occupational exposure. In each

1 study, the authors relied on TCDD measures in the cohort to back-calculate serum lipid or body
2 fat levels of TCDD using a simple one-compartment kinetic model based on the assumption of a
3 first-order decrease in the levels of exposure dose as a function of time. The assumed half-life of
4 TCDD used in the models varied from study to study. None of the studies sampled TCDD levels
5 from the entire cohort; for example, Ott and Zober collected samples from 138/243 workers
6 (57% of the cohort), which was the highest percentage of workers sampled among the three
7 studies. Steenland et al. (2001, [198589](#)) and Becher et al. (1998, [197173](#)) used the measured and
8 back-extrapolated TCDD concentrations to estimate the exposures that were associated with
9 various occupations within the cohort, and subsequently used this information to develop
10 exposure matrices (i.e., the TCDD load per unit time for an occupation) that then could be used
11 to estimate the cumulative dioxin dose for each cohort member. Ott and Zober (1996, [198408](#))
12 used regression procedures with data on time spent at various occupational tasks to estimate
13 TCDD levels for all members of the cohort. Following the estimation of worker exposures in
14 each of these three studies, the studies' authors divided these cohorts into exposure subgroups
15 based on the estimated TCDD levels.

16 In the 2003 Reassessment, EPA identified a POD based on a 1% response in cancer
17 mortality (ED₀₁) for the Steenland et al. (2001, [198589](#)), and the Ott and Zober (1996, [198408](#))
18 studies. EPA extrapolated from this POD to lower doses using a straight line drawn from the
19 POD to the origin—zero incremental dose, zero incremental response—to give a probability of
20 extra risk. Because there was insufficient evidence to support an assumption of nonlinearity,
21 EPA chose to develop these models using a linear model.

22

23 **5.2.3.1.1.1. Steenland et al. (2001, 198589).**

24 Steenland et al. (2001, [198589](#)) developed dose-response models based on TCDD
25 exposures and all cancer mortalities from eight plants in the NIOSH cohort (see Section
26 2.4.1.1.1.3 for study details). Serum lipid levels of TCDD in 1988 were measured in
27 193 workers at one of these plants. Steenland and coauthors relied on a first-order kinetic model
28 (assuming a constant 8.7 year half-life) to back-extrapolate to serum TCDD levels at the time of
29 the last occupational exposure. The study authors assigned exposure estimates to each of the
30 3,538 workers in the cohort based on a job-exposure matrix. This matrix was based on (1) an
31 estimated level of contact with TCDD, (2) the degree of TCDD contamination of the products

1 the workers produced, and (3) the fraction of a workday during which the worker likely
2 contacted the TCDD-contaminated products. They then estimated each worker's serum TCDD
3 levels as an area under the concentration curve (AUC) for lipid-adjusted serum levels over time.
4 The mortality analysis was conducted on 256 cancer decedents.

5 Several different dose-response models were fit to these data to provide estimates of fatal
6 cancer risk. The best-fitting model was a Cox regression exposure-response model using the
7 log(AUC) of TCDD lipid concentration (ppt-year) lagged by 15 years as the exposure metric.
8 Steenland and colleagues also developed a piecewise linear regression model with no lag, in
9 which two separate linear slopes were estimated. This analysis assumed a background exposure
10 of 0.5 pg/kg-day. The lipid concentrations were converted to body burdens by dividing by 4.
11 The central tendency estimate and lower bound ED₀₁s from the piecewise linear model and their
12 associated cancer slope factors for the most sensitive endpoint (male cancer mortality) are
13 presented in Table 5-1.

14 15 **5.2.3.1.1.2. *Becher et al. (1998, 197173).***

16 Based on the Hamburg cohort, Becher et al. (1998, [197173](#)) reported a dose-response
17 analysis for all fatal cancers combined (see Section 2.4.1.1.1.3.4 for study details). The mortality
18 analysis was conducted in 1992 on 124 cancer decedents. The exposure variable in the study
19 was the integrated blood levels for TCDD concentration over time (AUC, ng/kg-years), as
20 estimated by Flesch-Janys et al. (1998, [197339](#)); these were converted to body burdens by
21 dividing by 4. Estimates of the half-life of TCDD, based on the sample of 48 individuals with
22 repeated measures, were incorporated into a model that back-extrapolated TCDD exposures to
23 the end of the employment after accounting for the workers' ages and body fat percentages.
24 These estimated exposure measures were then applied to the entire cohort, which consisted of all
25 1,189 regular male employees who were employed for at least 3 months between 1952 and 1984
26 at the Boehringer chemical plant in Hamburg, Germany.

27 Becher et al. (1998, [197173](#)) used a Cox regression approach for the dose-response
28 modeling and developed three models: a multiplicative model, an additive model, and a power

1 model.³² The response variable in each model was the SMR for total cancer mortality. The
2 models were calculated with lag times of up to 20 years. The multiplicative model provided the
3 best fit; however, the study authors judged the fits for all three models to be acceptable. The
4 model results were used to calculate unit risk estimates derived as the risk of cancer death
5 through age 70 given a daily dose of 1 pg/kg body weight of TCDD minus the risk given no
6 exposure to TCDD. These calculations were based on background German cancer mortality
7 rates. The model results were used to calculate cancer risk estimates. The lower bound
8 estimates on the dose were not available for models published by Becher et al. due to the absence
9 of statistical parameter measures. The central tendency estimate ED_{01s} from the three statistical
10 models and their associated cancer slope factors are presented in Table 5-1.

11

12 **5.2.3.1.1.3. Ott and Zober (1996, 198408).**

13 In the 2003 Reassessment, EPA also developed a dose-response analysis based on a study
14 reported by Ott and Zober (1996, [198408](#)) for cancer incidence and mortality experienced by
15 243 men, who were exposed to TCDD in 1953 during an accident at the BASF plant in Germany
16 (see Section 2.4.1.2.1.2.1 for study details). The cohort was followed through 1992. TCDD
17 blood lipid levels were available for 138 of these men 30 years after the accident. These levels
18 were back-extrapolated and used to estimate the AUC for TCDD. Body burdens (ng/kg) were
19 estimated by dividing AUC by 4, and steady-state body burdens were estimated assuming a
20 constant half-life of approximately 7.1 years.³³ Ott and Zober (1996, [198408](#)) used Cox
21 proportional hazard approaches to estimate both cancer incidence and cancer mortality risk per

³²The “multiplicative model” set relative risk (RR) equal to $\exp(\beta d)$, where the dose d is the AUC. The “additive model” set $RR = 1 + \beta d$, and the “power model” set $RR = \exp(\beta \log(kd + 1))$. The values β and k are estimated parameters.

³³Based on the initial body burden (B_0) EPA estimated the body burden at time t using the following formula:

$B(t) = B_0 e^{-k_e t}$, where k_e is an elimination constant equal to $\ln(2)/(\text{half-life in years})$. This implies that the AUC at

time T after initial exposure is $AUC = \frac{B_0}{k_e} (1 - e^{-k_e T})$. T in this case was 39 years (time from the accident in 1953 to

the follow-up in 1992). Dividing by a lifetime of 71 years (mean age in 1954, 33 years, plus 38 years from 1954 to the followup in 1992) yields the lifetime mean body burden as:

$B_{mean} = \frac{B_0}{71k_e} (1 - e^{-k_e T})$. In the 2003 Reassessment, EPA converted the steady-state body burden to units of equivalent

initial dose by dividing by the constant $\frac{1}{71k_e} (1 - e^{-k_e T})$. With the given values for half-life and T, that constant is

0.1411 and 1/(the constant) is 7.09.

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1 unit TCDD dose.³⁴ Ott and Zober reported conditional risk ratios for cancer mortality that were
2 slightly larger than the conditional risk ratios for cancer incidence, which is counter-intuitive.
3 The risk of cancer mortality would be expected to be greater than the risk of cancer incidence.
4 The conditional risk ratio (and 95%CI) for all cancer mortality (1.22; 1.00–1.50) exceeded the
5 conditional risk ratio for all incident cancer cases (1.11; 0.91–1.35). Similarly, the conditional
6 risk ratios for digestive cancer mortality (1.46; 1.13-1.89) and respiratory cancer mortality (1.09;
7 0.70–1.68) were also both larger than the conditional risk ratios for all digestive cancers (1.39;
8 1.07–1.69) and all respiratory cancers (1.02; 0.65–1.59). As expected, in this cohort, incident
9 cases exceeded cancer mortality for total cancers (47 vs. 31), digestive cancers (12 vs. 11) and
10 respiratory cancers (13 vs. 11). Ott and Zober also reported that conditional risks for mortality
11 for all cancer and lung cancer associated with cigarette smoking were also higher than the
12 respective incidence risks. In their Cox regression analysis, Becher et al. (1998, [197173](#)) also
13 report that the regression coefficient for total cancer mortality (0.0096) was slightly larger than
14 the regression coefficient for total cancer incidence (0.0089). The finding of Ott and Zober and
15 Becher et al. that the risk of cancer mortality is greater than cancer incidence is possibly due to a
16 systematic difference in the reference population for incidence vs. the reference population for
17 mortality. The central tendency estimate and lower bound ED₀₁s from the modeling and their
18 associated cancer slope factors are presented in Table 5-1.

19

20 **5.2.3.1.2. Evaluation of Other Epidemiologic Studies Considered for OSF Derivation.**

21 Three additional epidemiologic studies that met the study inclusion criteria (see
22 Section 2.3) for use in dose response modeling as set forth in this document are evaluated in this
23 section for the estimation of cancer risk estimates. These studies were either published after the
24 Reassessment (Cheng et al. (2006, [523122](#)) and Collins et al., (1996, [197637](#))), or not used to
25 derive an OSF in the Reassessment (Warner et al., 2002, [197489](#)). Each study is summarized in
26 Section 2.4.1.

27

³⁴The model from Ott and Zober has risk proportional to $e^{\beta \times \text{dose}}$ with $\beta = \ln(1.22)$. The corresponding slope for the mean (steady-state) body burden is $7.0851 * \log(1.22) * 0.001$ (the 0.001 converts nanograms to micrograms).
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1 **5.2.3.1.2.1. *Cheng et al. (2006, 523122).***

2 As discussed in Section 2.4.1.1.1.4, Cheng et al. (2006, [523122](#)) analyzed the
3 relationship between TCDD dose and all cancer mortality for the same subset of NIOSH workers
4 as analyzed previously by Steenland et al. (2001, [198589](#)). In contrast to Steenland et al., Cheng
5 et al. (2006, [523122](#)) used the “concentration- and age-dependent elimination model”
6 (concentration- and age-dependent elimination [CADM], discussed in Section 3.3; see also
7 Aylward et al. (2005, [197114](#))), rather than a constant 8.7-year half-life, and calculated serum-
8 derived TCDD estimates for use in dose-response analysis. An important feature of CADM is
9 that it incorporates concentration- and age-dependent elimination of TCDD from the body,
10 meaning that the effective half-life of TCDD elimination varies based on exposure history, body
11 burden, and age of the exposed individuals. As discussed in Section 3.3, the use of the CADM
12 model to simulate TCDD kinetics in the NIOSH cohort results in time-integrated body burden
13 estimates four to five times greater than those obtained with the simple first-order model, with
14 smaller differences between the two methods at lower exposures.

15 Following the estimation of dose using the CADM-derived AUC values, Cheng and
16 colleagues (Cheng et al., (2006, [523122](#)); the “Cheng analysis”) derived dose-response estimates
17 for the NIOSH cohort using linear Cox regression for both lagged and un-lagged exposure on
18 various subsets of the data (high-exposures trimmed). The results for the lagged-exposure
19 analysis are summarized in Table 5-2. For comparison, the Cox regression coefficient from the
20 analysis conducted by Steenland et al. (2001, [198589](#)), which relied on a first-order elimination
21 rate model assuming a constant 8.7-year half-life, is also shown in the table. As in Steenland et
22 al. (2001, [198589](#)),³⁵ Cheng et al. (2006, [523122](#)) found a much stronger relationship between
23 cancer mortality and exposure metrics lagged 15 years compared to the relationships for
24 unlagged exposures. Cheng et al. (2006, [523122](#)) also noted that the dose-response relationship
25 plateaued above the 95th percentile of exposure. For exposures lagged 15 years, the regression
26 coefficient (β) of the linear slope derived by Cheng et al. (2006, [523122](#)) was 3.3×10^{-6} per
27 ppt-year lipid-adjusted serum TCDD, with a standard error of 1.4×10^{-6} (Table III of Cheng et
28 al. (2006, [523122](#))). The upper 5% of the exposure range (individuals $\geq 252,950$ ppt-year lipid
29 adjusted serum TCDD) was excluded in estimating this slope. Because this exclusion reduces

³⁵ Lagged exposures modeled only for log-transformed serum concentrations, not for untransformed serum concentrations in the piece-wise linear model.

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1 the upper portion of the response where the slope is shallow³⁶, this likely better represents the
2 slope in the region of the curve where the fatal cancer risk is increasing with dose, which is the
3 equivalent of dropping the highest dose in an animal bioassay or using a piece-wise linear model
4 as in Steenland et al. (2001, [198589](#)).

5 To develop cancer risks for TCDD, EPA used the modeling results of the Cheng analysis,
6 with conversion to oral intake using the Emond human PBPK model as follows. The slope (β)
7 from the Cheng analysis is the slope of the linear relationship between the natural logarithm of
8 the rate ratio (RR) and the cumulative fat TCDD concentration (fat-AUC). Conceptually, the
9 slope (β) is similar to an OSF, except that it is expressed in terms of fat-AUC rather than intake.
10 Also, the slope represents the incremental increase in cancer mortality (expressed as an RR)
11 above the background TCDD exposure experienced by the NIOSH cohort rather than above zero.
12 Using the upper 95% bound on β and assuming that the slope is the same below the NIOSH
13 cohort background exposure level (approximately 5 ppt/yr TCDD fat concentration), EPA
14 calculated risk-specific doses (as daily oral intakes) for TCDD for risk levels of concern to EPA.
15 The risk-specific doses were estimated from the Emond human PBPK model for the lifetime-
16 average TCDD fat concentrations corresponding to the fat-AUC predicted by the Cheng et al.
17 model for each of the risk levels of concern. The steps in this computation are as follows:

- 18
- 19 • Background cancer mortality risk estimate (R_0). EPA used an R_0 of 0.112 as reported by
20 Cheng et al. (2006, [523122](#))³⁷
- 21 • Total cancer mortality risk in the exposed group associated with a specified (extra) risk
22 level (RL) of fatal cancer (TR_{RL}). A TR_{RL} associated with any given extra risk level (e.g.,
23 0.01, 1×10^{-6}) can be calculated using the following relationship for extra risk:

$$ER = \frac{TR_{RL} - R_0}{1 - R_0} \quad (\text{Eq. 5-1})$$

³⁶ Steenland et al. (2001, [198589](#)); Steenland and Deddens (2003, [198587](#)) found a slightly negative slope for the higher exposures, stating that the phenomenon could be a result of exposure misclassification, depletion of susceptible individuals or saturation of receptor-mediated processes.

³⁷ In Table IV, Cheng et al. (2006, [523122](#)) report two estimates of background fatal cancer risk, R_0 , for males aged 75 years: 0.112 and 0.124. A R_0 estimate of 12.4% was used by Steenland et al. (2001, [198589](#)), and 11.2%, as estimated for all males in the 1999–2001 Surveillance Epidemiology and End Result data set. EPA chose to use the more recent estimate of 11.2% for the purpose of predicting risk in the current U.S. population.

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- Incremental cancer mortality risk in the exposed population based on a given extra risk (R_D). R_D , is calculated as the difference between the total risk and background risk and expressed in terms of RL and R_0 by combining Equations 5-2 and 5-1.

$$R_D = TR_{RL} - R_0 \quad (\text{Eq. 5-2})$$

$$R_D = RL \times (1 - R_0) \quad (\text{Eq. 5-3})$$

- Cumulative TCDD concentration in the fat compartment for a given extra risk (AUC_{RL}). AUC_{RL} is then calculated by taking the natural logarithm of Equation 3 from Cheng et al. (2006, [523122](#)), rearranging and substituting for RR ³⁸ ($RR = [R_D + R_0]/R_0$):

$$AUC_{RL} = \ln((R_D + R_0)/R_0)/\beta^* \quad (\text{Eq. 5-4})$$

where β^* is the central-tendency regression slope or the 95% upper bound (β_{95}) determined by summing the regression coefficient (β) and the product of 1.96 and the standard error of the regression coefficient, yielding an estimate of 6.0×10^{-6} per ppt-year lipid adjusted serum TCDD, as follows:

$$\beta_{95} = \beta + 1.96 * SE \quad (\text{Eq. 5-5})$$

- Continuous daily TCDD intake associated with a given extra risk [D_{RL}]. Because the fat concentrations generated by CADM are not linear with oral exposure at higher doses, a single oral slope factor to be used for all risk levels cannot be obtained; the response is approximately linear with fat concentrations and oral intake at lower doses. Instead, a risk-specific D_{RL} must be estimated by converting the respective AUC_{RL} to the corresponding lifetime daily intake, using an appropriate human toxicokinetic model. EPA has chosen to use the Emond human PBPK model for this purpose because the CADM configuration does not facilitate this process and so that the dose conversions are consistent with those used in the derivation of the RfD. A D_{RL} is obtained from the Emond model by finding the average lifetime daily intake corresponding to the AUC_{RL} in the fat compartment.³⁹

Note that there are two nonlinear steps in the estimation of risk-specific doses from the Cheng et al. model. First, fat-AUC (AUC_{RL}) and the incremental cancer mortality risk (R_D) do

³⁸ As defined by Cheng et al. (2006, [523122](#), p. 1063).

³⁹ Although the NIOSH cohort exposures are reported as LASC, they are treated as fat concentrations in the Cheng analysis because fat in all tissues are modeled as one compartment (hence equal) in CADM. The translation to oral intake in the Emond model is from the fat compartment, rather than the serum compartment, even though the serum and fat compartments are not equivalent, because the regression slope (β) in the Cheng analysis is in terms of the (equivalent) fat compartment.

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1 not have a linear relationship (see Eq. 5-5); however, the relationship becomes virtually linear
2 below an incremental risk of 10^{-3} (see Table 5-3). Second, TCDD fat concentration is not linear
3 with oral intake in the Emond human PBPK model (see Section 3); this relationship also is close
4 to linear below the 10^{-5} risk level. The resulting predicted cancer-mortality risk is approximately
5 linear with daily oral intake at low doses. Table 5-3 shows the AUC_{RL} and D_{RL} based on the
6 95% upper-bound regression slope (β_{95}) from the Cheng analysis for a number of risk levels of
7 interest to the EPA. For comparative purposes, EPA has also shown the equivalent oral slope
8 factor ($RL \div D_{RL}$) for those same risk levels. Table 5-4 also shows analogous results based on
9 the best estimate of regression coefficient ($\beta = 3.3 \times 10^{-6}$) for total fatal cancers from the Cheng
10 analysis.

11

12 **5.2.3.1.2.2. Warner et al. (2002, 197489).**

13 Warner et al. (2002, [197489](#)) is a study of 981 females exposed to elevated TCDD levels
14 following the Seveso accident of 1976 (see Section 2.4.1.1.1.4.2 for study details). The TCDD
15 exposure pattern involving a single period of elevated TCDD exposures followed by an extended
16 period of lower ambient level TCDD exposures and elimination is similar to that of the BASF
17 cohort (Ott and Zober, 1996, [198408](#)). TCDD levels, measured or estimated in blood lipids
18 shortly after the time of the accident, were available for all women. These women were divided
19 into four exposure groups of <20, 20–44, 44.1–100, and >100 ppt. In this cohort, 21 total
20 cancers have been observed; 15 of these were breast cancer cases and 3 were thyroid cancer
21 cases. Cox proportional hazards modeling showed that the hazard ratio for breast cancer
22 associated with a 10-fold increase in serum TCDD levels (\log_{10} (TCDD)) was significantly
23 increased to 2.1 (95% CI = 1.0–4.6). Rate ratios (95% CI) for cancer incidence in these 4 groups
24 were 1.0, 1.0 (0.2–5.5), 2.2 (0.5–10.8) and 2.5 (0.5–11.8). Using a Cox proportional hazards
25 model and assuming continuous exposure, the rate ratio was 1.7 (0.9–3.4) for each 10-fold
26 increase in serum TCDD; that is, a \log_{10} transformation of the exposure estimates in their
27 analysis was presented. They reported a test for trend of $p = 0.09$.

28 EPA attempted to estimate an ED_{01} from the modeled results of Warner et al. (2002,
29 [197489](#)) from the statistically significant hazard ratio for breast cancer. However, EPA had to
30 estimate the slope of the tangent to the log-linear relationship. Because the exponentiated slope
31 of a log-dose linear relationship is not constant but varies with dose, and because the lowest

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1 exposure measure was well-above the 1% response region of interest, EPA could not confidently
2 estimate the tangent to the log-dose linear relationship. The transformation of the \log_{10} dose
3 units to linear units of TCDD yielded an implausibly low ED_{01} and correspondingly high cancer
4 risk that was inconsistent with a visual inspection of the untransformed plot. EPA was not
5 confident in these values for health risk assessment because of uncertainties in the transformation
6 in the low response region of the original model. Thus, EPA did not derive an ED_{01} or oral slope
7 factor for this study.

8
9 **5.2.3.1.2.3. *Collins et al. (2009, 197627).***

10 Collins et al. (2009, [197627](#)) investigated the relationship between serum TCDD levels
11 and mortality rates in the NIOSH cohort (see Section 2.4.1.1.1.1.5 for study details). The
12 investigators completed an extensive dioxin serum evaluation of workers employed by the Dow
13 Chemical plant in Midland, Michigan, that made 2,4,5-trichlorophenol (TCP) from 1942 to 1979
14 and 2,4,5-T from 1948 to 1982. Collins et al. (2009, [197627](#)) developed historical TCDD
15 exposure estimates for all 1,615 workers using serum samples from 280 former workers that
16 were collected during 2004–2005. Investigators calculated a cumulative measure of exposure
17 using a simple one-compartment first-order pharmacokinetic model and elimination rates as
18 estimated from the BASF cohort (Flesch-Janys et al., 1996, [197351](#)). The follow-up interval for
19 these workers covered the period between 1942 and 2003. Thus, the study included 10 more
20 years of follow-up than earlier investigations of the entire NIOSH cohort. A key limitation of
21 this study is that the derivation of the SMRs and slope parameters did not include a lag period,
22 unlike other analyses of the NIOSH cohort (e.g., Cheng et al., 2006, [523122](#); Steenland et al.,
23 2001, [198589](#)).

24 Although results were largely negative, statistically significant mortality in the cohort
25 was found for soft-tissue sarcoma (SMR = 4.1, 95% CI = 1.1–10.5), based on only four deaths.
26 A regression coefficient of 0.05872 (standard error not reported), and a hazard ratio of 1.060
27 (95% CI = 1.017 to 1.106) were reported by Collins et al. (2009, [197627](#)) for soft-tissue sarcoma.
28 Although it met the dose-response study criteria, EPA could not calculate an upper bound on the
29 regression coefficient because the standard error was not given. In addition, EPA was unable to
30 estimate an extra-risk value because the reference population response was not specified. Thus,
31 EPA did not derive an ED_{01} or oral slope factor for this study.

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1 **5.2.3.2. Dose-Response Modeling Based on Animal Bioassay Data**

2 Figure 5-3 provides a summary of the process EPA has utilized to select and identify
3 candidate TCDD OSFs from key animal bioassays that were identified in Section 2.4.3 of this
4 document. For each in vivo animal cancer study that qualified for TCDD dose-response
5 assessment using the study inclusion criteria specified in Section 2.3.2, EPA first selected the
6 species/sex/tumor data set combinations that had been characterized as having statistically
7 significant increases in tumor incidence by either a pair-wise test between the treated group and
8 the controls or by a trend test showing increases in tumors with increases in dose. Next, EPA
9 used the Emond animal kinetic model discussed in Section 3 to estimate blood concentrations
10 corresponding to each study's average daily administered doses for use in dose response
11 modeling. Benchmark dose lower confidence bounds (BMDL₀₁s) were then estimated for the
12 blood concentrations by (1) using the multistage cancer model for each species/sex/tumor
13 combination within each study and (2) using a Bayesian Markov Chain Monte Carlo framework
14 that assumes independence of tumors, modeling all tumors together for each species/sex
15 combination within each study. The final selected models were subjected to goodness-of-fit tests
16 and visual inspections of fit to the raw data. Thus, for each sex/species combination within each
17 study, this process generated a BMDL₀₁ for each single tumor type and another BMDL₀₁ for the
18 combined tumors. Finally, using the Emond human kinetic model discussed in Section 3, human
19 equivalent doses (BMDL_{HEDS}) were then estimated for each of the BMDL₀₁s and, using a linear
20 extrapolation, OSFs were calculated by $OSF = 0.01/BMDL_{HED}$. The highest OSF for a
21 species/sex combination for either a single tumor type or all combined tumors was selected as a
22 candidate OSF for TCDD cancer assessment. These steps in Figure 5-3 are further described in
23 detail in the following sections.

24 25 **5.2.3.2.1. Selection of key data sets.**

26 Based on the study selection criteria outlined in Section 2.3.2 (see Figure 2-3), EPA
27 selected five animal bioassays for use in the cancer dose-response assessment for TCDD (see
28 Table 2-6 and Section 2.4.2 for detailed study descriptions). Four of these studies (Della et al.,
29 1987, [197405](#); Kociba et al., 1978, [001818](#); NTP, 1982, [594255](#); Toth et al., 1979, [197109](#)), were
30 evaluated in the 2003 Reassessment, while one study (NTP, 2006, [543749](#)) was published after
31 the 2003 Reassessment was released. The NTP (2006, [543749](#)) study was specifically called out

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1 by the NAS (2006, [198441](#))) report as cancer bioassay data that EPA should evaluate prior to
2 completing its TCDD dose-response assessment. As discussed below, EPA has chosen to
3 conduct dose-response modeling for a number of tumor types from each of the sex/species
4 combinations in these studies in order to maximize the amount of information available to
5 support OSF derivation. Because tumors occurred in multiple sites in the exposed animals, each
6 tumor type was considered separately (individual tumor models) and were also combined into
7 composite tumor incidence dose estimates (multiple tumor models).

8 The tumor incidence tables for these five bioassays are shown in Tables 5-5 through 5-14
9 (see Section 2.4.2 for details of these studies). The data in these tables are summarized from
10 each study's reference publication and are the species/sex/tumor incidence data used for TCDD
11 dose-response modeling in this report. EPA selected the animal bioassay data sets in Tables 5-5
12 through 5-14 because they had been characterized by the study authors as having statistically
13 significant increases in tumor incidence by either a pair-wise test between at least one treated
14 group and the controls or by a trend test showing increases in tumors with increases in dose. An
15 exception was made for cases where statistical significance was found in only one dose group
16 that was not the highest dose group, and there were zero responses in every other dose group
17 including controls; these datasets were not modeled. For example, in NTP (2006, [543749](#)), EPA
18 notes that while the uterine tumors were statistically significant at 46 ng/kg using a pair-wise
19 test, there were no uterine tumors in any other dose group, including the control and high dose
20 groups, and the trend test was not significant; EPA excluded this tumor type from the analysis.
21 In addition, datasets with combined tumors for the same site were given priority over subsets of
22 tumors for that site. For example, in the NTP (1982, [594255](#)) study on female mice, data on
23 combined hepatocellular adenomas or carcinomas were modeled, but not data on hepatocellular
24 adenomas alone (not statistically significant) or on hepatocellular carcinomas alone (statistically
25 significant trend and high dose group). In the case of the Kociba et al. (1978, [001818](#)) female rat
26 combined hepatocellular adenomas and carcinomas only, EPA used data from a reanalysis of the
27 pathology slides that was published by Goodman and Sauer (1992, [197667](#)); because the study
28 authors did not statistically analyze the revised tumor incidence data from their reanalysis, EPA
29 applied a Fischer's Exact Test to evaluate the statistical significance of those data. In the case of
30 the NTP (2006, [543749](#)) study only, information was available regarding the length of time that
31 the animals stayed on test (105 weeks); animals who died within the first year were censored

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1 from analysis in this document because animals who died within the first year were not
2 considered to have been alive long enough to develop tumors. Therefore, those animals were not
3 included in the denominators in Table 5-11. These adjusted incidence data were used in the
4 analysis of tumor dose-response for NTP (2006, [543749](#)) in this document. The tumor incidence
5 data in Tables 5-5 through 5-14 include

- 6
- 7 • nasal, tongue and adrenal tumors in males (Table 5-5), and liver, nasal and lung tumors in
8 females from the Kociba et al. (1978, [001818](#)) 2-year study of Sprague-Dawley rats
9 (Table 5-6),
- 10 • subcutaneous tissue, liver, adrenal and thyroid tumors in females (Table 5-7) and liver,
11 thyroid and adrenal tumors in males (Table 5-8), from the NTP (1982, [594255](#)) 2-year
12 study of Osborne-Mendel rats,
- 13 • subcutaneous tissue, hematopoietic system, liver and thyroid tumors in females
14 (Table 5-9), and lung and liver tumors in males, from the NTP (1982, [594255](#)) 2-year
15 study of B6C3F₁ mice (Table 5-10),
- 16 • liver, oral mucosa, pancreas and lung tumors in females from the NTP (2006, [543749](#)) 2-
17 year study of Sprague-Dawley rats (Table 5-11),
- 18 • liver tumors in males from the Toth et al. (1979, [197109](#)) 1-year study of Swiss/H/Riop
19 mice (Table 5-12), and
- 20 • liver tumors in males (Table 5-13) and females from the Della Porta et al. (1987, [197405](#))
21 52-week study of B6C3F₁ mice (Table 5-14).

22

23 For each cancer endpoint, the reported (administered) doses from each study were converted,
24 where necessary, to average daily doses in ng/kg-day (e.g., doses administered 5 days/week were
25 adjusted by multiplying by 5 and dividing by 7 to get average daily doses). These doses were
26 then subjected to kinetic modeling to generate blood concentrations for use in TCDD dose-
27 response modeling.

28

29 **5.2.3.2.2. Dose adjustment and extrapolation methods for selected data sets.**

30 **5.2.3.2.2.1. Dose metric estimation for dose-response modeling.**

31 Tables 5-5 through 5-14 show the blood concentrations that were used in TCDD dose-
32 response modeling of the animal bioassay data. Based on kinetic analysis (see Section 3), a
33 choice for whole blood concentration of TCDD was made for the purpose of dose extrapolation
34 between animals and humans. In order to estimate blood concentrations for each study selected,

1 the Emond PBPK model was run using ACSLX® software, version 2.5.0.6 (see Section 3).
2 Depending on the selected study, either rat or mouse versions of the model were used. In each
3 case, the simulation was performed using the exposure and observation durations, the body
4 weights, and the adjusted doses from the original studies. Details of PBPK model input
5 parameters are given for each study's m-file in Appendix C.2. In the case of Toth et al. (1979,
6 [197109](#)) study, which dosed the animals for a year and then followed up for the lifetime of the
7 animal, only the one-year simulation was performed. The m-files were used to run the
8 appropriate PBPK model to estimate time-averaged, maximum, and terminal (end of exposure)
9 blood concentration (see Appendix C.3). Other model simulated dose metrics such as
10 concentrations for liver, fat, Ah-receptor bound in liver, body burden, and the time at which the
11 maximum concentration was reached for each dose metric are also reported for illustrative
12 purposes in Appendix C.3. The complete results for each study modeled are shown in
13 Appendix C.3.

14

15 **5.2.3.2.2.2. Calculation of human equivalent doses (HEDs).**

16 Human equivalent doses (ng/kg-day), corresponding to each BMDL (ng/kg) were
17 calculated using the Emond human PBPK model (see Section 3) and are denoted as BMDL_{HEDS}.
18 The Emond human PBPK model was run for 70 years assuming a constant daily dose starting
19 from birth. The model concentrations were averaged over both the entire 70 year lifetime
20 (lifetime average) and over the five years surrounding the peak concentration (five-year average)
21 (see Section 3.3.1, describing first order body burden estimation). The human equivalent doses
22 were estimated by adjusting the daily dose model input until the time-averaged whole blood
23 concentration matched the associated alternative dose BMDL (derived earlier from animal PBPK
24 model). For animal studies which lasted longer than 540 days, the lifetime average was used; for
25 studies lasting less than 540 days, the five year average was used. The process was iterative and
26 continued until the modeled human concentration was within 1% of the BMDL. In general,
27 however, the concentrations matched to within 0.1%.

28

1 **5.2.3.2.3. Dose-response modeling approaches for rodent bioassays.**

2 **5.2.3.2.3.1. Modeling of individual tumors.**

3 EPA's BMDS Software, version 2.1 was used to estimate the BMDL₀₁s for each of the
4 species/sex/tumor combinations, using the blood concentrations and incidence data shown in
5 Tables 5-5 through 5-14. Each data set was modeled using the multistage cancer model, and a
6 BMDL₀₁ in blood concentration was estimated. The multistage model has been used by EPA in
7 the majority of its quantitative cancer assessments because it is statistically robust and able to
8 provide good fits to a wide range of dose-response patterns. It is also consistent with the
9 multistage nature of the carcinogenic process. The mathematical form of the multistage model is

10
11
$$P(d) = 1 - \exp[-(q_0 + q_1d + q_2d^2 + \dots + q_kd^k)]$$
 (Eq. 5-6)
12

13 where

14 $P(d)$ = lifetime excess risk (probability) of cancer at dose d
15 q_i = parameters estimated in fitting the model, $i = 1, \dots, k$.

16

17 To estimate the BMD₀₁s and BMDL₀₁s, BMDS was run with all parameters set to their
18 defaults; up to three degrees of freedom were specified for the dichotomous, multistage cancer
19 model; and a 95% confidence level. A 1% extra risk benchmark response (BMR) was used for
20 each tumor type, as this response level was judged to be sufficiently close to the observed
21 responses (see Section 5.2.3.2.6.11 for an expanded discussion). The BMDL₀₁ (ng/kg) was then
22 converted to a BMDL_{HED} (ng/kg-day) using the Emond human model, and an OSF in units of
23 (mg/kg-day)⁻¹ was calculated by, $OSF = 0.01/BMDL_{HED} \times 10^6$. Because of the nonlinearity of
24 blood concentration and ingested dose in the Emond Human PBPK model, the cancer risk is only
25 approximately linear with the TCDD blood concentration and low TCDD oral ingestion doses,
26 but is not linear with ingested TCDD at higher doses.⁴⁰ Thus, to use these estimates in human
27 health risk assessment, risk-specific TCDD oral intake levels corresponding to the target risk
28 levels should be calculated, using a procedure similar to that for the slope factors based on
29 epidemiologic data (see Table 5-3). In the following sections, results are presented for the

⁴⁰ This situation is analogous to that for the cancer risk modeling of epidemiologic data from the Cheng et al. (2006) analysis in Section 5.2.3.1.2.1.

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1 models that provided the best overall fit to the data as judged by comparison of likelihood ratios
2 for models that had an acceptable fit (chi-squared goodness of fit statistic $p > 0.05$).

3 4 **5.2.3.2.3.2. Multiple tumor (Bayesian) models.**

5 Statistically significant increased tumor incidences were observed at multiple sites in
6 male and/or female rats (Kociba et al., 1978, [001818](#); NTP, 1982, [594255](#); NTP, 2006, [543749](#))
7 and male and female mice (NTP, 1982, [594255](#)) following oral exposures to TCDD. With this
8 multiplicity of tumors, the concern is that a potency or risk estimate based solely on one tumor
9 site (e.g., the most sensitive site) may underestimate the overall cancer risk associated with
10 exposure to this chemical. Relevant approaches in the 2005 Cancer Guidelines (U.S. EPA, 2005,
11 [086237](#)) for characterizing total risk include the following: (1) analyze the incidence of tumor-
12 bearing animals, or (2) combine the potencies associated with significantly elevated tumors at
13 each site. The NRC (1994, [006424](#)) concluded that an approach based on counts of animals with
14 one or more tumors (tumor-bearing animals) would tend to underestimate overall risk when
15 tumor types occur independently, and thus an approach based on combining the risk estimates
16 from each separate tumor type should be used. On independence of tumors, NRC (1994,
17 [006424](#)) stated "...a general assumption of statistical independence of tumor-type occurrences
18 within animals is not likely to introduce substantial error in assessing carcinogenic potency."

19 Because potencies are typically upper bound estimates, summing such upper bound
20 estimates across tumor sites is likely to overstate the overall risk. Therefore, following the
21 recommendations of the NRC (1994, [006424](#)) and the 2005 Cancer Guidelines (U.S. EPA, 2005,
22 [086237](#)), a statistically valid upper bound on combined risk was derived, assuming
23 independence, in order to gain some understanding of the overall risk resulting from tumors
24 occurring at multiple sites. In the case of TCDD, tumors are thought to be independent across
25 the sites found in these three studies because: (1) they are in different organs and tissues,
26 specifically liver, lung, thyroid, subcutaneous tissue, oral cavity, tongue, pancreas, adrenal cortex
27 and the hematopoietic system; (2) different kinds of tumors were found, even within the same
28 organ (e.g., both cholangiocarcinomas and hepatocellular adenomas were found in female rat
29 livers in NTP (2006, [543749](#)); and (3) the tumors found in these studies were not progressive
30 (i.e., they did not metastasize to other sites in the body). It is important to note that this estimate

1 of overall potency describes the risk of developing tumors at any combination of the sites and is
2 not the risk of developing tumors at all sites simultaneously.

3 For modeling individual tumor data, the multistage model is specified as shown in the
4 previous section (see Eq. 5-6). Under the assumption of independence, the model for the
5 combined (or composite) tumor risk is still multistage, with a functional form that has the sum of
6 stage-specific multistage coefficients as the corresponding multistage coefficient.

7
8
$$P_c(d) = 1 - \exp[-(\sum q_{0i} + d\sum q_{1i} + d^2\sum q_{2i} + \dots + d^m\sum q_{mi})], \text{ for } i = 1, \dots, k, \quad (\text{Eq. 5-7})$$

9

10 where k = total number of sites.
11

12 The resulting equation for fixed extra risk (BMR) is polynomial in dose (when logarithms
13 of both sides are taken) and can be solved in a straightforward manner for the combined BMD.
14 However, the current version of BMDS cannot estimate confidence bounds for this combined
15 BMD.

16 Therefore, a Bayesian approach to finding confidence bounds on the combined BMD was
17 implemented using WinBUGS (Spiegelhalter et al., 2003, [594261](#)). WinBUGS software is freely
18 available and implements Markov Chain Monte Carlo (MCMC) computations. Use of
19 WinBUGS has been demonstrated for derivation of a distribution of BMDs for a single
20 multistage model (Kopylev et al., 2007, [194860](#)) and is easily generalized (Kopylev et al., 2009,
21 [198071](#)) to derive the distribution of BMDs for the combined tumor load, following the NRC
22 (1994, [006424](#)) methodology described above. The advantage of a Bayesian approach is that it
23 produces a distribution of BMDs that allows better characterization of statistical uncertainty. For
24 the current analysis, a diffuse (high variance or low tolerance) Gaussian prior restricted to be
25 nonnegative was used. The posterior distribution was based on three simulation chains with
26 50,000 burn-in (i.e., the initial 50,000 iterations were dropped) and a thinning rate of 20,
27 resulting in 150,000 interactions total. The median and 5th percentile of the posterior distribution
28 provided the BMD₀₁ (central estimate) and BMDL₀₁ (lower bound) for combined tumor load,
29 respectively.

30 The methodology above was applied to the statistically significant dose-response data
31 from Kociba et al. (1978, [001818](#)), NTP (1982, [594255](#)), and NTP (2006, [543749](#)) (see

1 Section 2.3.2 for data set selection criteria).⁴¹ As with the risk estimates generated for individual
2 tumor sites, the combined analysis used the internal dose metric, whole blood concentration (see
3 Section 3). For the combined tumors for each sex/species combination, a BMDL₀₁ in blood
4 concentrations was estimated. The BMDL₀₁ (ng/kg) was then converted to a BMDL_{HED}
5 (ng/kg-day) using the Emond human model, and an OSF in units of (mg/kg-day)⁻¹ was
6 calculated by, $OSF = 0.01/BMDL_{HED} \times 10^6$. Because of the nonlinearity of blood concentration
7 and ingested dose in the Emond Human PBPK model, the cancer risk is linear only with the
8 TCDD blood concentration and low TCDD oral ingestion doses, but is not linear with ingested
9 TCDD at higher doses; a single OSF cannot represent the entire range of risks for oral ingestion.
10 Thus, to use these estimates in human health risk assessment, risk-specific TCDD oral intake
11 levels corresponding to the target risk levels should be calculated using a procedure similar to
12 that for the slope factors based on epidemiologic data (see Table 5-3).

13

14 **5.2.3.2.4. Results of dose-response modeling for rodent bioassays.**

15 Table 5-15 presents the benchmark dose modeling results for both the individual tumors
16 and the combined tumors based on TCDD blood concentrations. The *p*-values in the table are
17 for a chi-square goodness of fit statistic with significance of $p > 0.05$. Goodness of fit was
18 acceptable at $p > 0.05$ for all models. The difference in log likelihood (dLL) statistic documents
19 the difference in log likelihoods between stages of the models in cases where the stage is
20 above 1; it shows the difference between the stage in the table and the lower stage. For example,
21 for the NTP (2006, [543749](#)) liver cholangiocarcinomas, twice the difference of 2.92 would be
22 >3.84 , the test statistic from the assumed chi-square distribution,⁴² with $p = 0.95$, justifying the
23 choice of 3 stages over 2 stages. The best fitting multistage models include: a 1-stage (linear)
24 model for all of the individual tumor data sets from Kociba et al. (1978, [001818](#)), NTP (1982,
25 [594255](#)), and Toth et al. (1979, [197109](#)), for liver carcinomas in females in Della Porta et al.
26 (1987, [197405](#)), as well as for the pancreatic and oral mucosa tumors in NTP (2006, [543749](#)); a

⁴¹ Because only one tumor site was statistically significantly elevated in both the Della Porta et al. (1987, [197405](#)) and Toth et al. (1979, [197109](#)) (i.e., only increased incidences of liver tumors were statistically significant elevated in both studies), a multi-tumor analysis was not conducted.

⁴²The chi-square distribution with 1 degree of freedom is the correct distribution only under standard conditions (e.g., no boundary parameters in null hypothesis). Thus, the correct distribution for the situation where the parameter of interest is on the boundary, as happens with testing for the order of the multistage model, and, possibly nuisance parameters (estimated parameters of the model), is very difficult to derive (Self and Liang, 1987, [594398](#)). Therefore the *p*-value of chi-square with one degree of freedom is used as the best available choice.

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1 2-stage model for the lung tumors in NTP (2006, [543749](#)) and for liver carcinomas in males from
2 Della Porta et al. (1987, [197405](#)); and a 3-stage model for the liver cholangiocarcinoma and liver
3 adenoma data sets from NTP (2006, [543749](#)). The multi-stage model fit was not significant ($p >$
4 0.1) in the NTP (1982, [594255](#)) study for lung tumors in the male mouse ($p = 0.09$), adrenal
5 cortex ($p = 0.06$) and thyroid follicular cell adenomas ($p = 0.06$) in male rats, and subcutaneous
6 tissue in female mice ($p = 0.09$), and was also not significant for liver carcinomas ($p = 0.019$) in
7 female mice in Della Porta et al. (1987, [197405](#)). For the Toth et al. (1979, [197109](#)) liver
8 tumors, the model fit to all of the data was poor, and the highest dose group was dropped in order
9 to achieve an acceptable fit ($p = 0.29$). The BMD_{01S} and $BMDL_{01S}$ (ng/kg) were estimated from
10 these multistage models for the individual tumors. BMD_{01S} and $BMDL_{01S}$ (ng/kg) were also
11 provided in Table 5-15 for the combined tumors for each sex/species combination within a study.
12 These were estimated from the distributions of BMD_{01S} produced by the Bayesian MCMC
13 simulation (see Section 5.2.3.1.2.3.2). The BMD_{01S} and $BMDL_{01S}$ (ng/kg) for the combined
14 tumors in Table 5-15 are the mean and lower 95% percentile values from these distributions,
15 respectively.

16

17 **5.2.3.2.4.1. *Individual tumor models.***

18 Table 5-16 shows the $BMDL_{HEDS}$ (ng/kg-day) that were estimated from the $BMDL_{01S}$ in
19 Table 5-15 using the Emond human model (see Section 5.2.3.1.2.2.2) and the OSFs calculated
20 by, $OSF = 0.01/BMDL_{HED} \times 10^6$ to convert the OSF to $(\text{mg/kg-day})^{-1}$ units. BMDS results,
21 details of the model fits and dose-response graphics for all endpoints are shown in Appendix F.
22 Although only the blood concentration results are presented in this section, for comparison
23 purposes, Appendix F also provides modeling results for the studies' administered average daily
24 doses. Table 5-16 lists the OSFs in decreasing value. It can be seen that liver tumors in male
25 mice yield the highest slope factors; OSF values are 5.9×10^6 and 5.2×10^6 per mg/kg-day in
26 NTP (1982, [594255](#)) and Toth et al. (1979, [197109](#)), respectively. The OSFs for the new NTP
27 (2006, [543749](#)) study in female rats are two orders of magnitude lower, ranging from 1.8×10^4 to
28 1.8×10^5 per mg/kg-day, representing the lowest OSFs for TCDD from the individual tumor
29 models.

30

1 **5.2.3.2.4.2. *Multiple tumor (Bayesian) models.***

2 Table 5-17 shows the BMDL_{HEDS} (mg/kg-day) that were estimated from the BMDL_{01S} in
3 Table 5-15 using the Emond human model (see Section 5.2.3.1.2.2.2) and the OSFs calculated
4 by, $OSF = 0.01/BMDL_{HED} \times 10^6$ to convert the OSF to (mg/kg-day)⁻¹ units. Table 5-17 lists the
5 OSFs in decreasing value. It can be seen that the combined liver and lung tumors in male mice
6 yield the highest OSF value of 9.4×10^6 per mg/kg-day from NTP (1982, [594255](#)), and the
7 combined adrenal, tongue and nasal tumors in male rats yield the lowest OSF value of 3.2×10^5
8 from Kociba et al. (1978, [001818](#)). The OSF for the combined liver, oral mucosa, lung, and
9 pancreatic tumors in female rats from the newer NTP (2006, [543749](#)) study is 4.4×10^5 .

10
11 **5.2.3.2.5. *Summary evaluation of slope factor estimates from rodent bioassays.***

12 To estimate a range of candidate TCDD OSFs from the animal data, dose-response
13 modeling of the five chronic rodent bioassays identified in Section 2.4.3 was conducted. Dose-
14 response modeling was performed using whole blood concentrations, and BMDL_{HED} values
15 (ng/kg-day) were derived for the 28 species/sex/endpoint data sets individually (see Table 5-16)
16 and for seven species/sex combined tumor data sets (see Table 5-17).

17 The highest OSFs that have been derived for these animal cancer bioassays using the
18 multistage models are from the multiple tumor analyses for NTP (1982, [594255](#); 2006, [543749](#))
19 and Kociba et al. (1978, [001818](#)), presented in Table 5-17, and from the individual tumor
20 analyses for Toth et al. (1979, [197109](#)) liver tumors and Della Porta et al. (1987, [197405](#)) liver
21 carcinomas in male mice, presented in Table 5-16. The most sensitive species and sex is male
22 mice, for which the estimated BMDL_{HED} for combined tumors is 1.1×10^{-3} ng/kg-day. This
23 result, which is derived under the assumption that multiple tumor types occur independently in
24 the exposed animals, is, as expected, lower than the BMDL_{HED} for the most sensitive individual
25 tumor.

26 Based on these results, EPA believes that a credible value for the BMDL_{HED} derived from
27 the animal studies lies in the range shown in Table 5-17 between 3.1×10^{-2} and
28 1.1×10^{-3} ng/kg-day. These values, which correspond to oral slope factor values of 3.2×10^5
29 and 9.4×10^6 per mg/kg-day, respectively, encompass the range at which elevated cancer risks
30 can be detected for the most sensitive species, sex, and endpoints in the animal bioassay data.

1 As noted above in Sections 5.2.3.1.2.2 and 5.2.3.1.2.3, the cancer mortality risk is strictly
2 linear only with TCDD blood concentration, such that a single OSF cannot represent the entire
3 range of risks for oral ingestion. The OSFs shown in Tables 5-16 and 5-17 are based on HEDs
4 corresponding to the BMDL₀₁, which are most representative of lower human exposure levels,
5 including ambient exposures. For higher exposures, the risks increase at a slower rate with
6 increasing dose and the corresponding OSFs are lower; in those cases, risk-specific doses can be
7 calculated as previously described (see Section 5.2.3.2.3.2).

8
9 **5.2.3.2.6. *Qualitative uncertainties in slope factor estimates from rodent bioassays.***

10 This section presents a qualitative discussion of the uncertainties associated with
11 calculating the OSF for TCDD from chronic animal bioassay data. Discussions on the feasibility
12 of conducting a quantitative uncertainty analysis for TCDD using dose-response methods are
13 provided in Section 6.4.2 of this document.

14
15 **5.2.3.2.6.1. Quality of studies relied upon for determining POD.**

16 EPA considers the overall quality and breadth of the studies used for the cancer dose-
17 response analysis to be excellent. All of the studies were published in the peer-reviewed
18 literature, and two of them were conducted by NTP (1982, [594255](#); 2006, [543749](#)).
19 Kociba et al. (1978, [001818](#)), Della Porta et al. (1987, [197405](#)) and Toth et al. (1979, [197109](#))
20 are older studies, but appear to have been conducted according to good laboratory practice
21 standards. The control and dose group sample sizes were relatively large, ~40–50 animals or
22 more per group for all of the studies. All five studies exposed the test animals via the oral route
23 to TCDD alone, as was stipulated in EPA’s study inclusion criteria. Collectively, these five
24 studies reported development of numerous cancer endpoints (tumors) in both sexes in two strains
25 of rats (Sprague-Dawley and Osborne-Mendel) and two strains of mice (i.e., B6C3F₁,
26 Swiss/H/Riop). The overall high quality of these studies and the strong, positive association
27 between TCDD exposure and cancer suggests that study quality is not a major contributing factor
28 to uncertainty in the risk estimates.

1 **5.2.3.2.6.2. Interpretation of results from studies relied upon for determining POD.**

2 As discussed in Section 3.4.3.2.1, questions arose about the interpretation of liver tumor
3 responses in female rats in the Kociba et al. (1978, [001818](#)) study. Three re-evaluations of the
4 slides have been reported (Goodman and Sauer, 1992, [197667](#); Kociba et al., 1978, [001818](#);
5 Squire, 1980, [594272](#)). The decision to use the Goodman and Sauer (1992, [197667](#)) evaluation
6 was based on their use of the most current tumor classification procedures. The incidence of
7 hepatocellular adenomas and carcinomas (individually and combined), however, did vary
8 (sometimes widely) for each dose group across the three evaluations. Although the state-of-the-
9 science is reflected in the Goodman and Sauer analysis, there is some uncertainty in the
10 interpretation of any post-hoc analysis. No issues have arisen with regard to the interpretation of
11 the NTP (1982, [594255](#); 2006, [543749](#)), Della Porta et al. (1987, [197405](#)) or Toth et al. (1979,
12 [197109](#)) tumor identification and classification.

13
14 **5.2.3.2.6.3. Consistency of results across chronic rodent bioassays.**

15 The existence of five high-quality chronic bioassays for TCDD increases confidence and
16 reduces uncertainty in the cancer OSFs. Considered together, these studies tested two species
17 and both sexes of mice and rats, and a wide range of well-characterized tumor types. All five
18 studies were consistent in observing increases (at some dose level) in rates of liver tumors (in
19 both species and sexes). While tumors at other sites were observed (and those sites varied across
20 study, species, and sex), the liver tumors were consistently the most sensitive indicators of
21 carcinogenic response (with respect to BMDL_{HED} estimates). Lung tumors were also
22 consistently observed across three of the studies, in male mice in the NTP (1982, [594255](#)) study
23 and in female rats in Kociba et al. (1978, [001818](#)) and NTP (2006, [543749](#)). As discussed above,
24 the two most sensitive single-tumor endpoints as judged by BMDL₀₁ values were associated with
25 elevated liver tumor risks, followed by lung, lymphoma or leukemia, thyroid and adrenal
26 cancers. The consistency of tumor types and sensitivities across endpoints and studies lends
27 confidence to the multistage modeling results.

28
29 **5.2.3.2.6.4. Human relevance of rodent tumor data.**

30 There is some concordance in the tumor responses observed in the rodent test species and
31 humans, however, the most sensitive tumor site in the animals, the liver, has not been associated

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1 with cancer from TCDD exposures in humans. On the other hand, lung cancer and leukemia are
2 found both in the animal studies and in epidemiologic studies of exposed workers. The
3 consistency across sex, species, and strains in the animal studies suggests that the occurrence of
4 several of these tumors, in particular, liver and lung tumors is not an idiosyncratic response of a
5 particular combination of species, strain, or sex. As discussed in Section 5.2.1, the likely AhR
6 related carcinogenic mechanism is credible for humans as well as for rodent species.

7 8 **5.2.3.2.6.5. Relevance of rodent exposure scenario.**

9 Three of the five chronic rodent bioassays exposed the test animals for ~2 years, the
10 majority of their lifespans. Toth et al. (1979, [197109](#)) exposed the animals only for one year, but
11 they were kept on the study for a second year before they were evaluated for cancer. The Della
12 Porta et al. (1987, [197405](#)) study also exposed the test animals for one year, and a dosing error
13 occurred during the study. At ages 31 to 39 weeks, 41 male mice and 32 female mice in the
14 2,500 ng/kg BW dose group were mistakenly administered a single dose of 25,000 ng/kg BW
15 TCDD. TCDD treatment for the 2,500 ng/kg BW dose group was halted for 5 weeks (beginning
16 the week after the 25,000 ng/kg BW dose was administered in error) and resumed until exposure
17 was terminated at 57 weeks. Thus, the large single dose and subsequent period without TCDD
18 exposure confounds the dose-response relationship for this study. In general, these lifetime
19 bioassays in animals have long been used by EPA to assess potential lifetime exposures and
20 effects in humans. However, in the case of TCDD, the half life of TCDD in the body for rats,
21 mice, and humans is very different (see Section 3). Thus, there is a significant amount of
22 uncertainty in the use of rat and mouse data to develop OSFs for human cancer risk assessment
23 of TCDD.

24 25 **5.2.3.2.6.6. Impact of background TCDD exposures.**

26 It is known that TCDD has been found in the feed used in animal bioassays, and that this
27 is a confounding factor, particularly in older studies. The effect of TCDD in the diets of test
28 species has the potential to be quite significant given the low levels of TCDD at which adverse
29 effects have been observed. Insofar as that is an issue, the risks associated with TCDD
30 exposures in the animal bioassays, and therefore the OSFs, would be biased high, which could be
31 the case for the NTP (1982, [594255](#)), Della Porta et al. (1987, [197405](#)), Kociba et al. (1978,

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1 [001818](#)) and Toth et al. (1979, [197109](#)) studies. The impact of this issue is that the newer study,
2 NTP (2006, [543749](#)), accounted for TCDD exposures in the animal feed. Thus, there is likely to
3 be less uncertainty in the TCDD dose-response information presented in NTP (1982, [594255](#);
4 2006, [543749](#)) than in the other four studies conducted before 1990.

5
6 **5.2.3.2.6.7. Choice of endpoint for POD derivation.**

7 As noted above, the liver tumor PODs represent the most sensitive single-tumor endpoint
8 across the five cancer bioassays. Thus, the liver cancer endpoints must be seriously considered
9 for derivation of a TCDD OSF. As discussed in the previous section, EPA has also developed
10 Bayesian dose-response estimates for combined tumors, which yield BMDL₀₁ values slightly
11 lower than those for any individual tumor type. Although it is the most conservative choice to
12 select the lowest combined tumor POD for OSF derivation, there are uncertainties associated
13 with the multiple tumor analysis. The assumption of independence of tumors across sites is
14 reasonable, particularly since the tumors from TCDD do not metastasize. However, the
15 independence assumption lacks hard evidence and needs further laboratory confirmation.

16
17 **5.2.3.2.6.8. Choice of animal-to-human extrapolation method.**

18 The analyses presented here have used the Emond human kinetic model for extrapolating
19 dose from animals to humans (as discussed in Section 3.4.2). The rationale for this choice is that
20 the blood concentration metric most accurately reflects the concentration of TCDD in the various
21 tissues. As discussed in Section 3.4.3.2.4, use of the blood concentration dose metric results in
22 critical dose estimates (HEDs) that are considerably lower (10- to more than 100-fold) than those
23 derived based on administered dose. This does not reflect bias in the blood-based measure;
24 rather it is a reflection of the nonlinear biokinetics of TCDD in the body. EPA has also explored
25 the impacts of using other dose metrics, including AhR-bound TCDD concentration calculated
26 based on the Emond model. As discussed in Section 3.4.3.2.6.2, this also results in HED
27 estimates much lower than those obtained based on administered dose.

28
29 **5.2.3.2.6.9. Choice of model for POD and model uncertainty for POD derivation.**

30 The bioassay-based cancer dose-response assessment in this section has used the
31 multistage model which is the standard model choice for such assessments and has been the basis

1 for most of EPA's cancer risk assessments. The multistage model is the standard because it is
2 the only available model form that allows for low-dose linearity while accommodating
3 curvilinearity at higher doses and can be readily implemented.

4 There is some model choice uncertainty associated with instances of lack of fit. When
5 the multistage model does not adequately describe the observed pattern of responses (typically
6 determined by examining the p -value for lack of fit), a decision must be made about possible
7 adjustments, including the dropping of higher dose groups thought to be less relevant to the
8 estimation of low-dose slopes. In this analysis, poorer fits (p -values less than 0.10) were
9 observed in five cases, four from NTP (1982, [594255](#)) and one from Della Porta et al. (1987,
10 [197405](#)) (see Table 5-15). The lowest BMDL₀₁ associated a low p -value ($p = 0.09$) was for the
11 lung tumors in the NTP (1982, [594255](#)) male mouse, the third lowest POD behind the liver
12 PODs in the individual tumor data sets. The other instances were for adrenal cortex and thyroid
13 follicular cell adenomas in male rats and for subcutaneous tissue in female mice in the NTP
14 (1982, [594255](#)) study and for liver carcinomas in female mice in Della Porta et al. (1987,
15 [197405](#)). In those instances, the p -values were 0.06, 0.06, 0.09, and 0.019, respectively. These
16 poorly fit data sets provide OSF estimates that are uncertain and also contribute to uncertainty in
17 the combined tumor PODs from NTP (1982, [594255](#)). The lowest BMDL₀₁ in the combined
18 tumors is for the male mice combined liver and lung tumors, thus estimates from this sex/species
19 combination from NTP (1982, [594255](#)) is highly uncertain and impacts its choice as a POD.

21 **5.2.3.2.6.10. Statistical uncertainty in model fits.**

22 Every model fit to a data set is associated with some inherent statistical uncertainty. For
23 this reason, bounds were calculated and used for OSF derivation (e.g., lower bounds on
24 benchmark doses, in this case the BMDL₀₁s). Those bounds account for uncertainties associated
25 with finite samples of test animals, both in terms of the number of dose groups and of the
26 number of animals per dose group. Valid and accepted statistical procedures have been applied
27 to ascertain the impact of those limitations on the estimates of interest. That being the case, the
28 statistical uncertainties associated with finite samples have been adequately addressed.

1 **5.2.3.2.6.11. Choice of risk level for POD derivation.**

2 The BMR level that has been used for the POD in deriving the cancer OSF is one percent
3 extra risk. A single BMR was chosen for consistency across studies. Also, a BMR of 1% was
4 judged to be near the range of the observations. For the TCDD animal cancer bioassay data,
5 although many of the first positive tumor incidence responses (relative to controls) are closer to
6 10% (some higher), some are as low as 2%. Furthermore, most of the BMD₀₁ values are within a
7 factor of 3 of the lowest tested dose, and the BMDL₀₁ values are generally less than a factor of 2
8 below the BMD. Table 5-18 presents a comparison of BMDs, BMDLs and slope factors for 1%,
9 5% and 10% BMRs from the multi-tumor analyses of NTP (1982, [594255](#); 2006, [543749](#)) and
10 Kociba et al. (1978, [001818](#)) and for selected single tumor data sets from Toth et al. (1979,
11 [197109](#)) and Della Porta et al. (1987, [197405](#)). In Table 5-18, the choice of BMR has little or no
12 impact on the slope factors based on TCDD blood concentration for the combined or single
13 tumor incidences selected as representative of each study.⁴³ In contrast, Table 5-19 presents a
14 comparison of Human Equivalent Dose BMDs, BMDLs and slope factors for 1, 5, and 10%
15 BMRs from these same datasets. Table 5-19 shows that, when converting the blood
16 concentration to the equivalent HED, a 2-fold to 4-fold decrease in the OSF is obtained when
17 using a BMR of 10% rather than 1%. This result is a consequence of the nonlinearity in the
18 Emond PBPK model at higher doses, where dose-dependent elimination of TCDD in the liver
19 results in a less-than-proportional increase in blood concentration relative to oral intake. At
20 lower exposure levels, blood concentration is proportional to oral intake. Therefore, EPA has
21 chosen the lower BMR of 1% as more representative of the low-dose risk.

22
23 **5.2.3.3. *EPA's Response to the NAS Comments on Choice of Response Level and***
24 ***Characterization of the Statistical Confidence Around Low Dose Model Predictions***

25 The NAS was concerned with the statistical power to determine the shape of the dose
26 response curve at low doses, well below observed dose-response information. EPA shares this
27 concern in that the shape of the dose-response curve in the low-dose region cannot be determined
28 with confidence when based on higher dose information.

⁴³ This will generally be the case for multistage model fits with 1st-degree coefficients greater than zero because the response at the BMDL is virtually linear at BMRs of 10% or less. For model fits dominated by higher-order coefficients, linearity of response at the BMDL begins at lower BMRs.

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1 When tumor data are used for dose-response modeling, a POD is obtained from the
2 modeled tumor incidences. When assessing carcinogenicity using a linear extrapolation
3 approach from a POD, a balance must be struck between staying within the range of the
4 observations and obtaining a representative estimate of the low-dose slope. Traditional cancer
5 bioassays, with approximately 50 animals per group, can typically support modeling down to an
6 increased incidence of 1–10%; epidemiologic studies, with larger sample sizes, below 1%. For
7 the TCDD animal cancer bioassay data, most of the low-dose tumor incidence responses are
8 under 10% (relative to controls), with some as low as 2%. For comparison purposes, BMDs,
9 BMDLs and OSFs from the animal cancer bioassay benchmark dose modeling assuming 1, 5,
10 and 10% extra risk are shown in units of blood concentrations and human equivalent doses in
11 Tables 5-18 and 5-19, respectively. After evaluating the magnitude of the uncertainty in
12 BMDL_{01S} against the impact of using BMDL_{10S}, EPA has chosen to use a 1% BMR in all cases,
13 determining that the uncertainty bounds on the BMDL₀₁ values are reasonable.

14 In the analysis of the animal cancer bioassays presented in this document, the multistage
15 cancer model was applied with a linear dose extrapolation to zero. EPA used a 1% excess risk
16 estimate, i.e., a BMDL₀₁, as the POD for development of candidate TCDD cancer oral slope
17 factors using a Bayesian multitumor approach (see Section 5.2.3.2. The advantage of a Bayesian
18 approach is that it produces a distribution of BMDs that allows better characterization of
19 statistical uncertainty.

20 Central tendency slope estimates and upper bound oral slope factor estimates are part of
21 the standard BMDS multistage cancer model and are included in each output file for the animal
22 bioassay single tumor analyses in Appendix F. Central tendency BMDs are also reported for the
23 results of the animal bioassay multitumor analysis (see Table 5-15). Central tendency slope
24 estimates are given for all the qualifying epidemiological studies as well (see Tables 5-1 and
25 5-4), where possible.

26

27 **5.2.3.4. EPA's Response to the NAS Comments on Model Forms for Predicting Cancer Risks**
28 **Below the POD**

29 The NAS offered extensive comments on the cancer dose-response modeling in the 2003
30 Reassessment. Although epidemiologic and rodent bioassay data are useful for the evaluation of
31 the dose-response curve within the range of the observed response data, they have traditionally

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1 not been useful sources of information for identifying a threshold or for estimating the shape of
2 the dose-response curve below the POD. Rather, mechanistic toxicological data have been the
3 evidentiary sources of choice for those types of analyses. As noted above, any quantitative
4 estimation of carcinogenic risk associated with TCDD exposure requires low-dose extrapolation
5 of experimental data. Unfortunately, the shape of the dose-response curve in the low dose region
6 is unknown.

7 Several of the analyses of epidemiological cohort data evaluated the fit of different dose-
8 response models to the data. Log-dose models accentuate the importance of low-dose low-
9 magnitude responses and can yield implausible results. The most relevant models used in these
10 studies are the untransformed-dose Cox regression models. Better results have been obtained in
11 the cohort analyses when the flattening of the hazard-ratio curve is taken into account. The latter
12 has been modeled explicitly by Steenland et al. (2001, [198589](#)), who use a piecewise linear
13 model and implicitly by Cheng et al. (2006, [523122](#)), who drop out a percentage of the high-dose
14 response data and fit a linear model to the remainder. Importantly, the analyses of the
15 epidemiologic cohorts presented in Section 5.2.3.1 are limited to evaluation and reanalyses of
16 published data as reported by the study authors. EPA does not have access to the raw data from
17 these epidemiologic studies and, therefore, could not conduct *de novo* analyses.

19 **5.2.3.4.1. Choice of extrapolation approach**

20 **5.2.3.4.1.1. TCDD and receptor theory.**

21 TCDD is considered to be a receptor-mediated carcinogen in animals. Nearly all TCDD
22 experimental data are consistent with the hypothesis that the binding of TCDD to the AhR is the
23 first step in a series of biochemical, cellular, and tissue changes that ultimately lead to toxic
24 responses observed in both experimental animals and humans (Part II, Chapter 2 of the 2003
25 Reassessment). Ligand-receptor binding, like any bimolecular interaction, obeys the law of mass
26 action as originally formulated by A.J. Clark (Limbird, 1996, [594276](#)). The law of mass action
27 predicts the fractional receptor occupancy at equilibrium as a function of ligand concentration.
28 Fractional occupancy (Y) is defined as the fraction of all receptors that are bound to ligand:

$$29 \quad Y = \frac{[TCDD - AhR]}{[AhR]_{TOT}} = \frac{[TCDD - AhR]}{[AhR] + [TCDD - AhR]} = \frac{[TCDD]}{[TCDD] + K_d} \quad (\text{Eq. 5-8})$$

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1 where [TCDD] is the concentration of the ligand, [AhR] is the concentration of the receptor and
2 [TCDD-AhR] is the amount of liganded receptor. The equilibrium dissociation constant K_d
3 describes the affinity of the interaction and is the concentration of TCDD that results in 50%
4 receptor occupancy. This simple equation defines a rectangular hyperbola, which is the
5 characteristic shape of the vast majority of biological dose-response relationships.

6 In certain cases, no response occurs even when there is some receptor occupancy. This
7 suggests that there may be a threshold phenomenon that reflects the biological “inertia” of the
8 response (Ariens et al., 1960, [594279](#)). In other cases, a maximal response occurs well before all
9 receptors are occupied, a phenomenon that reflects receptor “reserve” (Stephenson, 1956,
10 [594280](#)). Therefore, the law of mass action cannot by itself fully explain the effect or response
11 observed after TCDD interacts with AhR. The ligand-receptor complex is associated with a
12 signal transduction or effector system. In the case of the AhR, this effector system can be
13 considered to be the transcriptional machinery itself. The key feature of this formulation is that a
14 response is proportional, or a function of, the number of receptors occupied.

15 Furthermore, for a ligand such as TCDD that elicits multiple receptor-mediated effects,
16 one cannot assume that the binding-response relationship for a simple effect (such as enzyme
17 induction) will necessarily be identical to that for a different and more complex effect (such as
18 cancer). The cellular cascades of events leading to different complex responses (e.g., altered
19 immune function, developmental effects, or cancer) are different, and other rate-limiting events
20 likely influence the final biological outcome resulting in different dose-response curves. Thus,
21 even though TCDD binding to AhR is assumed to be the initial event leading to a spectrum of
22 biological responses, TCDD-AhR binding data may not always correlate with the dose-response
23 relationship observed for particular effects.

24 A receptor-based mechanism would predict that, except in cases where the concentration
25 of TCDD is already high (i.e., [TCDD]~ K_d), incremental exposure to TCDD will lead to some
26 increase in the fractional occupancy of AhR. However, as discussed above, it cannot be assumed
27 that an increase in receptor occupancy will necessarily elicit a proportional increase in all
28 biological response(s), because numerous molecular events contributing to the biological
29 endpoint are integrated into the overall response. That is, the final biological response could be
30 considered as an integration of a series of interdependent dose-response curves with each curve
31 dependent on the molecular dosimetry for each particular step. Dose-response relationships that

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1 will be specific for each endpoint must be considered when using mathematical models to
2 estimate the risk associated with exposure to TCDD. It remains a challenge to develop models
3 that incorporate all the complexities associated with each biological response as the modes of
4 action for various toxicological endpoints appear to vary greatly. For TCDD, extensive
5 experimental data from studies using animal and human tissues indicate that cell- or tissue-
6 specific factors determine the quantitative relationship between receptor occupancy and the
7 ultimate biological response. This would suggest that the parameters for each mathematical
8 model might only apply to a single biological response within a given tissue and species, making
9 extrapolation to other systems challenging.

11 **5.2.3.4.1.2. Low-dose extrapolation: threshold or no threshold?**

12 As indicated in the 2005 Cancer Guidelines,⁴⁴ toxicity reference values for human
13 noncancer endpoints have historically been estimated based on a no-observed-adverse-effect
14 level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) from animal bioassay studies.
15 This terminology suggests a biological population threshold beneath which no harm is
16 anticipated. Reference values such as the oral reference dose (RfD) or inhalation reference
17 concentration (RfC) are derived by applying uncertainty factors (UFs) to a POD. Depending on
18 the nature of available data and modeling choice, a POD can be selected from values other than
19 an NOAEL or LOAEL, such as an ED_x, or a benchmark dose (BMD) or its BMDL. An RfD is
20 described as “likely to be without appreciable risk” but the probabilistic language has not as yet
21 been operationalized. There is no quantitative definition of “appreciable” and no mechanism to
22 compute risk as a function of dose, so as to ascertain that the risk is indeed not appreciable. The
23 risk at the RfD is not calculated, and it cannot be calculated within the current UF framework.
24 Instead, a hazard quotient is computed as the ratio of a given exposure to the RfD, or a margin of
25 exposure is estimated as the ratio of the POD to the human exposure level.

26 Cancer endpoints are predominantly thought to have no population biological threshold.
27 Although the terminology “threshold/nonthreshold” is still common in cancer dose-response

⁴⁴As stated in the 2005 Cancer Guidelines (U.S. EPA, 2005, [086237](#)): “For effects other than cancer, reference values have been described as being based on the assumption of biological thresholds. The Agency’s more current guidelines for these effects (U.S. EPA, 1996, [594399](#); U.S. EPA, 1998, [030021](#)) however, do not use this assumption, citing the difficulty of empirically distinguishing a true threshold from a dose-response curve that is nonlinear at low doses.”

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1 discussions, the 2005 Cancer Guidelines propose a different terminology, whereby “nonlinear
2 models” are those whose dose-response *slope* is zero at or above zero. In the natural language,
3 and indeed in data analysis, it is difficult to distinguish the following situations:

- 4
- 5 • The response approaches zero as dose goes to zero, versus
- 6 • The response *slope* goes to zero as dose goes to zero (nonlinear model).
- 7

8 This use of “nonlinear” is acknowledged to be idiosyncratic.⁴⁵ The NAS review (NAS,
9 2006, [198441](#)) does not consistently apply the terminology from the 2005 Cancer Guidelines, nor
10 does it consistently distinguish the above two circumstances: “...the observed data are more
11 consistent with a sublinear response that approaches zero at low doses rather than a linear dose
12 response” (NAS, 2006, [198441](#)). The point of a nonlinear model in the sense of the 2005 Cancer
13 Guidelines is that the response *slope* approaches zero. Both linear and nonlinear *responses*
14 approach zero at low dose (in the absence of background). Since the terms “linear,” “sublinear,”
15 and “nonlinear” invite confusion in this context, the following terminology is used in this
16 document:

17

18 *Threshold Model:* There is some threshold dose $T > 0$ such that the probability of
19 response for any dose less than or equal to T is zero, and the probability is nonzero for
20 any dose greater than T .

21 *Linear/ Linear above Threshold Model:* For the linear model, the probability of response
22 is proportional to the dose. For the linear over threshold model, the probability of
23 response is zero for a dose below the threshold, and it is proportional to the excess dose
24 over the threshold otherwise. Note that under the EPA cancer guidelines, the linear
25 above threshold model is classified as a nonlinear model.

26 *Nonlinear Model:* Any model that is not linear.

27 *Supralinear/ Supralinear above Threshold Model:* For the supralinear model, the slope of
28 the probability of response decreases as dose increases; in other words, the second
29 derivative of the response curve is negative. For the supralinear above threshold model,

⁴⁵From the 2005 Cancer Guidelines (U.S. EPA, 2005, [086237](#)): “The term ‘*nonlinear*’ is used here in a narrower sense than its usual meaning in the field of mathematical modeling. In these cancer guidelines, the term ‘*nonlinear*’ refers to threshold models (which show no response over a range of low doses that include zero) and some nonthreshold models (e.g., a quadratic model, which shows some response at all doses above zero). In these cancer guidelines, a nonlinear model is one whose slope is zero at (and perhaps above) a dose of zero. Use of nonlinear approaches does not imply a biological threshold dose below which the response is zero.”

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1 the second derivative is negative above the threshold, and the response probability is zero
2 below the threshold.

3 *Sublinear/Sublinear above Threshold Model:* For the sublinear model, the slope of the
4 probability of response increases as dose increases; in other words, the second derivative
5 of the response curve is positive. For the sublinear above threshold model, the second
6 derivative is positive above the threshold, and the response probability is zero below the
7 threshold.

8 *Zero Slope at Zero Model:* The slope of the response curve is zero at or above dose zero.
9

10 All of these models may be understood in an individual or population sense. According
11 to the 2005 Cancer Guidelines, the trigger for applying the basic RfD methodology for cancer
12 endpoints is sufficient evidence for the “zero slope at zero” model for the population. By
13 definition, any sublinear, supralinear, or linear model *above the threshold* is a zero slope at zero
14 (“ZS@Z”) model.

15 The relation between individual and population models is not immediately evident.
16 Figure 5-4 shows dose-response curves of the probability of response vs. dose for different
17 models dose-response shapes. The left panel in Figure 5-4 shows a supralinear dose-response
18 curve; the rate of increase of the response probability goes down as dose increases, or in the strict
19 mathematical sense, the second derivative is negative. The middle panel shows a sublinear dose-
20 response curve; the second derivative is positive. In this case the slope at zero is zero (ZS@Z).
21 However, sublinearity, in the strict mathematical sense, by itself does not imply that the slope at
22 zero is zero. The probit dose-response model shown in the right graph is sublinear and has
23 positive slope at zero (the log-probit model is zero slope at zero).

24 If individuals in a population have different dose-response curves, then the population
25 dose-response curve is obtained by averaging all these dose-response curves over the population.
26 The shape of the population dose-response curve will generally be quite different from the
27 individual curves. Figure 5-5 is a simple depiction of the relationship of individual vs.
28 population dose response. The left panel in Figure 5-5 shows dose-response curves for seven
29 individuals, each with a supralinear dose-response curve above individual-specific thresholds.
30 Averaging these curves gives the dashed dose-response curve, which is nearly linear. The graph
31 on the right is similar, except that the individual dose-response curves are linear above individual
32 thresholds. The population curve is quadratic and zero slope at zero applies.

1 Of course these are not the only possibilities; in general, the population dose-response
2 curve depends on (1) the distribution of individual thresholds in the neighborhood of zero, (2) the
3 dose-response curve for each individual, and (3) the dose metric. Under EPA’s Cancer
4 Guidelines, the zero-slope-at-zero criterion applies strictly to ingested dose, but the other two
5 factors (distribution of individual thresholds and dose-response curve for each individual) need
6 to be established before a zero slope at zero dose can be established. Otherwise the default linear
7 extrapolation to zero approach applies.

8 On the nature or the distribution of individual thresholds, often referred to as the
9 population tolerance distribution, there is ongoing debate as to how receptor kinetics influence
10 the shape of that distribution. Even within an individual, there is a lack of consensus as to
11 whether receptor kinetics confer linear or sublinear attributes to downstream events, or whether
12 receptor kinetics, themselves, are linear, sublinear, or supralinear. Whatever the nature of the
13 form of receptor kinetics, it may have little or no influence on the ultimate population response.
14 The kinetics of receptors is in the domain of the individual, rather than the population. As
15 described previously, receptor kinetics are governed by the law of mass action, which leads to a
16 low-dose proportional response model, generally modeled by some form of Hill function, the
17 low-dose linear form being Michaelis-Menten kinetics. There is no *a priori* reason to believe
18 that the shape of the dose-response curve in an individual has any relationship to the shape of the
19 population response, particularly for quantal endpoints. Lutz and Gaylor (2008, [594297](#)) present
20 an argument for considering the population response in terms of the more traditional tolerance
21 distribution, which is likely the result of more variable factors than the shape of receptor kinetics.
22 Perhaps more to the point, receptor activation is only the first of many events in the path to the
23 apical event (a tumor in this example). Because there are undoubtedly numerous additional
24 downstream events that must occur before the apical effect is observed, there are many
25 opportunities for interindividual variability to become manifest in the tolerance distribution.
26 Even at the first step, a more likely contributor to interindividual variability than the shape of the
27 response is the dose resulting in the response, as measured by the ED₅₀ (K_m in the Michaelis-
28 Menten formulation), which shifts the response curve. Factors that influence shifts in response
29 curves are generally modeled as normal or log-normal distributions and may confer a log-normal
30 shape on the population tolerance distribution, particularly if there are a number of dependent
31 sequential steps or distinct subpopulations (Hattis and Burmaster, 1994, [594301](#); Hattis et al.,

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1 1999, [594299](#); Lutz, 1999, [594298](#)), although other distributions could be equally likely (Crump
2 et al., 2010, [380192](#)).

3 To see how the discussion over threshold/nonthreshold might play out for TCDD,
4 consider the equilibrium dissociation constant K_d for TCDD, which measures the binding affinity
5 of TCDD to the AhR. Lower values indicate higher binding affinity and (other things being
6 equal) greater risk. For Han/Wistar rats, the value $K_d = 3.9$ is reported (standard deviation not
7 given); human values are reported as $K_d = 9.6 \pm 7.8$ (*0.3 – 38.8 with 15 of 67 donors without*
8 *detectable binding*) (Connor and Aylward, 2006, [197632](#)). If AhR binding is the rate-limiting
9 step for carcinogenesis, then the majority of a human population may be less susceptible than
10 Han/Wistar rats, whereas a population threshold, if it exists, might still be well below the
11 Han/Wistar rat threshold, given the large variability in the human K_d estimate (see also Section
12 6.4.2.9). The NAS contends that an AhR-mediated mode of action indicates a threshold dose-
13 response relation (NAS, 2006, [198441](#)). Presumably, the value of the threshold, if it exists,
14 depends on the AhR binding affinity. Arguing for a population threshold in this case requires
15 two types of information:

- 16
- 17 1. The distribution of the individual thresholds induced by, among other things, the
18 individual K_d values; and
- 19 2. The dose-response function for values above the threshold induced by K_d .
- 20

21 Without this information, the shape of the population dose-response curve cannot be
22 determined with any confidence and the default linear relationship applies; response probability
23 is modeled as a linear function of dose, for dose near zero. However, from the 2005 Cancer
24 Guidelines: “When adequate data on mode of action provide sufficient evidence to support a
25 nonlinear mode of action *for the general population* (emphasis added) and/or any subpopulations
26 of concern, a different approach—a reference dose/reference concentration that assumes that
27 nonlinearity—is used.” In current terminology, the reference dose methodology applies if there
28 is sufficient evidence supporting a “zero slope at zero” model; otherwise, the linear nonthreshold
29 model applies by default.

30 In principle, the choice between the above models could fall within the purview of dose-
31 response modeling. However, standard statistical methods encounter well-known difficulties in

1 detecting thresholds. Without going into detail, suffice to say that the maximum likelihood
2 estimate of response probability when no responses are observed in a finite sample is always
3 zero. That said, some researchers have attempted to identify thresholds (Aylward et al., 2003,
4 [594305](#); Mackie et al., 2003, [594303](#)) or nonlinearity (Hoel and Portier, 1994, [198741](#)) by means
5 of parameter estimation of appropriate models. A review of 344 rodent bioassays on 315
6 chemicals led to the following conclusion by Hoel and Portier (1994, [198741](#)):

7
8 We have also found that the oft-held belief that genotoxic compounds typically
9 follow a linear dose-response pattern and that nongenotoxic compounds follow a
10 nonlinear or threshold dose response pattern is not supported by the data. In fact
11 we find the opposite with genotoxic compounds differing from linearity more
12 often than nongenotoxic compounds.
13

14 The choice between a linear and “zero slope at zero” model in current practice does not
15 fall under dose-response model fitting, it is made on the basis of a structured narrative as set
16 forth in the 2005 Cancer Guidelines (U.S. EPA, 2005, [086237](#)):

17
18 In the absence of sufficiently, scientifically justifiable mode of action information,
19 EPA generally takes public health-protective, default positions regarding the
20 interpretation of toxicologic and epidemiologic data: animal tumor findings are
21 judged to be relevant to humans, and cancer risks are assumed to conform with
22 low dose linearity. ... The linear approach is used when: (1) there is an absence of
23 sufficient information on modes of action or (2) the mode of action information
24 indicates that the dose-response curve at low dose is or is expected to be linear.
25 Where alternative approaches have significant biological support, and no
26 scientific consensus favors a single approach, an assessment may present results
27 using alternative approaches. A nonlinear approach can be used to develop a
28 reference dose or a reference concentration.
29

30 **5.2.3.4.1.3. Extrapolation method.**

31 The 2005 Cancer Guidelines (U.S. EPA, 2005, [086237](#)) emphasize that the method used
32 to characterize and quantify cancer risk from a chemical is determined by what is known about
33 the MOA of the carcinogen and the shape of the cancer dose-response curve.

34 The NAS was critical of EPA’s decision to apply linear low-dose extrapolation for
35 TCDD cancer assessment in the 2003 Reassessment and encouraged the use of a nonlinear
36 approach. The 2005 Cancer Guidelines state that a nonlinear approach should be used when

1 “there are sufficient data to ascertain the mode of action and conclude that it is not linear at low
2 doses and the agent does not demonstrate mutagenic or other activity consistent with linearity at
3 low doses.”

4 Receptor modeling theory (as outlined in the 2003 Reassessment, Part II, Chapter 8)
5 indicates that exogenous compounds which operate through receptor binding mechanisms, such
6 as TCDD, will follow a linear dose-response binding in the 1–10% receptor occupancy region.
7 This theory has been supported by empirical findings and suggests that the proximal biochemical
8 effects (such as enzyme induction) and transcriptional reactions for TCDD may also follow
9 linear dose-response kinetics. More distal toxic effects could take any one of multiple forms
10 (i.e., linear, sublinear, supralinear or threshold) depending on (1) the toxic mechanism;
11 (2) location on the dose-response curve; and (3) interactions with other processes such as
12 intracellular protein binding and cofactor induction/repression.

13 In the case of TCDD, many adverse effects experienced at low exposure levels have too
14 much data variability to distinguish on a statistical basis (goodness-of-fit) between dose-response
15 curve options, and whether the dose-response is linear, sublinear or supralinear. For tumor
16 responses, with the exception of squamous cell carcinoma of the oral mucosa and adenomas or
17 carcinomas of the pancreas, which were fit with a linear multistage model, the tumor endpoints
18 in the NTP (2006, [543749](#)) study using female Sprague-Dawley (S-D) rats are all best fit with a
19 sublinear model (i.e., the multistage model fits to tumor incidence data were second or third
20 degree; see Table 5-15 and Appendix F). For all tumor incidence data from three of the other
21 cancer bioassays that met the study inclusion criteria (Kociba et al., 1978, [001818](#); NTP, 1982,
22 [594255](#); Toth et al., 1979, [197109](#)), the multistage model fit was linear (first degree), when based
23 on either administered dose or modeled blood concentrations (see Appendix F). For Della Porta
24 et al. (1987, [197405](#)), the female liver carcinomas were linear (first degree), but the female liver
25 adenomas and the male liver carcinomas were best modeled using a second degree model (see
26 Table 5-15).

27 Another issue of potential importance when evaluating the shape of the dose-response
28 curve for low dose effects is the concept of “interacting background.” The concept of interacting
29 background refers to a pathological process in the exposed population that shares a causal
30 intermediate with the toxicant being evaluated. On this issue, a recent NAS committee (NAS,
31 2009, [594307](#)) contended that

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1 ...the current EPA practice of determining “nonlinear” MOAs does not account
2 for mechanistic factors that can create linearity at low dose. The dose-response
3 relationship can be linear at a low dose when an exposure contributes to an
4 existing disease process Crump et al., 1976, [003192](#); Lutz, 1990, [000399](#). Effects
5 of exposures that add to background processes and background endogenous and
6 exogenous exposures can lack a threshold if a baseline level of dysfunction occurs
7 without the toxicant and the toxicant adds to or augments the background process.
8 Thus, even small doses may have a relevant biologic effect. That may be difficult
9 to measure because of background noise in the system but may be addressed
10 through dose-response modeling procedures. Human variability with respect to
11 the individual thresholds for a nongenotoxic cancer mechanism can result in
12 linear dose-response relationships in the population (Lutz, 2001, [053426](#); NAS,
13 2009, [594307](#).

14
15 AhR activation could be considered a causal intermediate in several disease processes.
16 Recent studies have linked AhR activation in the absence of exogenous ligand to a multitude of
17 biological effects, ranging from control of mammary tumorigenesis to regulation of
18 autoimmunity (reviewed in Hahn et al., 2009, [548725](#)). While the level of background activation
19 of AhR by endogenous compounds (or exogenous compounds other than TCDD) in the human
20 population is unknown, given the ubiquitous nature of several of the known endogenous and
21 exogenous AhR ligands, it is reasonable to assume that a certain baseline level of AhR activation
22 exists in the population. The degree to which TCDD exposure augments this baseline level of
23 AhR activation is unknown.

24 The 2005 Cancer Guidelines (U.S. EPA, 2005, [086237](#)) recommend that the method used
25 to characterize and quantify cancer risk from a chemical be determined by what is known about
26 the mode of action of the compound and the shape of the cancer dose-response curve. The linear
27 approach is used if there is sufficient evidence supporting linearity or if the mode of action is not
28 understood (U.S. EPA, 2005, [086237](#)). In the case of TCDD, (1) the mode of action of TCDD-
29 induced carcinogenesis beyond potential AhR activation is unknown; (2) information is lacking
30 to determine the shape of the dose-response curves at low doses for various adverse endpoints
31 (including cancer) in humans or experimental animals; (3) there is undoubtedly a certain level of
32 interacting background (i.e., AhR activation by endogenous ligands) in the human population;
33 (4) many of the rodent cancer dose-response relationships (Kociba et al., 1978, [001818](#); NTP,
34 1982, [594255](#); Toth et al., 1979, [197109](#)) are consistent with low-dose linearity (first degree
35 multistage model fit) when based on either administered dose or modeled blood concentrations;

1 and (5) higher human interindividual variability compared to experimental rodents will tend to
2 shift the shape of the dose-response towards linear (relative to rodents). None of these
3 suggestions of linearity, however, is conclusive (see next section for additional detail). The true
4 shape of the dose-response curve remains unknown. Therefore, in the absence of sufficient
5 evidence to the contrary or evidence to support nonlinearity, to estimate human carcinogenic risk
6 associated with TCDD exposure EPA assumed a linear low-dose extrapolation approach.

7
8 **5.2.3.4.1.4. *Discussion of low-dose linearity.***

9 Any quantitative estimation of carcinogenic risk associated with TCDD exposure requires
10 low-dose extrapolation of high dose experimental and epidemiologic data. Unfortunately,
11 despite the availability of the extensive database on the biological effects of TCDD, the shape of
12 the dose-response curve in the low-dose region is not known. This situation is not unique to
13 TCDD. For most carcinogens the available biological data do not provide sufficient mechanistic
14 information to determine the shape of the dose-response relationship at doses below the levels
15 where direct experimental or epidemiologic data are available. EPA’s Guidelines for Carcinogen
16 Risk Assessment (2005, [086237](#)) recognize this situation and describe approaches the Agency
17 uses for dose response assessment in cancer risk assessments depending on the available
18 scientific database. EPA’s basic approach makes a distinction between “low-dose linear” and
19 “nonlinear” dose response patterns. This distinction is important to understand as it addresses
20 the potential response at low dose, not the empirical pattern of response seen in the available
21 (often high dose) tumor data. To put matters simply, under a low-dose-linear model, the
22 estimated risk due to the carcinogen exposure is approximately proportional to the dose received
23 (at low dose). In mathematical terms, a low-dose-linear model is one whose slope is greater than
24 zero at a dose of zero (U.S. EPA, 2005, [086237](#); footnote, p. 1-11). Importantly, a low-dose-
25 linear model need not be linear at higher doses, and this is consistent with upward curving
26 responses (e.g., linear-quadratic) and downward curving (plateauing) responses that may be seen
27 various cancer studies. In EPA’s terminology a “nonlinear” dose-response, refers to situations
28 where there is not a linear component in the response at low-dose. In this context, a “nonlinear
29 model” is one whose slope is zero at (and perhaps above) a dose of zero (ibid). Nonlinear
30 response patterns can include threshold models where there is no response below a defined dose

1 level, or other patterns where response at low dose otherwise decreases rapidly as compared to a
2 low-dose-linear model.

3 As stated in the previous section, the low-dose linear approach for the TCDD
4 carcinogenicity assessment in this document is based on EPA’s scientific baseline inference
5 (“default”) regarding dose-response modeling. EPA believes that the mode of action is not
6 known, so is using the default linear extrapolation approach specified by EPA’s cancer
7 guidelines.

8 Nonetheless, there are biological data on TCDD that help inform the appropriateness of
9 low-dose-linear risk extrapolation for this compound. Furthermore, there is utility in
10 summarizing scientific reasoning that supports the approach of low-dose linearity as an
11 appropriate scientific baseline inference (“default”) for carcinogen risk assessment.

12 The issues pertaining to low-dose linearity were discussed in the report of a recent state-
13 of-the-science workshop on issues in low-dose risk extrapolation held by U.S. EPA and Johns
14 Hopkins Risk Science and Public Policy Institute in 2007 (White et al., 2009, [622764](#)). This
15 report states:

16

17 The complex molecular and cellular events that underlie the actions of agents that
18 lead to cancer and noncancer outcomes are likely to be both linear and nonlinear.
19 At the human population level, however, biological and statistical attributes tend
20 to smooth and linearize the dose-response relationship, obscuring thresholds that
21 might exist for individuals. Most notable of these attributes are population
22 variability, additivity to preexisting disease or disease processes, and background
23 exposure–induced disease processes; measurement error also undoubtedly
24 contributes to this phenomenon. The linear appearance of the population-level
25 dose-response function does not presume that the dose-response relationship is
26 necessarily linear for individuals (Lutz, 1990, [000399](#); 2001, [053426](#); Lutz et al.,
27 2005, [087763](#)), but may reflect a distribution of individual thresholds. These
28 attributes are likely to explain, at least in part, why exposure-response models of
29 the relationship between cancer or noncancer health effects and exposure to
30 environmental toxicants with relatively robust human health effects databases at
31 ambient concentrations (e.g., ozone and particulate matter air pollution, lead,
32 secondhand tobacco smoke, radiation) do not exhibit evident thresholds, even
33 though the MOAs include nonlinear processes for key events NRC (2005);
34 U.S. EPA (2006, [088089](#); 2006, [157071](#); 2006, [090110](#)); U.S. DHHS (2004,
35 [056384](#)).

36

1 Original arguments in favor of low-dose linearity for carcinogen risk assessment
2 (including for ionizing radiation, as developed from human data) are based on the occurrence of
3 damage (often termed “hits”) to DNA and the inference that resulting mutations would
4 contribute to cancer development. These arguments envisioned direct damage to DNA;
5 however, based on subsequent advances in mechanistic understanding, damage to DNA by
6 “secondary” reactive molecules (not just direct hits to DNA by radiation or other agents) is also
7 considered to play a major role. TCDD is not thought to produce DNA damage directly.
8 However, DNA damage may result subsequent to increased formation of reactive molecules
9 (reactive oxygen species (ROS) and metabolites of endogenous compounds). Thus, the presence
10 of low-dose linearity by this pathway would depend on whether such reactive molecules were
11 produced at low dose and whether that increased formation was proportional to dose. If that
12 were the case for TCDD, which is still unknown, arguments in favor of low-dose linearity
13 remain similar to those for direct-acting agents.

14 The kinetics of ligand receptor binding, and then the attachment of a receptor/ligand
15 complex to a promoter region of DNA are biochemical processes where low-dose linearity can
16 occur. Simple receptor binding interactions are often modeled using Michaelis-Menten
17 relationships which are linear at low dose. Thus, the *early* key events in a process of a receptor-
18 mediated toxicity pathway may often be expected to be low-dose linear. However, as in any
19 toxicity process, the ultimate shape of the dose-response relationship for an apical⁴⁶ toxicity
20 endpoint will depend on all the processes involved, not just receptor kinetics. These issues were
21 considered by NRC (NAS, 2009, [594307](#)) which included as an indication for non-threshold
22 dose response: “The fact that in receptor-mediated events, even at very low doses a chemical can
23 occupy receptor sites and theoretically perturb cell functions (such as signal transduction or gene
24 expression) or predispose the cell to other toxicants that bind to or modulate the receptor systems
25 (such as organochlorines and the aryl hydrocarbon receptor or endocrine disruptors and
26 hormonal binding sites).” The role of these factors for TCDD has not been fully elucidated.

27 Two other factors supporting low-dose linearity discussed in the workshop described by
28 White et al. (2009, [622764](#)) are additivity to background processes (dose additivity) and the
29 magnitude of human heterogeneity.

⁴⁶ An apical endpoint is an observable outcome in a whole organism, such as a clinical sign or pathologic state, that is indicative of a disease state that can result from exposure to a toxicant (NAS, 2007).

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1 Concerning dose additivity, Crump et al. (1976, [003192](#)) argued in the context of a
2 carcinogenic response that if the carcinogenic process resulting from exposure to an exogenous
3 agent (e.g., TCDD) is already operant in causing background responses, then the effect of the
4 exposure is to augment this process in a dose-additive fashion. The additional response caused
5 by the exposure is expected to increase approximately linearly with exposure at low exposures
6 (i.e., be low-dose linear). The NRC Science and Decisions report (NAS, 2009, [594307](#))
7 examined the issue of additivity to background, in particular calling attention to a need for
8 systematic consideration of endogenous processes related to disease development as well as
9 additivity to other exogenous exposures.⁴⁷ While the baseline activity (unexposed to exogenous
10 agents) of AhR is not well understood, the effects of exogenous agents need to be considered in
11 terms of how they add on to or modulate baseline physiological processes instead of considering
12 TCDD or other exogenous ligands to be “acting in a vacuum.”

13 The issue of human heterogeneity relative to the rodents used in bioassays has been
14 discussed at length in the literature and will not be repeated here (see also relevant text in
15 Section 5.2.3.4.1.3). However, as discussed by NAS (2009, [594307](#)), even in situations where
16 processes thought to be nonlinear are precursors to the development of cancer in test animals, a
17 different situation may result in humans: “However, given the high prevalence of those
18 background processes, and given the multitude of chemical exposure and high variability in
19 human susceptibility, the results may still be manifested as low-dose linear dose-response
20 relationships in the human population.” The population dose-response will be influenced by
21 heterogeneities in the population that affect internal dose as well as response. First, even if there
22 is strong curvilinearity in the dose-response curve in the dose range of relevance to human
23 exposures, there may be large differences across individuals in the doses at which transitions in
24 the shape of the dose-response curve occur. Greater variability in response to exposures would
25 be anticipated in heterogeneous populations than in inbred laboratory species under controlled
26 conditions (due to, e.g., genetic variability, disease status, age, and nutrition). The effect of
27 increased heterogeneity will be a broadening of the dose-response curve (i.e., less rapid fall-off
28 of response with decreasing dose) in diverse human populations and, accordingly, a greater

⁴⁷ It may be noted that when there are multiple exogenous exposures, it may be difficult to ascertain which exposure came first. However, the point is that if a combination of endogenous and exogenous factors is operative in causing biological response, then an additional small, dose additive, exposure can be predicted to cause a proportionate change in response.

1 potential for risks from low-dose exposures (Lutz et al., 2005, [087763](#); Zeise et al., 1987,
2 [060867](#)). The degree to which heterogeneity must be increased to “linearize” sublinear
3 responses of varying degrees has not yet been established.

4 Interpreting the shape of animal bioassay dose-response model fits always involves
5 assumptions about the shape of the response in the unobserved range (i.e., low dose). Cancer
6 bioassays can provide relatively little information on actual dose-response patterns below the
7 point of departure. However, it is generally not possible to either exclude or affirm low-dose
8 linear components statistically based upon empirical modeling of the dose-response data.⁴⁸
9 Dose-response modeling can, however, be useful in describing the size of a linear component in
10 the response that is compatible with study data. As an example, NRC (NAS, 2006, [198441](#))
11 advised EPA to examine the results of the NTP (2006, [543749](#)) study as indicating nonlinearity
12 of the observed tumor response. Among the tumors seen in the NTP bioassay, the dose-response
13 shape for cholangiosarcoma is notably curvilinear in the dose range of the observed tumor
14 response. Figure 5-6 shows the multistage modeling of the cholangiosarcoma data from the NTP
15 bioassay. The BMDL is calculated at an extra risk of 0.01. Even though the MLE dose response
16 is nonlinear (1st-degree coefficient is zero), the dose-response curve pertaining to the statistical
17 upper bound on risk (calculated here as the 95% lower confidence bound on dose) is
18 approximately linear below the 0.01 benchmark level and roughly superposes on the EPA default
19 linear extrapolation (see Figure 5-6B). For the oral squamous cell carcinoma (SCC) tumor data
20 (plot not shown), the MLE dose-response curve itself displays low-dose linearity (1st-degree
21 coefficient is greater than zero) and the EPA low-dose linear extrapolation is indistinguishable
22 from the upper bound curve. These observations are consistent with the findings of
23 Subramaniam et al. (2006), that for the large majority of chemicals, straight line extrapolation of
24 risk from the BMDL provides slope factor values very similar to those obtained by using an
25 upper bound on the multistage model risk estimate. Furthermore, in this assessment, EPA has
26 chosen to derive oral slope factors based on combined tumor incidence whenever possible,
27 modeling them under an assumption of independence. A Bayesian analysis is used in this
28 document to develop PODs based on combined tumor risk across the significantly elevated
29 tumor types observed in this bioassay (see Section 5.2.3.2.3.2). As a result of this analysis, the

⁴⁸ EPA policy is to allow for low-dose linearity in the modeling of tumors if a non-linear MOA has not been established.

1 central estimate for the composite dose-response curve shows little curvilinearity and the MLE
2 dose-response curve is substantially linear below a 0.1 extra risk level (see Figure 5-7A and
3 5-7B; see also Section 5.2.3.2.6.11).

4 The results here provide a comparison of EPA’s linear (straight line) dose-response
5 estimates with the degree of linearity seen in the fitted dose-response curves and the statistical
6 upper bounds on these curves. To do this the fitted model needs to allow for the possibility of
7 both curvilinearity at high dose and linearity at low dose. The multistage model has these
8 properties, which is among its advantages for application in carcinogen risk assessment. Most
9 other models commonly used to fit data in the observed range do not have this property.⁴⁹

10 One other issue relative to the determination of linearity arises in the visual interpretation
11 of dose-response plots. The common practice of plotting receptor kinetics data on semi-
12 logarithmic plots for scale convenience has unfortunately led to difficulties in the interpretation
13 of the shape of these relationships. An example is presented using the modeling study of Kohn
14 and Melnick (2002, [199104](#)), which was cited by NRC (NAS, 2006, [198441](#)) in its review of
15 EPA’s dioxin assessment as an example of nonlinear behavior at low dose: “Response is a
16 function of the number of occupied and activated receptors, which typically exhibit steep dose-
17 response relationships. For example, Kohn and Melnick (2002, [199104](#)) modeled the shape of
18 the dose-response relationship for receptor-mediated responses, using the estrogen receptor and
19 various xenoestrogens as a model receptor and ligands, respectively. The model included a
20 variety of assumptions with regard to receptor number, ligand binding affinity, and partial
21 agonist activities, yet in every instance clear sublinear responses were observed at low doses.”
22 However, as shown in Figure 5-8, the apparent strong upward curvature of the low-dose
23 relationship is no longer seen when the results are plotted on an arithmetic scale. Instead, the
24 system may be seen as providing an example of close to linear behavior in the low-dose region.
25

⁴⁹ The standard Hill models do not: A Hill model is only linear at low dose when the Hill parameter is equal to 1 (and in that case the Hill model is linear over the full dose range until the high dose region of “saturation” where the k_m parameter results in downward curvature). Thus, while the Hill model is a valuable tool for fitting data in the observed experimental range, it is not helpful in illustrating the potential for low-dose response. However, some have considered a dose-additive version of the Hill model which would allow for low-dose linearity.

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1 **5.2.3.4.1.5. Consideration of nonlinear methods.**

2 While the 2005 Cancer Guidelines deem linear extrapolation to be most appropriate for
3 TCDD, EPA has carefully considered the NAS recommendation to provide risk estimates using
4 both linear and nonlinear methods.

5 The 2005 Cancer Guidelines state

6
7 For cases where the tumors arise through a nonlinear mode of action, an oral
8 reference dose or an inhalation reference concentration, or both, should be
9 developed in accordance with EPA's established practice for developing such
10 values ... This approach expands the past focus of such reference values
11 (previously reserved for effects other than cancer) to include carcinogenic effects
12 determined to have a nonlinear mode of action.
13

14 In this section, EPA presents two illustrative examples of RfD development for
15 carcinogenic effects of TCDD. Each of these examples focuses on data derived from animal
16 bioassays as described in Section 2.4.2.

17
18 **5.2.3.4.1.5.1. Illustrative RfDs based on tumorigenesis in experimental animals.**

19 TCDD has been shown to be a multisite carcinogen in both sexes of several species of
20 experimental animals. It also has been shown to be carcinogenic to humans. Most of the
21 available quantitative human epidemiologic data related to TCDD carcinogenesis are for all
22 cancer mortality. Mortality is a frank effect and is generally considered to be inappropriate for
23 RfD development, therefore, the illustrative example below utilizes available evidence from
24 experimental animals. Table 5-20 presents candidate PODs and RfDs for TCDD carcinogenicity
25 based on combined tumor responses from the animal bioassays described in Section 2.4.2. The
26 PODs from the NTP (2006, [549255](#); 2006, [543749](#)) and Kociba et al. (1978, [001818](#)) animal
27 studies were derived from Bayesian multitumor dose-response modeling (as described in
28 Section 5.2.3.2, Table 5-17) using a BMR of 1%. Because only TCDD-induced liver tumors
29 were reported by Toth et al. (1979, [197109](#)), the BMR of 1% (POD) from that study was
30 generated using a first degree linear multistage model (see Table 5-15). TCDD-induced liver
31 tumors were reported by Della Porta et al. (1987, [197405](#)), with the male mouse producing the
32 lowest BMR of 1% (POD) using a second degree linear multistage model (see Table 5-15).
33 Following BMD modeling, BMDL_{HEDS} were then estimated (see Tables 5-16 and 5-17) using the

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1 TCDD whole-blood-concentration dose metric from the Emond model as described in Section 3.
2 The illustrative RfDs were derived by dividing the $BMDL_{HEDS}$ by appropriate uncertainty
3 factors. In each instance, a total UF of 30 was applied, comprising factors of 3 for the
4 toxicodynamic component of the interspecies extrapolation factor (UF_A) and a factor of 10 for
5 human interindividual variability (UF_H).

6 As shown in Table 5-20, the illustrative RfDs for TCDD-induced tumors range from
7 $3.6E-11$ for liver and lung tumors in male mice (NTP, 1982, [594255](#)) to $1.0E-9$ for adrenal
8 cortex, tongue and nasal/palate tumors in male rats (Kociba et al., 1978, [001818](#)). This
9 illustrative RfD range for TCDD tumorigenesis falls within the range of candidate RfDs for
10 noncancer TCDD effects presented in Table 4-5.

11
12 **5.2.3.4.1.5.2.** Illustrative RfDs based on hypothesized key events in TCDD's MOAs for liver
13 and lung tumors.

14 As described in Section 5.1, most evidence suggests that the majority of toxic effects of
15 TCDD are mediated by interaction with the AhR. EPA considers interaction with the AhR to be
16 a necessary, but not sufficient, event in TCDD carcinogenesis. The sequence of key events
17 following binding of TCDD to the AhR and that ultimately leads to the development of cancer is
18 unknown. While the mode of action of TCDD in producing cancer has not been elucidated for
19 any tumor type, the best characterized carcinogenic actions of TCDD are in rodent liver, lung,
20 and thyroid. The hypothesized sequence of events following TCDD interaction with the AhR is
21 markedly different for each of these three tumor types. Additionally, no detailed hypothesized
22 mode of action information exists for any of the other reported tumor types.

23 The endpoints selected for this illustration were evaluated to provide insight into the
24 quantitative relationships between tumor development and precursor events in TCDD-induced
25 carcinogenesis. The endpoints described below may or may not be biologically adverse in
26 themselves; the intent herein was to consider TCDD-induced biochemical and cellular changes
27 that could lead to subsequent tumor development.

28 In the following exercise, illustrative RfDs were derived for key events in TCDD's
29 hypothesized modes of action in the liver and lung. No appropriate dose-response data were
30 identified for key events in TCDD's hypothesized MOA for thyroid tumors in a

1 sex/species/strain that has been shown to develop thyroid tumors (i.e., female B6C3F1 mice and
2 male and female Osborne-Mendel rats (NTP, 1982, [594255](#))).

3 As this is an illustrative exercise only, only studies that were originally identified in
4 Section 2 for potential noncancer dose-response modeling were evaluated here (see Section 2.4.2
5 for study details). There may be additional studies available in the literature that would further
6 inform the dose-response assessment of these endpoints.

7 Additionally, for animal model consistency, only results from studies conducted in
8 female S-D rats are presented here. The majority of the available information on TCDD
9 carcinogenicity (and TCDD carcinogenic precursor events) comes from studies conducted in
10 female S-D rats and the most recent TCDD carcinogenicity study was conducted in female S-D
11 rats (NTP, 2006, [197605](#)). While both Kociba et al. (1978, [001818](#)) and NTP (2006, [543749](#))
12 have conducted TCDD carcinogenicity studies in female S-D rats, different substrains were used;
13 this difference in substrain may have resulted in the different carcinogenic responses reported
14 from the two studies. While the carcinogenicity of TCDD in female S-D rats has been well
15 characterized, this animal model does not exhibit the full suite of tumor responses reported for
16 TCDD (for instance, female S-D rats have not been shown to develop thyroid tumors).
17 Additionally, the most sensitive single tumor response in female S-D rats from NTP (2006,
18 [543749](#)) is squamous cell carcinoma of the oral mucosa (see Section 5.2.3.2), a tumor type for
19 which no mode of action information exists. Therefore, the illustrative RfDs described below
20 may not be protective against all tumor types.

21 For each endpoint, PODs for illustrative cancer RfD development were identified as
22 described for the noncancer RfD derivation in Section 4. Briefly, for the endpoints identified
23 below, the NOAEL_{HEDS} and/or LOAEL_{HEDS} were determined based on EPA analysis of the
24 original data presented by the study author (see Section 2.4.2 for details) and by application of
25 the Emond PBPK models as described in Section 3.3.4. BMDL_{HEDS} were determined as
26 described in Section 4.2 for all data sets amenable to BMD modeling. Modeling outputs for the
27 endpoints are presented in Appendices E and G as noted in Table 5-21. The illustrative RfDs
28 were derived by dividing the POD by appropriate uncertainty factors as indicated in Table 5-21.

29 **5.2.3.4.1.5.2.1.** *Liver tumors.*

30 Figure 5-9 presents one hypothesized mode of action for TCDD-induced liver tumors in
31 rats. TCDD activation of the AhR leads to a variety of changes in gene expression, including

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1 increased CYP1A1 mRNA and subsequent increases in CYP1A1 activity. These alterations in
2 gene expression are hypothesized to lead to hepatotoxicity, followed by compensatory
3 regenerative cellular proliferation and subsequent tumor development. The details of the
4 mechanism of TCDD-induced hepatotoxicity have not been fully determined but both CYP
5 induction and oxidative stress have been postulated to be involved (Maronpot et al., 1993,
6 [198386](#); Viluksela et al., 2000, [198968](#)). Additionally, oxidative DNA damage has been
7 implicated in liver tumor promotion (Umemura et al., 1999, [198001](#)). The enhanced cell
8 proliferation arising from either altered gene expression or hepatotoxicity, or both, could be the
9 principal factor leading to promotion of hepatocellular tumors (Whysner and Williams, 1996,
10 [197556](#)).

11 A dose-response relationship exists for TCDD-mediated hepatotoxicity, and this parallels
12 the dose-response relationship for tumor formation (or formation of foci of cellular alteration as a
13 surrogate of tumor formation). However, the dose-response relationship for other
14 TCDD-induced responses such as enhanced gene expression is different from the dose-response
15 for tumor formation in terms of both efficacy and potency (see Popp et al. (2006, [197074](#)) for
16 review).

17 A representative endpoint for each of the hypothesized key events following AhR
18 activation for TCDD-induced liver tumors was identified and is shown in Figure 5-9. Illustrative
19 RfDs based on each representative endpoint are shown in Table 5-21.

20

21 **5.2.3.4.1.5.2.2. Lung tumors.**

22 Far less is known about TCDD's mode of action in the lung. Figure 5-10 presents two
23 hypothesized modes of action for TCDD-induced lung tumors in rats. The first hypothesized
24 mode of action of TCDD in the lung involves disruption of retinoid homeostasis in the liver.
25 Retinoic acids and their corresponding nuclear receptors, the RARs and the RXRs, work together
26 to regulate cell growth, differentiation, and apoptosis. It is hypothesized that TCDD, through
27 activation of the AhR, can affect parts of the complex retinoid system and/or other signaling
28 systems regulated by, and/or cross-talking with, the retinoid system (reviewed in (Nilsson and
29 Håkansson, 2002, [548746](#))). These effects are then hypothesized to lead to lung tumor
30 development, however the mechanisms underlying this hypothesis are not well-defined. The
31 second hypothesized mechanism for the carcinogenic action of TCDD in the lung is through

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1 induction of metabolic enzymes. Through activation of AhR and subsequent induction of
2 metabolizing enzymes (such as CYP1A1), TCDD may enhance bioactivation of other
3 carcinogens in lung (Tritscher et al., 2000, [197265](#)). However, there are few studies to support
4 this hypothesis.

5 Representative endpoints could only be identified for two of the hypothesized key events
6 following AhR activation for TCDD-induced lung tumors. These endpoints are presented in
7 Figure 5-10. Illustrative RfDs based on each of these two representative endpoints are shown in
8 Table 5-21. There is insufficient information to form any conclusions on the quantitative
9 progression to tumorigenicity or on the relative protection afforded by preventing the key events
10 shown.

11
12 **5.2.3.4.1.5.2.3.** *Limitations of illustrative RfDs based on hypothesized key events in TCDD's*
13 *MOAs for liver and lung tumors.*

14 A trend for increasing RfD values that follows the progression of endpoints towards the
15 production of tumors is evident. However, there are a number of factors that prevent making
16 strong conclusions based on this exercise. These limitations include the following

- 17
- 18 • This example addresses only two tumor types in one species, strain and sex (female S-D
19 rats), with little information available on the hypothesized mode of action for lung
20 tumors. No mode of action information is available for the most sensitive tumor type in
21 this animal model (squamous cell carcinoma of the oral mucosa). Therefore, it is
22 possible that the illustrative RfDs presented in this example would not be protective
23 against all tumor types in female S-D rats. Importantly, other animal models have been
24 shown to be more sensitive to TCDD-induced carcinogenesis based on combined tumor
25 analysis (see Section 5.2.3.2); an RfD based on tumorigenesis in this animal model may
26 not be protective against tumorigenesis in other, more sensitive, animal models (or, by
27 extension, in humans).
 - 28 • Several of the BMDLs are based on poorly-fitting models, such that the RfD is based on
29 a LOAEL (or LOEL), which is not a particularly good measure for comparison across
30 endpoints (e.g., LOAELs are dependent on dose spacing in bioassays). Furthermore, the
31 hepatotoxicity BMDL based on a dichotomous 10% BMR, is not directly comparable to
32 all the other BMDLs based on a continuous 1 standard-deviation BMR (Crump, 2002,
33 [035681](#)). In addition, as the earlier effects (CYP induction, cellular proliferation) are not
34 considered to be necessarily adverse in themselves, the BMR of 1 standard-deviation
35 from the mean may not be the best choice for determining a POD based on biological
36 significance. The use of the 1 standard-deviation BMR for the illustrative examples is
37 primarily for comparison on an equal-magnitude-of-response basis across endpoints.

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- 1 • The endpoints selected as representative of each hypothesized key event may not be the
2 most appropriate choices. These particular endpoints were chosen because they were the
3 most sensitive indicator (i.e., lowest POD) from the available data or were the only
4 available choice based on a lack of data for other effects related to the hypothesized key
5 event.
- 6 • The optimum timing of these events may not be reflected in the endpoints selected.
7 Almost certainly, changes in gene expression are early events, such that a single
8 exposure should be relevant, as in the mRNA changes reported after a single TCDD
9 exposure (Vanden Heuvel et al., 1994, [594318](#)), although it is not known whether the
10 magnitude of these changes would be altered after longer-term exposure, or whether
11 longer-term exposure would be more relevant to downstream events. Similarly, single
12 exposures for induction of CYP enzymes would seem to relevant as a measure of the
13 immediate effect, but it may be longer-term repeated CYP activity that is important for
14 longer-term downstream events; Table 5-21 shows a nominal order-of-magnitude
15 difference in effect levels for similar effect magnitudes (ca. 20-fold) from single
16 exposures (Kitchin and Woods, 1979, [198750](#)) and long-term exposures (53-weeks;
17 NTP, 2006, [543749](#)). The relevant exposure durations for oxidative stress and later
18 effects are longer term, so a measurement of oxidative stress at 90-days in a rodent may
19 be appropriate; Wyde et al. (2001, [198575](#)) suggest that induction of 8-oxo-dG DNA
20 adducts are a result of longer-term oxidative stress because of the lack of effect of single
21 exposures. Hepatotoxicity and hepatocellular proliferation events would appear at
22 successively later times, but the effective exposure levels would depend heavily on the
23 endpoints chosen to represent those events and the time at which they were measured.
24 The toxic hepatopathy endpoint reported in NTP (2006, [543749](#)), is a general measure of
25 mild to moderate liver toxicity, but is measured only at the end of the study when tumors
26 have already appeared. Hepatocyte hypertrophy, measured at 31 weeks may be more
27 duration-relevant, but may not indicate actual hepatocellular toxicity.
- 28 • The lowest of the tested doses may well be much higher, given that all animal diets are
29 contaminated to a certain extent by TCDD, resulting in initial TCDD body burdens in all
30 animals. Vanden Heuvel et al. (1994, [594318](#)) reported TCDD liver concentrations in
31 control animals almost as high as for the low-dose group, which could equate to a
32 significant increase in the actual exposure experienced by the low-dose group. A similar
33 effect on the low-dose group (0.45 ng/kg) in Kitchin and Woods (1979, [198750](#)) is
34 possible, although they did not report control animal tissue concentrations. Higher
35 exposure levels or longer-term exposures would not be affected to the same degree, as
36 administered TCDD levels would likely be large compared to initial body burden or low-
37 level feed stock exposure.

38
39 Given the limitations described above, establishing an unambiguous progression of
40 effects is extremely problematic given the lack of sufficient data. Identifying a RfD that could
41 be considered to be protective against tumorigenesis in humans based on these data and models
42 is subject not only to the determination of effective low doses for the RfDs in Table 5-21 but also

1 to the determination of effective exposures that could be considered to be protective of all other
2 tumor types in female S-D rats as well as all other animal models. The latter would entail
3 identifying precursors that are sufficient in themselves for progression to tumorigenesis for all
4 tumor types. Given the disparate sequence of hypothesized key events following TCDD-induced
5 AhR activation for the tumor types for which some information is available, AhR
6 binding/activation is the only key event that is likely to be shared across tumor types. No
7 appropriate quantitative data on AhR binding/activation by TCDD in relevant animal models
8 were located; therefore, an illustrative RfD based on TCDD AhR activation could not be
9 developed.

10 Simon et al. (2009, [594321](#)) present a similar analysis for the liver tumors observed in the
11 NTP (2006, [543749](#)) study, showing a progression of effects from early biochemical events to
12 irreversible liver toxicity, culminating in tumorigenesis. While illustrative of the putative tumor-
13 promoting MOA for TCDD, the limitations of using such an approach within the context of an
14 assessment of the overall carcinogenic risk of TCDD as detailed above still apply. Simon and
15 colleagues also present RfDs for liver tumors and several precursor endpoints. All the RfDs
16 presented in Simon et al. (2009, [594321](#)) are essentially equivalent and are 1 to 3 orders of
17 magnitude higher than the RfDs for equivalent endpoints presented in Table 5-21. These
18 discrepancies are partly due to the fact that the Emond PBPK models (Emond et al., 2004,
19 [197315](#); Emond et al., 2005, [197317](#); Emond et al., 2006, [197316](#); see also Section 3.3.4) used in
20 this document predicts lower TCDD intakes for similar tissue concentrations than the CADM
21 kinetic model (Aylward et al., 2005, [197014](#); Carrier et al., 1995, [197618](#)) used by Simon and
22 colleagues. However, a larger contributor to these discrepancies is the use of a chemical-specific
23 adjustment factor (CSAF) of 0.1 for the toxicodynamic component of the interspecies
24 uncertainty factor by Simon et al. (2009, [594321](#)), while EPA used an uncertainty factor of 3.
25 EPA does not find that the *in vitro* evidence presented by Simon et al. in support of a CSAF of
26 0.1 for interspecies toxicodynamics meets the burden of proof necessary for a reduction in this
27 uncertainty factor.

28

5.3. DERIVATION OF THE TCDD ORAL SLOPE FACTOR AND CANCER RISK ESTIMATES

EPA was able to derive candidate OSFs for all cancer mortality from human epidemiologic studies as well as for individual and combined tumor incidence from rodent cancer bioassays. Each of these studies was selected for TCDD dose-response modeling using the study inclusion criteria outlined in Section 2. The derivation of these OSFs can be found for the epidemiologic data in Section 5.2.3.1 and for the rodent bioassay data in Section 5.2.3.2.

The OSFs based on epidemiologic studies from three cohorts ranged from 3.75×10^5 to 2.5×10^6 per mg/kg-day (see Tables 5-1 and 5-3). For the animal data, OSFs based on individual tumors were developed for 28 study/sex/endpoint combinations, and the results ranged from 1.8×10^4 to 5.8×10^6 per mg/kg-day (see Table 5-16). The OSFs based on combined tumors were developed for 7 study/sex combinations, and the results ranged from 3.2×10^5 to 9.4×10^6 per mg/kg-day (see Table 5-17). Figure 5-11 demonstrates the range of these OSFs in units of per mg/kg-day. The human study OSFs are shown at the far left of the figure, and the rodent endpoints are arranged by species to the right. For comparison with the other studies, the OSF from Cheng et al. (2006, [523122](#)) is based on a 1×10^{-6} risk level (Table 5-3).

As recommended by expert panelists at EPA's 2009 Dioxin Workshop (U.S. EPA, 2009, [522927](#)) and in the 2005 Cancer Guidelines (U.S. EPA, 2005, [086237](#)), EPA has chosen to give higher consideration to the human epidemiologic data rather than the animal bioassay data in developing an OSF for TCDD. Candidate OSFs derived from the human data are consistent with the animal bioassay OSFs; specifically, the human OSFs fall within the same range as the animal bioassay OSFs. Because all the human and animal studies were considered to be of high quality and yielded similar ranges of OSFs, EPA has chosen to rely on the epidemiologic data for OSF derivation.

The strengths and limitations of the five epidemiological studies meeting the inclusion criteria for cancer dose-response modeling are summarized in Table 5-22. Among the human studies, the occupational TCDD exposures in the NIOSH and Hamburg cohorts are assumed to be reasonably constant over the duration of occupational exposure. In contrast, the TCDD exposure patterns in the Seveso and BASF cohorts are associated with industrial accidents; as a consequence, the exposure patterns are acute, high dose followed by low-level background exposure. Such exposure patterns similar to those experienced by the BASF and Seveso cohorts

1 have been shown to yield higher estimates of risk when compared to constant exposure scenarios
2 with similar total exposure magnitudes (Kim et al., 2003, [199146](#); Murdoch and Krewski, 1988,
3 [548718](#); Murdoch et al., 1992, [548719](#)). Thus, EPA has judged that the NIOSH and Hamburg
4 cohort response data are more relevant than the BASF and Seveso data for assessing cancer risks
5 from continuous ambient TCDD exposure in the general population.

6 The NIOSH (Cheng et al., 2006, [523122](#); Steenland et al., 2001, [198589](#)) and Hamburg
7 (Becher et al., 1998, [197173](#)) cohort studies report cumulative TCDD levels in the serum for
8 cohort members. The most significant difference among the Cheng et al. (2006, [523122](#))
9 analysis and those of Steenland et al. (2001, [198589](#)) and Becher et al. (1998, [197173](#)) is the
10 method used to back-extrapolate exposure concentrations based on serum TCDD measurements.
11 Steenland et al. (2001, [198589](#)) and Becher et al. (1998, [197173](#)) back-extrapolated exposures
12 and body burdens using a first-order model with a constant half-life. In contrast, Cheng et al.
13 (2006, [523122](#)) back-extrapolated body burdens using a kinetic modeling approach that
14 incorporated concentration- and age-dependent elimination kinetics.

15 Although all three of these are high-quality studies, the kinetic modeling used by Cheng
16 et al. (2006, [523122](#)) is judged to better reflect TCDD pharmacokinetics, as currently
17 understood, than the first-order models used by Steenland et al. (2001, [198589](#)) and Becher et al.
18 (1998, [197173](#)). EPA believes that the representation of physiological processes provided by
19 Cheng et al. (2006, [523122](#)) is more realistic than the assumption of simple first-order kinetics
20 and this outweighs the attendant modeling uncertainties. Furthermore, the use of kinetic
21 modeling is consistent with recommendations both by the NAS and the Dioxin Workshop panel.

22 However, as discussed in Section 3.3.2, the kinetic model that they employed does have
23 certain limitations, including the fact that it has been calibrated based on a relatively small
24 number of human subjects. In addition, their kinetic model does not allow body mass index
25 (BMI; and hence fat content) to vary with age, which may bias the model results. Nonetheless,
26 EPA prefers the increased technical sophistication of the dose estimates used in the cancer
27 mortality risk estimates derived from Cheng et al. (2006, [523122](#)) to those derived from
28 Steenland et al. (2001, [198589](#)).

29 **EPA, therefore, has decided to use the results of the Cheng et al. (2006, [523122](#))**
30 **study for derivation of the TCDD OSF based on total cancer mortality as calculated by**
31 **EPA using data and models from the Cheng et al. (2006, [523122](#)) study as described in**

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1 **Section 5.2.3.1.2.** Although the OSF is only strictly defined for exposures above the
2 background exposure experienced by the NIOSH cohort, which was assumed to be 0.5
3 pg/kg-day TCDD, or 5 pg/kg-day total TEQ, EPA assumes that the slope (risk vs. blood
4 concentration) is the same below those background exposure levels as it is above. Table 5-3
5 shows the oral slope factors at specific target risk levels (OSF_{RLS}) which range from
6 1.1×10^5 to 1.3×10^6 per (mg/kg-day). EPA recommends the use of an OSF of 1×10^6 per
7 (mg/kg-day) when the target risk range is 10^{-5} to 10^{-7} . Although EPA prefers the human
8 data, EPA also presents a number of OSFs derived from rodent bioassays. Most of these
9 animal studies are of note, because in general they were well-designed and conducted. In
10 particular, the NTP (2006, [543749](#)) study was recently conducted and represents the most
11 comprehensive evaluation of TCDD chronic rodent toxicity to date.

13 **5.3.1. Uncertainty in Estimation of Oral Slope Factors from Human Studies**

14 A fair amount of uncertainty is associated with the estimation of slope factor values and
15 cancer risk specific doses for TCDD based on the epidemiological studies. In some instances,
16 the influence of a given factor is theoretically amenable to analysis, but such investigation is
17 limited by the availability of sufficiently detailed data to support such an analysis. In other
18 cases, only very broad ranges can be placed on the uncertainty associated with a given feature of
19 the analysis, or uncertainties must be discussed qualitatively.

20 The following four sources of uncertainty are addressed in this section: uncertainty in
21 exposure estimates in the epidemiologic studies (see Section 5.3.1.1), uncertainty in the shape of
22 the dose-response curve (see Section 5.3.1.2), uncertainty in extrapolating risks below exposure
23 levels in the reference population (see Section 5.3.1.3), uncertainty in cancer risk estimates
24 arising from background DLC exposure (see Section 5.3.1.4) and uncertainty in cancer risk
25 estimates arising from occupational coexposures to DLCs (see Section 5.3.1.5). Section 5.3.2
26 explores other sources of uncertainty in the epidemiologic risk estimates including the use of
27 cancer mortality rather than cancer incidence data in the derivation of the oral slope factor,
28 possible influences of inter-individual variability in TCDD kinetics, and exposures to other
29 occupational carcinogens.

1 **5.3.1.1. Uncertainty in Exposure Estimation**

2 The major technical challenge within each of the epidemiological studies was developing
3 relevant and precise estimates of exposure. While Warner et al.(2002, [197489](#)) collected blood
4 samples relatively close to the time of the Seveso accident and could reasonably estimate peak
5 exposures based on these collected samples, in the case of the Becher et al. (1998, [197173](#)), Ott
6 and Zober (1996, [198408](#)), Steenland et al. (2001, [198589](#)), and Cheng et al. (2006, [523122](#))
7 studies, the major exposure issues included the following

- 8
- 9 • Selecting (an) appropriate dose metric(s) for dose-response modeling,
- 10 • Estimating serum TCDD levels for the entire cohort based on measurements from a
11 smaller number of the subjects in the cohort collected long after the occupational
12 exposures had occurred, and then assigning exposures to the remaining members of the
13 cohort based on qualitative job classifications.
- 14 • Estimating time-weighted average tissue doses (e.g., lipid-average serum concentration
15 over time) based on single samples taken at one point in time. (Except for the Becher et
16 al. (1998, [197173](#)) analysis where one of the study strengths was their estimate of TCDD
17 half life, which utilized repeated measurements from a subset of their cohort).

18

19 In the Becher et al. (1998, [197173](#)), Steenland et al. (2001, [198589](#)), and Cheng et al.
20 (2006, [523122](#)) studies, dose-response modeling was performed using ppt-years lipid-adjusted
21 serum concentration as the primary dose metric for TCDD; serum TCDD was the only direct
22 measurement of exposure or dose that was available. In addition, as discussed in Section 3.3.4,
23 serum concentration is a reasonable index of total tissue concentration (target organ dose), and
24 lipid-adjusted serum concentration provides a reasonable index of TCDD in the fatty components
25 of tissues. Ott and Zober (1996, [198408](#)) used ng/kg body weight at the time of the accident as
26 the primary dose metric, and U.S. EPA (2003, [537122](#)) later converted these to units of ppt-years
27 lipid-adjusted serum concentration.

28 The decision to use cumulative serum concentrations (ppt-years) as the primary dose
29 metric for carcinogenicity is based on the understanding that time weighted concentrations (over
30 a chronic exposure period) are the most appropriate dose measures for cancer risk assessment.
31 This may not be strictly true if cancer induction by TCDD is considered to be a “threshold
32 process.” However, as discussed in Section 5.2, there are reasonable grounds to believe that the

1 assumption of low-dose linearity is reasonable for TCDD, especially when calculating
2 population risks where the effects of interindividual variability must be taken into account.

3 In addition to the issue of low-dose thresholds, the rationale for using cumulative dose
4 metrics also can fail at high doses if the adverse response in question involves a step that is
5 saturable (e.g., where there is a maximum level of response that cannot be exceeded owing to a
6 rate-limited process). There is some evidence for such a phenomenon in the NIOSH cohort
7 where cancer risks in the highest exposure group (>50,000 ppt-years) appear to saturate, and the
8 response decreases at this level (Steenland et al., 2001, [198589](#)). Steenland et al. (2001, [198589](#))
9 suggest that the apparent saturation of dose-response in this cohort may be due, at least partially,
10 to exposure misclassification among the highest exposed individuals, rather than to an actual
11 reduction in response per unit exposure.

12 The uncertainty associated with differences in the exposure patterns is important to
13 consider across the five epidemiologic studies. Steenland et al. (2001, [198589](#)), Cheng et al.
14 (2006, [523122](#)), and Becher et al. (1998, [197173](#)) studied cohorts exposed to elevated TCDD
15 levels over a long period of time, while Ott and Zober (1996, [198408](#)) and Warner et al. (2002,
16 [197489](#)) studied cohorts exposed to TCDD levels significantly above background at one point in
17 time but the exposures and likely the TCDD body burdens declined significantly following these
18 periods of elevated exposure. Both these chronic and acute exposures can be analyzed in terms
19 of cumulative exposure to TCDD. Use of such a metric requires an assumption that the “actual”
20 cancer potency associated with a cumulative dose where much of the dose is received at a single
21 point in time and then gradually eliminated would be similar to the cancer potency of the same
22 cumulative dose received over a longer period of time and also gradually eliminated. While EPA
23 believes that such an assumption is not unreasonable, the experiment of Kim et al. (2003,
24 [199146](#)), which showed statistically significant increase in liver effects due to a peak TCDD
25 dose when compared to chronically-dosed Sprague-Dawley rats administered the same levels of
26 TCDD when measured as a cumulative dose, suggests that additional analyses of cumulative and
27 peak TCDD dose measures may need to be conducted.

28 There are uncertainties associated with the approaches used to estimate TCDD exposures
29 in the members of the occupational epidemiologic studies for which no measurement data were
30 available. To impute TCDD levels for workers without measured samples, all four occupational
31 epidemiologic studies matched workers for whom measured TCDD samples had never been

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1 reported to workers with measured TCDD levels based on job histories. The NIOSH cohort is
2 used to illustrate some of the uncertainties. In the NIOSH cohort, the subset of workers (roughly
3 5% of the total cohort) with blood serum data comprised surviving members of the cohort (in
4 1988), and therefore, their age distribution likely differed from the rest of the cohort. For each
5 worker in this subset, the following data were available: (1) job classification information,
6 (2) employment history, and (3) serum TCDD measures. All of the workers in this subset were
7 employed at a single plant where the work histories were less detailed than at other plants, and
8 many of the workers at this plant had the same job title and were employed during the same
9 calendar period. There is an assumption that workers with same job title and work history were
10 exposed to the same TCDD levels within a plant and across plants; this obviously does not
11 account for exposure heterogeneity.

12 Both Steenland et al. (2001, [198589](#)) and Cheng et al. (2006, [523122](#)) addressed the
13 potential for exposure measurement error in TCDD estimates and possible exposure
14 misclassification. For the highest exposure workers, Steenland et al. (2001, [198589](#)) and Cheng
15 et al. (2006, [523122](#)) found weak, “noisy,” and/or negative exposure-response relationships.
16 Steenland et al. (2001, [198589](#)) suggests that possible explanations for this observation include
17 the saturation of effects at the upper end of the dose-response curve, instability of the TCDD
18 exposure estimates based on the limited number of highly exposed individuals, and the increased
19 probability of exposure misclassification for workers whose job histories indicate the highest
20 exposures. As Steenland et al. (2001, [198589](#)) reported, some of the highest exposures might
21 have been inaccurately estimated because they occurred in workers exposed to short-term, high-
22 dose exposures during spill clean-up. Cheng et al. (2006, [523122](#)) used sensitivity analyses to
23 examine this measurement error issue and evaluated the potential for exposure misclassification
24 by using ln-transformed TCDD ppt-years. The authors removed all observations with exposures
25 within the lower and upper 1, 2.5, or 5th percentiles of the TCDD ppt-year distribution and also
26 removed observations within just the upper 1, 2.5, or 5th percentile of TCDD ppt-years. These
27 sensitivity analyses yielded results similar to those reported in the primary analysis. An
28 additional concern is that exposure errors might distort the exposure distribution in the
29 population, which generally spreads the response out over a wider dose range. This serves to
30 increase the variance of the regression model, altering both the POD and the corresponding OSF.

1 Becher et al. (1998, [197173](#)) only considered workers from a single plant but their
2 analysis included workers employed in five different job locations within the plant. The
3 influence of worker location on slope factor estimates does not appear to be further explored and
4 may represent a source of uncertainty.

5 To estimate long-term body burden metrics from the serum TCDD measurements,
6 Steenland et al. (2001, [198589](#)) employed simple first order kinetic elimination rate model with a
7 half-life of 8.7 years. Limitations of this approach include (1) the average elimination half-life
8 among the study subjects may not be 8.7 years given differences between the study population
9 and the Ranch Hand population from which the value was estimated, (2) use of a single-value
10 estimate fails to take into account the inherent variability in elimination half life among the
11 individual workers, and (3) it fails to take into account variations in elimination kinetics
12 throughout the lifetime of the exposed worker due to change in body fat, age, etc. The impact of
13 these potential sources of bias on the estimates of time-integrated body burden cannot be
14 quantitatively assessed. However, Steenland et al. (2001, [198589](#)) noted that modest changes in
15 elimination half-life (to 7.1 years) had only a very small impact on risk estimates.

16 Cheng et al. (2006, [523122](#)) estimated past body burdens using the CADM approach
17 (described in Section 3) (Aylward et al., 2005a, b) rather than a half-life estimate. As noted
18 above, the incorporation of concentration- and age-dependent elimination into this approach has
19 significant advantages over the use of a constant elimination half-life. However, as discussed in
20 Section 3.3, the CADM has only been subject to limited testing against human validation data
21 sets, so the degree to which its advantages are realized in practice cannot be easily assessed.
22 There are no available human data in the low dose region, the region of interest to this
23 assessment, to compare with the CADM (or Emond) model predictions.

24 Becher et al. (1998, [197173](#)) developed half life estimates based on multiple TCDD
25 blood measures in 48 individuals from this cohort. These half life estimates were then used to
26 back calculate TCDD concentrations at the end of each worker's employment, accounting for
27 age and percentage of body fat. This cohort-specific information may provide a better exposure
28 estimate than Steenland et al. (2001, [198589](#)) or Ott and Zober (1996, [198408](#)) who used similar
29 kinetic approaches. However, the comparison of the accuracy of the exposure estimates across
30 the cohorts is not easily assessed. There are several assumptions and important uncertainties
31 involved in modeling TCDD exposures in these cohorts. The study authors have invoked

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1 different kinetic assumptions when extrapolating measured levels of TCDD in sera backward in
2 time to estimate higher chronic or peak dosage (i.e., there is uncertainty in these back-
3 calculations that includes assumptions regarding elimination kinetics). There is also uncertainty
4 in applying such estimates to other members of the cohort based on similar characteristics (e.g.,
5 job category).

6

7 **5.3.1.2. Uncertainty in Shape of the Dose-Response Curve**

8 Another source of uncertainty is the nature of the dose-response curve in the low dose
9 region of interest for risk assessment for environmental exposures (e.g., <1 pg/kg-day). The
10 epidemiologic data are based on occupational studies in which exposures were often several
11 orders of magnitude higher than environmental exposures. In these studies, data from the low
12 dose region are quite sparse, and only one study examined uncertainty due to the low dose
13 region. Steenland and Deddens (2003, [198587](#)) attempted to analyze this region specifically by
14 fitting threshold curves to the NIOSH data in which there was no extra risk from exposure until
15 some specific level. However, this model did not fit as well as models without a threshold. In
16 general, the usual assumption of linearity in the low dose region seems reasonable when using
17 epidemiologic data given the lack of data in this region that precludes the rejection of linearity.

18 There is uncertainty in the extrapolation of the OSF to the low dose region (e.g.,
19 <5 pg/kg-day). EPA developed the cancer assessment in this document assuming the slope in the
20 low-dose region of the dose-response curve is linear; the decision was made due to the lack of
21 sufficient evidence to support an assumption of nonlinearity as outlined in the EPA's Cancer
22 Guidelines (U.S. EPA, 2005, [086237](#)). Similarly, there is uncertainty as to whether a threshold
23 exists for TCDD-induced toxicity leading to tumorigenesis and the dose associated with such a
24 threshold, if it exists, is unknown. EPA chose to model this dose-response without a threshold
25 because there is insufficient evidence to support an assumption of a threshold.

26 It also is noteworthy that the shapes of the exposure-response in several of these studies,
27 based on the published statistical models, is indicative of a response that tends to tail off or
28 "plateau" at high cumulative exposures to TCDD. This phenomenon has been seen in many
29 studies of occupational carcinogens, and may reflect a number of things including exhaustion of
30 people susceptible to cancer, saturation of biological pathways which are part of the pathway to

1 cancer, and increased error measurement of dose at high levels biasing dose-response towards
2 the null (Stayner et al., 2003, [054922](#)).

4 **5.3.1.3. Uncertainty in Extrapolating Risks below Reference Population Exposure Levels**

5 Another source of uncertainty in using human epidemiologic data is due to the lack of
6 completely unexposed populations; there are no human populations that have zero dioxin
7 exposure. The cancer exposure responses modeled in all epidemiologic cohorts, whether
8 primarily exposed via occupational or environmental exposures, can be evaluated with
9 confidence only above the lowest exposed group (i.e., the reference population). There are
10 substantial uncertainties associated with estimating cancer risks from background exposures of
11 TCDD and DLCs because these risks are aggregated in the overall background risk of the
12 referent population, to which outcomes of cohort subjects experiencing higher dioxin exposures
13 are compared. Therefore, the risk modeled from the epidemiologic data is unavoidably the
14 incremental risk above a background exposure to dioxins in the general environment (assumed to
15 be primarily from food intake). Typically, serum TCDD levels in the general populations in the
16 geographic locations and times at which the epidemiologic studies were undertaken have been
17 reported to be on the order of 5 to 20 ppt (Mocarelli et al., 1991, [199600](#))(WHO, 1998; Pinsky
18 and Lorber, 1998). Hence, the extra risks should be considered as those incurred by added
19 exposure above these background exposures, which then introduces uncertainty associated in the
20 cancer slope factor estimate at exposures below background levels. EPA assumes that the slope
21 of the risk curve below the background exposure experienced by the epidemiologic study cohorts
22 is the same as the (modeled) slope above those background exposure levels; data do not exist to
23 test this assumption.

24 Also, background TCDD/DLC exposures experienced by the epidemiologic study cohorts
25 have been estimated to be much larger (5 to 10-fold) than current background levels. Lorber et
26 al. (2009, [543766](#)) estimate that current U.S. intake rates are roughly 0.58 pg TEQ/kg-day at the
27 50th percentile and suggest that human TEQ ingestion exposures likely peaked in the 1970's.
28 Steenland et al. (2001, [198589](#)), presumably based in part on WHO (1998), estimated
29 background intake rates to be 5 pg TEQ/kg-day for the NIOSH cohort. As a result, the
30 assessment of cancer mortality risk at current background exposure levels is also subject to
31 extrapolation uncertainty.

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1
2 **5.3.1.4. Uncertainty in Cancer Risk Estimates Arising from Background DLC Exposure**

3 None of the slope factors presented in this document, whether based on epidemiologic
4 studies or animal bioassays, takes into account the impact of background exposure to DLCs.
5 Background DLC exposure can be estimated for only one of the animal cancer bioassays NTP
6 (2006, [543749](#)). Background TCDD and DLC exposure for the rats in the NTP (2006, [543749](#))
7 does not appear to have been significant, with respect to the magnitude of administered doses
8 (see Section 5.3.2.1). However, given the trend towards lower exposures to TCDD in recent
9 years, the TCDD/DLC exposure may have been much higher in the older studies (e.g., Kociba et
10 al., 1978, [001818](#); NTP, 1982, [543764](#); Toth et al., 1979, [197109](#)). The impact of background
11 TCDD/DLC exposure on the cancer risk modeling of any of the bioassay data would be to
12 increase the dose term associated with each response; consequently, increasing the magnitude of
13 the BMDL, with a proportional reduction in the magnitude of the slope factor, although the
14 effect would probably be small (see Section 5.3.2.1). Note that the shift in dose increases the
15 estimated low doses proportionately more than the higher doses, potentially obscuring the
16 relationship between dose and response in the low dose region.

17 Background dioxin exposure for the epidemiologic cohorts, however, could have been
18 substantial with respect to the TCDD exposures in the reference populations used in the
19 modeling. As an example, the background dioxin intake the NIOSH cohort, which is the basis
20 for the oral slope factor described previously in this section (5.3), was estimated to be
21 0.5 pg/kg-day for TCDD and 10 times higher (5 pg/kg-day) for total TEQ (Steenland et al., 2001,
22 [197433](#))(WHO, 1998). WHO (1998) estimated that TCDD comprised only about 5 to 10% of
23 total TEQ from exposure to DLCs in food, based on DLC exposure estimates and TEFs available
24 at that time. Eskenazi et al. (2004, [197160](#)) estimated that TCDD was 20% of total TEQ in the
25 serum of the reference population in the Seveso Women's Health Study from measurements
26 taken in 1976. Based on more recent estimates (Lorber et al., 2009, [543766](#)), TCDD is about
27 10% of total TEQ in human serum in the United States. Steenland et al. (2001, [198589](#)) assumed
28 a (cumulating) background exposure of 5-6 ppt TCDD and 50 ppt total TEQ per year in serum
29 for their analysis of the NIOSH cohort cancer mortality response. The resulting cumulative
30 background exposures, particularly for total TEQ, are large compared to the lower cumulative
31 occupational exposures over the life-time of the cohort (birth to death or end of follow-up).

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1 Crump et al. (2003, [197384](#)), based on Steenland et al. (2001, [198589](#)), assumed a cumulative
2 background serum concentration of 3,000 ppt-year for total TEQ (50 ppt/year × 60 years), which
3 is much larger than the lower NIOSH cohort occupational TCDD exposures. The latter, when
4 grouped in cumulative TCDD serum-concentration septiles Steenland et al. (2001, [197433](#)),
5 range from 260 to 850 ppt-yr in the first few septiles. Conceivably, the much larger background
6 exposure could have a somewhat larger effect on the slope factor than for the relatively lower
7 background exposure in the animal bioassays. Because the Cheng et al. (2006, [523122](#))
8 modeling does not account for background TEQ, the resulting slope factor is biased high. None
9 of the published analyses of the NIOSH cohort data (Cheng et al., 2006, [523122](#); Crump et al.,
10 2003, [197384](#); Steenland et al., 2001, [198589](#)) present an analysis that addresses the effect of
11 background TEQ exposure on the modeled risk.⁵⁰ Given the data and modeling results currently
12 available, the EPA could not find an approach for expressing the quantitative impact with any
13 accuracy or confidence.

14

15 **5.3.1.5. Uncertainty in Cancer Risk Estimates Arising from Occupational DLC Coexposures**

16 The slope factor estimates are based on an assumption that occupational exposure was
17 entirely to TCDD, with no explicit consideration of the risk attributable to occupational DLCs.
18 Because TCDD typically occurs as a component of a mixture with other DLCs that are assumed
19 to affect cancer risk through dose addition, the assumption that the exposures are entirely TCDD
20 could lead to a positive bias in the slope factor estimates derived from these epidemiologic
21 studies, if the estimates are confounded by other exposures to DLCs and the TEQ dose is larger
22 than the fraction accounted for by TCDD alone. The magnitude of the potential bias can be
23 estimated in a general way through the estimation of risks for plausible mixtures of DLCs and
24 TCDD exposures in the cohort with the same composition as the Steenland et al. (2001, [198589](#))
25 and Cheng et al. (2006, [523122](#)) studies, but the detailed data required to perform such an
26 analysis on the NIOSH cohort are not available. In addition to the slope factor estimated for
27 TCDD, Becher et al. (1998, [197173](#)) also evaluated the slope based on TEQs. They found a
28 dose-response effect for TCDD but not for TEQ (excluding TCDD) which suggests that
29 confounding by DLCs did not occur.

⁵⁰ Steenland et al. (2001, [197433](#)) present a TEQ analysis but for a scenario where total TEQ is 10 times the TCDD exposure for both background and occupational exposure.

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5.3.2. Other Sources of Uncertainty in Risk Estimates from the Epidemiological Studies

Other aspects of the Steenland et al. (2001, [198589](#)), Cheng et al. (2006, [523122](#)), Becher et al. (1998, [197173](#)), and Ott and Zober (1996, [198408](#)) studies that are not directly associated with TCDD or DLCs may contribute uncertainty to the cancer slope factor estimates. This section lists several of these and discusses their potential directional bias in slope. General issues associated with potential confounding effects also were discussed in the 2003 Reassessment (U.S. EPA, 2003, [537122](#)).

All of the studies that meet the criteria (with the exception of Warner et al., 2002, [197489](#)) measure cancer mortality rather than cancer incidence. This likely biases the slope factor downward relative to a slope calculated for cancer incidence, the typical basis of EPA cancer slope factors. In the NIOSH cohort, roughly one-third of the fatal cancers were identified as lung cancer. Because of the high case mortality rate associated with lung cancer during the period of cohort evaluation (e.g., the 5-year relative survival rates for lung cancer were less than 10% before 1973 and were less than 15% before 1995 (Horner et al., 2009), the slope factor estimated for cancer mortality might not be much lower than that calculated for cancer incidence. This assumes that the outcome of a cancer incident (i.e., cancer mortality) is independent of occupational TCDD exposure levels. Estimation of cancer incidence in the general population associated with TCDD exposure would require assumptions related to the relative survival and age-specific cancer risks in the exposed population compared to the NIOSH cohort or the Hamburg cohort; insufficient data are available to support such an analysis.

The routes of TCDD exposures in the occupational cohorts include dermal and inhalation exposures (Steenland et al., 1999, [197437](#)), the U.S. population is assumed to be primarily exposed through the intake of TCDD and DLCs in foods). Given the persistence of TCDD in the body, differences in exposure routes may not be significant, but route-specific effects can not be precluded. The directional bias on the slope factor that is associated with this uncertainty is not known.

Occupational exposures to other carcinogens could lead to uncertainty in the slope factor. For example, in addition to TCDD, the Hamburg cohort was also exposed to hexachlorocyclohexane (HCH), which IARC classified as possibly carcinogenic to humans, and lindane, which EPA (2001) stated had “suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential.” While such co-exposures would not bias the exposure

1 metric (i.e., not dose additive), to the extent that they were correlated with TCDD exposure, the
2 cancer mortality risk attributed to TCDD would be overestimated, biasing the slope factor high
3 because all cancers are attributed to TCDD. To examine this, Cheng et al. (2006, [523122](#))
4 assessed the impact of possible confounding by conducting excluding individual plants in the
5 modeling. If the estimated cancer risks as a function of exposure did not change too much when
6 specific facilities were left out, then confounding was deemed unlikely. Cheng et al. (2006,
7 [523122](#)) likewise found little variation in risks based on these analyses.

8 There is adequate evidence to believe age, gender, and body fat content all can have a
9 significant impact on elimination kinetics and consequent cancer risks associated with TCDD
10 exposure (U.S. EPA, 2003, [537122](#)). While the authors evaluating the Hamburg cohort
11 accounted for such impacts in their kinetic analysis, interindividual kinetic differences were not
12 considered in evaluations of other cohorts.

13 There may be gender differences that affect susceptibility to TCDD exposure. The
14 cohorts analyzed by Steenland et al. (2001, [198589](#)), Cheng et al. (2006, [523122](#)), Ott and Zober
15 (1996, [198408](#)) and Becher et al. (1998, [197173](#)) were comprised almost exclusively of men.
16 This precluded systematically addressing differences between males and females in these studies.
17 Further, because EPA could not develop an estimate from the Warner et al. (2002, [197489](#))
18 cohort, none of the studies analyzed here for cancer dose-response contained a significant
19 percentage of women. Thus, the generalizability of the slope factor estimates to women is
20 uncertain.

21 Finally, of these cancer cohorts only the Seveso cohort included children. The unique
22 sensitivities of infants, toddlers, and children cannot be addressed based on information in the
23 occupational cohorts, although the increases in cancer risk in the Seveso cohort, to date, appear
24 to be modest. Aside from differences in exposure patterns and body fat content, the unique
25 developmental status of children may result in a substantially different profile of cancer risks
26 (and magnitudes of those risks) than can be addressed by simply compensating on the basis of
27 differences in body weight, food intake, etc. Further, because EPA could not develop an
28 estimate from the Warner et al. (2002, [197489](#)) cohort, none of the studies for cancer dose-
29 response analyzed contained a significant percentage of women. Thus, the generalizability of the
30 slope factor estimates to women and children is uncertain.

1 A number of other factors are routinely evaluated in cancer epidemiology studies, but
 2 appear likely to have little impact on the direction of the slope factor; however, they likely
 3 increase overall variability either in the dose or response. These include smoking and lifestyle
 4 factors. Intraindividual variation in TCDD kinetics and susceptibility also could affect the
 5 relationship between exposure and cancer risk. In each of these cases, it is difficult to determine
 6 the directional bias these factors introduce into the derivation of the slope factor, unless
 7 somehow they are correlated with with occupational dioxin exposures.

8
 9 **5.3.2.1. Effect of Added Background TEQ on TCDD Dose-Response**

10 A source of uncertainty for TCDD dose-response modeling is the impact that background
 11 exposures of TCDD and other DLCs might have on the modeling output. As mentioned
 12 previously in Text Box 4-1, NTP (2006, [543749](#)) presented measurements of TCDD in the fat of
 13 control animals. To study the potential impact of background TCDD and total TEQ on the
 14 cancer dose-response modeling for the NTP (2006, [197605](#)) study, EPA has estimated
 15 background levels of TCDD and TEQ (based on total TCDD, PeCDF and PCB126) from the
 16 mixture study to serve as surrogates for background exposures in the TCDD-only study (limit of
 17 detection too high for control level measurements). Background doses were estimated as:

18
 19
$$Chemical_i(B) = \frac{Chemical_i(fat_{MC}) \times TEF_i}{TCDD(fat_{TCDD})} \times Dose_{TCDD} \quad (Eq. 5-9)$$

20
 21 where

- 22 Chemical_i(B) = estimate of background exposure to Chemical i in ng/kg units of TCDD
 23 blood concentrations at 105 weeks, for i = TCDD, PeCDF and PCB126.
 24 Chemical_i(fat_{MC}) = mean pg/g of Chemical i in the fat tissues of the control animals at
 25 105 weeks in mixtures study (NTP, 2006, [543749](#)).
 26 TCDD(fat_{TCDD}) = mean pg/g of TCDD in the fat tissues of the 3 ng/kg dose group at
 27 105 weeks in the TCDD study (NTP, 2006, [197605](#)).
 28 Dose_{TCDD} = 2.56 ng/kg TCDD blood concentration for the 3 ng/kg dose group in the
 29 TCDD study (from the Emond rat PBPK modeling of NTP, 2006,
 30 [197605](#))
 31 TEF_i = Toxicity Equivalence Factor for Chemical i (from Van den berg et al.
 32 (2006, [543769](#))).

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1 Assuming simple proportionality of blood TCDD concentrations between controls and
2 low-dose (3 ng/kg) animals, the TEF-adjusted ratio of each congener (Chemical *i*) in control-
3 animal fat to low-dose-animal fat is multiplied by the modeled TCDD blood concentration for
4 the low-dose animals to obtain an equivalent background exposure in the dose metric (ng/kg
5 whole blood) used to calculate all the OSFs in this assessment. For total TEQ, the estimates
6 across the three congeners are summed. The total TEQ estimates are biased somewhat high
7 because they are based on terminal (2-year) measurements rather than representing lifetime
8 averages. Background exposures are then added to the modeled TCDD blood concentrations for
9 several different background exposure scenarios (see Table 5-23) prior to conducting
10 Benchmark-Dose (BMD) modeling.

11 BMD modeling was conducted for the cholangiocarcinoma endpoint in the TCDD study
12 (NTP, 2006, [197605](#)). This was done for scenarios that added the following estimated TCDD or
13 TEQ background doses to the TCDD study doses: background TCDD only, total estimated TEQ,
14 twice the total TEQ and ten times the background TCDD (see Table 5-23). These doses may
15 bound the potential background exposures as TCDD has been thought to represent about 10% of
16 all TEQs at environmental levels (WHO, 1998). Table 5-24 shows that, as expected, adding to
17 the exposure term increases the BMDL (and decreases the OSF) and also shifts the shape of the
18 dose-response slope slightly towards sublinear (see Appendix I). However, at these background
19 exposure levels relative to the administered dose levels, there is very little quantitative impact on
20 the cancer dose-response modeling for the NTP (2006, [197605](#)) study. Even with the most
21 extreme assumption that background TCDD is only 10% of total background TEQ, the BMDL
22 changes by only 12%. Assuming that background exposures were higher for older studies (e.g.,
23 Kociba et al., 1978, [001818](#); NTP, 1982, [594255](#)), the impact would be somewhat higher, but
24 unless the background exposures were substantially higher than the lower tested doses (ca.
25 1–10 ng/kg-day), a significant change in the dose-response modeling results would not be
26 expected.⁵¹

27 However, as discussed previously, background TEQ exposures were likely very high
28 with respect to the lower occupational TCDD exposure levels as reported in the epidemiologic
29 studies. Table 5-25 shows the relative increase in exposure levels (as cumulative serum TCDD

⁵¹ Note that the situation is different for single-exposure studies where accumulated body burden from background exposures could be higher than the lowest administered dose (see Tex Box 4.1 in Section 4.4).

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1 concentrations) for the NIOSH cohort septiles assuming that total background TEQ is 10 times
2 background TCDD and that 50 ppt TEQ per year is accumulated in serum (Crump et al., 2003,
3 [197384](#); Steenland et al., 2001, [198589](#)). Although definitive quantitative analyses have not yet
4 been published or designed, the impact on modeled TCDD risk from these studies could be
5 substantial. The expectation for the direction of the effect would be the same as for the animal
6 bioassays; adding to the exposure magnitude without changing the response would decrease the
7 unit risk.

9 **5.3.3. Approaches to Combining Estimates from Different Epidemiologic Studies**

10 Meta-analyses and pooled analyses are two common approaches for combining
11 epidemiologic study data. Meta-analyses are a useful way to combine epidemiologic data from
12 different studies and derive a common estimate of effect, particularly when there are a large
13 number of comparable studies that are fairly homogenous as to make them possible to combine.
14 A meta-analysis often involves a weighted average of effect measures, dose-response
15 coefficients, or ED₀₁s.

16 Unlike a meta-analysis, a pooled analysis combines the original exposure and health
17 outcome data across multiple studies, enabling a fit of new models to the data which were not
18 used in the original publications. Whereas a pooled analysis of the four different cohorts
19 considered here would be useful to explore the functional form and fit of models (either
20 statistical or multistage) across all four cohorts, this would entail a lengthy undertaking and is not
21 being contemplated here, due in part to concerns about the confidence in the results of such an
22 undertaking.

24 **5.3.3.1. The Crump et al. (2003, [197384](#)) Meta-analysis**

25 Crump et al. (2003, [197384](#)) published a meta-analysis that incorporated data from the
26 three studies EPA used in the quantitative dose-response modeling presented in the 2003
27 Reassessment (U.S. EPA, 2003, [537122](#)). These three study populations were the NIOSH
28 (Steenland et al., 2001, [197433](#)), the Hamburg (Becher et al., 1998, [197173](#)), and the BASF (Ott
29 and Zober, 1996, [198408](#)) cohorts. The data for the NIOSH study included six additional years
30 of follow-up and improved TCDD exposure estimates that had not been applied to EPA's dose-
31 response modeling in the 2003 Reassessment. This study examined the relationship between

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1 TCDD exposure and all-cancer mortality. SMR statistics that had been used in all three studies
2 were applied.

3 The Crump et al. (2003, [197384](#)) analysis was based on published data, and therefore,
4 selection of the dose metric was limited to how aggregated data had been presented in the
5 publications. For the NIOSH component of the analysis, the exposure data were based on
6 worker-specific data and specific processes performed at each plant (Steenland et al., 2001,
7 [197433](#)). The previous approach assigned workers that had broad categories of exposure
8 duration with the same cumulative serum level, and did not take into account the particular plant
9 or the job assignment within the plant. The Crump et al. (2003, [197384](#)) approach did take into
10 account when exposure occurred in relation to the follow-up interval. The TCDD exposure
11 metric used was a cumulative serum lipid concentration (CSLC). For the Hamburg cohort,
12 Crump et al. (2003, [197384](#)) used an average value from the exposure ranges provided in Flesch-
13 Janys et al. (1998, [197339](#)). For the BASF cohort, arithmetic averages for the dose categories
14 were converted to TCDD CSLC intakes by dividing them by 0.25 (average body fat of 25%) and
15 a decay rate that corresponded to a half-life of 7 years.

16 The outcome variable for the dose-response modeling was all cancer mortality, and
17 CSLC was the independent variable. Crump et al. (2003, [197384](#)) performed a series of trend
18 tests to determine the lowest dose for which a statistically significant trend in SMR could be
19 shown and all other lower doses. These tests also examined the highest dose in which there was
20 no statistically significant trend using data from this dose and all other lower doses. Estimates of
21 ED₁₀, ED₀₅, and ED₀₁ for TEQ with respect to the lifetime probability of dying from cancer were
22 calculated. This calculation assumed a first-order elimination process with a half-life of
23 7.6 years, a 50% systemic uptake of ingested dioxin, that dioxin concentration in serum lipid is a
24 suitable measure for dioxin concentration in all lipid, and that all dioxin is sequestered in lipid
25 (which comprises 25% of body weight). Age-specific mortality rates in the presence of dioxin
26 exposure were then generated. Life-table methodology was used to calculate lifetime risks of
27 cancer mortality.

28 Based on the modeling results, the hypothesis of a baseline SMR of 1.0 was rejected, and
29 the linear model produced an SMR estimate of 1.17 (95% CI = 1.04–1.30) from these studies.
30 The dose-response curves for the three studies were not homogeneous. Namely, the points from
31 the BASF cohort fell below the predicted curve. Because the heterogeneity was not judged to be

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1 extreme by different statistical tests, however, the investigators used a common model in a
2 combined analysis of the data from the three studies. The linear model provided an adequate fit
3 of the data, and the slope associated with CSLC-ppt was 6.3×10^{-6} (95% CI = 8.8×10^{-7} to
4 1.3×10^{-5}). Based on goodness of fit analysis, the preferred estimate of ED₀₁ was 45 pg/kg-day,
5 which was six times higher than the estimate of 7.7 pg/kg-day derived by Steenland et al. (2001,
6 [198589](#)).

8 **5.3.3.2. EPA's Decision Not to Conduct a Meta-analysis**

9 From a statistical perspective, meta-analyses may not be very reliable when applied to a
10 small number of studies. Crump et al. (2003, [197384](#)) used only three studies. Had EPA
11 undertaken a meta-analysis for the studies that met its criteria, most of the weight would come
12 from the two large studies on the NIOSH and Hamburg cohorts. However, such an analysis
13 relies on an assumption of a normally distributed between-study effect. This normality
14 assumption cannot be assessed with only three observations, yet the meta-analysis estimate is
15 highly sensitive to this distributional assumption (Higgins et al., 2009, [594339](#)). Because of this
16 limitation and the imprecision of the between-study variance estimate, statisticians often
17 recommend forgoing meta-analysis in favor of discussing the individual studies when few
18 studies are available (Cox, 2006, [594342](#); Higgins et al., 2009, [594339](#)). Based on these
19 considerations, EPA decided not to undertake a meta-analysis in this document.

20 As noted previously, Crump et al. (2003, [197384](#)) has conducted a meta-analysis of the
21 three cohorts considered here, i.e., the NIOSH, Hamburg, and BASF cohorts. However, Crump
22 et al. modeled SMR data in which the cohorts were compared to the general population, rather
23 than on internal exposure-response analyses as relied upon in this document. Their analysis
24 included a total of 15 different SMRs from the three studies. A prior analysis of the dose-
25 responses by Becher et al. (1998, [197173](#)) was used (i.e., the categorical SMR analysis by
26 Flesch-Janys et al. (1998, [197339](#))). Additionally, a prior analysis of the NIOSH cohort
27 (Steenland et al., 1999, [197437](#)) in which SMRs were calculated was used. Crump et al. (2003,
28 [197384](#)) found that a linear dose-response gave a good fit to the data, and used that for deriving
29 an ED₀₁. However, they found that a supra-linear dose-response provided a better fit to the data,
30 but rejected the supra-linear model (a power model) because of an infinite slope at zero dose. In
31 the original publications by Becher et al. (1998, [197173](#)) and Steenland et al. (2001, [198589](#)),

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1 both observed a supra-linear dose-response trend. Crump et al. (2003, [197384](#)) concluded that
2 the ED₀₁ was 45 pg/kg-day, six times higher than the ED₀₁ of 7.7 pg/kg-day calculated by
3 Steenland et al. (2001, [198589](#)) using the same dietary units (pg/kg-day).

1 **Table 5-1. Cancer slope factors calculated from Becher et al. (1998, [197173](#)),**
 2 **Steenland et al. (2001, [197433](#)) and Ott and Zober (1996, [198408](#)) from 2003**
 3 **Reassessment Table 5-4**
 4

Study	ED ₀₁ (LED ₀₁) (ng/kg)	Cancer slope factor per ng/kg-day above background ^a (UCL)
Hamburg cohort Power model Becher et al. (1998, 197173)	6 (N.A.)	5.1 (N.A.)
Hamburg cohort Additive model Becher et al. (1998, 197173)	18.2 (N.A.)	1.6 (N.A.)
Hamburg cohort Multiplicative model Becher et al. (1998, 197173)	32.2 (N.A.)	0.89 (N.A.)
NIOSH cohort Piecewise linear model Steenland et al. (2001, 198589)	18.6 (11.5)	1.5 (2.5)
BASF cohort, from Ott and Zober (1996, 198408), multiplicative	50.9 (25.0)	0.57 (1.2)

5
 6 ^aAssumes 25% of body weight is lipid; in humans 80% of dioxin dose is absorbed from the normal
 7 diet; the TCDD half-life is 7.1 years in humans. Background all cancer mortality rate calculated
 8 through lifetable analysis to 75 years. Summary results are for male all cancer risk, because the
 9 male lifetime (to 75 years) all cancer risk is greater than for females, leading to correspondingly
 10 higher cancer slope factors. As detailed in Part III, Chapter 8, $RelRisk(ED_{01}) = 0.99 +$
 11 $0.01/Risk_{(0\ dose)}$. Based on the manner in which the dose-response data were calculated using Cox
 12 regression rate ratio analyses, risks are given as cancer slope factors for 1 pg/kg-day above
 13 background, assumed 5 ppt TCDD in lipid.
 14 UCL = upper confidence limit.
 15

16 Source: U.S. EPA (U.S. EPA, 2003, [537122](#); Part III, Chapter 5, Table 5-4)

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Table 5-2. Cox regression coefficients and incremental cancer-mortality risk for NIOSH cohort data

Model	Cox regression coefficient estimate (ppt-year)⁻¹	Incremental risk^a
Steenland et al.(2001, 197433) (unlagged exposures)		
Piecewise linear	1.5×10^{-5}	7.0×10^{-4}
Cheng et al. (Cheng et al., 2006, 523122) (exposures lagged 15 years)		
Linear, lower 95% of observations	3.3×10^{-6} ^b	1.2×10^{-4}
Linear, full data	1.7×10^{-8} ^c	6.3×10^{-7}

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^aCompared to internal reference population (lowest exposure group), with a cancer mortality rate of 0.214; assumes background exposure of 5 ppt per year serum-lipid TCDD concentration.

^b $p \leq 0.05$.

^c $p \leq 0.05$.

^dNot statistically significant ($p > 0.05$).

Source: Cheng et al. (2006, [523122](#); Table IV).

1 **Table 5-3. Comparison of fat concentrations, risk specific dose estimates and**
 2 **associated oral slope factors based on upper 95th percentile estimate of**
 3 **regression coefficient^a of all fatal cancers reported by Cheng et al. (2006,**
 4 **[523122](#)) for selected risk levels**
 5

Risk level (RL)	AUC _{RL} (ppt-yr)	FAT _{RL} (ng/kg)	Risk specific dose ^b (D _{RL}) (ng/kg-day)	Equivalent oral slope factors (OSF _{RL}) per (mg/kg-day)
1×10^{-2}	1.262×10^4	1.803×10^2	8.79×10^{-2}	1.1×10^5
5×10^{-3}	6.432×10^3	9.189×10^1	3.14×10^{-2}	1.6×10^5
1×10^{-3}	1.307×10^3	1.867×10^1	2.88×10^{-3}	3.5×10^5
5×10^{-4}	6.546×10^2	9.352×10^0	9.56×10^{-4}	5.2×10^5
1×10^{-4}	1.311×10^2	1.873×10^0	1.29×10^{-4}	7.8×10^5
5×10^{-5}	6.558×10^1	9.368×10^{-1}	5.52×10^{-5}	9.1×10^5
1×10^{-5}	1.312×10^1	1.874×10^{-1}	8.94×10^{-6}	1.1×10^6
5×10^{-6}	6.559×10^0	9.370×10^{-2}	4.25×10^{-6}	1.2×10^6
1×10^{-6}	1.312×10^0	1.874×10^{-2}	8.08×10^{-7}	1.2×10^6
5×10^{-7}	6.559×10^{-1}	9.370×10^{-3}	4.00×10^{-7}	1.3×10^6
1×10^{-7}	1.312×10^{-1}	1.874×10^{-3}	7.92×10^{-8}	1.3×10^6

6
 7 ^a Based on regression coefficient of Cheng et al. (2006, [523122](#), Table III), excluding observations in the upper 5%
 8 range of the exposures; where reported $\beta = 3.3 \times 10^{-6}$ ppt-years and standard error = 1.4×10^{-6} . Upper 95th
 9 percentile estimate of regression coefficient (β_{95}) calculated to be $6.04 \times 10^{-6} = (3.3 \times 10^{-6}) + 1.96 \times (1.4 \times 10^{-6})$;
 10 background cancer mortality risk is assumed to be 0.112 as reported by Cheng et al. (2006, [523122](#)).

11 ^bTo calculate the extra cancer risk (ER) and OSF for any TCDD daily oral intake (D):

- 12 5. For D in ng/kg-d, look up the corresponding fat concentration (ng/kg = ppt) from the conversion chart
- 13 (nongestational lifetime dose metrics) in Appendix C.4.1.
- 14 6. Calculate the AUC in ppt-yrs by multiplying the fat concentration by 70 years.
- 15 7. Calculate Extra Risk (ER) using the following equation:
- 16 ER = $[\exp(\text{AUC} \times 6.04\text{E-}6) \times 0.112 - 0.112] \div 0.888$.
- 17 8. Calculate the OSF $(\text{mg/kg-d})^{-1} = 1\text{E}6 \times (\text{ER} \div \text{D})$.

18 Example for risk at the RfD: D = 7×10^{-4} ng/kg-d; fat concentration = 6.93 ng/kg;

19 AUC = 70 years \times 6.93 ppt = 485 ppt-year;

20 ER = $\exp(485 \text{ ppt-year} \times 6.04\text{E-}6 (\text{ppt-yr})^{-1}) \times 0.112 - 0.112) \div 0.888 = 3.7 \times 10^{-4}$

21 OSF = $1\text{E}6 \text{ ng/mg} \times (3.7 \times 10^{-4} \div 7 \times 10^{-4} \text{ ng/kg-d}) = 5.3 \times 10^5 (\text{mg/kg-d})^{-1}$.

1 **Table 5-4. Comparison of fat concentrations, risk specific dose estimates and**
 2 **associated central tendency slope estimates based on best estimate of**
 3 **regression coefficient^a of all fatal cancers reported by Cheng et al. (2006,**
 4 **[523122](#)) for selected risk levels**
 5

Risk level (RL)	AUC _{RL} , (ppt-yr)	FAT _{RL} (ng/kg)	Risk specific dose (D _{RL}) (ng/kg-day)	Central tendency slope estimates (mg/kg-day) ⁻¹
1×10^{-2}	2.312×10^4	3.303×10^2	2.21×10^{-1}	4.5×10^4
1×10^{-3}	2.393×10^3	3.419×10^1	6.97×10^{-3}	1.4×10^5
1×10^{-4}	2.402×10^2	3.431×10^0	2.74×10^{-4}	3.7×10^5
1×10^{-5}	2.403×10^1	3.432×10^{-1}	1.74×10^{-5}	5.7×10^5
1×10^{-6}	2.403×10^0	3.432×10^{-2}	1.50×10^{-6}	6.7×10^5
1×10^{-7}	2.403×10^{-1}	3.432×10^{-3}	1.46×10^{-7}	7.0×10^5

6
 7 ^aBased on regression coefficient of Cheng et al (2006, [523122](#); Table III) excluding observations in the upper 5%
 8 range ($\geq 252,950$ ppt-year lipid adjusted serum TCDD) of the exposures; where reported $\beta = 3.3 \times 10^{-6}$ ppt-years;
 9 background cancer mortality risk is assumed to be 0.112 as reported by Cheng et al. (2006, [523122](#)).

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 12 **Table 5-5. Kociba et al. (1978, [001818](#)) male rat tumor incidence data^a and**
 13 **blood concentrations for dose-response modeling**
 14

	Vehicle control (ng/kg)	Low dose (ng/kg)	Medium dose (ng/kg)	High dose (ng/kg)
Morphology: topography	0	1.56	7.16	38.72
Stratified squamous cell carcinoma of hard palate or nasal turbinates	0/85	0/50	0/50	4/50 ^b
Stratified squamous cell carcinoma of tongue	0/85	1/50	1/50	3/50 ^b
Adenoma of adrenal cortex	0/85	0/50	2/50	5/50 ^b

15
 16 ^aSource: Kociba et al.(1978, [001818](#); Table 4).

17 ^bStatistically significant by Fischer Exact Test ($p < 0.05$).

1 **Table 5-6. Kociba et al. (1978, [001818](#)) female rat tumor incidence data^a and**
 2 **blood concentrations for dose-response modeling**
 3

	Vehicle control (ng/kg)	Low dose (ng/kg)	Medium dose (ng/kg)	High dose (ng/kg)
Morphology: topography	0	1.55	7.15	38.56
Hepatocellular adenoma(s) or carcinoma(s)	2/86	1/50	9/50 ^a	18/45 ^b
Stratified squamous cell carcinoma of hard palate or nasal turbinates	0/86	0/50	1/50	4/49 ^b
Keratinizing squamous cell carcinoma of lung	0/86	0/50	0/50	7/49 ^b

4
 5 ^aSource: Kociba et al. (1978, [001818](#); Table 5). Incidence for Hepatocellular adenomas or carcinomas is from
 6 Goodman and Sauer (Goodman and Sauer, 1992, [197667](#); Table 1); EPA calculated statistical significance as the
 7 study authors did not provide this.

8 ^bStatistically significant by Fischer Exact Test ($p < 0.05$).
 9

10
 11 **Table 5-7. NTP (1982, [594255](#)) female rat tumor incidence data^a and blood**
 12 **concentrations for dose-response modeling**
 13

	Vehicle control (ng/kg)	Low dose (ng/kg)	Medium dose (ng/kg)	High dose (ng/kg)
Morphology: topography	0	1.96	5.69	29.75
Subcutaneous tissue: fibrosarcoma	0/75	2/50	3/50	4/49 ^b
Liver: neoplastic nodule or hepatocellular carcinoma	5/75 ^c	1/49	3/50	14/49 ^b
Adrenal: cortical adenoma, or carcinoma or adenoma, NOS	11/73 ^c	9/49	5/49	14/46 ^b
Thyroid: follicular-cell adenoma	3/73 ^c	2/45	1/49	6/47

14
 15 ^aSource: NTP (1982, [594255](#); Table 10).

16 ^bStatistically significant by Fischer Exact Test ($p < 0.05$).

17 ^cStatistically significant trend by Chochran-Armitage test ($p < 0.05$).

1 **Table 5-8. NTP (1982, [594255](#)) male rat tumor incidence data^a and blood**
 2 **concentrations for dose-response modeling**
 3

	Vehicle control (ng/kg)	Low dose (ng/kg)	Medium dose (ng/kg)	High dose (ng/kg)
Morphology: topography	0	1.96	5.70	29.87
Liver: neoplastic nodule or hepatocellular carcinoma	0/74 ^b	0/50	0/50	3/50
Thyroid: follicular-cell adenoma or carcinoma	1/69 ^b	5/48 ^c	8/50 ^c	11/50 ^c
Adrenal cortex: adenoma	6/72	9/50	12/49 ^b	9/49

4
 5 ^aSource: NTP(1982, [594255](#); Table 9).

6 ^bStatistically significant trend by Chochran-Armitage test ($p < 0.05$).

7 ^cStatistically significant by Fischer Exact Test ($p < 0.05$).

8
 9
 10 **Table 5-9. NTP (1982, [594255](#)) female mouse tumor incidence data^a and**
 11 **blood concentrations for dose-response modeling**
 12

	Vehicle control (ng/kg)	Low dose (ng/kg)	Medium dose (ng/kg)	High dose (ng/kg)
Morphology: topography	0	1.95	5.84	32.06
Subcutaneous tissue: fibrosarcoma	1/74 ^b	1/50	1/48	5/47 ^c
Hematopoietic system: lymphoma or leukemia	18/74 ^b	12/50	13/48	20/47 ^c
Liver: hepatocellular adenoma or carcinoma	3/73 ^b	6/50	6/48	11/47 ^c
Thyroid: follicular-cell adenoma	0/69 ^b	3/50	1/47	5/46 ^c

13
 14 ^aSource: NTP (1982, [594255](#); Table 15).

15 ^bStatistically significant trend by Chochran-Armitage test ($p < 0.05$).

16 ^cStatistically significant by Fischer Exact Test ($p < 0.05$).

1 **Table 5-10. NTP (1982, [594255](#)) male mouse tumor incidence data^a and**
 2 **blood concentrations for dose-response modeling**
 3

	Vehicle control (ng/kg)	Low dose (ng/kg)	Medium dose (ng/kg)	High dose (ng/kg)
Morphology: topography	0	0.77	2.27	11.24
Lung: alveolar/bronchiolar adenoma or carcinoma	10/71 ^b	2/48	4/48	13/50
Liver: hepatocellular adenoma or carcinoma	15/73 ^b	12/49	13/49	27/50 ^c

4 ^aSource: NTP (1982, [594255](#); Table 14).

5 ^bStatistically significant trend by Cochran-Armitage test ($p < 0.05$).

6 ^cStatistically significant by Fischer Exact Test ($p < 0.05$).

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10 **Table 5-11. NTP (2006, [197605](#)) female rat tumor incidence data^a and blood**
 11 **concentrations for dose-response modeling^b**
 12

System: morphology: topography	Vehicle control (ng/kg)	Low dose (ng/kg)	Low-med dose (ng/kg)	Median dose (ng/kg)	Med-high dose (ng/kg)	High dose (ng/kg)
	0	2.56	5.69	9.79	16.57	29.70
Liver: cholangiocarcinoma	0/49 ^c	0/48	0/46	1/50	4/49	25/53 ^c
Liver: hepatocellular adenoma	0/49 ^c	0/48	0/46	0/50	1/49	13/53 ^c
Oral mucosa: squamous cell carcinoma	1/49 ^c	2/48	1/46	0/50	4/49	10/53 ^c
Pancreas: adenoma or carcinoma	0/48 ^c	0/48	0/46	0/50	0/48	3/51
Lung: cystic keratinizing epithelioma	0/49 ^c	0/48	0/46	0/49	0/49	9/52 ^c

13 ^aSource: NTP (2006, [197605](#); Table A3a).

14 ^bIncidence adjusted for animals <365 days on study.

15 ^cStatistically significant by Poly-3 Test ($p < 0.05$).

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1 **Table 5-12. Toth et al. (1979, [197109](#)) male mouse tumor incidence data^a and**
 2 **blood concentrations for dose-response modeling**
 3

	Vehicle control (ng/kg)	Low dose (ng/kg)	Medium dose (ng/kg)	High dose (ng/kg)
Morphology: topography	0	0.57	14.21	91.21
Liver tumors	7/38	13/44	21/44 ^b	13/43

4
 5 ^aSource: Toth et al. (1979, [197109](#); Table 1).

6 ^bStatistically significant by Chi² Test ($p < 0.01$).

7
 8
 9 **Table 5-13. Della Porta et al. (1987, [197405](#)) male mouse tumor incidence**
 10 **data^a and blood concentrations for dose-response modeling**
 11

	Vehicle control (ng/kg)	Low dose (ng/kg)	High dose (ng/kg)
Morphology: topography	0	38.00	67.77
Hepatocellular carcinoma	5/43	15/51 ^b	33/50 ^b

12
 13 ^aSource: Della Porta et al. (1987, [197405](#); Table 4).

14 ^bStatistically significant by Chi² Test ($p < 0.05$).

15
 16
 17 **Table 5-14. Della Porta et al. (1987, [197405](#)) female mouse tumor incidence**
 18 **data^a and blood concentrations for dose-response modeling**
 19

	Vehicle control (ng/kg)	Low dose (ng/kg)	High dose (ng/kg)
Morphology: topography	0	37.59	66.97
Hepatocellular adenoma	2/49	4/42 ^b	11/48 ^b
Hepatocellular carcinoma	1/49	12/42 ^b	9/48 ^b

20
 21 ^aSource: Della Porta et al. (1987, [197405](#); Table 4).

22 ^bStatistically significant by Chi² Test ($p < 0.05$).

Table 5-15. Comparison of multi-stage modeling results across cancer bioassays using blood concentrations

Study	Species	Sex	Morphology: topography	Multi-stage modeling: ^a stage, GoF <i>p</i> -value, LL difference	BMD ₀₁ (ng/kg)	BMDL ₀₁ (ng/kg)
Della Porta et al. (1987, 197405)	Mouse	Male	Hepatocellular carcinoma	2, <i>p</i> = 0.52	7.14	1.17
		Female	Hepatocellular adenoma	2, <i>p</i> = 0.86	14.49	2.34
			Hepatocellular carcinoma	1, <i>p</i> = 0.019	2.30	1.54
Kociba et al. (1978, 001818)	Rat	Male	Stratified squamous cell carcinoma of hard palate or nasal turbinates	1, <i>p</i> = 0.81	5.76	2.79
			Stratified squamous cell carcinoma of tongue	1, <i>p</i> = 0.47	6.09	2.60
			Adenoma of adrenal cortex	1, <i>p</i> = 0.78	3.25	1.85
			Combined tumors Bayesian analysis		1.57	0.96
		Female	Hepatocellular adenoma(s) or carcinoma(s)	1, <i>p</i> = 0.24	0.70	0.50
			Stratified squamous cell carcinoma of hard palate or nasal turbinates	1, <i>p</i> = 0.97	4.51	2.34
			Keratinizing squamous cell carcinoma of lung	1, <i>p</i> = 0.63	3.14	1.79
			Combined tumors Bayesian analysis		0.51	0.37
NTP (1982, 594255)	Rat	Female	Subcutaneous tissue: fibrosarcoma	1, <i>p</i> = 0.18	3.13	1.38
			Liver: neoplastic nodule or hepatocellular carcinoma	1, <i>p</i> = 0.22	1.17	0.74
			Adrenal: cortical adenoma, or carcinoma or adenoma, NOS	1, <i>p</i> = 0.34	1.61	0.81
			Thyroid: follicular-cell adenoma	1, <i>p</i> = 0.57	3.38	1.55
			Combined tumors Bayesian analysis		0.46	0.31
		Male	Liver: neoplastic nodule or hepatocellular carcinoma	1, <i>p</i> = 0.85	6.14	2.70
			Thyroid: follicular-cell adenoma or carcinoma	1, <i>p</i> = 0.06	1.21	0.70
			Adrenal cortex: adenoma	1, <i>p</i> = 0.06	3.98	1.22
Combined tumors Bayesian analysis		0.74	0.44			

Table 5-15. Comparison of multi-stage modeling results across cancer bioassays using blood concentrations (continued)

Study	Species	Sex	Morphology: topography	Multi-stage modeling: ^a stage, GoF <i>p</i> -value, LL difference	BMD ₀₁ (ng/kg)	BMDL ₀₁ (ng/kg)
NTP (1982, 594255) cont.	Mouse	Female	Subcutaneous tissue: fibrosarcoma	1, <i>p</i> = 0.93	3.40	1.69
			Hematopoietic system: lymphoma or leukemia	1, <i>p</i> = 0.98	1.14	0.61
			Liver: hepatocellular adenoma or carcinoma	1, <i>p</i> = 0.34	1.49	0.83
			Thyroid: follicular-cell adenoma	1, <i>p</i> = 0.09, no improvement with higher orders	3.05	1.44
			Combined tumors Bayesian analysis		0.44	0.29
		Male	Lung: alveolar/bronchiolar adenoma or carcinoma	1, <i>p</i> = 0.09	2.53	0.41
			Liver: hepatocellular adenoma or carcinoma	1, <i>p</i> = 0.93	0.21	0.14
			Combined tumors Bayesian analysis		0.16	0.11
NTP (2006, 197605)	Rat	Female	Liver: cholangiocarcinoma	3, <i>p</i> = 0.99, dLL = 2.93	7.57	4.13
			Liver: hepatocellular adenoma	3, <i>p</i> = 0.93, dLL = 2.10	10.22	6.53
			Oral mucosa: squamous cell carcinoma	1, <i>p</i> = 0.27	2.20	1.39
			Pancreas: adenoma or carcinoma	1, <i>p</i> = 0.64	10.52	4.63
			Lung: cystic keratinizing epithelioma	2, <i>p</i> = 0.51, dLL = 3.55	8.30	5.24
			Combined tumors Bayesian analysis		1.18	0.78
Toth et al. (1979, 197109)	Mouse	Male	Liver: tumors	1, <i>p</i> = 0.29	0.37	0.21

^aAnalysis uses a chi-square goodness of fit statistic for differences in the log likelihoods (*p* > 0.05).

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Table 5-16. Individual tumor points of departure and slope factors using blood concentrations

Study	Tumor Site (Sex/Species)	BMDL _{HED} (ng/kg-day)	OSF (per mg/kg-day)
NTP (1982, 594255)	Liver: adenoma or carcinoma (male mice)	1.7E-03	5.8E+6
Toth et al. (1979, 197109)	Liver tumors (male mice)	1.9E-03	5.2E+6
NTP, (1982, 594255) ^a	Lung: adenoma or carcinoma (male mice)	8.7E-03	1.1E+6
Kociba et al. (1978, 001818)	Liver: adenoma or carcinoma (female rats)	1.2E-02	8.6E+5
NTP (1982, 594255)	Hematopoietic: lymphoma or leukemia (female mice)	1.6E-02	6.4E+5
NTP (1982, 594255) ^a	Thyroid: follicular cell adenoma (male rats)	1.9E-02	5.2E+5
NTP (1982, 594255)	Liver: neoplastic nodule or hepatocellular carcinoma (female rats)	2.1E-02	4.8E+5
NTP (1982, 594255)	Adrenal: cortical adenoma or carcinoma or adenoma, NOS (female rats)	2.4E-02	4.1E+5
NTP (1982, 594255)	Liver: adenoma or carcinoma (female mice)	2.5E-02	4.0E+5
Della Porta et al. (1987, 197405)	Hepatocellular carcinoma (male mice)	3.1E-02	3.2E+5
NTP (1982, 594255) ^a	Adrenal cortex: adenoma (male rats)	4.5E-02	2.2E+5
Della Porta et al. (1987, 197405) ^a	Hepatocellular carcinoma (female mice)	4.9E-02	2.0E+5
NTP (1982, 594255)	Subcutaneous fibrosarcoma (female rats)	5.4E-02	1.8E+5
NTP (2006, 197605)	Oral mucosa: squamous cell carcinoma (female rats)	5.5E-02	1.8E+5
NTP (1982, 594255) ^a	Thyroid: adenoma (female mice)	5.7E-02	1.7E+5
NTP (1982, 594255)	Thyroid: follicular cell adenoma (female rats)	6.5E-02	1.5E+5
NTP (1982, 594255)	Subcutaneous fibrosarcoma (female mice)	7.4E-02	1.4E+5
Kociba et al. (1978, 001818)	Lung: carcinoma (female rats)	8.0E-02	1.2E+5
Kociba et al. (1978, 001818)	Adenoma of adrenal cortex (male rats)	8.5E-02	1.2E+5
Della Porta et al. (1987, 197405)	Hepatocellular adenoma (female mice)	9.4E-02	1.1E+5
Kociba et al. (1978, 001818)	Nasal/Palate: carcinoma (female rats)	1.2E-01	8.2E+4
Kociba et al. (1978, 001818)	Tongue: carcinoma (male rats)	1.4E-01	7.0E+4
NTP (1982, 594255)	Liver: neoplastic nodule or hepatocellular carcinoma (male rats)	1.5E-01	6.6E+4
Kociba et al. (1978, 001818)	Nasal/Palate: carcinoma (male rats)	1.6E-01	6.3E+4
NTP (2006, 197605)	Liver: cholangiocarcinoma (female rats)	2.9E-01	3.5E+4
NTP (2006, 197605)	Pancreas: adenoma or carcinoma (female rats)	3.4E-01	2.9E+4
NTP (2006, 197605)	Lung: cystic keratinizing epithelioma (female rats)	4.1E-01	2.4E+4
NTP (2006, 197605)	Liver: hepatocellular adenoma (female rats)	5.6E-01	1.8E+4

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Table 5-17. Multiple tumor points of departure and slope factors using blood concentrations

Study	Sex/species: tumor sites	BMDL_{HED} (ng/kg-day)	OSF (per mg/kg-day)
NTP (1982, 594255)	Male mice: liver adenoma and carcinoma, lung	1.1E-03	9.4E+6
NTP (1982, 594255)	Female mice: liver adenoma and carcinoma, thyroid adenoma, subcutaneous fibrosarcoma, all lymphomas	5.3E-03	1.9E+6
NTP (1982, 594255)	Female rats: liver neoplasitic nodules, liver adenoma and carcinoma, thyroid follicular cell adenoma, adrenal cortex adenoma or carcinoma	5.7E-03	1.8E+6
Kociba et al. (1978, 001818)	Female rats: liver adenoma carcinoma, oral cavity, lung	7.3E-03	1.4E+6
NTP (1982, 594255)	Male rats: thyroid follicular cell adenoma, adrenal cortex adenoma	9.6E-03	1.0E+6
NTP (2006, 197605)	Female rats: liver cholangiocarcinoma, hepatocellular adenoma, oral mucosa squamous cell carcinoma, lung cystic keratinizing epithelioma, pancreas adenoma, carcinoma	2.3E-02	4.4E+5
Kociba et al. (1978, 001818)	Male rats: adrenal cortex adenoma, tongue carcinoma, nasal/palate carcinoma	3.1E-02	3.2E+5

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Table 5-18. Comparison of cancer BMDs, BMDLs, and slope factors for combined or selected individual tumors for 1, 5, and 10% extra risk

Study	Species	Sex	BMD ₀₁ (ng/kg)	BMDL ₀₁ (ng/kg)	SF ₀₁ (ng/kg) ⁻¹	BMD ₀₅ (ng/kg)	BMDL ₀₅ (ng/kg)	SF ₀₅ (ng/kg) ⁻¹	BMD ₁₀ (ng/kg)	BMDL ₁₀ (ng/kg)	SF ₁₀ (ng/kg) ⁻¹
Kociba (1978, 001818) ^a	Rat	Female	4.9E-01	3.8E-01	2.7E-02	2.5E+00	1.9E+00	2.7E-02	4.9E+00	3.8E+00	2.7E-02
		Male	1.5E+00	9.6E-01	1.0E-02	7.2E+00	4.8E+00	1.0E-02	1.5E+01	9.6E+00	1.0E-02
NTP (1982, 594255) ^a	Rat	Female	4.4E-01	3.2E-01	3.2E-02	2.2E+00	1.6E+00	3.2E-02	4.4E+00	3.2E+00	3.2E-02
		Male	6.9E-01	4.5E-01	2.2E-02	3.5E+00	2.2E+00	2.2E-02	6.9E+00	4.5E+00	2.2E-02
	Mouse	Female	4.3E-01	3.0E-01	3.4E-02	2.1E+00	1.5E+00	3.4E-02	4.3E+00	3.0E+00	3.4E-02
		Male	1.5E-01	1.1E-01	9.4E-02	7.7E-01	5.4E-01	9.4E-02	1.5E+00	1.1E+00	9.4E-02
NTP (2006, 197605) ^a	Rat	Female	1.1E+00	7.8E-01	1.3E-02	4.8E+00	3.6E+00	1.4E-02	8.2E+00	6.6E+00	1.5E-02
Della Porta et al. (1987, 197405) ^b	Mouse	Male	7.1E+00	1.2E+00	8.5E-03	1.4E+01	5.0E+00	1.0E-02	2.0E+01	9.7E+00	1.0E-02
		Female	2.3E+00	1.5E+00	6.5E-03	1.0E+01	6.8E+00	7.3E-03	2.1E+01	1.4E+01	7.1E-03
Toth et al., (1979 197109) ^c	Mouse	Male	3.7E-01	2.1E-01	4.8E-02	1.9E+00	1.1E+00	4.7E-02	3.9E+00	2.2E+00	4.6E-02

^aCombined tumors, Bayesian analysis.

^bHepatocellular carcinomas for both males and females.

^cHepatocellular carcinomas.

TCDD blood concentrations from Emond rodent PBPK models.

SF = BMR ÷ BMDL_{BMR}, where BMR = 0.01, 0.05, or 0.10.

Table 5-19. TCDD human-equivalent dose (HED) BMDs, BMDLs, and oral slope factors (OSF) for 1, 5, and 10% extra risk

Study	Species	Sex	BMD ₀₁ (ng/kg-d)	BMDL ₀₁ (ng/kg-d)	OSF ₀₁ (ng/kg-d) ⁻¹	BMD ₀₅ (ng/kg-d)	BMDL ₀₅ (ng/kg-d)	OSF ₀₅ (ng/kg-d) ⁻¹	BMD ₁₀ (ng/kg-d)	BMDL ₁₀ (ng/kg-d)	OSF ₁₀ (ng/kg-d) ⁻¹
Kociba (1978, 001818) ^a	Rat	Female	1.1E-02	7.4E-03	1.4E+00	1.3E-01	8.6E-02	5.8E-01	3.8E-01	2.59E-01	4.0E-01
		Male	5.9E-02	3.1E-02	3.3E-01	6.6E-01	3.6E-01	1.4E-01	1.8E+00	9.7E-01	1.0E-01
NTP (1982, 594255) ^a	Rat	Female	9.7E-03	5.8E-03	1.7E+00	1.1E-01	6.6E-02	7.6E-01	3.2E-01	1.9E-01	5.2E-01
		Male	1.9E-02	9.7E-03	1.0E+00	2.2E-01	1.1E-01	4.5E-01	6.2E-01	3.3E-01	3.1E-01
	Mouse	Female	9.1E-03	5.4E-03	1.9E+00	1.1E-01	6.0E-02	8.3E-01	3.0E-01	1.8E-01	5.7E-01
		Male	1.9E-03	1.2E-03	8.3E+00	2.2E-02	1.3E-02	3.8E+00	6.4E-02	3.8E-02	2.7E+00
NTP (2006, 197605) ^a	Rat	Female	4.1E-02	2.3E-02	4.4E-01	3.6E-01	2.4E-01	2.1E-01	7.9E-01	5.7E-01	1.8E-01
Della Porta et al. (1987, 197405) ^b	Mouse	Male	5.2E-01	3.1E-02	3.2E-01	1.7E+00	3.8E-01	1.3E-01	2.8E+00	1.0E+00	1.0E-01
		Female	9.2E-02	4.9E-02	2.0E-01	1.1E+00	6.0E-01	8.3E-02	2.9E+00	1.7E+00	5.9E-02
Toth et al. (1979, 197109) ^c	Mouse	Male	5.1E-03	1.9E-03	5.3 E+00	6.7E-02	2.7E-02	1.9E+00	2.0E-01	8.5E-02	1.2 E+00

^aCombined tumors, Bayesian analysis.

^bHepatocellular carcinomas for both males and females.

^cHepatocellular carcinomas.

HEDs from Emond human PBPK model corresponding to blood concentration BMDs and BMDLs in Table F3-1.

OSF = BMR ÷ BMDL_{BMR}, where BMR = 0.01, 0.05, or 0.10.

Table 5-20. Illustrative RfDs based on tumorigenesis in experimental animals

Study	Species, strain (sex)	Protocol	Endpoint	BMDL _{HED} ^a (ng/kg-day)	RfD ^b (mg/kg-day)
NTP (1982, 594255)	Mouse, B6C3F1, male	2-year gavage; n = 50	Liver adenoma and carcinoma, lung	1.1E-3	3.6E-11
Toth et al. (1979, 197109)	Mouse, Swiss/H/Riop, male	1-year gavage (1-year average); n = 38-44	Liver tumors	1.9E-3	6.3E-11
NTP (1982, 594255)	Mouse, B6C3F1, female	2-year gavage; n = 50	Liver adenoma and carcinoma, thyroid adenoma, subcutaneous fibrosarcoma, all lymphomas	5.3E-3	1.7E-10
NTP (1982, 594255)	Rat, Osborne-Mendel, female	2-year gavage; n = 50	Liver neoplastic nodules, thyroid follicular cell adenoma, liver adenoma and carcinoma, adrenal cortex adenoma or carcinoma	5.7E-3	1.9E-10
Kociba et al. (1978, 001818)	Rat, S-D, female	2-year dietary; n = 50	Liver adenoma carcinoma, oral cavity, lung	7.3E-3	2.4E-10
NTP (1982, 594255)	Rat, Osborne-Mendel, male	2-year gavage; n = 50	Thyroid follicular cell adenoma, adrenal cortex adenoma	9.6E-3	3.2E-10
Della Porta et al. (1987, 197405)	Mouse, B6C3F1, male	1-year gavage; n = 40-50	Hepatocellular carcinoma	3.1E-02	1.0E-9
NTP (2006, 197605)	Rat, S-D, female	2-year gavage; n = 53	Liver cholangiocarcinoma, hepatocellular adenoma, oral mucosa squamous cell carcinoma, lung cystic keratinizing epithelioma, pancreas adenoma, carcinoma	3.1E-2	1.0E-9
Kociba et al. (1978, 001818)	Rat, S-D, male	2-year dietary; n = 50	Adrenal cortex adenoma, tongue carcinoma, nasal/palate carcinoma	3.1E-2	1.0E-9

^aBMR = 0.01.

^bUF = 30; UF_A = 3, UF_H = 10.

Table 5-21. Illustrative RfDs based on hypothesized key events in TCDD's MOAs for liver and lung tumors

Key event	Endpoint and exposure duration	NO(A)EL _{HED} (ng/kg-day)	LO(A)EL _{HED} (ng/kg-day)	BMDL _{HED} ^a (ng/kg-day)	RfD ^b (mg/kg-day)	Study
Liver tumors						
Changes in gene expression	CYP1A1 mRNA, 1 day	1.8E-05	3.4E-04	2.3E-03 ^c (Appendix H)	6E-13 ^{d,e}	Vanden Heuvel et al. (1994, 594318)
Changes in gene expression	Benzo(a)pyrene hydroxylase (BPH) activity (CYP1A1), 1 day	9.2E-04	6.0E-03	4.6E-04 ^{c,d} (Appendix H)	2E-11 ^{d,e}	Kitchin and Woods (1979, 198750)
	EROD (CYP1A1), 53 weeks	none	1.4E-01	9.5E-03 ^c (Appendix H)	3E-10 ^e	NTP (2006, 197605)
Oxidative stress	DNA single-strand breaks, 90 days	none	3.3E-02	2.2E-02 ^c (Appendix H)	7E-10 ^e	Hassoun et al. (2000, 197431)
	TBARS, 90 days	–	–	4.4E-02 (Appendix H)	2E-09 ^e	Hassoun et al. (2000, 197431)
	Cytochrome C reductase, 90 days	–	–	8.8E-02 (Appendix H)	3E-09 ^e	Hassoun et al. (2000, 197431)
Hepatotoxicity	Toxic hepatopathy, 2 years	none	1.4E-01	1.8E-01 ^c (Appendix E)	5E-09 ^f	NTP (2006, 197605)
	Hepatocyte hypertrophy, 31 weeks	9.3E-02	3.3E-01	8.8E-03 (Appendix E)	3E-10 ^e	NTP (2006, 197605)
Hepatocellular proliferation	Labeling index, 31 weeks	none	1.4E-01	6.6E-02 ^c (Appendix H)	2E-09 ^e	NTP (2006, 197605)
Lung tumors						
Metabolic enzyme induction	EROD (CYP1A1), 53 weeks	none	1.4E-01	2.9E-04 ^c (Appendix H)	1E-11 ^e	NTP (2006, 197605)
Retinoid homeostasis	Hepatic retinol and retinyl palmitate, 90 days	none	1.1E+00	1.7E-01 ^c (Appendix E)	6E-09 ^e	Van Birgelen et al. (1995, 198052)

^aBMR for continuous endpoints—1 standard deviation; for quantal endpoints—10%.

^bBolded NOAEL, LOAEL, or BMDL is selected POD; poorly-fitting BMDLs above the LOAEL not used.

^cPoor BMD model fit or no good model fit.

^dCould be higher depending on the effect of background exposure (see Section 5.3.2.1).

^eUF = 30; UF_A = 3; UF_H = 10.

^fUF = 300; UFA = 3; UFH = 10; UFL = 10.

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Table 5-22. Comparison of principal epidemiological studies

Strengths	Weaknesses	Study
<p>Cumulative TCDD levels in the serum were estimated on an individual-level basis for the 3,538 workers.</p> <p>Evaluated effect of lag periods (0 and 15 years).</p> <p>Measured and back-extrapolated TCDD concentrations to refine and quantify job exposure matrices, which were then used to estimate dioxin cumulative dose for each member of their entire cohort.</p> <p>Internal cohort comparisons (Cox regression model).</p> <p>Background exposure estimated.</p>	<ul style="list-style-type: none"> • Exposure to other chlorinated hydrocarbons (dioxin like compounds). • Extrapolation of dose from a small subset (roughly 5%, $n = 170$) of the cohort. • Serum fat or body fat levels of TCDD were back-calculated using a simple first-order model. Half-life of TCDD is variable but simulated as a constant. Changes in the lipid fraction of body weight or presence/absence of genetic differences in humans that alter the distribution and metabolism of TCDD were not considered. • Serum lipid levels of TCDD in 1988 were measured only at one of the eight plants in the study. No follow-up measures. The estimates of dose are based on blood samples taken decades after exposure. 	<p>NIOSH cohort Steenland et al. (2001, 197433)</p>
<p>Cumulative TCDD levels in the serum were estimated on an individual-level basis for the 3,538 workers.</p> <p>TCCD dose estimates were simulated with a kinetic model that included considerations of exposure intensity and age-dependent body weight and fat levels.</p> <p>Evaluated effect of lag periods (0 and 15 years).</p> <p>Background exposure estimated.</p> <p>Stratified risk estimates for smoking and nonsmoking.</p> <p>Race and age adjustments.</p> <p>Internal cohort noted an inverse-dose response for high-exposure groups and thus excluded the data resulting in stronger associations.</p>	<ul style="list-style-type: none"> • Extrapolation of dose from a small subset (roughly 5%, $n = 170$) of the cohort. • The authors reported the CADM model provided an improved fit over the one-compartmental model, but no evidence was reported regarding any formal test of statistical significance. • Serum lipid levels of TCDD in 1988 were measured only at one of the eight plants in the study. No follow-up measures. The estimates of dose are based on blood samples taken decades after exposure. • Exposure to other chlorinated hydrocarbons (dioxin like compounds). • No consideration for recent exposures to TCDD, changes in the lipid fraction of body weight or presence/absence of genetic differences in humans that alter the distribution and metabolism of TCDD could cause misclassification. 	<p>NIOSH cohort Cheng et al. (2006, 523122)</p>

Table 5-22 Comparison of principal epidemiological studies (continued)

Strengths	Weaknesses	Study
<ul style="list-style-type: none"> • Repeated TCDD measures in serum in 48 individuals. Used to estimate half-life for study cohort. Took into account the age and body fat percentage of the workers. Measured and back-extrapolated TCDD concentrations to quantify exposures for the remaining cohort members using 5 different working areas of the plant. • Evaluated effect of lag periods up to 20 years. • Multiple statistical models used to evaluate fatal cancer slope estimates. • Background exposure estimated. 	<ul style="list-style-type: none"> • Exposure to other chlorinated hydrocarbons (dioxin like compounds), HCH, and lindane. • Extrapolation of dose from a small subset (roughly 4%, $n = 1,189$) of the cohort. • Serum fat or body fat levels of TCDD were back-calculated using a simple first-order model. Presence/absence of genetic differences in humans that alter the distribution and metabolism of TCDD were not considered. • Serum lipid levels of TCDD for only 275 workers. 	Becher et al. (1998, 197173); Hamburg Cohort
<ul style="list-style-type: none"> • Both internal and external analyses. • Adjustment for age, BMI, and smoking. • Both cancer incidence and cancer mortality data available, although results somewhat discordant, with steeper dose-response seen for cancer mortality. 	<ul style="list-style-type: none"> • Acute dose due to accident may not be comparable to chronic dose accumulated over a long time, as in most environmental exposures. • Relatively small number of cancer deaths compared to NIOSH and Hamburg cohorts ($n = 31$). • Serum TCDD levels measured 30 years after accident, requiring extrapolation back in time to estimate cumulative dose over time. • Serum TCDD levels measured only on a sample of the cohort (138 out of 243), requiring assumptions about similarities in exposure scenario for other workers to estimate their exposure 	Ott and Zober (1996, 198408)

Table 5-22 Comparison of principal epidemiological studies (continued)

Strengths	Weaknesses	Study
<ul style="list-style-type: none"> • TCDD levels measured in all 891 members of this female cohort. • Most TCDD measurements based on observed levels in stored serum at the time of the accident in 1976, no extrapolation needed to estimate past levels. • Internal analyses. • Evaluates female cancer incidence, other studies evaluate male cancer mortality. • Presumed adjustment for age and potential breast cancer confounders (15 of 21 cancers were breast cancer). 	<ul style="list-style-type: none"> • Acute dose due to accident may not be comparable to chronic dose accumulated over a long time, which is typical of most environmental exposures. • Did not evaluate different lag periods. • Not clear if any adjustment for confounders. • Small number of cancers ($n = 21$). • Doses known in 1976, require assumptions about excretion over time to estimate cumulative dose (9 year half life assumed), presumed metric of primary interest. No more recent TCDD concentration data used. • Reported \log_{10} transformation of the exposure estimates in their regression analysis. 	Warner et al. (2002, 197489)

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Table 5-23. Added background TEQ exposures to blood TCDD/TEQ concentrations in rats^a

Background TEQ added				
None	Est. TCDD only ^b	Est. TEQ ^c	2× Est. TEQ ^d	10× Est. TCDD ^e
0	0.064	0.19	0.38	0.64
2.56	2.62	2.75	2.94	3.20
5.69	5.75	5.88	6.07	6.33
9.79	9.85	9.98	10.1	10.5
16.6	16.7	16.8	17.0	17.2
29.7	29.8	29.9	30.1	30.3

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^aBackground exposures estimated from NTP (2006, [543749](#)); rat TCDD concentrations from NTP (2006, [197605](#)).

^bEstimated from TCDD fat concentration measurements in NTP (2006, [543749](#)).

^cEstimated from combined TCDD, PeCDF, and PCB-126 fat concentration measurements in NTP (2006, [543749](#)).

^dAssumes that measured congeners comprise 50% of actual TEQ exposure.

^eAssumes that TCDD comprises 10% of total background TEQ exposure.

1 **Table 5-24. Effect of added background TEQ exposure on BMDL₀₁ for**
 2 **cholangiocarcinomas in rats (NTP, 2006, [197605](#))**
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Background TEQ ^a	Added exposure (ng/kg blood TEQ)	BMDL ₀₁ ^b (ng kg blood)
None ^c	0	4.14
Est. TCDD only	0.064	4.19
Est. TEQ	0.19	4.30
2× Est. TEQ	0.38	4.45
10× Est. TCDD	0.64	4.65

4 ^aScenarios as in Table 5-20.

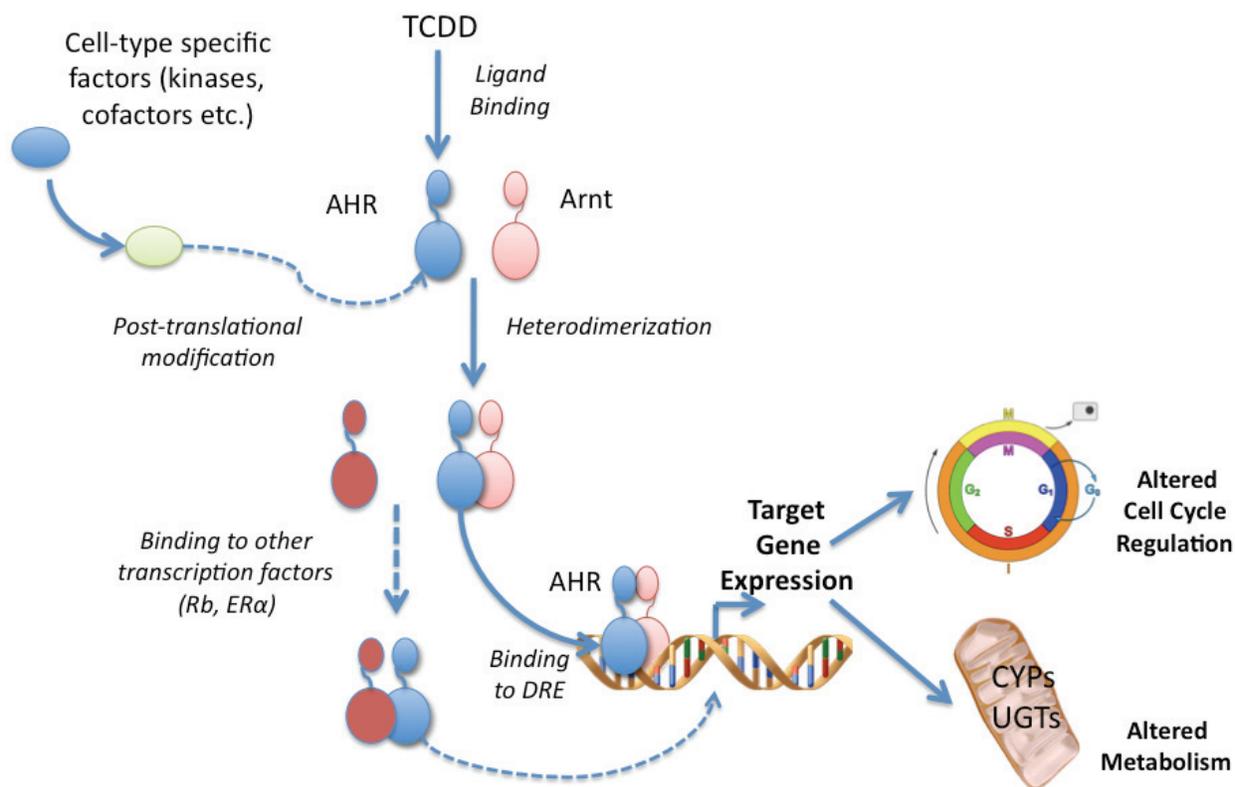
5 ^bMultistage model results from BMDS version 2.1.1 (see Appendix I for modeling details).

6 ^cSame result as for the single tumor modeling presented previously in this section.
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10 **Table 5-25. NIOSH cohort septile data with added TEQ background^a**
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Septile	TCDD serum level (ppt-yr)	TCDD + background TEQ (ppt-yr)	Relative increase (%)
1	260	2,960	1,040
2	402	3,102	770
3	853	3,553	320
4	1,895	4,595	140
5	4,420	7,120	60
6	12,125	14,825	20
7	59,838	62,538	5

12 ^aSeptile data from Steenland et al. (2001, [197433](#)); cumulative background TEQ estimate from Crump et al.
 13 (2003, [197384](#)); both based on estimates by WHO (1998).
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 2 **Figure 5-1. Mechanism of altered gene expression by AhR.** The regulation of
 3 gene expression by TCDD in mammalian cells requires binding of the xenobiotic
 4 to the aryl hydrocarbon receptor (AhR). The AhR is part of a multi-protein
 5 complex that includes heat shock proteins and various kinases and other post-
 6 translational modifying factors. Upon ligand binding, the AhR heterodimerizes
 7 with the aryl hydrocarbon receptor nuclear translocator (Arnt) and binds to dioxin
 8 response elements (DREs) found in target genes. Alternatives to DRE-dependent
 9 gene expression exist whereby the AhR complex associates with other
 10 transcription factors and results in a cross-talk between these systems. The
 11 culmination of regulation of AhR targets genes (both increases and decreases in
 12 transcription) results in an alteration in cellular phenotypes, including changes in
 13 intracellular metabolism and changes in cell cycle regulation.

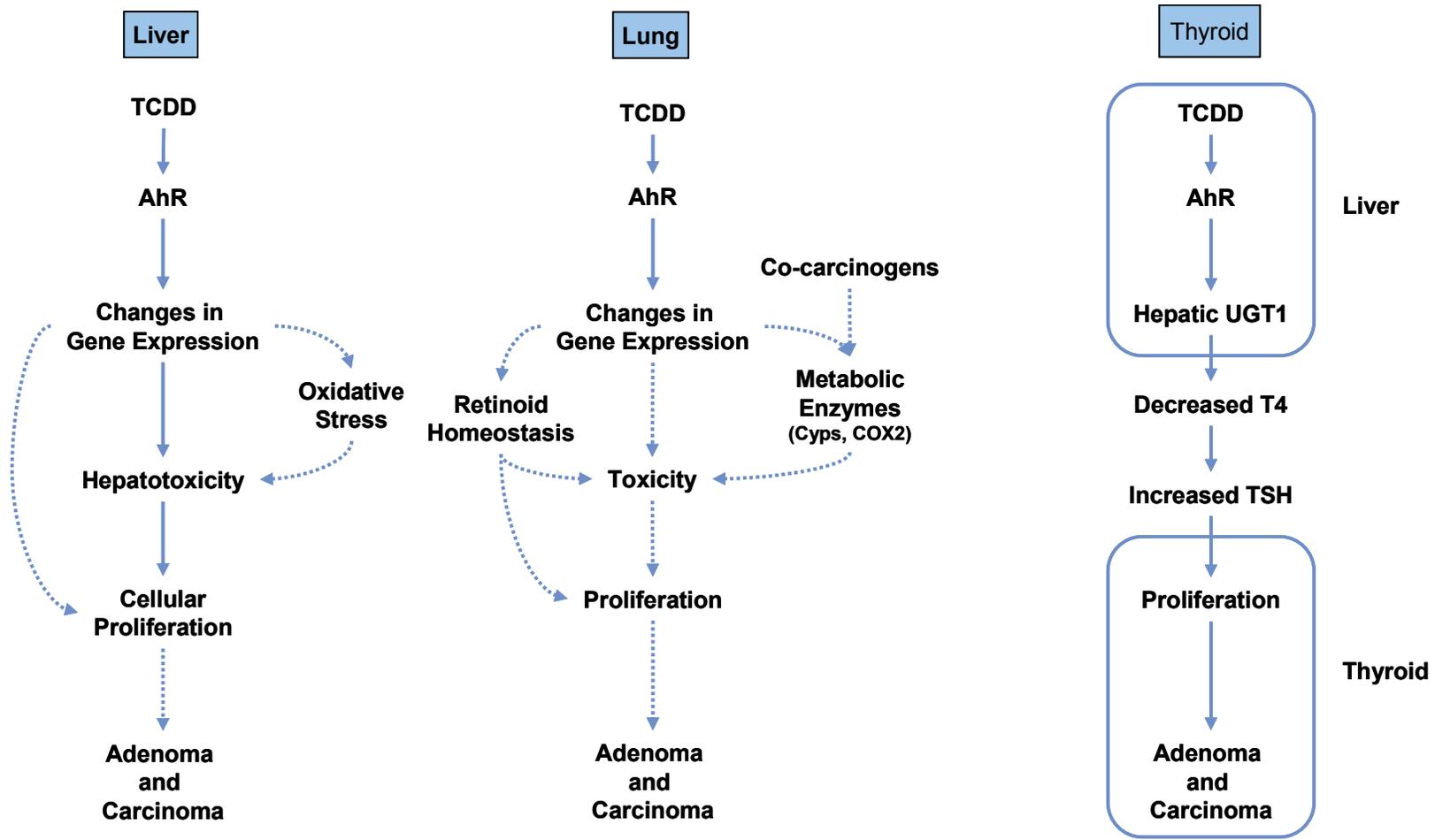


Figure 5-2. TCDD's hypothesized modes of action in site-specific carcinogenesis. See text for details. In each instance, the solid arrows depict pathways that are well-established and are associated with low uncertainty. The dashed arrows represent connections that are less established and are associated with higher uncertainty.

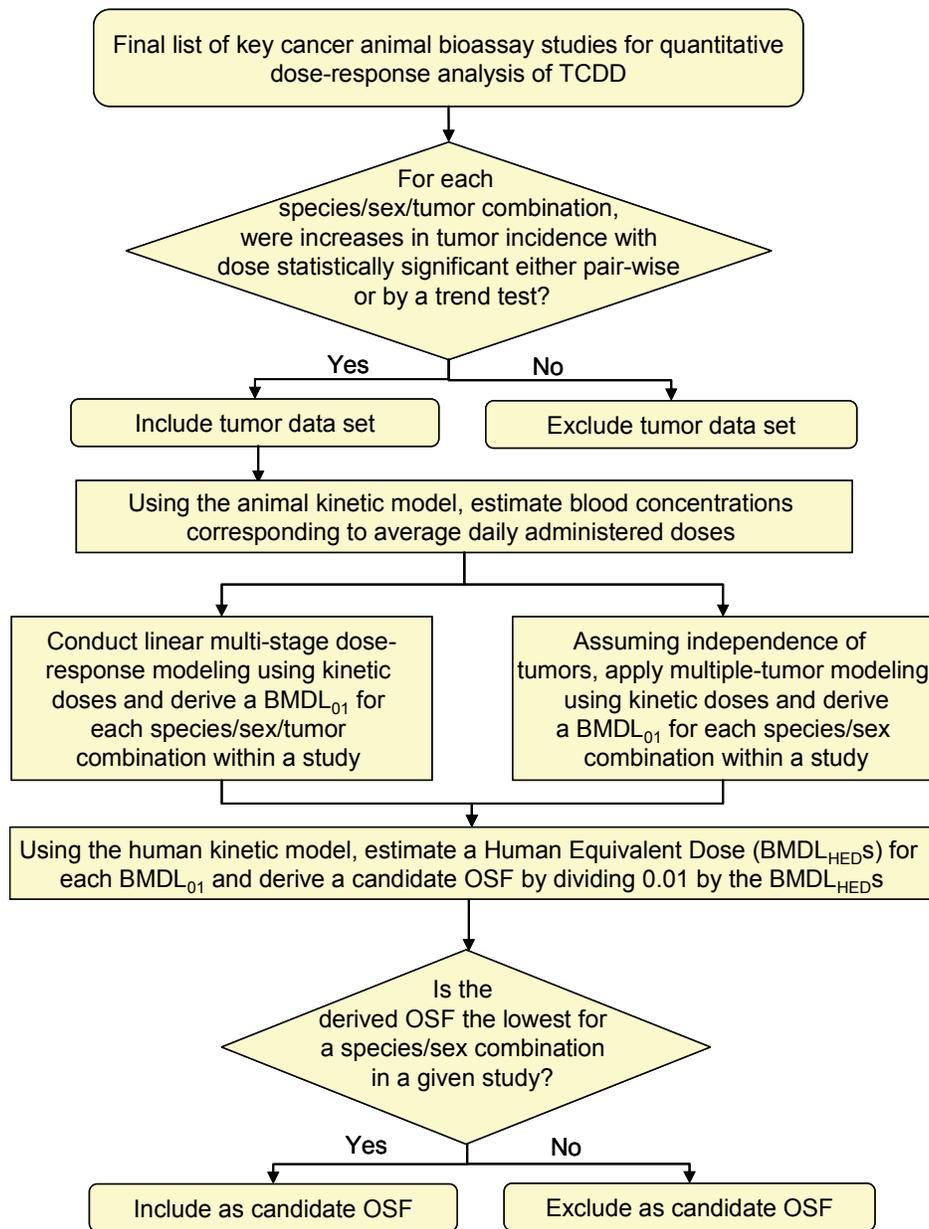
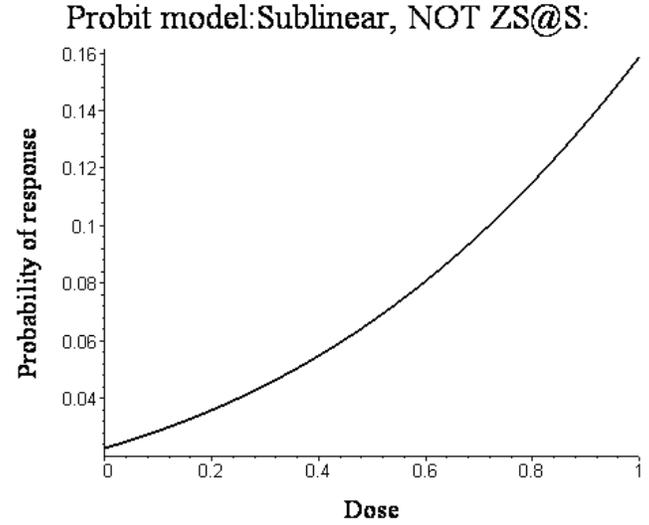
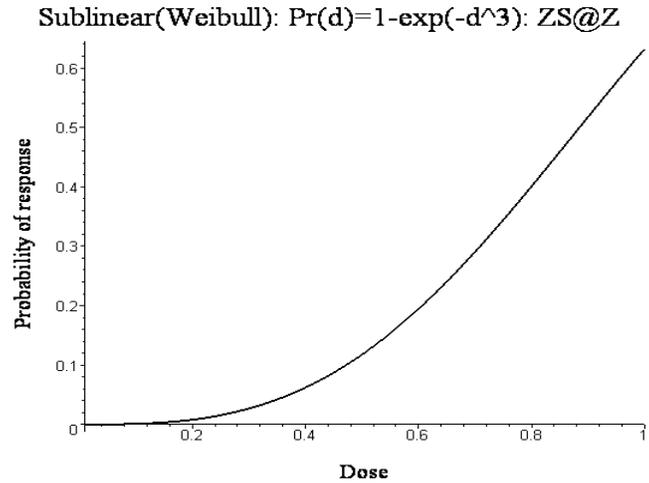
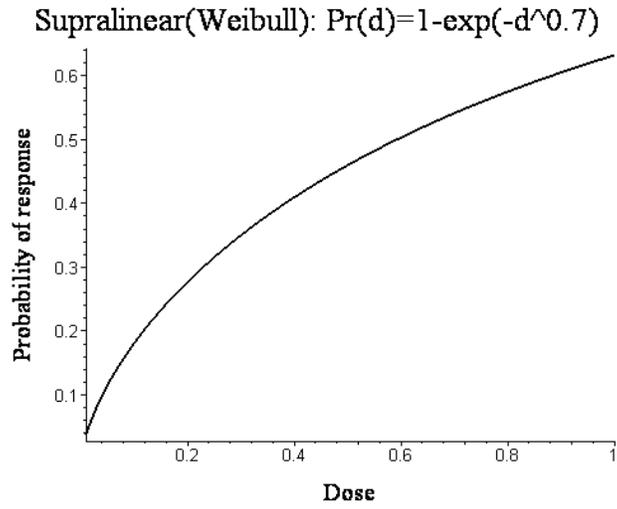


Figure 5-3. EPA’s process to select and identify candidate OSFs from key animal bioassays for use in the cancer risk assessment of TCDD.

For each cancer study that qualified for TCDD dose-response assessment using the study inclusion criteria, EPA first selected the species/sex/tumor combinations with statistically significant increases in tumor incidence by either a pair-wise test between the treated group and the controls or by a trend test showing increases in tumors with increases in dose. Next, EPA used an animal kinetic model to estimate blood concentrations corresponding to the study average daily administered doses for use in dose response modeling. BMDL₀₁’s were then estimated for the blood concentrations by, (1) using the linearized multistage model for each species/sex/tumor combination within each study, and (2) using the linearized multistage model within a Bayesian Markov Chain Monte Carlo framework that assumes independence of tumors and modeling all tumors together for each species/sex combination within each study. Using the human kinetic model, human equivalent doses (BMDL_{HEDS}) were then estimated for each of the BMDL₀₁s and oral slope factors were calculated by $OSF = 0.01/BMDL_{HED}$. The lowest OSF for a species/sex combination for either a single tumor type or all tumors combined was selected as a candidate OSF for TCDD risk assessment.

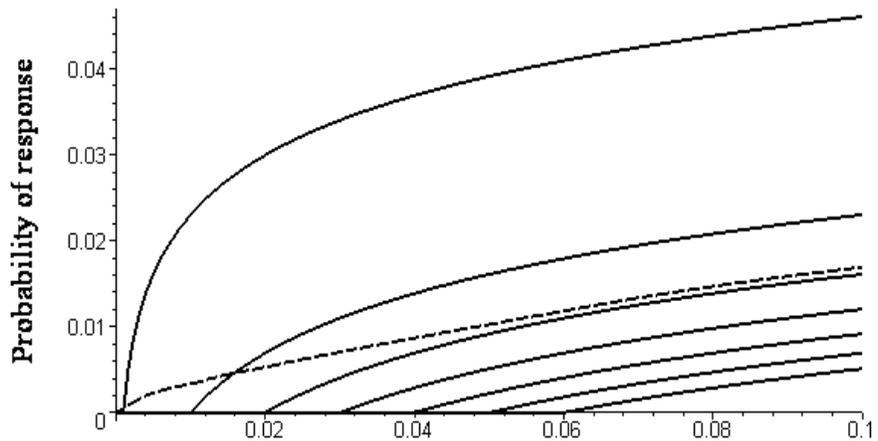
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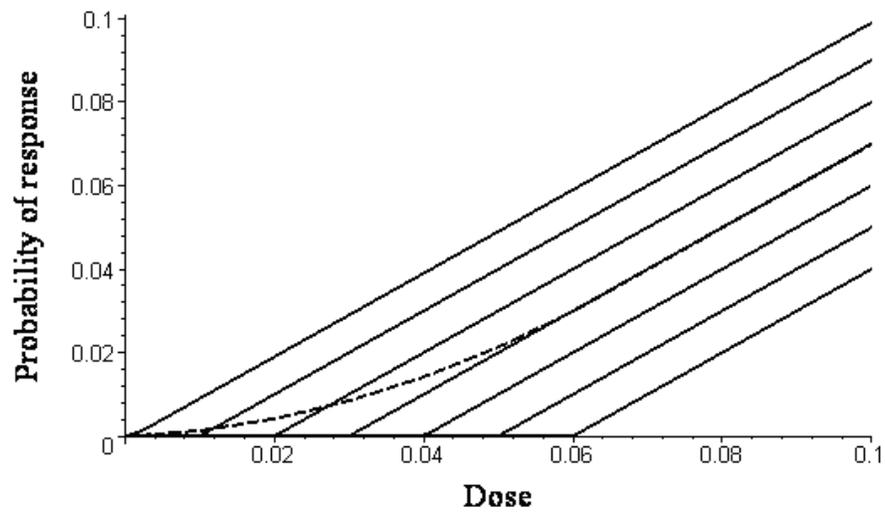
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Figure 5-4. Dose-response model shape

Individuals with supralinear above threshold, and population DR curve



Individuals with linear above threshold, and population DR curve

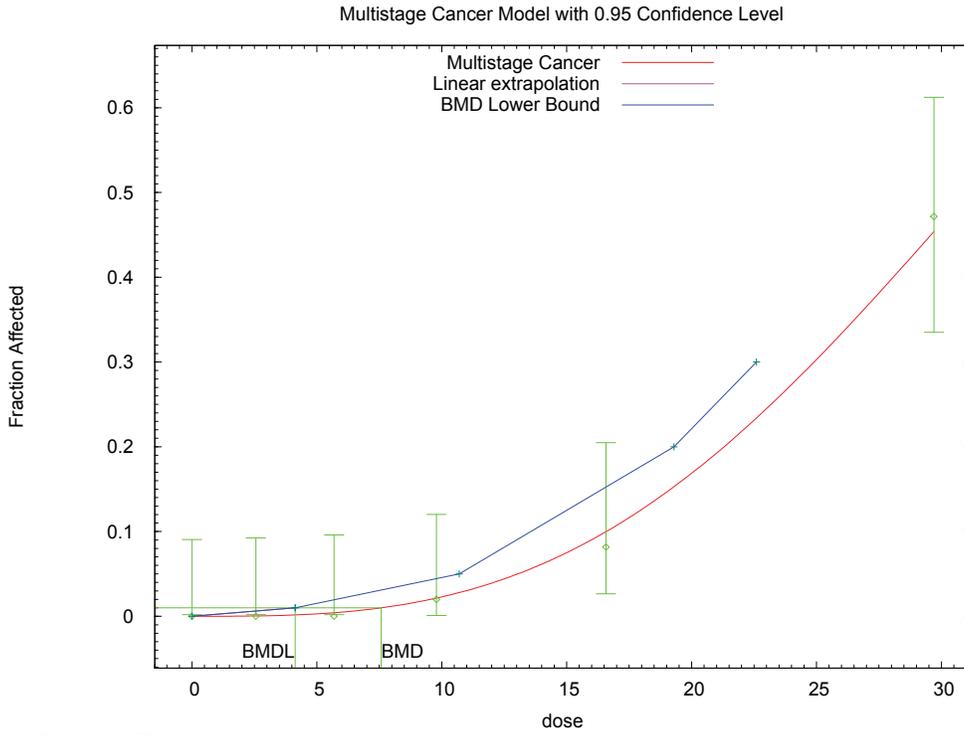


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Figure 5-5. Comparison of individual and population dose-response curves; a simple illustration.

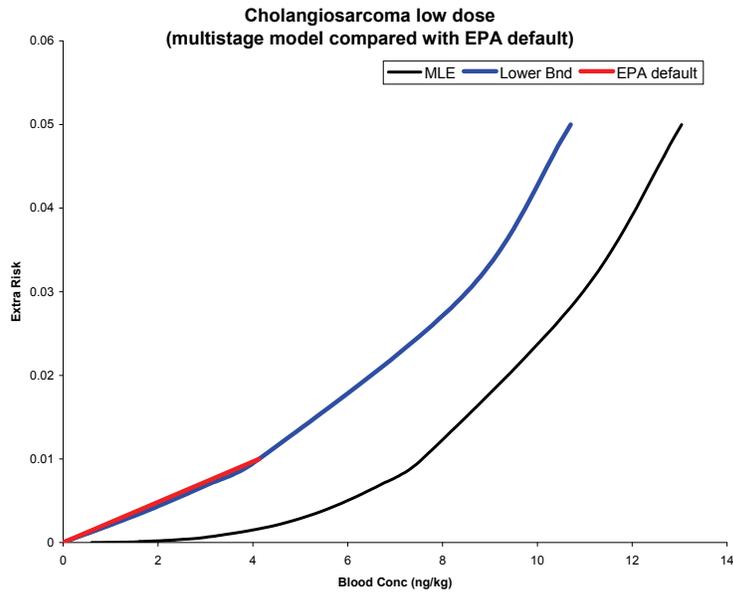
1 A. Full response range

2



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3 B. Low-dose region

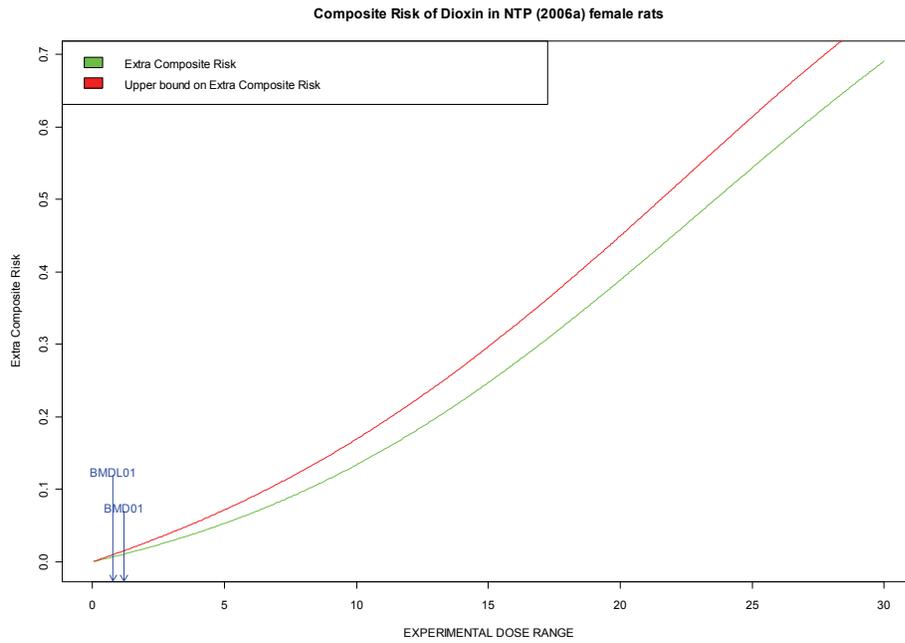


4 **Figure 5-6. Multistage benchmark dose modeling of NTP (2006, 197605)**
5 **cholangiosarcoma data.**

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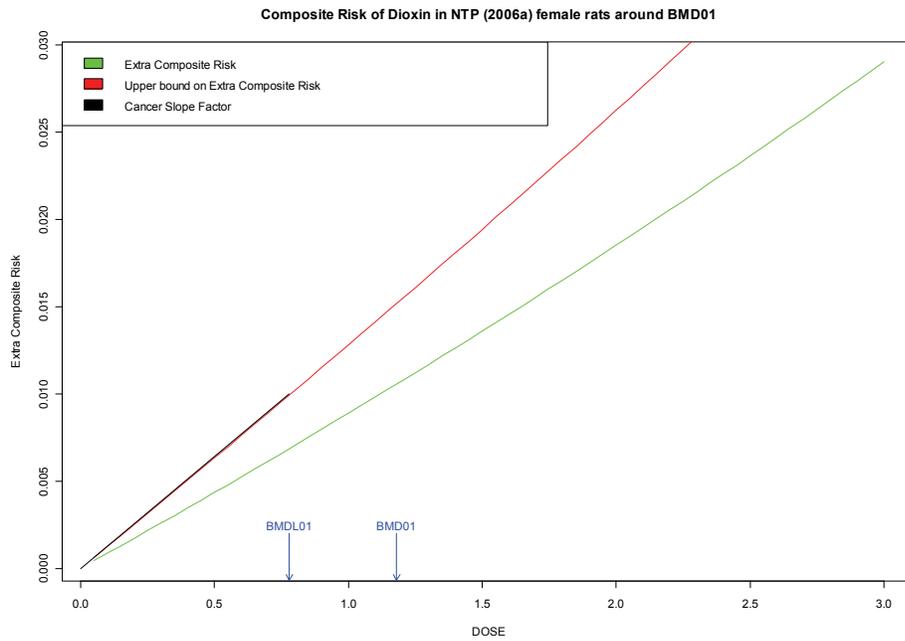
A. Full response range



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B. Low-dose region



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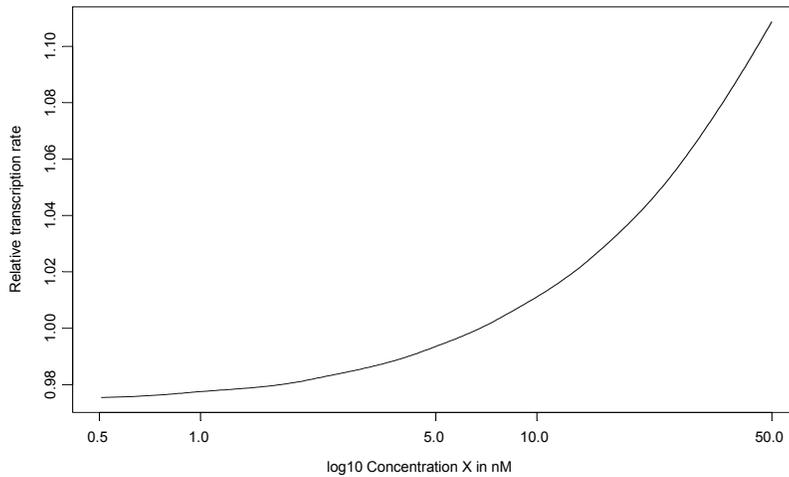
6

Figure 5-7. Multistage benchmark dose modeling of NTP (2006, [197605](#)) combined tumor data.

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1 A.

Kohn and Melnick (2002) Figure 5 on log Scale (KR.X=3300)

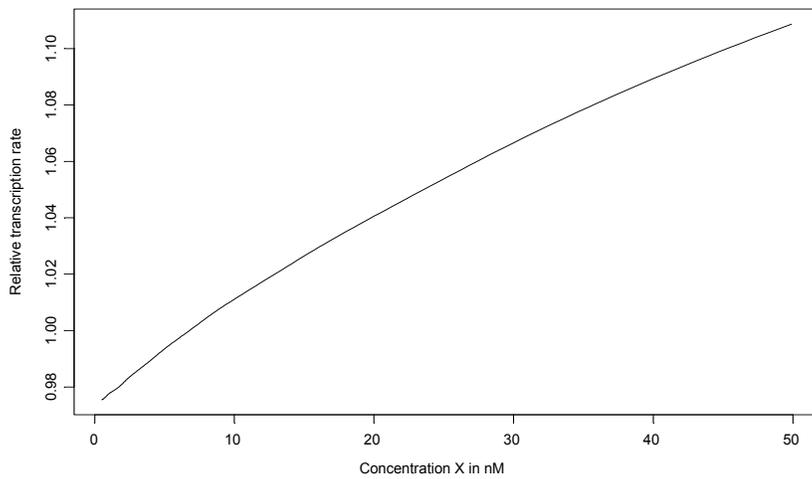


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12 B.

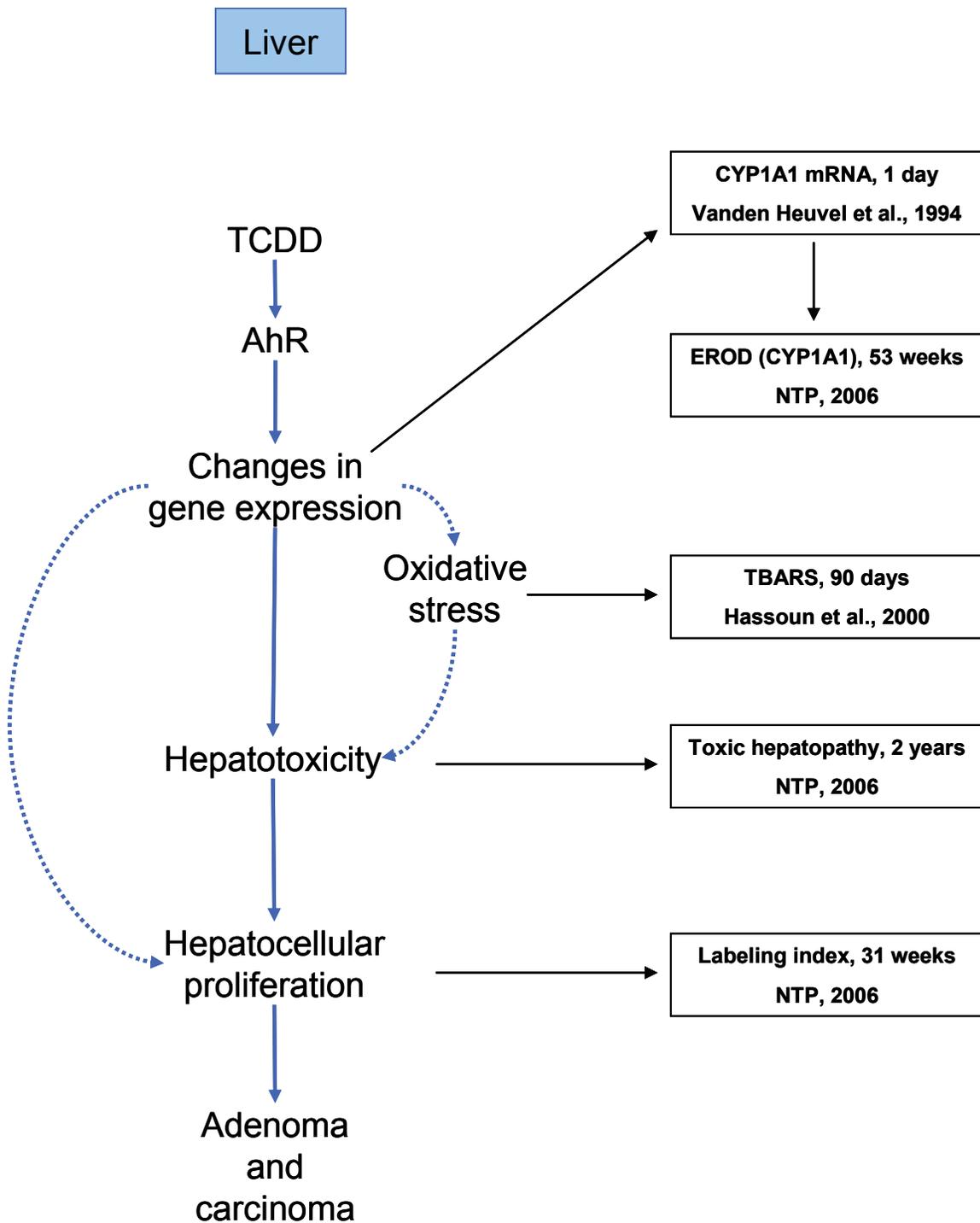
Kohn and Melnick (2002) Figure 5 on Arithmetic Scale (KR.X=3300)



21

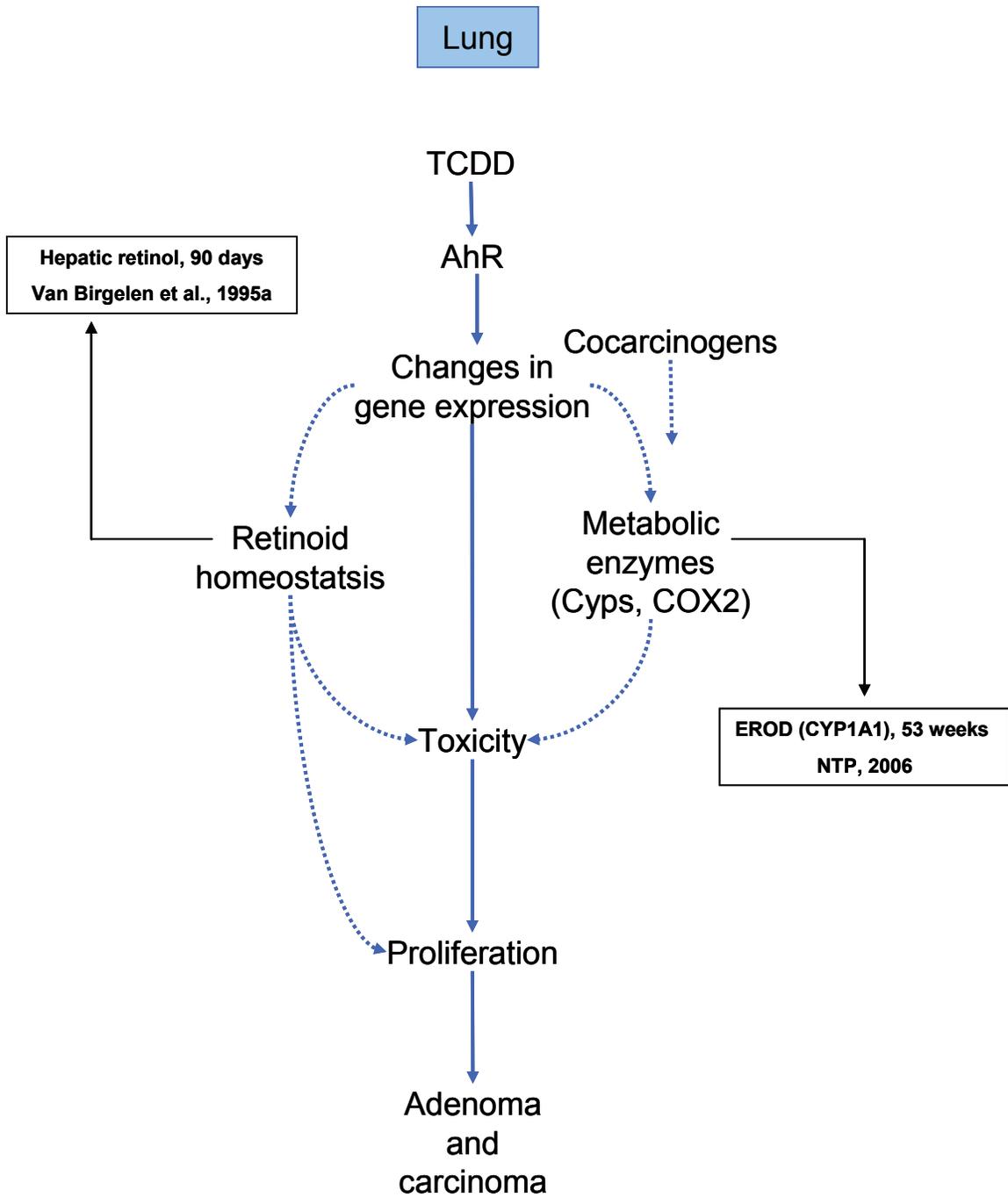
22

23 **Figure 5-8. Estrogen receptor-mediated response-modeling plot from Kohn**
24 **and Melnick (2002, [199104](#)): low-dose region shown.**



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Figure 5-9. Representative endpoints for each of the hypothesized key events following AhR activation for TCDD-induced liver tumors.



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Figure 5-10. Representative endpoints for two hypothesized key events following AhR activation for TCDD-induced lung tumors.

Cancer Slope Factors for 2,3,7,8-TCDD

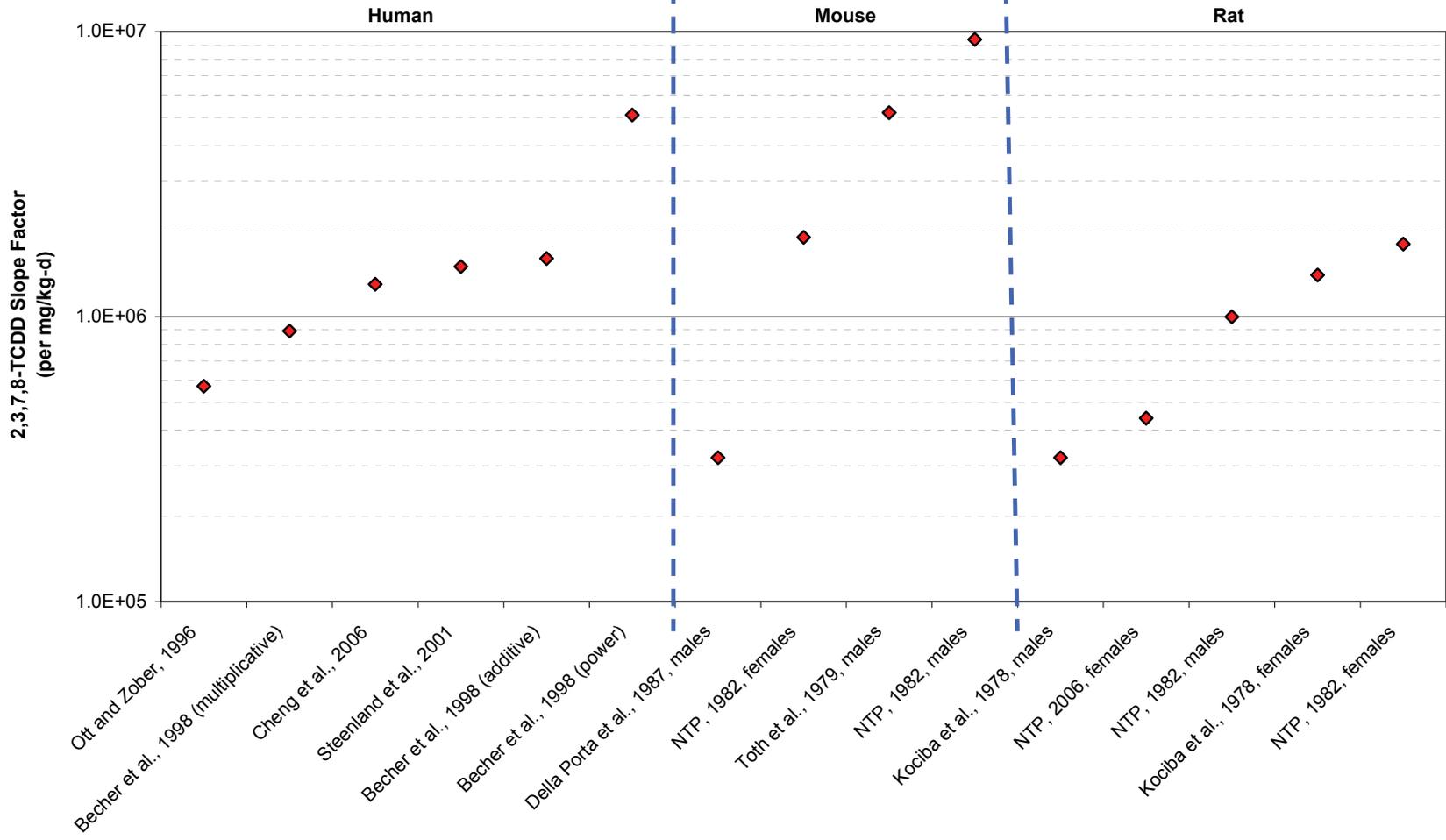


Figure 5-11. Candidate oral slope factor array.

1 **6. FEASIBILITY OF QUANTITATIVE UNCERTAINTY ANALYSIS**
2 **FROM NAS EVALUATION OF THE 2003 REASSESSMENT**

3
4
5 **6.1. INTRODUCTION**

6 This section focuses on the third area for improvement in the 2003 Reassessment that was
7 identified by the National Academy of Sciences (NAS) review committee (NAS, 2006, [198441](#)),
8 i.e., improving transparency, thoroughness, and clarity in *quantitative uncertainty analysis*.
9 Although the NAS committee summarized the shortfalls in the 2003 Reassessment categorically,
10 the elaborations within their report often contain the qualification “if possible” and do not take a
11 position with regard to the feasibility of many of its suggestions. With appreciation for the
12 extent of information available for dioxin, the goal of this section is to circumscribe the
13 feasibility of a data-driven quantitative uncertainty analysis for TCDD dose-response
14 assessment. Following brief highlights of the evolution of quantitative uncertainty analysis for
15 such applications, this section lays out definitions of key terms, reviews EPA’s position
16 regarding cancer and noncancer endpoints, summarizes the NAS critique, and evaluates the
17 feasibility of quantitative uncertainty analysis for TCDD within the framework of EPA’s
18 noncancer RfD and cancer slope factor dose-response methodologies.

19
20 **6.1.1. Historical Context for Quantitative Uncertainty Analysis**

21 The basic methods of probabilistic risk assessment (PRA) were developed in the
22 aerospace program in the 1960s, and they found their first full-scale application in the
23 U.S. Nuclear Regulatory Commission’s (U.S. NRC’s) *Reactor Safety Study of 1975*—including
24 accident consequence analysis and uncertainty analysis (U.S. NRC, 1975, [543729](#)). This study,
25 commonly referred to as the Rasmussen Report after its lead author, is considered to be the first
26 modern PRA. In the aftermath of the 1979 Three Mile Island accident, a new generation of
27 PRAs appeared in which some of the methodological problems of the 1975 study were avoided.
28 These advances were reflected in the Commission’s *Fault Tree Handbook* (U.S. NRC, 1981,
29 [543730](#)) and PRA guide ((U.S. NRC, 1983, [543732](#)), which shored up and standardized much of
30 the risk assessment methodology. An extensive chapter of the latter was devoted to uncertainty
31 and sensitivity analysis. These documents formed the basis for standards and guidelines

1 established by other agencies, including the U.S. Department of Energy (U.S. DOE, 1992,
2 [543733](#)) and National Aeronautics and Space Administration (NASA, 2002, [543734](#)).

3 In 1991, a set of U.S. NRC studies known as NUREG 1150 used structured expert
4 judgment to quantify uncertainty and set new standards for uncertainty analysis, in particular
5 with regard to expert elicitation (U.S. NRC, 1991, [543736](#)). This was followed by a joint
6 U.S.-European Union (EU) program for quantifying uncertainty in accident consequence models.
7 Expert judgment methods were further elaborated in those evaluations, as well as screening,
8 dependence modeling and sensitivity analysis (EC, 2009, [543738](#)). Studies building off of this
9 work have performed a large-scale uncertainty analysis of European consequence models and
10 provided extensive guidance on identifying important variables; selecting, interviewing and
11 combining experts; propagating uncertainty; inferring distributions on model parameters; and
12 communicating results, as documented by Goossens et al. (1996, [548727](#); 1997, [543752](#); 1998,
13 [548726](#); 2001, [548730](#); 2001, [548731](#); 2001, [548732](#); 2001, [548735](#); 2001, [548737](#); 2001,
14 [548738](#); 2001, [548734](#)) and others (Brown et al., 1997, [543739](#); Harper et al., 1995, [202317](#);
15 2002, [198124](#)).

16 The National Research Council (NRC) has been a persistent voice in urging the
17 government to enhance its risk assessment methodology beginning with its report on risk
18 assessment in the federal government (NRC, 1983, [194806](#)). The Council’s 1989 report,
19 *Improving Risk Communication*, inveighed against minimizing the existence of uncertainty and
20 noted the importance of considering the distribution of exposure and sensitivities in a population
21 (NRC, 1989, [000858](#)). The issue of uncertainty was a clear concern in subsequent reports,
22 including those assessing human exposure to airborne pollutants (NRC, 1991, [037823](#)). Building
23 on its evaluation of *Issues in Risk Assessment* (NRC, 1993, [078637](#)), the landmark study *Science*
24 *and Judgment in Risk Assessment* (NRC, 1994, [006424](#)) gathered many of these themes in a plea
25 for quantitative uncertainty analysis as “the only way to combat the false sense of certainty
26 which is *caused* by a refusal to acknowledge and (attempt to) quantify the uncertainty in risk
27 predictions.” A subsequent report, *Estimating the Public Health Benefits of Proposed Air*
28 *Pollution Regulations* (NRC, 2002, [035312](#)), identified three barriers to the broad acceptance of
29 recent EPA health benefit analyses: (1) the large amount of uncertainty inherent in these
30 analyses, (2) the manner in which EPA deals with this uncertainty, and (3) “... projected health
31 benefits are often reported as absolute numbers of avoided death or adverse health outcomes

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1 without a context of population size or total numbers of outcomes.” The Council encouraged
2 EPA to “explore alternative options for incorporating expert judgment into its probabilistic
3 uncertainty analyses.”

4 In an early 2009 report, *Science and Decisions: Advancing Risk Assessment*, the NRC
5 committee on improving risk analysis encouraged EPA to harmonize approaches for cancer and
6 noncancer dose-response assessment (NRC, 2009, [194810](#)), which involves uncertainty issues
7 discussed in this section. Even more recently, EPA released a draft white paper, *Using*
8 *Probabilistic Methods to Enhance the Role of Risk Analysis in Decision Making* (U.S. EPA,
9 2009, [522927](#)). Although not focused specifically on quantitative uncertainty analysis, there is
10 overlap with the issues treated here, and relevant insights are anticipated from ongoing efforts in
11 this area.

13 **6.1.2. Definition of Terms**

14 For purposes of this study, the following definitions are adopted:⁵²

15
16 *Uncertainty Characterization.* This consists of a *Structured Uncertainty Narrative* and, if
17 the uncertainty is supported by quantitative models, *Quantitative Uncertainty Analysis*.

18 *Structured Uncertainty Narrative.* This identifies the assumptions conditional on which
19 uncertainty is to be characterized and delineates the type of arguments with supporting
20 evidence that buttress these assumptions.

21 *Quantitative Uncertainty Analysis.* This is a quantification of the uncertainty attending
22 the use of quantitative models. It applies to a mathematical model of physical
23 phenomena, some of whose parameter values are not known with certainty. A joint
24 distribution is assigned to uncertain model parameters and propagated through the model
25 to yield a joint distribution over the model output. Thus, a quantitative uncertainty
26 analysis always has a joint distribution over model outputs as its result.

27 *Joint Distribution/Marginal Distribution.* For a set of uncertain quantities, a joint
28 distribution is an assignment of probabilities (or probability densities) for each possible
29 combination of values of these quantities. Each uncertain quantity has a marginal
30 distribution, that is, an assignment of probabilities (or probability densities) to each
31 possible value of that quantity. Assigning a marginal distribution to each quantity is not
32 equivalent to assigning a joint distribution to the set of quantities, unless the quantities
33 are independent; in this case the joint distribution is just the product of the margins.

⁵²Many of these definitions are standard terms in probability and statistics, as described in Saltelli et al. (2000, [543756](#)), Cox (2006, [594342](#)), Kurowicka and Cooke (2006, [543758](#)), and NRC (2007, [543748](#)); some are reflected in current Agency practice (U.S. EPA, 2009, [522927](#)).

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1 *Qualitative/Informal Uncertainty Analysis.* This assembles the arguments and evidence
2 and provides an assessment of their plausibility in terms of verbal modifiers. The
3 meaning of verbal modifiers such as “likely/unlikely” or “plausible/implausible” in the
4 natural language⁵³ is indeterminate and context dependent. The way in which these
5 qualifiers combine in the natural language requires critical attention from a quantitative
6 viewpoint. (For example, if A is likely and B is likely and C is likely, is A and B and C
7 likely?) It is sometimes claimed that the probability formalism does not capture the way
8 people reason with uncertainty, and many alternatives have been proposed.⁵⁴

9 This is not the place to discuss foundational issues, except to remark that the practitioner
10 wishing to depart from the standard probability formalism should carefully explore the
11 whole range of alternatives and critically examine the operational meaning of the
12 primitive notions.

13 *Sensitivity Analysis.* If a quantitative model uses “nominal values” (approximations of the
14 real values) for various input parameters, a sensitivity analysis is performed by choosing
15 different values for these parameters and re-running the model to assess the impact of
16 changes in these parameters on model output. Applicable methods include one- and
17 two-at-a-time methods, design of experiments and Morris’s method (Saltelli et al., 2000,
18 [543756](#)). They aim at estimating first- and perhaps higher-order effects with a minimal
19 number of model runs, by systematically varying the nominal values. In large
20 uncertainty analyses, sensitivity analysis is used to screen variables for in-depth
21 uncertainty quantification, and thus is part of a quantitative uncertainty analysis
22 (Kurowicka and Cooke, 2006, [543758](#)). As a note, the NAS committee report (NRC,
23 2006) does not distinguish between uncertainty and sensitivity analysis. In fields which
24 have not developed a tradition in uncertainty quantification, the spread of values
25 generated by a sensitivity analysis is sometimes presented as a representation of
26 uncertainty (Murphy et al., 2004, [543741](#)). The question of whether this is or is not the
27 case is moot so long as the uncertainty on model input parameters is not quantified.
28 Systematically varying input values is not the same as sampling input parameter values
29 from their uncertainty distributions. In any event, a systematic approach to parameter
30 variation is essential; simply choosing a few values of interest and generating different
31 output is of limited scientific benefit and inevitably raises questions of selection bias.
32 That said, if alternative values are commonly used and therefore recommend themselves,
33 then running these through the models can help sensitize users to parameter variations
34 and their impacts on model outputs.

⁵³*Natural language* denotes any discourse in which the meaning of the words is not formalized; rather, these words are just “as they come in off the street” with whatever meaning a participant may give them.

⁵⁴Before the advent of personal computers, various shorthand techniques were developed for computing system risk. In control theory, schemes of ‘interval probabilities’ were proposed which could be propagated through a system to yield bounds on system reliability. Whereas these bounds originally reflected accuracy of shorthand approximations of complex formulae, their offspring have been proposed as quantifications of uncertainty. Alternative notions of uncertainty are also proposed with the goal of simplifying the assessment and computational burden or capturing putative features of uncertainty which are overlooked in probability theory. These include possibility theory, fuzzy numbers, qualitative algebra, imprecise probabilities, belief functions, certainty factors, and the like. Nonmonotonic reasoning systems attempt to capture reasoning about knowledge, or reasoning from partial knowledge; they include default logic, defeasible logic, abductive logic, and autoepistemic logic, to name a few.

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1 *Cognitive Uncertainty.* This concerns uncertainty regarding what is the case. Not
2 knowing “what is the case” may be conceived as uncertainty over the set of all
3 possibilities, sometimes expressed as ‘uncertainty over the set of possible worlds.’
4 Uncertainty over possible worlds may be represented formally as probability; that is, the
5 uncertainty of a given situation is represented as a number between zero and one, and the
6 uncertainty of either of two mutually exclusive situations is the sum of the uncertainties
7 of each situation.⁵⁵ Two interpretations or operationalizations of the probability
8 formalism are current: the objective or frequentist interpretation and the subjective or
9 Bayesian interpretation. These interpretations are not mutually exclusive, as subjective
10 probabilities can and often do track relative frequencies.

11 *Volitional Uncertainty.* This concerns uncertainty regarding what to do. In the natural
12 language, being unsure which course of action to choose is also called “uncertainty.”
13 Insofar as uncertainty on the best course of action can be translated into a claim about the
14 state of the world, volitional uncertainty can be translated into cognitive uncertainty. For
15 example, a regulatory body charged with setting a speed limit is obliged to make a
16 decision. The decision may be cautious or reckless, well or poorly motivated, wise or
17 foolish; but it cannot be true or false. Since the decision makes no claim about the state
18 of the world, it cannot be uncertain in the cognitive sense. The uncertainty cannot be
19 analyzed by sampling from some distribution. However, if the decision is based on the
20 claim that the chosen speed limit minimizes accidents while maintaining a prescribed
21 traffic volume, that claim may be uncertain and may be subjected to quantitative
22 uncertainty analysis. A discretionary decision of a regulatory body may entrain cognitive
23 uncertainty, but it becomes amenable for quantitative uncertainty analysis only when it is
24 linked to a claim about the state of the world.

25 *Aleatoric/Epistemic Uncertainty.* This terminology has become standard in the technical
26 uncertainty analysis literature, and it has been called *Variability/Uncertainty* in some
27 areas, particularly dealing with human populations. A variable whose uncertainty is
28 aleatoric for a given population takes different, uncertain, values for each member of the
29 population. If its uncertainty is epistemic, it takes the same uncertain value for all
30 members of the population. Issues involving uncertainty and variability or epistemic and
31 aleatory uncertainty translate into issues of dependence, when conducting a quantitative
32 uncertainty analysis (see Section 6.1.3.3). In its *Science and Judgment* report, NRC
33 (1994, [006424](#)) correctly remarks that “the amount of variability is generally itself an
34 uncertain parameter.” It is natural to ask whether a given uncertainty is aleatoric or
35 epistemic, whereas it is awkward to ask whether this uncertainty is uncertain or
36 variable—which explains the preference for the epistemic/aleatoric terminology.
37

⁵⁵These are known collectively as Kolmogorov’s probability axioms. The additivity of probability for exclusive alternatives states, e.g., that the probability of an unseen object being red or green is the sum of the probability that it is red and the probability that it is green. This of course assumes that “red” and “green” are clearly defined, such that nothing can be simultaneously red and green. Many alternative representations of uncertainty contest this additivity property.

6.1.3. Key Elements of a Quantitative Uncertainty Analysis

The uncertainty propagation can be performed by some rough estimation, as for example the delta method (Oehlert, 1992, [543742](#)), or in rare cases it can be performed analytically, as in simple error propagation.⁵⁶ Most often, however, it will be performed using Monte Carlo simulation. A joint distribution is assigned to the parameters of a quantitative model and then propagated through the model by sampling repeatedly from this joint distribution, computing model output and generating a distribution of model output. Every uncertainty analysis is conditional on initial assumptions. A “complete” uncertainty analysis is an unattainable goal; the best that can be done in practice is to identify and motivate the assumptions that are used. This section is not a how-to guide, but a to-do list of key elements of any quantitative uncertainty analysis.⁵⁷

6.1.3.1. Quantitative Model

The starting point of any quantitative uncertainty analysis is a mathematical model or procedure for calculating quantities of interest. A structured narrative explains the choice of quantitative models. If some values of input parameters for this calculation are not known with certainty, then the question arises: “What is the uncertainty attending the use of this model?” This is the question a quantitative uncertainty analysis seeks to answer.

6.1.3.2. Marginal Distributions over Model Parameter

If the model parameters are directly measurable with sampling error, then the sampling distribution may itself be used in the quantitative uncertainty analysis. If the model parameters are fit to data that are sampled from a known or hypothesized distribution, then by resampling this distribution and refitting the model, distributions over the model parameters may be constructed. Physically-based simulation models, such as pharmacokinetic models or environmental transport models, may be solved analytically if equilibrium reaction rates (the

⁵⁶Simple measurement error is often represented by adding a normally distributed random variable with mean zero to a “true” value. If several measurements are performed in succession, and the errors on each measurement are assumed to be independent, then the error induced by adding the measurement results is also a normally distributed random variable whose mean is zero and whose variance is the sum of the variances on the individual measurements.

⁵⁷These key elements of quantitative uncertainty analysis are discussed in many publications such as Saltelli et al. (2000, [543756](#)), Cox (2006, [594342](#)), Kurowicka and Cooke (2006, [543758](#)), NRC (2007, [543748](#)) and EPA (2009, [522927](#)).

1 transfer coefficients) are constant. If these rates are not constant, as when concentrations are
2 near saturation levels, then simulating the pharmacokinetics or transport is indicated. Structured
3 expert judgment has been applied for uncertainty quantification within the engineering
4 community since the time of the Rasmussen Report (U.S. NRC, 1975, [543729](#)). More recently,
5 this approach has been “test-driven” by EPA in assessing health effects of fine particulates
6 (Walker et al., 1999, [198615](#)), and its potential application has been identified in the Agency’s
7 *Guidelines for Carcinogen Risk Assessment*, commonly referred to as the Cancer Guidelines
8 (U.S. EPA, 2005, [086237](#)).⁵⁸

9
10 **6.1.3.3. *Dependence between Parameter Uncertainties: Aleatoric and Epistemic (Uncertainty***
11 ***and Variability)***

12 Two uncertain quantities are independent if knowledge about one of them does not alter
13 our uncertainty regarding the other. The quantities are dependent if they are not independent.
14 The role of dependence modeling in quantitative uncertainty analysis must be addressed. To
15 illustrate, cigarette smoking and body fat are both thought to influence biomarkers for toxic
16 response to dioxin exposure, such as ethoxyresorufin-*O*-deethylase (EROD) activity (Pereg et al.,
17 2002, [199797](#)). In an individual sampled at random from a target population, both percent body
18 fat and whether (and how much) he or she smokes are uncertain.⁵⁹ However, these uncertainties
19 are not independent, inasmuch as smokers tend to have less body fat (Vanni et al., 2009,
20 [543754](#)).

21 Issues involving uncertainty and variability, or epistemic and aleatory uncertainty,
22 translate into issues of dependence when conducting a quantitative uncertainty analysis. For
23 example, a constant used to estimate the biokinetic behavior of dioxin may be uncertain. If it is
24 believed to be the same for every member of the population, the uncertainty is termed

⁵⁸The EPA (2005, [086237](#)) cancer guidelines state: “In many of these scientific and engineering disciplines, researchers have used rigorous expert elicitation methods to overcome the lack of peer-reviewed methods and data....” These cancer guidelines are flexible enough to accommodate the use of expert elicitation to characterize cancer risks, as a complement to the methods presented in the cancer guidelines. According to NRC (2002, [035312](#)), the rigorous use of expert elicitation for the analyses of risks is considered to be quality science.”

⁵⁹Because dioxins generally distribute to body fat/lipid, the percent body fat is often used to estimate body burden; a default value of 25% is common (Connor and Aylward, 2006, [197632](#)). However, body fat percentage varies widely between individuals, from a minimum essential level (e.g., 2% for men, 10% for women) to obesity (e.g., 38% or more for men, 42% for women). Considering that current estimates suggest 30% of the U.S. population are obese, an uncertainty analysis of dioxin risk in this population should sample individuals from their gender/body fat distribution and correlate this with other known or suspected covariates influencing toxic response (such as diet, smoking, natural and endogenous ligands, disease, and age).

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1 “epistemic.” In a quantitative uncertainty analysis, this factor would be sampled from its
2 uncertainty distribution on each Monte Carlo run and used for *all* members of the population.
3 Body fat, in contrast, is aleatoric. We do not sample one value from the body fat distribution and
4 use this value for *all* members of the population on each Monte Carlo run. Instead we sample a
5 body fat value for each individual on each run. Because body fat is correlated with other
6 relevant variables (e.g., smoking, gender, age, and socioeconomic status), all of these variables
7 should be sampled in a manner that reflects their dependences. Kinetic constants whose
8 uncertainty is epistemic are completely correlated across individuals: if the value is 0.5 for one
9 individual, it is 0.5 for everyone. Body fat values vary from individual to individual, and they
10 are correlated through a host of other variables.

11

12 **6.1.3.4. Model Uncertainty**

13 All models, being idealizations, are false; on this there is no uncertainty to quantify.
14 However, the choice of model may constrain the ability to represent uncertainty in observable
15 phenomena. Thus, in a linear low-dose model, uncertainty over a cancer slope factor may be
16 quantified, but uncertainty regarding changes in slope at distinct low-dose regimes cannot be
17 captured. When the model choice imposes severe and potentially unwelcome constraints on
18 uncertainty quantification, this must be addressed. Distributions over model parameters may be
19 selected and evaluated based on their ability to reflect uncertainty distributions over observable
20 phenomena predicted by the models.⁶⁰ In such cases, the uncertainty propagated through the
21 quantitative model is not strongly model-dependent. In other cases, multiple model alternatives
22 may be applied, whose “probability of being the true model” is known or assumed. Since
23 different models can always be regarded as specializations of more general models, the
24 distinction between parameter and model uncertainty is sometimes more apparent than real. For
25 example, as illustrated in the EPA Benchmark Dose Software (BMDS) (U.S. EPA, 2000,
26 [052150](#)), the multistage and Weibull dose-response models both contain the model $\text{Pr}(x) = \gamma +$
27 $(1 - \gamma)(1 - e^{-\beta^1 x})$ as a submodel, to which they collapse if other parameters are zero (multistage)
28 or one (Weibull). Recalling that the function $1/(1 + x)$ is first-order equivalent to $(1 - x)$ for

⁶⁰ Such techniques were first used on a large scale in the U.S. NRC-EU joint uncertainty analysis of consequence models for accidents at nuclear power plants, see Goossens et al. (1996, [548727](#); 2001, [548737](#); 2001, [548738](#); 2001, [548731](#); 2001, [548732](#); 2001, [548735](#)) (Bock et al., 1998, [548752](#)).

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1 small x, the same may be said for logistic models as well. In this case, these models could easily
2 be parameterized within one family, rendering the choice between them a choice of parameter
3 values. Similarly, the choice between sub-, supra-, and linear models is sometimes reduced to
4 parameter estimation within a more general class of model (Hoel and Portier, 1994, [198741](#)).

5 In other cases, the reduction of model uncertainty to parameter uncertainty is less natural.
6 For example, according to the “chemoprotection model” of Greenlee et al. (2001, [015400](#)),
7 dioxin’s binding to the aryl hydrocarbon receptor (AhR) inhibits proliferation in tumor cells and
8 thus suppresses mammary tumors. Dose-dependent protection and cancer induction can both be
9 true, each applying to different tissues. Although mathematical models exhibiting these twin
10 features have been suggested (e.g., Kohn and Melnick, 2002, [199104](#)), these models are not yet
11 readily estimable from data, and the choice between them is referred to the structured narrative.

12 13 **6.1.3.5. *Sampling Method***

14 All sampling on a computer is “pseudo random.” Significant issues arise in choosing a
15 method for sampling high-dimensional distributions with dependence. If evaluating the
16 quantitative model is very time consuming, various “quasi random” schemes may be applied,
17 including Latin hypercube sampling, importance sampling, and Hammersley sampling. These
18 methods involve trade-offs between economy and accuracy of the dependence modeling.

19 20 **6.1.3.6. *Method for Extracting and Communicating Results***

21 When a large quantitative uncertainty analysis has been performed, the method for
22 identifying important contributors and communicating this information to users is not
23 straightforward. Analysts have proposed many ways to quantify the uncertainty contribution of
24 one variable, or set of variables, on others,⁶¹ and the analyst’s decision at this juncture may
25 strongly impact the “take-home” message from the study. An importance measure that averages

⁶¹A few examples may suffice. The standard Pearson correlation coefficient measures the linear dependence between two variables, positive or negative. The rank or Spearman correlation coefficient measures the monotone dependence. The correlation ratio measures the (unsigned) variance contribution of an explanatory variable on a target variable. The regression coefficient measures the expected change in standard (not natural!) units of a target variable, per standard unit change in an explanatory variable, and assumes this expected change is independent of the values of the explanatory variables. Multiple correlation measures the correlation between a given variable and its best linear predictor based on another set of variables. The partial correlation of two variables given a set of other variables is their correlation after discounting the influence of the other variables. The correlation ratio, multiple correlation, and the regression coefficient are not symmetric; the correlation ratio and multiple correlation are always non-negative (Kurowicka and Cooke, 2006, [543758](#); Saltelli et al., 2000, [543756](#)).

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1 over an entire sample space may obscure the features of real interest. For example, the drivers of
2 cancer induction at low doses might be different from the drivers at high doses. If the drivers of
3 low-dose cancer induction are of interest, then importance measures that average over all doses
4 should not be considered.

6 **6.2. EPA APPROACHES FOR ORAL CANCER AND NONCANCER ASSESSMENT**

7 Different types of toxicity information have historically been used in EPA’s oral cancer
8 and noncancer dose-response assessments, although efforts to harmonize these approaches are
9 ongoing. For oral exposures, noncancer endpoints are commonly assessed using the RfD
10 methodology to derive “an estimate (with uncertainty spanning perhaps an order of magnitude) of
11 a daily oral exposure to the human population (including sensitive subgroups) that is likely to be
12 without an appreciable risk of deleterious effects during a lifetime.” In contrast, cancer
13 endpoints are commonly assessed using a dose-response function with the probability of excess
14 risk above background modeled as a linear function of dose, for doses down to zero. The RfD
15 method relies on a POD. The cancer dose-response method uses a POD if the linear model is
16 chosen. From the Cancer Guidelines, cancer endpoints can also be assessed using the RfD
17 methodology if the proof burden is satisfactorily met (as described in Section 5.2.3.4.1.2).

18 Toxicity reference values have typically been derived for human noncancer endpoints
19 based on a no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level
20 (LOAEL) from animal bioassay studies. This terminology suggests a biological population
21 threshold beneath which no harm is anticipated. Reference values such as the oral RfD or
22 inhalation reference concentration are derived by applying uncertainty factors (UFs) to a POD.
23 Depending on the nature of available data and modeling choice, a POD can be selected from
24 values other than a NOAEL or LOAEL, such as an ED_x (effective dose eliciting x percent
25 response), or a benchmark dose (BMD) or its lower confidence bound (BMDL). The BMD is
26 the dose that induces a benchmark response (BMR), which is often chosen to represent a 5 or
27 10% increase in excess risk above background. The POD is divided by one or more uncertainty
28 factors that represent knowledge gaps (see Section 6.4.1.2 for details on specific types of UFs).

29 An RfD is described as “likely to be without appreciable risk” but the probabilistic
30 language has not as yet been operationalized. A quantitative definition of “appreciable” has not
31 been articulated, and methods to compute risks above the RfD as a function of dose have not

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1 been designated for use by the EPA; thus, it is not current practice to ascertain that the risk is
2 indeed not appreciable. In addition, different participants in discussions over
3 threshold/nonthreshold models for dioxin may have different perspectives regarding how to
4 define “appreciable risk.” Under the current POD/UF framework, dose-response functions are
5 not provided for calculating the actual risk at or above the RfD. Instead, to provide a “risk
6 indicator” for use in screening for health hazards, a hazard quotient (HQ) is computed as the
7 ratio of a given oral exposure to the RfD, or a margin of exposure (MOE) is estimated as the
8 ratio of the POD to the human exposure level.

9 For the cancer endpoint, an oral cancer slope factor may be derived for human health risk
10 assessment, typically based on tumor incidence data from an animal bioassay or on cancer
11 incidence or deaths from an epidemiologic study. In the EPA Cancer Guidelines, cancer is
12 predominantly thought to have no population biological threshold and a linear extrapolation to
13 zero is applied from the POD based on extra risk above background, i.e., the probability of the
14 endpoint decreases linearly in dose from the POD to zero or to a population background level. In
15 the absence of sufficient information on the cancer mode of action (MOA), the linear model is
16 applied as a default. The linear model also can be applied when there is sufficient MOA
17 evidence supporting this choice for low-dose cancer induction. Cancer endpoints could also be
18 evaluated using a “nonlinear” model. In this case, the proof burden clearly rests on the nonlinear
19 model; there must be sufficient evidence to override the health-protective default or
20 scientifically-based choice of a linear model, as described in the Cancer Guidelines. These
21 Guidelines state, “When adequate data on mode of action provide sufficient evidence to support
22 a nonlinear mode of action *for the general population* (emphasis added) and/or any
23 subpopulations of concern, a different approach—a reference dose/reference concentration that
24 assumes that nonlinearity—is used.” In current terminology, the RfD methodology applies to the
25 cancer endpoint if there is sufficient evidence supporting a “zero slope at zero” model;
26 otherwise, the linear nonthreshold model applies by default. (See Section 5.2.3 for a detailed
27 discussion of linear vs. nonlinear extrapolations below the observed data, population vs.
28 individual thresholds, and how the Cancer Guidelines are applied in choosing dose-response
29 model forms for risk assessment.)

1 **6.3. HIGHLIGHTS OF NAS REVIEW COMMENTS ON UNCERTAINTY**
2 **QUANTIFICATION FOR THE 2003 REASSESSMENT**

3 The NAS (2006, [198441](#); 2006, [543760](#)) identified a number of uncertainty
4 characterization issues for the 2003 Reassessment; key sources of uncertainty for which
5 quantification is suggested are highlighted in Table 6-1. The discussion in this section focuses
6 on comments related to dose response.

7 There are several nuances in the NAS position relative to the need for substantial
8 improvement in transparency, thoroughness, and clarity in quantitative uncertainty analysis for
9 the 2003 Reassessment. These nuances concern whether the nonlinear model (note that the NAS
10 committee uses “sublinear” and “nonlinear” interchangeably) is scientifically better supported
11 than the linear model, and if the sublinear model is better supported, whether this is based on
12 data or on apodictic knowledge (knowledge without uncertainty) of the MOA. The NAS
13 committee does not distinguish between individual and population dose-response models;
14 however the criteria from the EPA Cancer Guidelines clearly apply to population models.
15 Assuming that the AhR-mediated MOA implies a threshold for each individual, the step to a
16 population “zero slope at zero” model requires the following, as identified and discussed in detail
17 in Section 5.2.3.:

- 18
- 19 1. The distribution of the individual thresholds induced by the MOA, and
 - 20 2. The dose-response function for values above the thresholds.
- 21

22 This information can either come from data or from known information of the MOA, but
23 the burden of proof clearly rests on the nonlinear model. This section summarizes the NAS
24 committee’s overall positions. Responses to specific suggestions are given in Section 6.4 and
25 summarized in Section 6.5. Several excerpts of specific comments from NAS (2006, [198441](#))
26 illustrate key issues.

27 The NAS committee favors the nonlinear model with a threshold:
28

1 ...the committee concludes that, although it is not possible to scientifically prove
2 the absence of linearity at low doses, the scientific evidence, based largely on
3 mode of action, is adequate to favor the use of a nonlinear model that would
4 include a threshold response over the use of the default linear assumption.
5 *(p. 122)*
6

7 The committee does not state whether the threshold applies to the population, or whether each
8 individual has his/her own threshold.

9 The NAS also comments on whether the nonlinear model should be used to compare with
10 the linear default:

11
12 Because the committee concludes that the data support the hypothesis that the
13 dose-response relationship for dioxin and cancer is sublinear, it recommends that
14 EPA include a nonlinear model for cancer risk estimates but also use the current
15 linear models for comparative purposes. *(p. 16)*
16

17 The committee does not suggest what the (population/individual) threshold might be, nor how it
18 might be supported on the basis of data. Rather, the apodictic knowledge that there *is* a
19 (population/individual) threshold places the dioxin risk assessment within the RfD framework,
20 using a HQ or MOE as the basis for indicating the potential risks from exposure. The committee
21 further asks for a quantitative characterization of the range of uncertainty:

22
23 The committee determined that the available data support the use of a nonlinear
24 model, which is consistent with receptor-mediated responses and a potential
25 threshold, with subsequent calculations and interpretation of MOEs. EPA's sole
26 use of the default assumption of linearity and selection of ED₀₁ as the only POD
27 to quantify cancer risk does not provide an adequate quantitative characterization
28 of the overall range of uncertainties associated with the final estimates of cancer
29 risk. *(p. 24)*
30

31 Regarding the Cancer Guidelines' requirement of sufficient evidence to use a nonlinear
32 approach for cancer risk assessment, the committee indicates that quantitative evidence will not
33 decide the linearity/nonlinearity (nonthreshold/threshold) issue, but knowledge (without
34 uncertainty) of the MOA should be used:
35

1 Quantitative evidence of nonlinearity below the point of departure (POD), the
2 ED₀₁⁶² will never be available because the POD is chosen to be at the bottom end
3 of the available dose-response data. ... EPA should give greater weight to
4 knowledge about the mode of action and its impact on the shape of the
5 dose-response relationship. (p. 178)
6

7 The comment continues, with the committee implicitly acknowledging that there is no
8 evidence arguing against linearity, but that the lack of evidence should not justify using the linear
9 model.

10 The committee considers that the absence of evidence that argues against linearity
11 is not sufficient justification for adopting linear extrapolation, even over a dose
12 range of one to two orders of magnitude or to the assumption of linearity through
13 zero, which would not normally be applied to receptor-mediated effects. (p. 178)
14

15 In addition, the committee recommended that EPA explore both linear and nonlinear
16 approaches to TCDD cancer assessment:

17
18 On the whole, the committee concluded that the empirical evidence supports a
19 nonlinear dose response below the ED₀₁, while acknowledging that the possibility
20 of a linear response cannot be completely ruled out. The Reassessment
21 emphasizes the lack of such nonlinear models, hence its adoption of the approach
22 of linear extrapolation below the POD level. Although this approach remains
23 consistent with the cancer guidelines...., EPA should acknowledge the qualitative
24 evidence of a nonlinear dose response in a more balanced way, continue to fill in
25 the quantitative data gaps, and look for opportunities to incorporate mechanistic
26 information as it becomes available. The committee recommends adopting both
27 linear and nonlinear methods of risk characterization to account for the
28 uncertainty of dose-response relationship shape below ED₀₁ (p. 72).
29

30 In this document, EPA has applied its own guidance on cancer risk assessment and
31 adopted linearity (and an assumption of no threshold) as a health-protective default approach in
32 the absence of sufficient evidence of MOA involving a threshold for all tumors resulting from
33 TCDD exposures (volitional uncertainty). (Note that the NAS report appears to view the
34 absence of evidence as imposing a burden of proof on the linear model [cognitive uncertainty];
35 see Sections 5.2.3.4.1.2 and 6.2 regarding the burden of proof.) In addition, the NAS
36 committee's request to apply nonlinear methods for the cancer assessment is addressed, in

⁶² Effective dose (ED) is the dose corresponding to a X% increase (in this case a 1%) in an adverse effect such as a cancer endpoint, relative to the control response.

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1 Section 5.2.3.4.1.4 of this document. That evaluation describes the application of nonlinear
2 methods to TCDD data and presents two illustrative examples of RfD development for
3 carcinogenic effects: one based on tumorigenesis in experimental animals, and the other on
4 hypothesized key events in TCDD's MOAs for liver and lung tumors.

5 The thrust of the NAS remarks regarding transparency, thoroughness and clarity in
6 quantitative uncertainty analysis relevant to dose-response can be summarized as follows:

- 7
- 8 1. The uncertainty of cancer risks due to dioxin exposure should be quantified.
- 9 2. Dioxin cancer risk should be treated either as a threshold phenomenon, thus following the
10 basic RfD methodology, or should be modeled using a sublinear dose-response function
11 below the observed data, with the linear model used for comparison.
- 12 3. The POD should be subjected to quantitative uncertainty analysis.

13 A similar point of view has been indicated by others.⁶³ Detailed suggestions regarding specific
14 improvements for quantitative uncertainty analysis in the 2003 Reassessment are outlined in the
15 next section and summarized in Section 6.5.

16

17 **6.4. FEASIBILITY OF CONDUCTING A QUANTITATIVE UNCERTAINTY**

18 **ANALYSIS FOR TCDD**

19 This section focuses on uncertainty analysis for TCDD dose response, which involves a
20 range of issues as highlighted in Table 6-1.

21

22 **6.4.1. Feasibility of Conducting a Quantitative Uncertainty Analysis under the RfD**

23 **Methodology**

24 This discussion applies to all noncancer endpoints of TCDD, and to cancer endpoints
25 insofar as they fall under the RfD methodology. An RfD is obtained through the following steps:

- 26
- 27 1. Choose a POD, then
- 28 2. Apply uncertainty factors (UFs) to account for knowledge shortfalls.
- 29

⁶³For example, from Popp et al. (2006, [197074](#)). "Overall, the evidence indicates that (1) TCDD causes cancer via a receptor-mediated process; (2) this dose-response is non-linear; and (3) a threshold region exists for TCDD-induced cancer below which adverse effects are unlikely to occur."

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1 The method of uncertainty factors harkens back to the engineering practice of safety
2 factors (Lehman and Fitzhugh, 1954, [003195](#)). To illustrate, if the reference load for an
3 engineered structure is X, then engineers might design the structure to withstand load 3X, using a
4 safety factor of 3 to create a margin of safety. If the structure functions in a corrosive
5 environment, another factor could be multiplied to guarantee safety for that condition, and
6 another factor could be applied for heat, another for vibrations, and so on. The choice of values
7 is simply based on good engineering practice, i.e., reflecting what has worked in the past.
8 Although safety factors are still common in engineering, they are giving way to probabilistic
9 design in many applications. The reason is that compounding safety factors quickly leads to
10 overdesigning. Compounding safety margins for spaceflight systems may render them too heavy
11 to fly. As our understanding of a system increases, it becomes possible to guarantee the requisite
12 safety by leveraging our scientific understanding of the materials and processes. That of course
13 requires formulating clear probabilistic safety goals and developing the techniques to
14 demonstrate compliance.

15 The engineering community has never sought to account for uncertainty by treating
16 safety factors as random variables and assigning them (marginal) distributions; such an approach
17 would not counteract the overdesigning inherent in safety factors. Many authors, including the
18 recent national committee for *Science and Decisions* (NRC, 2009, [194810](#)), have advocated just
19 such a probabilistic approach to the apparent “overdesigning” of the RfD when multiple UFs are
20 used in its derivation.

21 The NAS committee that evaluated the 2003 Reassessment does not discuss how to
22 perform uncertainty analysis. But their call for substantial improvement in quantitative
23 uncertainty analysis with TCDD falling under the RfD framework entails examining the
24 *feasibility* of quantitative uncertainty analysis within this framework. (Note that the EPA
25 Integrated Risk Information System (IRIS) database uses uncertainty factors without
26 probabilistic interpretations; some context for this is offered in Section 6.4.1.2.)

27 28 **6.4.1.1. *Feasibility of Conducting a Quantitative Uncertainty Analysis for the Point of*** 29 ***Departure***

30 The POD plays a role in both the noncancer RfD methodology and the cancer
31 dose-response methodology. The POD can be selected from various options, such as a NOAEL

1 or LOAEL, a BMDL, or an ED_x. The feasibility of quantitative uncertainty analysis for each of
2 these three options is considered below.

3 By definition, the NOAEL is the highest of the tested doses in a toxicological experiment
4 that is judged not to have caused an adverse effect (with dose expressed as a dose rate, in
5 mg/kg-day). A quantitative uncertainty analysis for a NOAEL or LOAEL encounters the
6 following problem. In an experiment involving a small, positive response, the probability of
7 seeing no response can be calculated using a binomial model with the number of exposed
8 animals and the observed number of responses. However, in an experiment with no response,
9 the probability of having observed a response cannot be calculated without assuming a response
10 probability. Such an assumption could not be based on observed data. The probability of a
11 higher NOAEL or higher LOAEL can be computed, but not that of a lower NOAEL or LOAEL.
12 In other words, the probability that an experiment with a positive result may have yielded a null
13 response can be estimated, but not the probability that an experiment with a null response might
14 have yielded a positive response.⁶⁴

15 In addressing uncertainty quantification for a BMDL or ED_x, two questions must be
16 distinguished regarding the response:

17

- 18 1. What is the distribution of possible doses that causes an x% increase over background?
- 19 2. What is the distribution for possible values of increase over background caused by a
20 given dose?

21

22 The BMD is defined as the dose that realizes a BMR. It is an estimate from bioassay data
23 that requires choosing a BMR and fitting a dose-response curve. The BMR, being a choice, is
24 not amenable to quantitative uncertainty analysis, but the choice can be motivated in a structured
25 narrative. The BMDL is the lower confidence limit on the dose that realizes a BMR (e.g., 95%)
26 that can be found based on the uncertainty in the parameters of the dose-response relationship.
27 Thus, the BMDL is addressed to the first question above, and represents in this case the
28 95% lower confidence band of the distribution of possible doses causing an x% increase over
29 background. In the standard approach, the uncertainty captured by the BMDL is sampling

⁶⁴The probability associated with a null response is often estimated by fitting a dose-response model to the data.
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1 uncertainty *conditional* on the truth of the dose-response model. Different models might fit the
2 data equally well yet lead to different BMDLs.

3 The BMDL is also influenced by the constraints imposed on the parameter fitting.
4 Suppose that the slope is expected to be greater than one, and that the maximum likelihood
5 estimate of the slope is slightly greater than one. Since the constraint is not binding, the
6 constrained and unconstrained model would have the same Akaike Information Criterion and
7 would be equivalent in this sense. However, computing the BMDL with the slope constraint can
8 lead to very different values than without this constraint. In the latter case, slope values less than
9 one contribute to the uncertainty in the dose causing the BMR (see Cooke, 2009, [543763](#)).

10 The ED_x can also be taken as a POD. It is similar in spirit to the BMD; however, as used
11 here, the term ED_x applies when the dose causing an x% extra risk over background has actually
12 been observed, not estimated from a fitted dose-response model.⁶⁵ The observations are subject
13 to sample fluctuations, and if the experiment on which the ED_x is based were repeated, different
14 values might be found. It is helpful to consider a numerical example. Suppose a background
15 response rate of 10% is established based on many observations of nonexposed individuals. In a
16 given experiment, involving say 100 individuals given dose *d*, 14 individuals responded. The
17 percent increase *x* over background (extra risk) is found by solving:

18
19
$$14/100 = 10/100 + x \times 90/100, \text{ or } x = 4.4\%.$$

20
21 We conclude that $d = ED_{4.4}$. We may assume that if the experiment were repeated with 100 new
22 individuals sampled independently from the whole population, the response would be given by a
23 binomial distribution with parameters (14, 100). The number of responses might be greater or
24 smaller than four, there is a 16% chance of observing 10 or fewer responses. The response to
25 dose *d* would not be distinguished from the background in that case, and a higher dose would be
26 used for the POD.

27 The uncertainty analysis of ED_x as the POD involves addressing the second question
28 above, without a quantitative dose-response model. A quantitative uncertainty analysis is
29 hampered, however, by the possibility that dose *d* would produce a response less than or equal to

⁶⁵This definition of ED_x is adopted to distinguish the modeled response (BMD) and the observed response (ED_x), and it is more restrictive than usages common in the literature.

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1 the background, in which case the POD is indeterminate—another experiment with a different
2 dose would be chosen as the POD. A true quantitative uncertainty analysis of ED_x as the POD
3 would thus require a full bioassay experimental design, with binomial sampling of response rates
4 at each dose level in the assay. Absent that, quantitative uncertainty analysis is not possible.

5 The interplay of choice and estimation ingredients in the POD depends on the type of
6 POD. The main features of the above discussion are captured in Table 6-2. This table notes that
7 the BMDL captures the uncertainty caused by sampling fluctuations *given* that the dose-response
8 model is true. Other methods are available to compute the BMDL using (1) model-independent,
9 observable uncertainty; (2) nonparametric Bayesian dose-response models; or (3) Bayesian
10 model averaging (Cooke, 2009, [543763](#)). Only the ED_x can be subject to a quantitative
11 uncertainty analysis, and then only if a full bioassay data set is available.

13 **6.4.1.2. Feasibility of Conducting a Quantitative Uncertainty Analysis with Uncertainty** 14 **Factors**

15 Uncertainty factors are chosen based on a structured narrative characterizing knowledge
16 shortfalls involving the following issues:

- 18 1. Interspecies extrapolation (UF_A: from animal data to humans).
- 19 2. Intraspecies extrapolation (UF_H: to account for human interindividual variability,
20 considering sensitive subgroups).
- 21 3. LOAEL to NOAEL extrapolation (UF_L: to estimate the dose corresponding to no adverse
22 effect, from a reported LOAEL).
- 23 4. Subchronic to chronic extrapolation (UF_S: to estimate effects of chronic exposures, from
24 a subchronic study).
- 25 5. Database deficiency (UF_D: to extrapolate from an incomplete data set, e.g., in terms of
26 endpoints assessed or study design, i.e., from a poor to a sufficient or rich data context).

27
28 The standard chronic RfD can represent a sensitive human (H) response to a toxic
29 substance under chronic (C) exposure conditions. Suppose a BMDL POD were based on animal
30 (A) data from a subchronic (S) study. The database for that chemical could be rich (R), e.g.,
31 involving multiple (and at least sensitive) species/strains, both sexes, multiple life stages, with
32 multiple endpoints observed under sound study designs. Or the data could be poor (P), with
33 limited measurements from only a subchronic animal study (ASP) forming the basis for

1 estimating a general reference value for humans (including sensitive subgroups) under chronic
2 exposure conditions. For that case, the UF method would be applied as follows:

$$RfD = \frac{ASP}{UF_A \times UF_S \times UF_D \times UF_H} \quad (\text{Eq. 6-1})$$

3
4
5
6 where UF_A , UF_S , UF_D , and UF_H are the uncertainty factors for extrapolating from animals to
7 humans (UF_A), subchronic to chronic exposure conditions (UF_S), without adequate endpoint
8 coverage (UF_D), and considering sensitive human subpopulations (UF_H). It is possible to assign
9 distributions to the UFs in Eq. 6-1, and to perform a Monte Carlo analysis to produce a
10 quantitative uncertainty distribution over the dose or value likely to be without appreciable risk
11 to humans for chronic exposures. Many authors have proposed such an approach,⁶⁶ and the
12 recent NRC (2009, [194810](#)) report on science and decisions encourages EPA to move in this
13 direction.

14 The idea of using a Monte Carlo analysis to develop quantitative uncertainty distributions
15 for the RfD is simple, but the data on which the UFs are based and the assumptions that would
16 need to be made should be further explored. For example, it is assumed that the extrapolation
17 from subchronic to chronic exposure (UF_S) is the same whether applied to animals or humans,
18 and whether applied to sufficient (rich) or deficient (poor) data contexts. Swartout et al. (1998,
19 [093460](#)) noted “Within the current RfD methodology, UF_S does not consider differences among
20 species, endpoints, or severity of effects; the same factor is applied in all cases.” In addition, due
21 to the paucity of relevant human data, the same authors suggested the use of other endpoints as
22 surrogates in estimating the extrapolation from animals to humans, UF_A . Further, few data exist

⁶⁶There has been considerable work on giving a probabilistic interpretation of the UFs, including by Abdel-Rahman and Kadry (1995), Vermeire et al. (1999), Baird et al. (1996), Swartout et al. (1998, [093460](#)), Slob and Pieters (1998, [087256](#)), Evans and Baird (1998), Calabrese and Gilbert (1993), Calabrese and Baldwin (1995), Hattis et al. (2002, [548720](#)), Kang et al. (2000, [548722](#)), and Pekelis et al. (2003, [548723](#)). These evaluations can be considered to frame what might be called a *random chemical* approach. Several authors adduce properties based on log normal distributions. Insightful studies by Kodell and Gaylor (1999);(Gaylor and Kodell, 2000, [548724](#)) suggest that uncertainty factors are independent log normal variables. Combining uncertainty factors involves multiplying the median values, and combining the “error factors” according to the formula $K_{S \times H} = \exp[1.6449 \times \sqrt{(\sigma_S^2 + \sigma_H^2)}]$, where σ_S^2 , σ_H^2 are the variances of $\ln(UF_S)$ and $\ln(UF_H)$. Thus $UF_S \times UF_H$ is a lognormal variable with $\text{Median}(UF_S \times UF_H) = \text{Median}(UF_S) \times \text{Median}(UF_H)$, and 95th percentile given by $\text{Median}(UF_S \times UF_H) \times K_{S \times H}$. If U_S and U_H each have an error factor or 10, then the error factor of $UF_S \times UF_H$ is not 100 but 25.95. Several authors suggest that multiplying uncertainty factors might over-protect. Recent proposals from the National Research Council reflect the random chemical concept, and they inherit its problems (NRC, 2009, [194810](#)).

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1 in humans to accurately portray the interindividual variability represented by UF_H . Much of the
2 data gathered to date on distributions of UFs have aggregated across other extrapolations; that is,
3 data from subchronic to chronic ratios are aggregated over different species and different data
4 contexts. Finally, it may be noted that an important issue is the data on which empirical
5 distributions of response ratios are based. Brand et al. (1999, [007629](#); 2001, [543765](#)) studied the
6 sampling behavior of response ratios and raised concerns with regard to their informativeness.

7 Detailed analyses of the data underlying a Monte Carlo uncertainty analysis of Eq. 6-1
8 would afford the possibility of verifying at least some of the assumptions and numerical
9 estimations such an analysis must make. Even if the assumption that the same UF_S is applicable
10 for all species, endpoints, and effect severities is thought to be biological plausible, the question
11 of whether a given set of chemicals reflects this assumption, and hence they are suitable for a
12 Monte Carlo analysis of Eq. 6-1, can only be decided by data evaluation. Data are the ultimate
13 arbiter of whether quantitative uncertainty analysis with uncertainty factors, as currently
14 envisioned, has sufficient evidentiary support.

16 **6.4.1.3. *Uncertainty Reduction Using Quantitative Data for Species Extrapolation***

17 Expressing dose in units of exposure that are more closely related to target tissue than to
18 contact with administered feed (or an environmental medium) can reduce uncertainty in
19 extrapolations of dose, route or species. This concept underlies EPA's establishment of the
20 Inhalation Reference Concentration Methodology (U.S. EPA, 1994, [006488](#)). Under this
21 method, species differences in tissue exposure for inhalation toxicants serve as the basis for
22 interspecies adjustments of dose. Likewise, the International Programme on Chemical Safety
23 (IPCS) has established guidance for chemical-specific adjustment factors (IPCS, 2005), which
24 also uses a measure of internal exposure (dose) to normalize (e.g., make equivalent) the dose
25 between species. Certain more recent IRIS values also reflect such an approach, with
26 data-derived extrapolation factors replacing default adjustments. Under such approaches, the
27 relationship between external exposure and target tissue exposure is determined in each species,
28 and the applied doses are normalized on the basis of the same level of the internal tissue
29 exposure. One distinction between the two approaches is that the IPCS (2005) approach is
30 based on the attainment of the same levels of the toxicant in the blood (the central compartment)
31 rather than in the actual target tissue (a consideration based in part on the fact that typically the

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1 only data available to evaluate a human toxicokinetic model will be venous blood
2 concentrations, rather than concentrations in a responding tissue or organ). Further, it has been
3 shown that species differences in internal dosimetry are more a function of species differences
4 in blood solubility than differences in tissue solubility—that is, once distributed to blood,
5 species differences in tissue exposure are less likely to be based on species differences in tissue
6 solubility.

7 The approach to development of interspecies extrapolation factors for inter- and
8 intraspecies extrapolation of effective dose for the oral RfD for dioxin, which is described in
9 Sections 3 and 4 of this document, is in agreement with both of these approaches. All tissues in
10 the body are exposed to dioxin via the bloodstream. Even in instances where the specific target
11 tissues for observed effects may be other than the tissue where the effect is observed (e.g.,
12 effects mediated through the endocrine system), this biologically-based approach remains valid
13 and reduces uncertainty in dose extrapolation. The approach to extrapolation of dosimetry—on
14 the basis of circulating levels of dioxin in blood—makes optimal use of human
15 exposure-response data, human biomonitoring data, and toxicokinetic modeling to estimate
16 equivalent exposures for humans and test species without requiring that the target tissue be
17 conclusively identified. The decision to base animal-to-human extrapolation on circulating
18 levels of dioxin in blood, as predicted by a well-evaluated PBPK model, reduces some potential
19 sources of uncertainty.

20

21 **6.4.1.4. *Conclusion on Feasibility of Quantitative Uncertainty Analysis with the RfD***
22 ***Approach***

23 A quantitative uncertainty analysis of the POD is not feasible for PODs based on
24 NOAELs or LOAELs. For the BMDL, such an analysis is not appropriate because the BMDL is
25 already a quantile from an uncertainty distribution of the BMD. However, this uncertainty
26 distribution can be obtained in different ways that capture different aspects of uncertainty.
27 Quantitative uncertainty analysis is feasible if the POD is based on the ED_x (as defined above)
28 and is supported by a full set of bioassay data. A quantitative uncertainty analysis based on a
29 probabilistic interpretation of uncertainty factors in their present form invokes strong
30 assumptions. The data on which the distributions of uncertainty factors are based could be used
31 to check at least some of these assumptions.

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1 **6.4.2. Feasibility of Conducting a Quantitative Uncertainty Analysis for TCDD under the**
2 **Dose-Response Methodology**

3 Quantitative uncertainty analysis starts with a mathematical model and seeks to quantify
4 the uncertainty attending the use of this model. Dose-response relations are mathematical
5 models expressing the probability of response as a mathematical function of dose. For several
6 decades, the uncertainty attending the use of dose-response models has been an abiding concern
7 in many sectors, including the chemical and nuclear industries as well as the public health sector.
8 Given a set of animal bioassay data, quantifying dose-response uncertainty may be approached in
9 different ways. The differences reflect different types of uncertainty that are captured. A recent
10 evaluation enumerates the following possible methodologies (Bussard et al., 2009, [543770](#)):
11

12 **Benchmark Dose Modeling (BMD):** Choose the ‘best’ model, and assess
13 uncertainty assuming this model is true. Supplemental results can compare
14 estimates obtained with different models, and sensitivity analyses can investigate
15 other modeling issues.

16 **Probabilistic Inversion with Isotonic Regression (PI-IR):** Define
17 model-independent ‘observational’ uncertainty, and look for a model that captures
18 this uncertainty by assuming the selected model is true and providing for a
19 distribution over its parameters.

20 **Non-Parametric Bayes (NPB):** Choose a prior mean response (potency)
21 curve (potentially a “non-informative prior”) and a precision parameter to express
22 prior uncertainty over all increasing dose-response relations, and update this prior
23 distribution with the bioassay data.

24 **Bayesian Model Averaging (BMA)** (as considered here): Choose an
25 initial set of models, and then estimate the parameters of each model with
26 maximum likelihood. Use classical methods to estimate parameter uncertainty,
27 given the truth of the model. Determine a probability weight for each model
28 using the Bayes Information Criterion, and use these weights to average the model
29 results.
30

31 The first of the above methods involves standard classical statistical methods and
32 captures sampling uncertainty conditional on the truth of the model used. The other methods are
33 “exotic” in the sense that they attempt to capture uncertainty that is not conditional on the truth
34 of a given model. All have been subjected to peer review and published, but they do not enjoy
35 the wide usage of the standard classical methods. The Bayesian models involve subjective
36 choices of prior distributions. Insofar as the final result is largely independent of the choice of

1 prior, these methods conform to the current starting point of focusing on data-driven methods
2 and not appealing to structured expert judgment. (Structured expert judgment can also be
3 considered an exotic method; an explanation of this approach falls outside the scope of this
4 report.)

5 A quantitative uncertainty analysis of TCDD capturing uncertainty in extrapolating data
6 from animal bioassays to human reference values together with consideration of epidemiological
7 data from studies of workers (routine exposures) or the general public (including dietary
8 exposures and those reflecting discrete poisonings or accidental releases) would raise many
9 issues. The major issues are summarized below.

10
11 **6.4.2.1. Feasibility of Quantitatively Characterizing the Uncertainties Encountered when**
12 **Determining Appropriate Types of Studies (Epidemiological, Animal, Both, and**
13 **Other)**

14 The risk assessor must choose the data set(s) that will serve as a starting point for
15 dose-response modeling. With respect to TCDD, a wealth of animal bioassay data exist in the
16 scientific literature, across species ranging from rats, mice, guinea pigs, and hamsters to mink,
17 dogs and monkeys, and a variety of tissues, organs, and systems. In addition, a considerable
18 amount of human data is available from clinical/case reports, accidental releases, and
19 occupational exposures, including epidemiological data for several cohorts. As detailed in
20 Sections 2, 4 and 5, some of the main sources of uncertainty in the TCDD epidemiological data
21 include the healthy worker effect, confounding and exposure misclassification. Epidemiological
22 data are usually attended with large uncertainties regarding the doses actually received by
23 individuals. The difficulty in characterizing individual-level exposures largely stems from
24 having limited internal measures of TCDD exposure, as biomonitoring data may only be
25 available for one point in time or on a subset of the exposed population. Although there is little
26 direct evidence of strong confounding in the cohorts of TCDD and dioxin-like compounds, some
27 of the confounders that have been evaluated in a few of the epidemiological studies include
28 gender, body mass index, age, cigarette and alcohol consumption, and hair and eye color
29 (Baccarelli et al., 2005, [197053](#); 2006, [197036](#); Eskenazi et al., 2002, [197168](#); 2002, [197164](#);
30 Pereg et al., 2002, [199797](#)). As discussed in Section 5 on TCDD carcinogenicity, an additional
31 limitation of the epidemiological evidence includes the lack of organ specificity, as many of the

1 studies have shown associations between TCDD exposure and all-cause mortality. With
2 disagreement in the literature over the nature, scope, and quality of the epidemiological data for
3 TCDD, given the lack of precedent for a multisite carcinogen without particular sites
4 predominating, some have urged caution in the interpretation of the epidemiological data based
5 on small relative risks Popp et al. (2006, [197074](#)).

6 Despite these uncertainties, the EPA Cancer Guidelines express a clear preference for
7 epidemiological studies over animal data. The question here is whether quantitative uncertainty
8 analyses based on either a collection of bioassay data or on several epidemiological studies can
9 be combined in some overall uncertainty assessment. Diverse human studies are sometimes
10 combined into a meta-analysis, and the issues arising in this regard are instructive. A primary
11 challenge of meta-analytical approaches is combining heterogeneous effects that may result from
12 studies of different populations, study designs or analytical techniques. The question of whether
13 uncertainty arising from combining such different studies can be taken into account in
14 quantitative uncertainty analysis is similar to that of accounting for uncertainty due to missing
15 covariates in Cox regression (see Section 6.4.2.2).

16 Existing standard statistical tools are insufficient to address this issue, as they quantify
17 uncertainty in model parameters estimated from data. However, exotic methods, such as
18 Bayesian methods, probabilistic inversion, or structured expert judgment may be applicable.
19 These methods can be applied when a quantitative model *predicts* other phenomena, even though
20 these phenomena could not be used to estimate the model. The question of whether such
21 methods could remain sufficiently tethered to data, or whether structured expert judgment is
22 unavoidable, is a subject for future research.

24 **6.4.2.2. *Uncertainty in TCDD Exposure/Dose in Epidemiological Studies***

25 Uncertainties in epidemiological studies arise from a variety of study characteristics.
26 There are many types of epidemiological study designs which determine the data structure,
27 including intervention trials, case-control studies, cohort studies and cross-sectional studies. A
28 variety of mathematical models some of these can be used to analyze epidemiological data; some
29 of these includes Cox proportional hazard, Poisson regression, linear and logistic regression.
30 The model outputs are based on different measures of association such as rate ratios, risk ratios,
31 odds ratios, and standardized mortality ratios (SMRs, ratio of observed to expected deaths).

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1 Exposure uncertainties often concern back-casted exposures based on current serum lipid
2 concentrations, estimated/self reported dietary habits, fish consumption, placenta lipid
3 concentrations, and other measures.

4 Uncertainty in exposure is often dealt with by coarsely grouping a cohort into exposed
5 and unexposed groups. The output of such a study can be coarse grained in a similar way;
6 instead of computing dose-dependent risk estimates, standard mortality ratios might be used to
7 compare the exposed and unexposed groups. Packages computing the outputs routinely produce
8 confidence intervals that reflect sampling fluctuations (e.g., can indicate the potential for chance
9 to explain the association), assuming truth of the model. Additional uncertainty could be
10 factored in with exotic methods. A significant issue in epidemiological studies is the effect of
11 omitted covariates. Omitted covariates in Cox regression will bias the estimates of effects of
12 included covariates. If the omitted covariates are independent of the included covariates, the bias
13 is toward zero in absolute value (Bretagnolle and Huber-Carol, 1988, [543772](#)); if the omitted
14 covariates are not independent, little can be inferred.

15 With regard to individual studies, it might be possible to identify specific opportunities
16 for uncertainty quantification. This is illustrated here using the study of Steenland et al. (2001,
17 [198589](#)) of more than 3,500 male workers exposed to TCDD-contaminated products at eight
18 U.S. chemical plants. Each worker was assigned an exposure score based on an estimated level
19 of contact with TCDD, the degree of TCDD contamination of product at each plant over time,
20 and the fraction of a workday in contact with the product. For 170 workers, the serum TCDD
21 levels were also measured. The serum levels were back-extrapolated to the last time of exposure
22 using a constant biological half life, and regressed on the exposure scores. This regression
23 model was used to predict the dose in all workers, and predicted dose was correlated with cancer
24 mortality. Figure 6-1 shows a scatter plot of back-casted versus predicted TCDD serum levels
25 for the 170 workers on which the regression was based.

26 Given a predicted TCDD level, the uncertainty on the back-casted TCDD value could be
27 inferred from such data by various techniques. A key question is whether the actual cancer
28 mortalities among 170 back-casted workers are randomly placed in the conditional distribution
29 given predicted TCDD. Imagine, in other words, that the mortalities among the 170 back-casts
30 are colored red in Figure 6-1. At any given level of TCDD prediction, are the red points evenly
31 distributed, or are they shifted to the right? In principle, the correlation between mortality and

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1 back-casted TCDD level, given the predicted level, could be estimated. This amounts to
2 estimating heteroscedasticity in the regression model.⁶⁷ Then, for each of the 3,538 workers,
3 given his predicted TCDD level, we could sample a back-casted TCDD level, appropriately
4 correlating with mortality, and recompute the dose response analysis. Repeating this many times
5 we could build up a distribution for excess lifetime cancer mortality risk.

6 It is instructive to step through similar issues with regard to biological half life,
7 background and body fat. The Steenland et al. (2001, [197433](#)) analysis assumed a constant
8 TCDD biological half life (8.7 years). A distribution over this half life could plausibly be
9 developed from published sources. Assuming this half life is constant for all workers, but
10 uncertain (epistemic uncertainty), this distribution could easily supplement the previous
11 distribution: first sample a half life (to be applied to all workers), then estimate the regression
12 model for this half life, and sample back-casted TCDD levels given each worker's exposure
13 score, taking account of correlation with mortality. This works if the half life uncertainty is
14 epistemic. However, since the half life is estimated from data, it is more reasonable to suppose
15 that the half life varies from worker to worker (aleatoric uncertainty). Here again the correlation
16 with mortality must be taken into account, indeed it seems reasonable to suppose that the
17 256 cancer deaths involved workers with longer half lives. However, there is no way ex post of
18 determining the biological half life in the deceased workers.

19 The potential impact of uncertainty regarding background exposure and body fat is likely
20 similar to the uncertainty of estimating the half life of TCDD. Steenland et al. (2001, [197433](#))
21 held the background level constant at the median level (6.1 ppt, range 2.0 to 19.7) for
22 79 nonexposed workers from whom blood was also drawn (see also Section 6.4.2.4). The full
23 distribution of TCDD levels for these nonexposed workers could be used as well. Is it
24 reasonable to suppose that responsive workers (i.e., those exhibiting the response) have
25 background levels that are sampled randomly from this distribution, or might they not plausibly
26 come from the high end of the distribution? The analysis also assumed a constant percentage of
27 body fat (30%), whereas body fat percentage varies in the general population, e.g., for men this
28 has been reported to range from 2 to 38% or more (see Footnote in Section 6.1.3.3). The body

⁶⁷ Heteroscedasticity occurs when the variance of the dependent variable in a regression analysis varies across the data, violating the assumption of equal variance commonly used in many regression models.

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1 fat distribution in the worker population could have been ascertained, but again the question
2 arises, are the responsive workers sampled randomly from this distribution?

3 These three factors, variable half life, variable background, and variable body fat
4 percentage, might combine to make the effective dose level among the responsive workers
5 significantly higher than would appear in a study that assumes these factors to be constant.
6 However, such concerns cannot be addressed in a quantitative uncertainty analysis, unless cancer
7 mortality can be correlated with these variables. In an optimal study design, this information
8 could be retrieved from the data. However, in most observational epidemiological studies such
9 data are not available, and it might be possible to estimate these correlations in some other
10 defensible manner, in which case the effect of exposure uncertainty could be quantified and
11 propagated. Such an analysis would involve substantial effort and should not be undertaken
12 under assumptions that are themselves implausible. Protocols for epidemiological studies do not
13 currently require such uncertainty quantification. In any event, Steenland et al. (2001, [197433](#))
14 should be recognized for conscientiously identifying these key issues.

16 **6.4.2.3. *Uncertainty in Toxicity Equivalence (TEQ) Exposures in Epidemiological Studies***

17 Toxicity equivalence factors (TEFs) are used to infer the health effects of dioxin-like
18 compounds based on their relative potencies compared to TCDD. These factors are not known
19 with certainty, and the question arises whether uncertainty in TEFs can be incorporated into a
20 quantitative uncertainty analysis. The process of deriving TEFs applied by the World Health
21 Organization (WHO, 2005, [198739](#)) is described in Van den Berg et al. (2006, [543769](#)).
22 Distributions of relative potencies (REPs) were developed from the scientific literature, with
23 preference for in vivo studies, as supplemented by in vitro studies. An expert panel used a
24 consensus process to select a TEF value for each congener, in half log steps “Thus, the TEF is a
25 central value with a degree of uncertainty assumed to be at least \pm half a log, which is one order
26 of magnitude. However, it should be realized that TEF assignments are usually within the 50th to
27 75th percentile of the REP distribution, with a general inclination toward the 75th percentile in
28 order to be health protective” (Van den Berg et al., 2006, [543769](#)) (see Figure 6-2 of this
29 document).

30 The WHO considers the uncertainty in TEFs to span one order of magnitude (presumably
31 log uniformly distributed). It would be tempting to use the distributions in Figure 6-2 to quantify

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1 uncertainty in the TEFs in a quantitative uncertainty analysis. However, the issue of dependence
2 in this case is daunting. For example, should values of 1,2,3,7,8,-pentachlorodibenzofuran and
3 2,3,4,7,8-pentachlorodibenzofuran be sampled independently? The choice of dependence
4 structure will have a large effect. As described by (Van den Berg et al., 2006, [543769](#)), the
5 differences in REPs reflect differences in dosing regimens, species, endpoints, mechanisms, and
6 calculation methods. In a quantitative uncertainty analysis one must insure that these are not
7 double counted.

8
9 Reasons for significant differences in REPs for the same congener can be caused
10 by the use of different dosing regimens (acute vs. subchronic), different endpoints,
11 species, and mechanisms (e.g., tumor promotion caused by at least two different
12 mechanisms as for mono-*ortho*-substituted PCBs), as well as different methods
13 used for calculating REPs. Thus, different methodological approaches used in
14 different studies clearly provide uncertainties when deriving and comparing REPs.
15 If future study designs to derive REPs were more standardized and similar, the
16 variation in REPs when using the same congener, endpoint, and species might be
17 expected to be smaller (Van den Berg et al., 2006, [543769](#)).

18
19 Although the TEFs themselves and the distributions underlying them are based on expert
20 judgment, it is possible to incorporate these into a quantitative uncertainty analysis; however, it
21 is not simply a matter of taking the distributions in Figure 6-2 to predict the results, with
22 uncertainty, of exposure to dioxin-like compounds. The issues of dependence and double
23 counting must first be addressed. Inasmuch as the distributions are the result of expert judgment,
24 this would reasonably involve structured expert judgment as well. (Procedures for this type of
25 assessment have been developed and applied, and it would entail a significant level of effort.)

26 27 **6.4.2.4. Uncertainty in Background Feed Exposures in Bioassays**

28 TCDD is not produced intentionally but rather is formed as a byproduct of volcano
29 eruptions, forest fires, manufacturing of steel and certain chemicals (including some pesticides
30 and paints), pulp and paper bleaching, exhaust emissions, and incineration. It enters the food
31 supply primarily via aerial transport and deposition of emissions, and it bioaccumulates in animal
32 fat. In general, food of animal origin contributes to about 80% of the overall human exposure.
33 For example, Schechter et al. (1997, [198396](#)) measured dioxins in pooled food samples collected
34 in 1995 from supermarkets across the United States. Reported as parts per trillion (ppt) toxicity

1 equivalences (TEQs), fresh water fish had the highest level (1.43); followed by butter (1.07);
2 hotdog/bologna (0.54); ocean fish (0.47); cheese (0.40); beef (0.38); eggs (0.34); ice cream
3 (0.33); chicken (0.32); pork (0.32); milk (0.12); and vegetables, fruits, grains, and legumes
4 (0.07). More recent exposure studies indicate dietary levels have decreased over time. Values
5 reported for the early 2000s by Lorber et al. (2009, [543766](#)), in ppt TEQ, are: fish (0.33); beef
6 (0.12); dairy, other than milk (0.079); eggs (0.06); pork (0.036); poultry (0.018); other meat
7 (0.058); and milk (0.012).

8 These results illustrate that a person’s dietary intake of dioxins depends on the relative
9 intake of foods with high or low levels of contamination, and human background levels will vary
10 accordingly. The same applies to experimental animals in bioassays, although in those cases the
11 background intake can in principle be controlled. Some of the effects of TCDD and other AhR
12 agonists in enhancing the early initiation stages of cancers are considered to occur as a result of
13 prenatal exposures that are not included in the standard National Toxicology Program (NTP)
14 bioassay protocol (Brown et al., 1998, [051311](#); Muto et al., 2001, [548713](#)). Further, to enhance
15 reproducibility and keep statistical fluctuations to a minimum, the standard NTP assays are
16 deliberately run on groups of animals that are relatively uniform genetically, fed uniform diets,
17 and have the minimum possible exposures to toxicants other than the agent(s) being tested. This
18 tends to reduce the potential for observing the consequences of potential interactive effects that
19 might occur in the diverse human population with its variety of dietary and other exposures to a
20 wide range of potentially interacting substances and conditions.

21 A critical question is the extent to which the background exposure influences the
22 dose-response curve, and how this background should be taken into account. One idea,
23 articulated in the recent NRC (2009, [194810](#)) report on science and decisions, involves an
24 “interacting background.”⁶⁸ This can be implemented by computing a virtual dose B which,
25 according to the selected dose-response model, would explain a chosen fraction of the
26 background response. If the chosen model for dose δ is $f(\delta)$, the model can be adapted to

⁶⁸“Effects of exposures that add to background processes and background endogenous and exogenous exposures can lack a threshold if a baseline level of dysfunction occurs without the toxicant and the toxicant adds to or augments the background process. Thus, even small doses may have a relevant biologic effect. That may be difficult to measure because of background noise in the system but may be addressed through dose-response modeling procedures” (NRC, 2009).

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1 account for an interacting background by writing $f^*(\delta) = f(\delta + B) - f(B)$. This can alter the
2 model's behavior at zero dose.

3 For example, if $f(\delta) = \delta^n / (\delta^n + EC_{50}^n)$, the derivative $d(f)/d(\delta)$ is $n\delta^{n-1}EC_{50}^n / (\delta^n + EC_{50}^n)^2$,
4 which goes to zero as $\delta \rightarrow 0$, if $n > 1$. However, replacing δ with $(\delta + B)$ evidently changes the
5 derivative at zero to $nB^{n-1}EC_{50}^n / (B^n + EC_{50}^n)$. This model is not yet estimable from data, as we
6 have no way of choosing from the available animal data the fraction of background response to
7 be explained by the model when applied to humans (although judgments could be made if we
8 had better information about the details of the processes that are involved in causing various
9 human health effects). However, as a conceptual model, it serves to remind us that the manner
10 of accounting for background exposures can influence a model's behavior in the low-dose
11 region. (Note that sensitivity analyses can be done showing the consequences of assuming
12 different amounts of interacting background within the context of a specific nonlinear model.)
13

14 **6.4.2.5. Feasibility of Quantifying the Uncertainties Encountered When Choosing Specific** 15 **Studies and Subsets of Data (e.g., Species and Gender)**

16 Species, strain, gender, life stage, and other characteristics of experimental animals are
17 selected for a given study based on previous knowledge (e.g., of the species sensitivity,
18 availability of strains having little genetic variation for the endpoints in question, relevance of
19 the MOA, and degree to which the endpoints are similar for humans). Many other decisions are
20 made in designing a bioassay study; will the animals be sacrificed at the termination of the study
21 (if not a lifetime study), or will they be allowed to live out their natural lives? What dosing
22 regimen should be applied? How will the animals be fed and handled? Although such questions
23 may engender uncertainty in the minds of the experimenters, and reviewers; such uncertainty is
24 not amenable for quantitative uncertainty analysis unless and until there are quantitative models,
25 with parameters estimable from data, that can predict the effect of these choices on the response
26 function.
27

28 **6.4.2.6. Feasibility of Quantifying the Uncertainties Encountered when Choosing Specific** 29 **Endpoints for Dose-Response Modeling**

30 Standard experimental protocols guide the selection of exposure/dosing conditions for a
31 given bioassay, including the amount, delivery vehicle, route, timing, dosing frequency and

1 duration, and dose spacing. The goal is to find the dose range where the experimental animals
2 begin to respond adversely, to help anchor the lower end of the dose-response relationship, and
3 to avoid multiple experiments in which all or none of the animals respond. A common
4 recommendation is that the dose levels be chosen such that the increments in probability of
5 response are roughly equal. Hence, the choice of endpoint, dose spacing, and number of animals
6 should be made with these factors in mind. Of particular importance is the number of animals at
7 each dose level in relation to the choice of endpoint and probability of response. Using more
8 animals at the lower dose levels increases the probability of seeing some animals respond; on the
9 other hand, it will give higher weight to the low-dose responses in model fitting and uncertainty
10 quantification. Including many low-dose groups in a study with no expected response can
11 produce a bias in the event of model mis-specification (see Text Box 6-1). The conclusion with
12 regard to the feasibility of this quantitative uncertainty analysis echoes that of the previous
13 paragraph: such uncertainty is not amenable for quantitative analysis unless and until there are
14 quantitative models, with parameters estimable from data, that predict the effect of these choices
15 on the response function.

16

17 **6.4.2.7. Feasibility of Quantifying the Uncertainties Encountered when Choosing a Specific**
18 **Dose Metric (Trade-Off between Confidence in Estimated Dose and Relevance of**
19 **MOA)**

20 The concept of dose is not straightforward. To review, the Cancer Guidelines provide the
21 following taxonomy:

22

- 23 • *Exposure* is contact of an agent with the outer boundary of an organism.
- 24 • *Exposure concentration* is the concentration of a chemical in its transport or
25 carrier medium at the point of contact.
- 26 • *Dose* is the amount of a substance available for interaction with metabolic
27 processes or biologically significant receptors after crossing the outer boundary of
28 an organism.
- 29 • *Potential dose* is the amount ingested, inhaled, or applied to the skin.
- 30 • *Applied dose* is the amount of a substance presented to an absorption barrier and
31 available for absorption (although not necessarily having yet crossed the outer
32 boundary of the organism).

33

Text Box 6-1. Model Mis-Specification and Maximum Likelihood Estimation.

The maximum likelihood estimate (MLE) is widely used in statistics because of its attractive properties: *If* the true model generating the data is from the class whose parameters are being estimated, *then* under regularity conditions, the expected MLE converges to the true value, and its variance converges to zero. The caveat against what is called “mis-specification” is very important and easily overlooked. An illustration can be extracted from the NTP (2006a) data for female rat tumor incidence of cholangiocarcinoma, representative of the data which persuaded the NAS committee that the cancer dose response for dioxin was “sublinear.”

NTP (2006a) Female Rat Tumor Incidence Data for Cholangiocarcinoma					
Blood concentration (ng/kg)	2.56	5.69	9.79	16.57	29.70
Number exposed	48	46	50	49	53
Number responding	0	0	1	4	25
Relative frequency	0	0	0.02	0.08	0.47

The Hill model with MLE in this case has zero slope at zero. The default Linear Low Dose (LLD) model fits a Hill model to doses with positive responses, but it extrapolates linearly from the lowest observed nonzero response frequency. Both models have the same two parameters, but the parameter values of the Hill model used in the LLD model are different from those in Hill model fit to all doses, including doses with zero response. Although the null responses are expected on the LLD model, the Hill model has greater log likelihood since it gives higher probability to the null responses (see below).

NTP (2006a) Female Rat Tumor Incidence Data for Cholangiocarcinoma: Low-Dose Linear and Hill Models					
Blood concentration (ng/kg)	2.56	5.69	9.79	16.57	29.70
Number exposed	48	46	50	49	53
Response probability: Linear Low Dose (LLD)	0.005	0.012	0.014	0.09	0.47
Response probability: Hill model	0.00009	0.0017	0.013	0.09	0.47
Probability of cohort null response: LLD	0.77	0.58			
Probability of cohort null response: Hill	0.99	0.92			
Log Likelihood	LLD		2.46		
	Hill		2.16		

Suppose, for the sake of illustration, that the data were generated with the response probabilities from the LLD model. The Hill model would be mis-specified in this case, as the model generating the data is not a Hill model. Because of the small cohort size, the probability of null responses is such that the Hill model has greater likelihood than the LLD model with probability (based on bootstrapping) about 0.43, even though the latter, by construction, is the true model. Averaging over many simulated responses from the LLD model, the Hill model underestimates the response probabilities for doses 2.56 and 5.69 by factors of 7.5 and 2.1 respectively. In the event of such mis-specification, the bias in the Hill model would be aggravated by including more 50-rat experiments with doses lower than 2.56.

- 1 • *Absorbed dose* is the amount crossing a specific absorption barrier (e.g., the
2 exchange boundaries of skin, lung, and digestive tract) through uptake processes.
- 3 • *Internal dose* is a more general term, used without respect to specific absorption
4 barriers or exchange boundaries. *Delivered dose* is the amount of the chemical
5 available for interaction by any particular organ or cell

6
7 Due to their greater causal proximity to the affected organs, using the absorbed dose or
8 internal dose would yield statistically more powerful results and enable more precise predictions
9 than potential dose. If it is not possible to measure these or they were not measured during the
10 conduct of the study (as is commonly the case), then other available dose metrics, such as
11 potential dose or exposure, are used. Due to toxicokinetic variability, different individuals
12 receiving the same exposure may not have the same absorbed dose. Hence, use of either
13 exposure or exposure concentration adds variability to the predicted results. The dose metric
14 should be selected that (1) has the most proximate possible causal relation to the production of an
15 adverse health endpoint, and (2) can be readily related to the units of (external) exposure that
16 will be the basis for assessing human exposures.

17 18 **6.4.2.8. Feasibility of Quantifying the Uncertainties Encountered When Choosing Model** 19 **Type and Form**

20 The EPA (2009, [522927](#)) draft white paper on probabilistic methods notes: “There is no
21 consensus on any one well-accepted general methodology for dealing with model uncertainty,
22 although there are various examples of efforts to do so.” Model uncertainty was introduced in
23 Section 6.1.3.4. Many statistical techniques are available to evaluate model adequacy or to
24 choose a “best” model. Although it is tempting to qualify such deliberations as “uncertainty that
25 a model is true,” one must remember that all models, being idealizations, are false. Ultimately,
26 one is interested in uncertainty with regard to observable phenomena, not with regard to models.
27 Models are merely tools for describing the phenomena. Nonetheless, the choice of a model
28 constrains the ways in which uncertainty can be represented, so the question is how to deal with
29 these constraints. A recent study of uncertainty modeling in dose response (Cooke, 2009,
30 [543763](#)) addresses precisely this issue and provides technical details to frame possible options.

31 Before exploring exotic approaches to model uncertainty (i.e., those not yet widely used
32 in dose-response analyses), one feature in the standard statistical treatment of uncertainty must

1 be appreciated. Consider a model based on experimental data, typically bioassay data, in which
2 a certain number of study subjects are exposed to varying doses of a test substance, and in which
3 the numbers of subjects exhibiting a response are tallied. Values for the parameters in the model
4 are chosen by the principle of maximal likelihood: those values are chosen which render the data
5 as likely as possible. According to standard practice, a model is chosen that best fits the data
6 according to one of the accepted criteria, such as reduced R^2 , or the Akaike Information
7 Criterion. There might be many incompatible models that are nearly as good.

8 One can ask the following: If the experiments on which the model is based were repeated,
9 sampling the same number of experimental subjects from the distribution posited by the model,
10 how much could our parameter estimates change? This is described by a joint distribution over
11 the model's parameters, which captures sampling uncertainty under the assumption that the
12 model is true. Now, all models are false, and as our sample sizes grow the lack of fit in the
13 model becomes increasingly apparent. At the same time, the sample fluctuations in parameter
14 estimates—*assuming the model is true*—become smaller and smaller. In very large
15 epidemiological studies, standard statistical methods can produce razor-thin confidence bands in
16 this way, which fail to capture experts' uncertainty regarding observable phenomena.⁶⁹

17 The exotic methods sketched in the beginning of Section 6.4.2 may be viewed as attempts
18 to deal with this feature. Probabilistic inversion methods were deployed on a large scale in the
19 joint U.S. NRC-EU uncertainty analyses noted in Section 6.1. Distributions over model
20 parameters are intended to capture an antecedently defined uncertainty over observable
21 phenomena predicted by the model. This method was applied in dispersion and deposition
22 modeling and further environmental transport models (including uptake) for radionuclides. In
23 most cases, the observable uncertainty was based on structured expert judgment, but it has also
24 been based on binomial uncertainty in bioassay studies. A potential drawback is that it may not
25 prove possible to capture the observable uncertainty in this way with a classically best-fitting
26 model, and new models may be required.

27 Nonparametric Bayesian methods arose in the biomedical and reliability fields. They
28 start with a prior distribution over all nondecreasing dose-response functions, and update these

⁶⁹See, for example, Tuomisto et al. (2008, [548715](#), Table 6) for a comparison of experts' uncertainty in health effects of fine particulates with uncertainties derived from sampling uncertainty from large epidemiological studies. Although the experts generally agree with the studies' central estimates, their uncertainty bands are often much wider than those surrounding the published estimates.

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1 with observations from a bioassay. No further assumptions regarding parametric form are
2 introduced, but the prior distribution remains important for doses outside the range of
3 observations. Bayesian model averaging starts with a prior distribution over a set of candidate
4 models, and updates this distribution with bioassay data. The method is flexible and intuitive,
5 though attenuation of the effect of the prior on the posterior must be verified.

6 All these approaches represent attempts to capture “extramodel uncertainty,” that is,
7 uncertainty that is not conditional on the truth of the model. This is an active research area, and
8 improvements in methods for capturing extramodel uncertainty in quantitative uncertainty
9 analysis are anticipated. A major effort with regard to TCDD dose-response would be indicated
10 when the strengths and weakness of the exotic methods are well understood.

11 12 **6.4.2.9. Threshold MOA for Cancer**

13 The NAS committee avers that knowledge of the AhR binding MOA implies that there is
14 a response threshold for TCDD cancer induction. The differences between individual and
15 population thresholds are not discussed, but the following two possibilities are distinguishable:

- 16
17 1. The threshold is the same for each individual; since human variability in AhR binding
18 affinity is rather large (see Section 5.2.3.3), this entails that the threshold is not affected
19 by the binding affinity.
20 2. The threshold varies across individuals and is related to the individual AhR binding
21 affinity.
22

23 These two positions are different. As shown in Section 5.2.3 it is quite possible that each
24 individual in a population has a threshold, whereas the population dose-response relation is
25 linear. Because the NAS committee does not distinguish which of these positions it holds, the
26 feasibility of quantitative uncertainty analysis is examined here for both.

- 27
28 i. Quantitative uncertainty analysis concerns a mathematical model. In case (1), this model
29 would show how the existence of the AhR binding would induce a threshold,
30 independently of the strength of the binding. Assessing the feasibility of quantitative
31 uncertainty analysis must await the elaboration of such a model.
32 ii. In case (2), it must be shown that the distribution of thresholds, and the dose-response
33 function above the threshold, are able to induce a population “zero slope at zero dose”
34 (ZS@Z) model. Recall, the burden of proof is on this (ZS@Z) model. Scoping the

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1 population variability with regard to AhR-mediated mechanisms in general, and dioxin
2 sensitivity in particular, is an active area of research. It involves phenotyping human
3 AhR-mediated responsiveness and relating this to polymorphisms in the human
4 population. Harper et al. (2002, [198124](#)) report that a 10-fold variation in binding
5 affinity of AhR for TCDD in human placental tissue did not reveal any polymorphisms,
6 suggesting that the relation between phenotypical and genotypical variation is tenuous.
7 Tuomisto et al. (1999, [548717](#)) demonstrate large variations in efficacy in two rat strains
8 whose binding affinity is similar (Long-Evans, $K_d = 3.4$, Han/Wistar, $K_d = 3.9$ (as also
9 discussed in Connor and Aylward, 2006, [197632](#))), and they also show that this variation
10 is endpoint-specific. The responses in both strains are similar for cytochrome P450
11 (CYP)1A1 induction, but very dissimilar for thymus atrophy, serum bilirubin, and
12 mortality. Toide et al., (2003, [548792](#)) suggest that common biochemical measures of
13 EROD activity might be mediated by CYP1B1 and CYP1A2. The differences in serum
14 bilirubin at doses around 10 $\mu\text{g}/\text{kg}$ are about a factor of 30. Han/Wistar rats seldom die at
15 this dose, while mortality of Long Evans rats is about 50%. The mechanisms are not
16 understood.

17
18 Although the mass action dose-response model does not have a threshold, it is possible
19 that certain enzymes block the receptor binding, and until these are overwhelmed, no response
20 occurs. The availability of such enzymes may vary from individual to individual, and may or
21 may not covary with the dissociation constant, K_d . Pursuing these lines of research may result in
22 a convincing demonstration of a population (ZS@Z) model. Such a model would express the
23 individual threshold in terms of parameters that could be estimated with uncertainty from the
24 data.

25 26 **6.4.2.10. Feasibility of Quantifying the Uncertainties Encountered when Selecting the BMR**

27 The NAS committee explicitly requested that the uncertainty attending the choice of a
28 BMR be quantified. Although selecting relevant alternative values for the BMR may provide
29 information of interest, it does not constitute a quantitative analysis of uncertainty. The
30 alternative values must be sampled from some uncertainty distribution. Since this concerns
31 volitional uncertainty, there is no underlying distribution from which to sample, unless the
32 choice of BMR is related to some claim about the state of the world.

33 However, in response to the NAS concerns, this document provides some limited
34 quantitative comparisons of BMR choices. BMDs, BMDLs and OSFs from the animal cancer
35 bioassay benchmark dose modeling assuming 1, 5, and 10% extra risk are compared in units of
36 blood concentrations and human equivalent doses in Tables 5-18 and 5-19, respectively. In

1 addition, MLE and upper bound slope factor estimates based on Cheng et al. (2006, [523122](#)) are
2 presented (see Tables 5-3 and 5-4). For the noncancer effects, key animal study PODs
3 (ng/kg-day) are shown based on different dose metrics: administered dose, first-order body
4 burden HED, and blood concentration (see Tables 4-3 and 4-4).

6 6.5. CONCLUSIONS REGARDING THE FEASIBILITY OF QUANTITATIVE 7 UNCERTAINTY ANALYSIS

8 In this section the main conclusions regarding the feasibility of quantitative uncertainty
9 analysis are summarized in relation to specific suggestions made by the NAS committee (see
10 Section 6.5.1). Following this, a suggested research agenda for moving forward in this area is
11 provided (see Section 6.5.2).

13 6.5.1. Summary of NAS Suggestions and Responses

14 On page 130 of their report (NAS, 2006, [198441](#)), NAS makes specific suggestions
15 regarding uncertainty quantification. These are reformatted and presented in italics below.
16 Following each suggestion, a summary of the discussion in this section is given, with reference
17 to the section in which it is addressed.

19 *EPA should have addressed quantitatively the following sources of uncertainty:*

- 21 • *Basis for risk quantification:*
 - 22 1. *bioassay data,*
 - 23 2. *occupational cohort data.*

24
25 **Response:** (1) Classical statistical methods yield distributions on model parameters
26 which reflect sample fluctuations, assuming that the model is true. This type of
27 uncertainty is taken into account in the BMDL. Exotic methods can account for
28 uncertainty which is not conditional on the truth of a model, at least for bioassay data
29 (see Section 6.4.2). (2) For epidemiological data, the dose reconstruction often involves
30 assumptions which may support data driven uncertainty analysis, if sufficient data can
31 be retrieved. Examples discussed above include back-casted TCDD level, biological
32 half life, body fat and background (see Section 6.4.2.2). Uncertainty in the choice of
33 bioassay data sets or choice of occupational cohort data sets is volitional, and is not
34 quantified by sampling an input distribution. To be amenable for quantitative
35 uncertainty analysis, the choice must be linked to a statement about the state of the
36 world (see Section 6.1.1).

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- 1 • *Epidemiology data to use:*
 - 2 1. *risk estimate developed with data aggregated from all suitable studies,*
 - 3 2. *risk estimate or estimates developed using each study individually.*
- 4 • *Factors affecting extrapolation from occupational to general population cohorts,*
5 *including differences in baseline health status, age distribution, the healthy worker*
6 *survivor effect, and background exposures.*

7
8 **Response:** (1) Quantitative uncertainty analysis based on meta-analysis data poses
9 challenges owing to differences in study protocols. Exotic methods might take us further,
10 the question is whether the restriction to data driven methods (as opposed to expert
11 judgment or Bayesian methods) could be maintained (see Sections 6.4.2.2 and 6.4.2.3).
12 (2) If the general population is characterized by distributions over known confounders
13 whose coefficients are estimated from the epidemiological studies, then uncertainty over
14 these coefficients can be extracted with the methods mentioned in Section 6.4.2.1.
15 Uncertainty due to missing covariates is intractable for data driven uncertainty analysis
16 (see Section 6.4.2.2).

- 17 • *Bioassay data to use:*
 - 18 1. *risk estimate developed with the single data set implying the greatest risk (that is,*
19 *single study, tumor site, gender),*
 - 20 2. *risk estimate developed with multiple data sets satisfying an a priori set of*
21 *selection criteria.*

22
23 **Response:** (1) Uncertainty in choice of data sets is volitional and is not quantified by
24 sampling an input distribution. To be amenable for quantitative uncertainty analysis the
25 choice must be linked to a statement about the state of the world (see Section 6.1.1).
26 (2) The issue here is similar to the meta-analysis addressed in (2.a).

- 27 • *Dose-response model:*
 - 28 1. *linear dose response,*
 - 29 2. *nonlinear dose.*

30
31 **Response:** (1) When low dose extrapolation is done using a linear model by default, the
32 uncertainty is volitional. To be amenable for quantitative uncertainty analysis, the choice
33 must be linked to a statement about the state of the world (see Section 6.1.1). The ED_x as
34 POD for the linear extrapolation can be subjected to quantitative uncertainty analysis, if
35 based on sufficient bioassay data. (2) With respect to nonlinear dose response, it is
36 possible that human thresholds exist, and that the distribution of thresholds can be
37 characterized in the human population. In as much as the mechanisms for this are not yet
38 understood, there is no quantitative model expressing threshold as a function of
39 parameters which could be estimated, with uncertainty, from data. This currently limits
40 the application of uncertainty quantification (see Section 6.4.2.9).

- 1 • *Dose metric:*
 - 2 1. *average daily intake,*
 - 3 2. *area under the blood concentration-time curve,*
 - 4 3. *lifetime average body burden,*
 - 5 4. *peak body burden,*
 - 6 5. *other.*

7
8 **Response:** (1-5) The dose metric is chosen to maximize causal proximity to the endpoint,
9 while maintaining the link to measured exposure (see Section 6.4.2.7). There may be
10 uncertainty with regard to which metric is optimal. If an inappropriate metric is chosen
11 in a bioassay study, this would be expressed in noisier responses which would tend to
12 suppress the dependence of endpoint on dose. A data driven quantitative uncertainty
13 analysis of dose metric would require a mathematical model expressing endpoints as a
14 function, inter alia, of dose metric, with parameters estimated from data.

- 15 • *Dose metric—biological measure:*
 - 16 1. *free dioxin,*
 - 17 2. *bound dioxin.*

18
19 **Response:** (1–2) The issue is whether all TCDD available for AhR binding, or only the
20 bound TCDD, should be used as a dose metric. Binding affinity is determined by more
21 factors than genetic polymorphisms and these other factors are poorly understood (see
22 Section 6.4.2.9). A quantitative uncertainty analysis must await the formulation of a
23 quantitative model expressing binding affinity in terms of parameters which can be
24 estimated from data.

- 25 • *POD:*
 - 26 1. *ED₁₀,*
 - 27 2. *ED₀₅,*
 - 28 3. *ED₀₁*

29
30 **Response:** (1–3) Uncertainty in choosing a POD is volitional. Uncertainty in the value
31 of an ED_x can be quantified in a data driven manner if sufficient bioassay data is at hand
32 (see Section 6.4.1.1).

- 33 • *Value from ED distribution to use:*
 - 34 1. *ED,*
 - 35 2. *lower confidence bound value for the ED (LED),*
 - 36 3. *upper confidence bound for the ED (UED).*
- 37

1 **Response:** (1–3) Given that uncertainty on the POD is quantified, a distribution of the
2 slopes of a linear low dose extrapolation is readily derived, and hence a distribution of a
3 risk specific dose.

- 4 • *Where alternative assumptions or methodologies could not be ruled out as implausible or*
5 *unreasonable, EPA could have estimated the corresponding risks and reported the*
6 *impact of these alternatives on the risk assessment results. The potential impacts of four*
7 *sources of uncertainty are discussed below.*

- 8 1. *The full range of plausible parameter values for the dose-response functions used*
9 *to characterize the dose-response relationship for the three occupational cohort*
10 *studies selected by EPA (Becher et al., 1998, [197173](#); Ott and Zober, 1996,*
11 *[198408](#); Steenland et al., 2001, [197433](#))).*
- 12 2. *Use of other points of departure, not just the ED₀₁ (or LED₀₁), to develop a CSF.*
- 13 3. *Alternative dose-response functional forms as well as goodness of fit of all*
14 *models, especially at low doses.*
- 15 4. *Uncertainty introduced by estimation of occupational exposures.*

16
17 **Response:** (1) The study of Steenland et al. (2001, [197433](#)) was selected to illustrate the
18 possibilities and limitations of quantitative uncertainty analysis for this type of study (see
19 Section 6.4.2.2). (2) The possibilities for uncertainty quantification with regard to the
20 POD are discussed in Section 6.4.1.1 and in the POD bullet above. (3) Goodness of fit at
21 any measured dose is evaluated in standard packages. There may be different models
22 with comparable goodness of fit at observed doses which differ strongly at doses outside
23 the measured range. Extra model uncertainty, that is, uncertainty which is not conditional
24 on the truth of any given model, is addressed by the exotic methods (see Section 6.4.2).
25 (4) The feasibility of quantifying uncertainty in occupational exposure is study specific.
26 The example of Steenland et al. (2001, [197433](#)) was discussed in some detail (see
27 Section 6.4.2.2). In general, the problem is not so much quantifying the exposure
28 uncertainty, but in quantifying the dependence between the endpoints and the exposure
29 uncertainty.
30

31 **6.5.2. How Forward? Beyond RfDs and Cancer Slope Factors to Development of** 32 **Predictive Human Dose-Response Functions**

33 Uncertainty quantification is an emerging area in science. There are many examples of
34 highly vetted and peer-reviewed uncertainty analyses based on structured expert judgment.
35 Under this process, experts in effect synthesize a wide diversity of information in generating
36 their subjective probability distributions. Where considerable data exist for an environmental
37 pollutant, such as for the well-studied TCDD, it is natural to ask whether these extensive data can
38 be leveraged more directly in uncertainty quantification. This is an area where research could be
39 focused. The requisite knowledge does not yet exist, but there are promising lines of attack. It is

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1 therefore not a question of convening blue-ribbon panels to reveal the proper approach; instead
2 multiple approaches should be encouraged, to try out new ideas and share experiences.

3 An important idea that has been pioneered in Europe is to organize bench-test exercises
4 where different approaches are applied to a common problem. This focuses the discussion on
5 real issues and builds a community of capable practitioners. Such initiatives have proven much
6 more productive than simply supporting individual researchers to explore their ideas.

7 Areas for which bench-test exercises might be appropriate include:

- 8
- 9 • Testing “exotic” methods for capturing model uncertainty;
- 10 • Combining bioassay and epidemiological data for uncertainty quantification;
- 11 • Assessing applicability of structured expert judgment, e.g., for low-dose extrapolation;
12 and
- 13 • Conducting dependence modeling, dependence inference, and dependence elicitation
14 (such as with regard to TEFs).
15

16 Looking beyond compounds for which considerable data exist, there will always be a
17 need to evaluate new substances. The target will be a simple method that:

- 18
- 19 1. Can yield predictions of toxicological indicators with uncertainty via a valid probabilistic
20 mechanism;
- 21 2. Could evolve from approaches based on similarities (such as a random chemical model)
22 under which the new substance could be seen as a random sample from a reference
23 distribution of chemicals considered sufficiently similar, e.g., in terms of structure,
24 physicochemical properties, and biological activity (potency); and
- 25 3. Is consistent with current risk assessment science and approaches, peer-reviewed and
26 accepted as EPA policy.
27

28 This last feature is important because advancements in risk assessment approaches should
29 extend logically from current methodology based on data analysis and scientific methods. For
30 example, the discussion surrounding uncertainty factors suggests that a probabilistically valid
31 inference system could substantially differ from the current system. Nonetheless, to meld with
32 current practice, it must initialize on the current system and allow this system to evolve in a
33 measured fashion. Ideally, methodological changes should be undertaken in a forum where such
34 issues are being addressed and not within an assessment of a single chemical.

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1 Additional research topics relevant to dioxin that could further inform health assessments
2 include population variability of biokinetic constants, threshold mechanisms for the mass action
3 model, and low-frequency polymorphisms (e.g., less than 1%). Further data and improved
4 methodologies in these areas, combined with developments illustrated elsewhere in this report,
5 will help reduce uncertainties and strengthen our understanding of potential health implications
6 of environmental contaminants.

1
2

Table 6-1. Key sources of uncertainty

<p>Selection of endpoint and of species/strain, gender, life stage, other subject characteristics</p> <ul style="list-style-type: none"> - critical effect - sensitivity (e.g., species, life stage) - human relevance
<p>Selection of key study(ies): human data and bioassays (strength, inclusion criteria)</p> <ul style="list-style-type: none"> - epidemiological studies, clinical/case reports (exposure estimate) - adequacy of study design, statistical power (exposure term, histopathology) - human relevance of bioassays (TK, MOA, endpoint) - data uncertainty, confidence in data; database deficiencies
<p>Use of TK, dosimetry; body burden; species differences, cross-species extrapolation</p> <ul style="list-style-type: none"> - bioavailability, dose dependence - half life, life stage, body fat, other compartments, age, other factors - body burden (peak, steady state, lifetime average) - physiologically-based pharmacokinetic (PBPK) modeling - scaling (human equivalents), adjustments (default and nondefault; with TD)
<p>Selection of dose metric</p> <ul style="list-style-type: none"> - intake (averaging time) - background (what place on the dose-response curve) - free vs. receptor-bound TCDD - tissue-specific concentration - lipid-normalized level
<p>Selection of POD</p> <ul style="list-style-type: none"> - selection (e.g., NOAEL/LOAEL, BMDL, ED_{01, 05, 10}) - derivation method (e.g., BMD) - choice of model form (e.g., Hill, Weibull) - statistical uncertainty at/confidence in POD
<p>Selection of dose-response model (e.g., biologically based, multistage) and of BMR</p> <ul style="list-style-type: none"> - biological plausibility, MOA - model type and form, alternative functional forms - range of plausible parameter values - goodness of fit, especially at low doses
<p>Selection of low-dose extrapolation approach</p> <ul style="list-style-type: none"> - linear/nonlinear - threshold/nonthreshold
<p>Human population variability</p> <ul style="list-style-type: none"> - subpopulations (e.g., occupational, general public, sensitive groups) - polymorphisms - life stage, other features - individual vs. population threshold
<p>Characterization of risk/effect</p> <ul style="list-style-type: none"> - adversity of effect (vs. in normal range of variation and adaptation) - uncertainty factors (TK; TD; chemical-specific vs. default; justification) - consistency of methods for endpoints with common MOA - back-extrapolation from occupational data - MOE, RfD; beyond a point estimate for SF

3
4

PBPK = physiologically-based pharmacokinetic; SF = slope factor; TD = toxicodynamic;
TK = toxicokinetic. (Other acronyms are as defined elsewhere within this section.)

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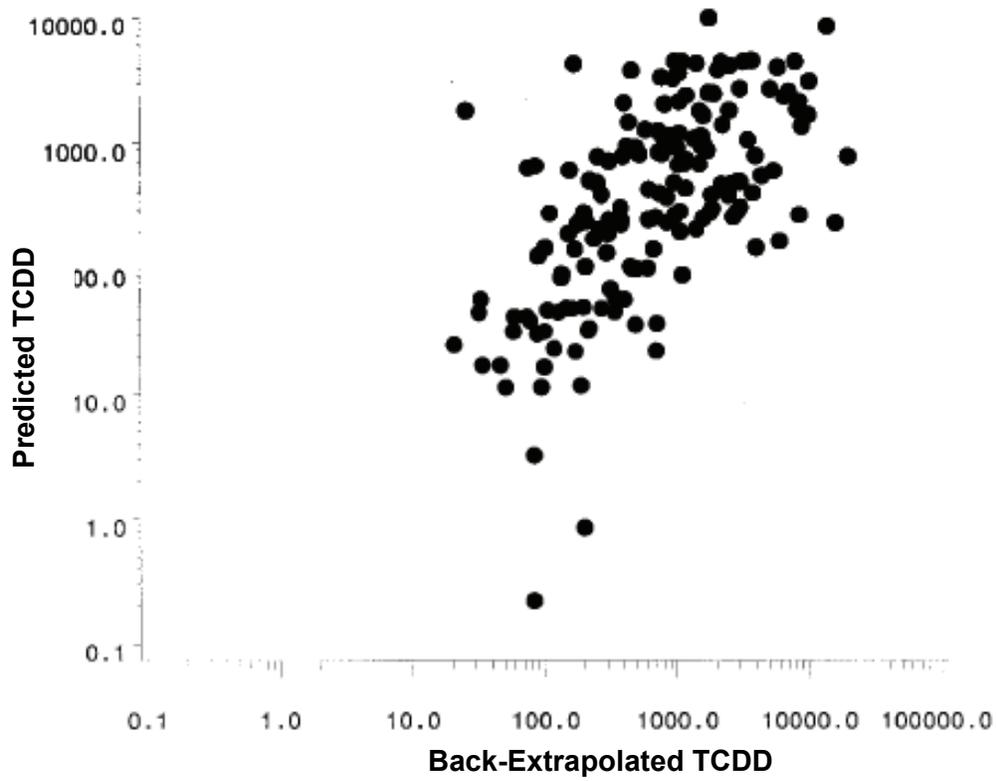
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Table 6-2. PODs and amenability for uncertainty quantification

POD	Data profile	Choice	Uncertainty quantification
LOAEL	Experimental dose level from set of exposure-response data	Choose set of exposure-response measurements	No
NOAEL	Experimental dose level from set of exposure-response data	Choose set of exposure-response measurements	No
BMDL	Estimate from bioassay data	Choose BMR, choose dose-response relation	No, the BMDL is a quantile of an uncertainty distribution assuming that the dose-response model is true
ED _x	Estimate from set of exposure-response data	Choose bioassay experiments to estimate ED _x	Yes, if full bioassay data are available

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Figure 6-1. Back-casted vs. predicted TCDD serum levels for a worker subset.

Source: Steenland et al. (2001, [197433](#)).

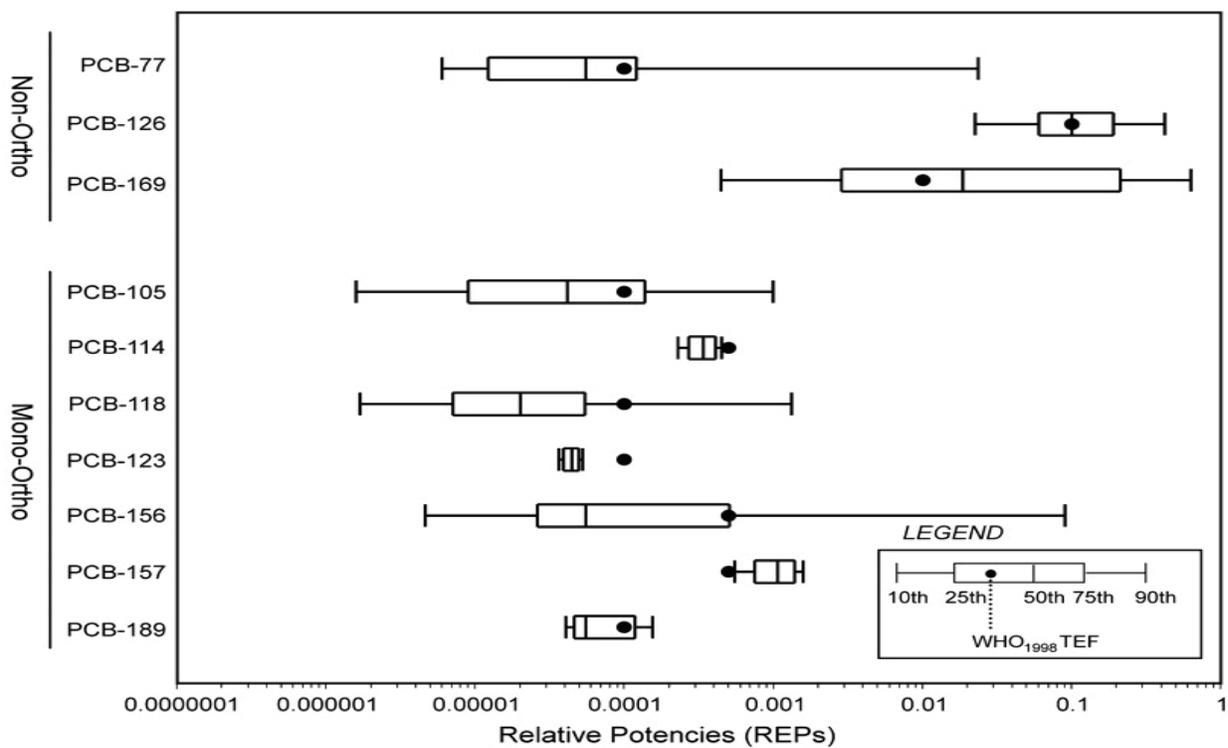
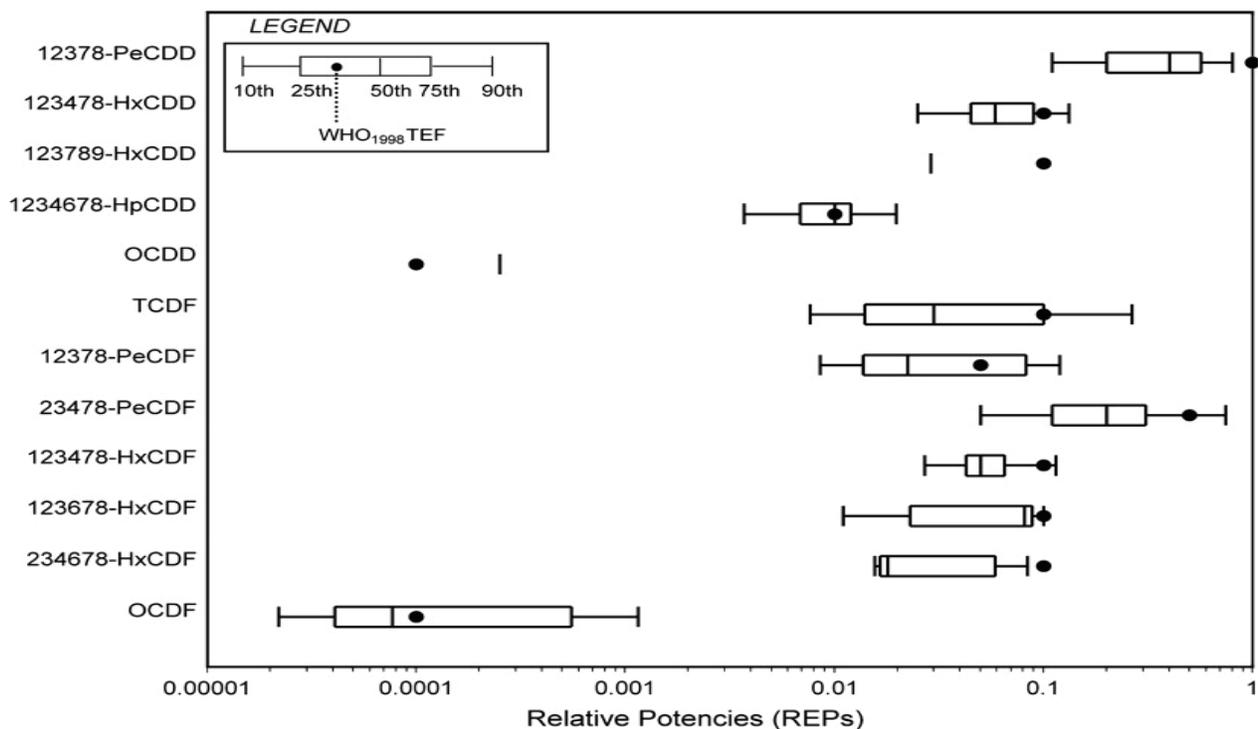


Figure 6-2. Distribution of in vivo unweighted REP values in the 2004 database.

Source: Van den Berg et al. (2006, [543769](#)), reprinted with permission from Haws et al. (2006, [198416](#)).

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