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Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) and Related Compounds

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

NOTICE

THIS DOCUMENT IS A PRELIMINARY DRAFT. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

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DISCLAIMER

This document is a draft for review purposes only and does not constitute U.S. Environmental Protection Agency policy. It has been provided for review to the National Academy of Sciences (NAS). While the NAS review is being conducted and until a final agency assessment has been released, the draft dioxin reassessment (2003 version or other draft versions) remains draft, does not represent a final position, and is not intended to serve as the basis or rationale for regulatory and other policy action. However, EPA will continue its work to reduce human exposure to dioxin.

While the NAS review is underway and no final reassessment has been issued, in meeting their regulatory responsibilities, the agency will continue its current practice of utilizing the best available data that meet the EPA Information Quality Guidelines and the government-wide Information Quality Guidelines issued by OMB. T he Agency will consider all such data and associated uncertainty to determine the strength of the evidence in proposing regulatory actions related to dioxin and dioxin-like compounds.

Exposure and Human Health Reassessment of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) and Related Compounds

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Ah	aryl hydrocarbon
AHF	altered heptacellular foci
AhR	aryl hydrocarbon receptor
ALK	alkaline phosphatase
ALT	alanine aminotransferase
Arnt	aryl hydrocarbon receptor nuclear translocator
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BaP	benzo[a]pyrene
BDD	brominated dibenzodioxin
BDF	polybrominated dibenzofuran
BMD	benchmark dose
BW	body weight
CDC	Centers for Disease Control and Prevention
CDD	chlorinated dibenzodioxin
CFD	chlorinated dibenzofuran
CI	confidence interval
CTL	cytotoxic T lymphocyte
CYP1A1	cytochrome P4501A1 enzyme
CYP1A2	cytochrome P4501A2 enzyme
CYP1B1	cytochrome P4501B1 enzyme
DFP (subscript)	dioxins, furans, PCBs
DEN	diethylnitrosamine
DHT	5α-dihydrotestosterone
DNA	deoxyribonucleic acid
ED	effective dose
ED_{01}	effective dose at the 1% response level
EDC/VC	ethylene dichloride/vinyl chloride
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
EPA	U.S. Environmental Protection Agency
FSH	follicle-stimulating hormone
g	gram
GD	gestation day
GGT	gamma glutamyl transferase
HAH	halogenated aromatic hydrocarbons
HCDD	hexachlorodibenzo-*p*-dioxin
HIF	hypoxia-inducible factor
HpCDD	heptachlorodibenzo-*p*-dioxin

hr	hairless
IARC	International Agency for Research on Cancer
ID	immunosuppressive dose
IgA	immunoglobulin A
I-P	initiation-promotion
IPCS	International Programme on Chemical Safety (WHO)
I-TEQ	international TEF scheme adopted by EPA in 1989
kg	kilogram
L	liter
LABB	lifetime average body burden
LED_{01}	lower bound of the effective dose at the 1% response level
LH	luteinizing hormone
LMS	linearized multistage
LOAEL	lowest-observed-adverse-effect level
MOE	margin of exposure
mRNA	messenger ribonucleic acid
MRL	minimal risk level (ATSDR)
NHANES	National Health and Nutrition Examination Survey
NHATS	National Human Adipose Tissue Survey
ng	nanogram
NIOSH	National Institute for Occupational Safety and Health
NTP	National Toxicology Program
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
OCDD	octachlorodibenzo-*p*-dioxin
pg	picogram
PAH	polycyclic aromatic hydrocarbon
PBPK	physiologically based pharmacokinetic
PBDD	polybrominated dibenzodioxin
PBDF	polybrominated dibenzofuran
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
PCP	pentachlorophenol
PCQ	polychlorinated quaterphenyl
PeCDD	pentachlorodibenzo-*p*-dioxin
PeCDF	pentachlorodibenzo-*p*-furan
PK	pharmacokinetic
POD	point of departure
POTW	publicly-owned treatment works

ppt	part per trillion
PVC	polyvinyl chloride
REP	relative potency
RfD	reference dose (EPA)
RR	relative risk
SAB	U.S. EPA's Science Advisory Board
SMR	standardized mortality ratio
SRBC	sheep red blood cells
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
TDG	thyroid binding globulin
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TCP	trichlorophenol
TDI	tolerable daily intake
TEF	toxic equivalency factor
TEQ	toxic equivalent
TEQ-WHO_{94}	1994 WHO extension of the I-TEF scheme to include 13 dioxin-like PCBs
TEQ-WHO_{98}	1998 WHO update to the previously established TEFs for dioxins, furans, and dioxin-like PCBs
TPA	tetradecanoyl phorbol acetate
TNP-LPS	trinitrophenyl-lipopolysaccharide
TSH	thyroid stimulating hormone
URL	unit risk level
WHO	World Health Organization

~	approximately
>	greater than
<	less than
≥	greater than or equal to
≤	less than or equal to
µg	microgram

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1. INTRODUCTION

This document presents an integrated summary of available information related to exposure to and possible health effects of dioxin and related compounds. It also presents a short risk characterization, which is a concise statement of dioxin science and the public health implications of both general population exposures from environmental "background"[1] and incremental exposures associated with proximity to sources of dioxin and related compounds. Even though this document is a summary of key findings developed in the exposure and health assessment portions (Parts I and II, respectively) of the U.S. Environmental Protection Agency's (EPA *or* Agency) dioxin reassessment, it is meant to be detailed enough to stand on its own for the average reader. Readers are encouraged to refer to the more detailed documents, cited below, for further information on the topics covered here and to see complete literature citations.

Estimating Exposure to Dioxin-Like Compounds: This document, hereafter referred to as Part I, the Exposure Document, is divided into 3 volumes: (1) Sources of Dioxin-Like Compounds in the United States; (2) Properties, Environmental Levels, and Background Exposures; and (3) Site-Specific Assessment Procedures.

Health Assessment for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and Related Compounds: This document, hereafter referred to as Part II, the Health Document, contains two volumes with nine chapters covering pharmacokinetics, mechanisms of action, epidemiology, animal cancer and various noncancer effects, toxic equivalency factors (TEFs), and dose-response.

Parts of this integrative summary and risk characterization go beyond individual chapter findings to reach general conclusions about the potential impacts of dioxin-like compounds on human health. This document specifically identifies issues concerning the risks that may be occurring in the general population at or near population background exposure levels. It

[1]The term "background exposure" has been used throughout this reassessment to describe exposure that regularly occurs to members of the general population from exposure media (food, air, soil, etc.) that have dioxin concentrations within the normal background range. Most (> 95%) background exposure results from the presence of minute amounts of dioxin-like compounds in dietary fat, primarily from the commercial food supply. The origin of this background exposure is from three categories of sources: naturally formed dioxins, anthropogenic dioxins from contemporary sources, and dioxins from reservoir sources. The term "background exposure" as used in this document should not be interpreted as indicating the significance or acceptability of risk associated with such exposures.

articulates the strengths and weaknesses of the available evidence for possible sources, exposures, and health effects, and it presents assumptions made and inferences used in reaching conclusions regarding these data. The final risk characterization provides a synopsis of dioxin science and its implications for characterizing hazard and risk for use by risk assessors and managers inside and outside the EPA and by the general public.

This document (Part III) is organized as follows:

1. Introduction. This chapter describes the purpose/organization of and the process for developing the report, defines dioxin-like compounds in the context of the EPA reassessment, and explains the toxic equivalence (TEQ) concept.

2. Effects Summary. This chapter summarizes the key findings of the Health Document and provides links to relevant aspects of exposure, mechanisms, and dose-response.

3. Mechanisms and Mode of Dioxin Action. This chapter discusses the key findings on effects in terms of mode of action. It uses the "Mode-of-Action Framework" recently described by the World Health Organization/(WHO) International Programme on Chemical Safety (IPCS) Harmonization of Approaches to Risk Assessment Project and contained in the Agency's draft guidelines for carcinogen risk assessment as the basis for the discussions.

4. Exposure Characterization. This chapter summarizes the key findings of the Exposure Document and links them to the effects, mechanisms, and dose-response characterization.

5. Dose Response Characterization. This chapter summarizes approaches to dose-response that are found in the Health Document and provides links to relevant aspects of exposure and effects.

6. Risk Characterization. This chapter presents conclusions that are based on an integration of the exposure, effects, mechanisms, and dose-response information. It also highlights key assumptions and uncertainties.

The process for developing this risk characterization and companion documents has been open and participatory. Each of the documents has been developed in collaboration with

scientists from inside and outside the Federal Government. Each document has undergone extensive internal and external review, including review by EPA's Science Advisory Board (SAB). In September 1992, early drafts of all the background chapters underwent external peer review. This was followed by extensive revision and re-review of the epidemiology chapter in September 1993. In September 1994, drafts of each document, including an earlier version of this risk characterization, were made available for public review and comment, which included a 150-day comment period and 11 public meetings around the country to receive oral and written comments. These comments, along with those of the SAB, have been considered in the drafting of this final document. The dose-response chapter of the Health Document underwent peer review in 1997; an earlier version of this Integrated Summary and Risk Characterization underwent development and review in 1997 and 1998, and comments have been incorporated.

In addition, as requested by the SAB, a chapter on toxic equivalency has been developed and underwent external peer review in parallel with the Integrated Summary and Risk Characterization in July 2000. Review by the SAB of the dose-response chapter, the toxic equivalency chapter, and the Integrated Summary and Risk Characterization occurred in November 2000. The report of that review was submitted to the EPA Administrator on May 31, 2001. These sections of the document, as well as a few of the other background chapters in Parts I and II, have been revised to reflect the comments of the SAB and the public. The comprehensive set of background documents and this integrative summary and risk characterization are now being published as final reports to replace previous dioxin assessments as the scientific basis for EPA decision making.

1.1. DEFINITION OF DIOXIN-LIKE COMPOUNDS

As defined in Part I of this document, this assessment addresses specific compounds in the following chemical classes: polychlorinated dibenzo-*p*-dioxins (PCDDs or CDDs), polychlorinated dibenzofurans (PCDFs or CDFs), polybrominated dibenzo-*p*-dioxins (PBDDs or BDDs), polybrominated dibenzofurans (PBDFs or BDFs), and polychlorinated biphenyls (PCBs); these chemicals are described as "dioxin-like." Dioxin-like refers to the fact that these compounds have similar chemical structure and physical-chemical properties, and they invoke a common battery of toxic responses. Because of their hydrophobic nature and resistance towards metabolism, these chemicals persist and bioaccumulate in fatty tissues of animals and humans.

The CDDs include 75 individual compounds; CDFs include 135 different compounds. These individual compounds are referred to technically as congeners. Likewise, the BDDs include 75 different congeners, and the BDFs include an additional 135 congeners. Only 7 of the 75 congeners of CDDs or of BDDs are thought to have dioxin-like toxicity: those with

chlorine/bromine substitutions in, at a minimum, the 2, 3, 7, and 8 positions. Only 10 of the 135 possible congeners of CDFs or of BDFs are thought to have dioxin-like toxicity; also those with substitutions in the 2, 3, 7, and 8 positions. This suggests that 17 individual CDDs/CDFs and an additional 17 BDDs/BDFs exhibit dioxin-like toxicity. The database on many of the brominated compounds regarding dioxin-like activity has been less extensively evaluated, and these compounds are not explicitly considered in this assessment. (For a review of this topic see Birnbaum et al., 2003.)

There are 209 PCB congeners, only 12 of which are thought to have dioxin-like toxicity: PCBs with four or more lateral chlorines, with one or no substitution in the ortho position. These compounds are sometimes referred to as coplanar, meaning that they can assume a flat configuration, with rings in the same plane. Similarly configured polybrominated biphenyls (PBBs) are likely to have similar properties. However, the database on these compounds with regard to dioxin-like activity has been less extensively evaluated, and these compounds are not explicitly considered in this assessment. Mixed chlorinated and brominated congeners of dioxins, furans, and biphenyls also exist, increasing the number of compounds potentially considered dioxin-like within the definitions of this assessment. The physical/chemical properties of each congener vary according to the degree and position of chlorine and/or bromine substitution. Very little is known about occurrence and toxicity of the mixed (chlorinated and brominated) dioxin, furan, and biphenyl congeners. Again, these compounds are not explicitly considered in this assessment.

Generally speaking, this assessment focuses on the 17 CDDs/CDFs and a few of the coplanar PCBs that are frequently encountered in source characterization or environmental samples. The Agency recognizes that other dioxin-like compounds exist in the chemical classes discussed above (e.g., brominated or chlorinated/brominated congeners) or in other chemical classes (e.g., polyhalogenated naphthalenes or benzenes, azo- or azoxybenzenes), but this evaluation focuses on the two dozen chlorinated congeners that are generally considered to be most associated with environmental and human health risks.

The chlorinated dibenzodioxins and dibenzofurans are tricyclic aromatic compounds with similar physical and chemical properties. Certain of the PCBs (the so-called coplanar or mono-ortho coplanar congeners) are also structurally and conformationally similar. The most widely studied of this general class of compounds is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). This compound, often simply called "dioxin," represents the reference compound for this class of compounds. The structure of TCDD and several related compounds is shown in Figure 1-1. Although sometimes confusing, the term "dioxin" is often also used to refer to the complex mixtures of TCDD and related compounds emitted from sources or found in the environment or

in biological samples. It can also be used to refer to the total TCDD "equivalents" found in a sample. This concept of toxic equivalency is discussed extensively in Part II, Chapter 9, Section 9.4, and is summarized below.

1.2. TOXIC EQUIVALENCY FACTORS

CDDs, CDFs, and PCBs are commonly found as complex mixtures when detected in environmental media and biological tissues or when measured as environmental releases from specific sources. Humans are likely to be exposed to variable distributions of CDDs, CDFs, and dioxin-like PCB congeners that vary by source and pathway of exposures. This complicates the human health risk assessment that may be associated with exposures to variable mixtures of dioxin-like compounds. In order to address this problem, the concept of toxic equivalency has been considered and discussed by the scientific community, and TEFs have been developed and introduced to facilitate risk assessment of exposure to these chemical mixtures.

On the most basic level, TEFs compare the potential toxicity of each dioxin-like compound in the mixture to the well-studied and understood toxicity of TCDD, the most toxic member of the group. The use of the TEF methodology has been EPA policy since 1987, when the Agency "adopted an interim procedure, based on dioxin 'toxicity equivalence' factors (TEFs), for estimating the hazard and dose-response of complex mixtures containing CDDs and CDFs in addition to 2,3,7,8-TCDD" (EPA 1987, 1989a). The background and historical perspective regarding this procedure is described in detail in Part II, Chapter 9, Section 9.1, 9.2, and in Agency documents (U.S. EPA, 1987, 1989a, 1991a). This procedure involves assigning individual TEFs to the 2,3,7,8-substituted CDD/CDF congeners and dioxin-like PCBs. To accomplish this, scientists have reviewed the toxicological databases and considered chemical structure, persistence, and resistance to metabolism and have agreed to ascribe specific "order of magnitude" TEFs for each dioxin-like congener relative to TCDD, which is assigned a TEF of 1.0. The other congeners have TEF values ranging from 1.0 to 0.00001. Thus, these TEFs are the result of scientific judgment of a panel of experts who used all of the available data, and they are selected to account for uncertainties in the available data and to avoid underestimating risk. In this sense, they can be described as "public health-conservative" values.

It is important to understand that this process results in values that represent the scientific judgment of experts working with specified criteria. As described below, these values rely more heavily on in vivo than in vitro data and on chronic or subchronic exposures rather than acute exposures. Attempts to replicate or critique individual TEF values on the basis of distributional analysis of relative potency (REP) estimates from individual endpoints or all data have been undertaken (Finley et al., 2003), suggesting possible benefits from the analysis of REP

distributions. It remains important, however, to recognize the emphasis placed by WHO on the above noted weighting factors and on the expert scientific judgment used to derive the existing TEF values.

The TEQ concept is applied by multiplying the TEF of each congener present in a mixture by the respective mass concentration and the products are summed to represent the 2,3,7,8-TCDD TEQ of the mixture, as determined by equation 1-1.

$$TEQ \cong \sum_{i-n}\left(Congener_i \times TEF_i\right)+\left(Congener_j \times TEF_j\right)+\ldots\ldots\left(Congener_n \times TEF_n\right) \qquad (1\text{-}1)$$

The TEF values for PCDDs and PCDFs were originally adopted by international convention (U.S. EPA, 1989a). Subsequent to the development of the first international TEFs for CDD/CDFs, these values were further reviewed and/or revised and TEFs were also developed for PCBs (Ahlborg et al., 1994; van den Berg et al., 1998). A problem arises in that past and present quantitative exposure and risk assessments may not have clearly identified which of three TEF schemes was used to estimate the TEQ. This reassessment introduces a new uniform TEQ nomenclature that clearly distinguishes between the different TEF schemes and identifies the congener groups included in specific TEQ calculations. The nomenclature uses the following abbreviations to designate which TEF scheme was used in the TEQ calculation:

1. I-TEQ refers to the International TEF scheme adopted by EPA in 1989 (U.S. EPA, 1989a). See Table 1-1.

2. TEQ-WHO_{94} refers to the 1994 WHO extension of the I-TEF scheme to include 13 dioxin-like PCBs (Ahlborg et al., 1994). The TEF values for the dioxins and furans are identical to the I-TEQ. See Table 1-2.

3. TEQ-WHO_{98} refers to the 1998 WHO update to the previously established TEFs for dioxins, furans, and dioxin-like PCBs (van den Berg et al., 1998). There are numerous changes in the TEF values for the dioxins, furans and PCBs. See Table 1-3.

The nomenclature also uses subscripts to indicate which family of compounds is included in any specific TEQ calculation. Under this convention, the subscript D is used to designate dioxins, the subscript F to designate furans, and the subscript P to designate PCBs. For example, "TEQ_{DF}-WHO_{98}" would be used to describe a mixture for which only dioxin and furan congeners were determined and where the TEQ was calculated using the WHO_{98} scheme. If PCBs had also

been determined, the nomenclature would be "TEQ_{DFP}-WHO_{98}." Note that the designations TEQ_{DF}-WHO_{94} and I-TEQ_{DF} are interchangeable, as the TEFs for dioxins and furans are the same in each scheme. Note also that in the current draft of this document, I-TEQ sometimes appears without the D and F subscripts. This indicates that the TEQ calculation includes both dioxins and furans.

This reassessment recommends that the WHO_{98} TEF scheme be used to assign toxic equivalency to complex environmental mixtures for assessment and regulatory purposes. Later sections of this document describe the mode(s) of action by which dioxin-like chemicals mediate biochemical and toxicological actions. These data provide the scientific basis for the TEF/TEQ methodology. In the 20-year history of the TEF/TEQ concept, the approach has evolved, and decision criteria supporting the scientific judgment and expert opinion used in assigning TEFs have become more transparent. Numerous states and countries and several international organizations have studied and consequently adopted this approach to evaluating complex mixtures of dioxin and related compounds (Part II, Chapter 9, Section 9.2). It has become the accepted methodology, although the need for research to explore alternative approaches is widely endorsed. Clearly, basing risk on TCDD alone or assuming that all chemicals are equally as potent as TCDD is inappropriate on the basis of available data. Although uncertainties in the use of the TEF methodology have been identified and are described later in this document and in detail in Part II, Chapter 9, Section 9.5, one must examine the use of this method in the broader context of the need to evaluate the potential public health and environmental impact of complex mixtures of persistent, bioaccumulative chemicals.

It can be generally concluded that the use of TEF methodology for evaluating complex mixtures of dioxin-like compounds decreases the overall uncertainties in the risk assessment process, as compared to alternative approaches. Use of the latest consensus values for TEFs assures that the most recent scientific information informs this "useful, interim approach" (U.S. EPA, 1989a; Kutz et al., 1990) to dealing with complex environmental mixtures of dioxin-like compounds. As stated by the EPA's SAB (U.S. EPA, 1995), "The use of the TEFs as a basis for developing an overall index of public health risk is clearly justifiable, but its practical application depends on the reliability of the TEFs and the availability of representative and reliable exposure data." EPA will continue to work with the international scientific community to update these TEF values to ensure that the most up-to-date and reliable data are used in their derivation and to evaluate their use on a periodic basis.

A chemical is assigned a TEF value on the basis of all the available data comparing the REP of a chemical to 2,3,7,8-TCDD. REP values are obtained from individual studies available in the peer-reviewed literature. In addition, there are weighting criteria that place more emphasis

on REP values from chronic and subchronic studies that examine toxic endpoints (van den Berg et al., 1998). There is a broad range in the quantity and quality of the data available for individual congeners. For example, the TEF for PCB 126 is based on over 60 REP values from in vivo endpoints that examine responses as diverse as enzyme induction, developmental toxicity, immunotoxicity, hepatic toxicity, alterations in hormones, and tumor promotion, whereas the TEF for 3,4,4',5-tetrachlorobiphenyl (PCB 81) is based on REP values for in vitro CYP1A induction and QSAR calculations. Fortunately, the uncertainty in the PCB 81 TEF based on limited data has minimal effect on the risk characterization of complex mixtures of dioxin-like compounds since it does not contribute significantly to human TEQ exposures.

Five congeners contribute approximately 80% of the total TEQ in humans: 2,3,7,8-TCDD; 1,2,3,7,8-PCDD; 1,2,3,6,7,8-HxCDD; 2,3,4,7,8-PCDF; and PCB 126 (see Part I, Volume 2 and Section 4.4.3 of this document). With the exception of 1,2,3,6,7,8-HxCDD, the TEFs for these chemicals are based on a number of different endpoints examined in multiple studies performed in different laboratories (Table 1-4). The TEF for 1,2,3,6,7,8-HxCDD is based heavily on a two-year bioassay in which rats were exposed to a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD. The TEFs for 2,3,4,7,8-PCDF and PCB 126 are similar to the mean REP value for all in vivo endpoints and are similar to their REPs for tumor promotion. The TEF for 1,2,3,7,8-PCDD is based largely on its REP for tumor promotion in rats, supported by studies of its biochemical effects in a subchronic mouse study (DeVito et al., 1997).

From these data, it is clear that the chemicals that contribute approximately 80% to the total human TEQ are well studied and that the assigned TEFs provide reasonable estimates of the relative potency of these chemicals. In contrast, although some chemicals in the TEF methodology have minimal data sets with which to reliably assess their relative potency, they do not contribute substantially to the background human blood TEQ.

The ability of the TEF methodology to predict the biological effects of mixtures containing dioxin-like chemicals has been evaluated in a number of experimental systems. These studies generally demonstrate that the assumption of additivity provides a reasonable estimate of the dioxin-like potential of a mixture (Part II, Chapter 9, Section 9.4). Hamm et al. (2003) demonstrated that a mixture of TCDD, PeCDD, TCDF, 1-PeCDF, 4-PeCDF, OCDF, and PCBs 77, 126 and 169 at doses approximating the relative abundance in the food supply, as described by Birnbaum and DeVito (1995), induced a similar spectrum of reproductive toxicity in rat offspring as does TCDD, and that the TEF methodology did reasonably well at predicting the dose-response relationship of the mixture. A close relationship was evident for maternal EROD enzyme induction between TCDD and the equivalent TEQ mixture, with a slightly lowered dose-response for fetal effects from the mixture (~2 fold lower), attributed to decreased transfer of

mixture components to the offspring. A recent statistical modeling exercise of EROD enzyme induction in the NTP bioassays (Toyoshiba et al., 2004) reported that from a statistical standpoint the consensus WHO_{98} TEFs were "significantly different from the maximum likelihood-based estimates, but not very different in actual magnitude." Graphing of the non-log-scaled summary data reported in Toyoshiba et al. (2004) reveals differences of less than 2 - 3 fold from predicted TEQ-based activities, for individual congeners and the mixture. There are examples of nonadditive interactions between dioxins and nondioxins. Both greater-than-additive and less-than-additive interactions have been observed in these studies. In general the nonadditive interactions between the dioxins and nondioxins have been observed at doses that are considerably higher than present background human exposures (Part II, Chapter 9, Section 9.4).

There are a number of natural chemicals that bind and activate the aryl hydrocarbon (Ah) receptor (AhR) and induce some dioxin-like effects. It has been proposed by some scientists that these chemicals contribute significantly to total TEQ exposures and that these exposures far outweigh those from PCDDs, PCDFs, and PCBs (Safe, 1995a). There are several limitations to these analyses, as detailed in Part II, Chapter 9, Section 9.3.5. The hypothesis is built on AhR binding studies and a few other in vitro studies that compared natural ligands to the dioxin-like chemicals. Under these circumstances, neither biological half-life nor toxicity profile is considered.

The in vivo data on the natural AhR ligands is limited to enzyme induction and a single developmental study. Few if any toxicology studies demonstrating clear dioxin-like toxicities have been published. The natural AhR ligands are rapidly metabolized and result in both transient tissue concentrations and transient effects. More recent data demonstrate that these potent in vitro AhR agonists (e.g., indolo[2,3-b]carbazole) neither elicit dioxin-like toxicity nor alter the effects of dioxin in vivo (Pohjanvirta et al., 2002). This may occur because of short persistence times in target organs or inadequate/inappropriate conformational changes induced as a result of AhR-ligand binding (Henry and Gasiewicz, 2003). The natural ligands also have their own distinct biological effects that are independent of the AhR, and it is not clear as to the role of the AhR in the biological effects of these chemicals. Because of the relative concentration of these compounds in the daily diet, their in vitro binding characteristics, and the limited toxicological information in vivo, this issue requires further research in order to better understand the uncertainty surrounding the relative potential health effects of dioxin and related chemicals as compared to natural AhR ligands.

One of the limitations of the use of the TEF methodology in risk assessment of complex environmental mixtures is that the risk from nondioxin-like chemicals is not evaluated in concert with that of dioxin-like chemicals. Another limitation of the TEF methodology is the application

of TEFs to nonbiological samples. The fate and distribution of PCDDs, PCDFs, and PCBs are not necessarily related to their TEFs. Thus, the use of the TEF for assessing potential hazard and risk based on dioxin-like compounds passing through nonbiological media must be done cautiously. Fate and transport of the mixture and likelihood and route of exposure will have important impacts on such assessments. Future approaches to the assessment of environmental mixtures should focus on the development of methods that will allow risks to be predicted when multiple mechanisms are present from a variety of contaminants coming into contact with humans and other environmental receptors through multiple routes.

There are a number of uncertainties in the application of the TEF methodology which are discussed in greater detail in Part II, Chapter 9. In 1998, the U.S. EPA and the U.S. Department of the Interior sponsored a workshop on the use of the TEF methodology in ecological risk assessment. This workshop involved panel members from academia, industry and state and federal governments. This panel concluded that "the uncertainties associated with using RePs or TEFs are not thought to be larger than other sources of uncertainty within the [ecological] risk assessment process (e.g., dose-response assessment, exposure assessment, and risk characterization)" (U.S. EPA, 2001a). In addition, despite the uncertainties in the TEF methodology, the use of this methodology decreases the overall uncertainty of the risk assessment. The panel had difficulty in quantitatively expressing the uncertainty in the TEF methodology. While the panel supported the use of the TEF methodology, they also recommended continued research focusing on a better understanding of the uncertainty in the TEF methodology.

1.3. UNDERSTANDING EXPOSURE/DOSE RELATIONSHIPS FOR DIOXIN-LIKE COMPOUNDS

Risk assessment requires the scaling of exposure/dose across endpoints and across species. Given the many responses to TCDD and its congeners, the selection of dose metrics for use in quantitative risk assessments is a complex problem. The biochemical and toxicological responses to TCDD and related chemicals are initiated by their interaction with the Ah receptor. Some responses, such as enzyme induction, require short periods (minutes to hours) of AhR activation. Other responses, such as cancer, require prolonged (months to many years) activation of this pathway. Still other responses, such as the developmental toxicities, require receptor activation during specific windows of sensitivity. Because of the different mechanisms involved in these diverse responses, it is unlikely that a single dose metric will be adequate for all of these endpoints.

A number of studies have proposed a variety of dose metrics for a number of different responses. These studies have taken different approaches, ranging from simple curve-fitting exercises (Hurst et al., 2000; van Birgelen et al., 1996) to more complex physiologically based pharmacokinetic (PBPK) modeling approaches (Jusko et al., 1995; Andersen et al., 1997; Kohn et al., 1993; Portier and Kohn, 1996). Area under the curve (AUC) has been used traditionally in the drug literature as a dose metric of choice when the dose and the time related to effects in humans are known.

The choice of dose metric not only considers mechanistic data but must consider pragmatic approaches as well. The use of the dose metric plays a role in its choice. Because of differences in lifespan and uncertainties in the windows of sensitivity for various endpoints, lifetime AUC may not be a useful dose metric for cross-species extrapolation in the risk assessment of dioxin and related compounds. For instance, reported interspecies differences in rat liver versus human lung cancer risks based on lifetime AUC are heavily influenced by different lifespans of humans (~70 yrs) versus rats (~2 years), and are mitigated though the use of peak levels or average concentrations (Aylward et al., 1996). Notably, there are no interspecies differences in risk calculations between humans and rats when applying average body burden to the same endpoint, all cancers combined, coupled with more detailed exposure data from the epidemiology studies (see Table 5-4). Because cross-species scaling is not required when the analysis is confined to humans, lifetime AUC has been used in the analysis of human cancer data on TCDD (Becher et al., 1998) and may be a useful dose metric when applied to accidental or occupational exposures.

The choice of dose metric is also dependent on the data available. A number of dose metrics, such as AhR occupancy, induction of CYP1A2, and decreases in epidermal growth factor (EGF) receptor (EGFR) have been proposed on the basis of PBPK models (Jusko et al., 1995; Andersen et al.,1997; Kohn et al., 1993; Portier and Kohn, 1996). Although these dose metrics have been useful in hypothesis testing in experimental systems, they are not useful in animal-to-human extrapolations due to the difficulty in measuring these parameters in humans. In the following section, the strengths and weaknesses of a variety of proposed dose metrics are presented.

1.3.1. Administered Dose

In experimental studies, animals are administered a defined dose through a variety of routes. A default method used by EPA (U.S. EPA, 1992a, 1996) to estimate the human equivalent dose when scaling across species is to use allometric scaling based on the following equation:

$$Dose_{human} = Dose_{rat} (BW_{rat}/BW_{human})^{0.25} \quad (1\text{-}2)$$

where BW is the body weight in kilograms and Dose is the daily administered dose in rats or the scaled human daily dose expressed as mg/kg/day, or in the case of TCDD ng/kg/day. This method, in the absence of data to select a more appropriate dose metric, is thought to scale administered dose in such a way as to result in equivalent effective doses in humans and experimental animals (U.S. EPA, 1992). Using this equation, a dose of 1 ng TCDD/kg/day in a 0.35 kg rat would result in a scaled human dose of 0.27 ng TCDD/kg/day for a 70 kg human. If this scaling method applies to TCDD and related chemicals, then 1 ng TCDD/kg/day in the rat should produce similar effective doses in a human exposed to 0.27 ng TCDD/kg/day, some 3.8 times lower. However, this method fails to take into account differences in the elimination half-life of the chemical in the two species. In the case of dioxin-like compounds, this is an important consideration.

Assuming similar sensitivity between rats and humans at the tissue level, effective doses should be a function of tissue concentration. Tissue concentrations of TCDD and related chemicals are directly related to the concentration of TCDD in the body. The steady-state concentration of TCDD in the body, or steady-state body burden, can be estimated in rats and humans using the following equation.

$$\text{Steady-state body burden (ng/kg)} = \frac{[\text{Dose (ng TEQ/kg)} * \text{half-life (days)}]}{\text{Ln}(2)} * F \quad (1\text{-}3)$$

where Dose is the daily administered dose, F is the fraction absorbed, and $t_{½}$ is the species-specific half-life of TCDD. In the present example, we will assume that the species-specific half-life of TCDD is 25 days for rats and 2593 days for humans. We also assume for this illustration that F is 50% for both human and animal studies. The fraction absorbed varies from ~50–100% of administered dose, depending on dosing matrix (pellets, oil, food, breast milk; greater variability from soil) and study species. For standardization elsewhere in Part III, Risk Characterization, the Agency has adopted 50% absorption from animal food pellets and 80%

from human dietary intake (see Part II, Chapter 1; Poiger and Schlatter, 1986; Abraham et al., 1996). The fraction absorbed linearly impacts the calculation of resulting body burden, with 80% absorption leading to a 1.6-fold higher value than 50% absorption.

Starting with an administered dose of 1 ng/kg/day in rats and the scaled human dose of 0.27 ng/kg/day, the steady-state body burdens are presented in Table 1-5. The steady-state body burden of TCDD using the scaled human dose is approximately 28 times that of the steady-state body burden in the rat (Table 1-5). Using equation 1-3 to estimate equivalent steady-state body burdens (i.e., 18 ng/kg), a human equivalent administered dose comparable to 1 ng/kg/day administered to the rat was estimated at 0.0096 ng/kg/day, over 100 times less.

Clearly, the default scaling method results in an estimated human equivalent dose that produces much greater estimated human tissue concentrations (505 ng/kg) than the rat's tissue concentration (18 ng/kg). The default scaling approach accounts for a difference of ~ 3.7 times, based on allometric considerations, yet the half-life of TCDD in humans alone is approximately 100-fold greater than in rats. This exercise suggests that administered dose may not provide a useful dose metric for cross-species extrapolation even if the dose is scaled using the EPA default methodology. However, administered dose can be used to compare chronic exposures between human populations in order to describe potential human health risks, because the species differences in half-life would not exist in this case. Adjustments will still need to be made, however, to compare short-term exposures expressed as intake as a function of body weight per day to more typical daily intake values in the general population.

1.3.2. Area Under the Curve

AUC is frequently used as a dose metric for reversible responses of pharmaceutical agents. Typically, these agents have half-lives on the order of minutes to hours. In addition, the pharmacological actions of the drug and the length of time of the response is clearly defined in both animals and humans. For example, for anesthetics, sleep time is used as the length of time for determining the AUC. In essence, plasma concentrations are readily determined and the time span is easily defined. In contrast, TCDD has a prolonged half-life in both humans and experimental animals and some of the adverse effects that are of concern in the hazard characterization are not reversible responses. Because of these differences it is unclear whether the AUC is the best dosemetric.

Mechanistic considerations suggest that AUC may be a useful dose metric for carcinogenesis. TCDD and related chemicals are thought to induce tumors through promotional mechanisms as opposed to acting as direct initiators. The promotional effects of TCDD and related chemicals are associated with altered gene expression, resulting in alterations in growth

and differentiation. This promotional process requires sustained tissue concentrations of TCDD sufficient to maintain increased gene expression. One recent study examined AUC as a dose metric for the tumor promotional responses of TCDD. Kim et al. (2003) compared AUC and peak concentrations in rats as a dose metric for liver tumor promotion. Animals receiving a single high exposure to TCDD had greater numbers of altered hepatic foci than animals receiving repeated low dose exposures, even though the AUC was equivalent between the two exposures. These data suggest that the peak concentrations of TCDD may play a significant role in TCDD carcinogenicity and that future dose-response modeling exercises should incorporate measures of dose timing and peak concentrations.

It is possible that AUC could be an appropriate dose metric for cancer in humans, and it may also involve the incorporation of a threshold concentration (Hays et al., 1997). However, the use of AUC for species extrapolation for TCDD is more complicated. Although blood or plasma concentrations of TCDD can be determined in both humans and animals, the determination of the time span for which the AUC is to be calculated is much less certain. For some of the toxic responses to TCDD, such as induction of cleft palate, the window of sensitivity is clearly defined in rodents and humans. For other responses, such as the developmental reproductive alterations observed in male rats, the window of sensitivity has been narrowed to exposures between gestational day 15 and 20 in the rats, but the human window of sensitivity is uncertain. For many of the chronic toxic effects of TCDD, the length of time required to induce the response remains uncertain in both experimental animals and humans. In order to apply AUC for species comparisons of sensitivity to TCDD, one must have a better understanding of the species differences in the windows of sensitivity to the various biological effects of TCDD.

In addition, differences in lifespan also must be considered. Brody and Reid (1967) proposed that the biological activity of a drug is related to its plasma concentrations. If animals and humans had the same plasma concentrations for their entire lives, the human AUC would be greater because humans have a longer half-life of elimination for TCDD. However, because the plasma concentrations would be the same, according to Brody and Reid (1967), the responses should be similar. Hence, in order to use AUC for chronic toxicities, such as cancer, a correction for the difference in lifespan must be applied. Typically, this involves the derivation of a lifetime average serum lipid concentration, which is calculated by dividing the AUC by the time period of exposure (Aylward et al., 1996). An estimation of the average daily AUC is directly related to steady-state body burdens. Hence, once the AUC is corrected for life-span differences, these values are equivalent to steady-state body burdens.

Although AUC may not be an appropriate dose metric for animal-to-human extrapolations, it may be a useful tool for comparing populations exposed to high concentrations

of dioxins over a short period of time to the background population. Becher et al. (1998) and Steenland et al. (2001) used this approach to examine dose-response relationships for cancer in occupationally exposed cohorts. One difficulty in determining AUC is the accuracy of the intake measurements. Past exposures through the diet are uncertain, although they have been estimated (Pinsky and Lorber, 1998). Future exposures are thought to be decreasing, although the exact magnitude of this decrease is uncertain. Hence, determination of AUC carries a number of uncertainties that must be considered.

1.3.3. Plasma or Tissue Concentrations

Brodie and Reid (1967) have argued that the response to a drug is determined by the amount bound to its biological receptor, and because the drug-receptor complex is in dynamic equilibrium with the free drug in the plasma, the biological response of a drug will be related to its plasma concentrations. There is no reason to believe that this relationship will not be true for TCDD and related chemicals. However, there are several data gaps that may prohibit the use of plasma or blood concentrations for species extrapolation. First, few animal studies have determined blood or plasma concentrations of TCDD, particularly in the subchronic, chronic, and lifetime exposures. PBPK models can be used to estimate blood concentrations and should provide reasonable estimates of these values. In contrast, the human exposure data are based predominantly on blood, serum, or plasma dioxin concentrations.

One limitation of the human data is that it is mostly presented on a lipid-adjusted basis. Hence, in order to compare the human and animal plasma or blood concentrations, one would have to first estimate the blood concentrations in the animals using a PBPK model. Then, either the animal data would have to be expressed as a lipid basis or the human data would have to be expressed as a wet-weight basis. In either case, assumptions of the percent lipid in the blood would have to be applied, as would a number of other assumptions typically used in the construction of PBPK models. Recent work by Salvan et al. (2001) has attempted to account for some of these assumptions in an analysis of cancer mortality in the National Institute for Occupational Safety and Health (NIOSH) cohort (Steenland et al., 1999, 2001) using data on age-related body mass index (BMI) and historical background exposures and tissue half-lives from the Ranch Hand cohort (Michalek and Tripathi, 1999).

The use of tissue concentrations as a dose metric has also been examined by van Birgelen et al. (1996) and Hurst et al. (1998, 2000). van Birgelen et al. presented data demonstrating that target tissue concentrations provided an accurate prediction of enzyme induction regardless of the exposure scenario (i.e., acute vs. subchronic). Similarly, Hurst et al. (2000) presented data demonstrating that fetal tissue concentrations of TCDD on gestation day 16 predicted decreases

in sperm counts, delays in puberty in males, urethra-phallus distance, and the incidence of vaginal threads in rats prenatally exposed to TCDD on either gestational day 9 or 15. These data suggest that target tissue concentrations may be a reasonable dose metric for these responses. Although target tissue concentrations may aid in estimating risks, these data are unlikely to be collected in humans in sufficient numbers to be useful, particularly for fetal concentrations.

Plasma (or serum) concentrations are also a useful tool for comparing exposures in different human populations. Application of plasma concentration as a dose metric for species extrapolation requires some level of assumptions, as described above, but reasonable comparisons could be made, particularly for steady-state in humans and animals. Comparing plasma or blood concentrations following acute exposures in experimental animals directly to steady-state human blood or plasma concentrations is problematic.

One problem with the use of plasma, blood, or target tissue concentrations as a dose metric is the limitations of current human PBPK models to predict these values on the basis of changes in intake patterns. Further work will be required to develop such models.

1.3.4. Steady-State Body Burdens

Body burden is defined as the concentration of TCDD and related chemicals in the body and is typically expressed as ng/kg body weight. In animals, these values are calculated from studies at or approaching steady-state. These values are calculated on the basis of knowledge of the species-specific half-life and the exposure or they are estimated on the basis of the TCDD tissue concentration, the size of the tissues, and the weight of the animal. In humans the values are typically presented as steady-state body burdens and are estimated on the basis of an intake rate and the half-life of TCDD in humans. Alternatively, body burdens in humans are estimated on the basis of lipid-adjusted serum or adipose tissue TCDD or TEQ concentrations (See Part I, Volume 2, Chapter 4).

Steady-state body burdens provide a useful dose metric for several reasons. First, tissue and blood concentrations are directly related to body burdens. Thus, body burdens are surrogates for tissue concentrations. Second, the differences in the half-life of TCDD between species are accounted for, because these body burdens are estimated at steady-state conditions. Third, DeVito et al. (1995) have demonstrated that for a multitude of in vitro, biochemical, and toxic responses, including chloracne and cancer, species have similar rates of responses when dose is expressed on a body burden basis. Finally, body burdens provide flexibility, because they can be estimated on the basis of either intake rates or on measured tissue concentrations.

Use of steady-state body burdens also has some limitations. In order to estimate steady-state body burdens from lipid-adjusted tissue concentrations, an assumption of the percent body

fat must be used. In the reassessment, a value of 25% has been used for humans. It should be noted that there are human populations with body fat compositions as low as 10% and greater than 35%. Also, when estimating the body burden on the basis of intake rates and half-lives, the uncertainty of these parameters should be considered. In the reassessment, the estimated current steady-state body burden of approximately 5 ng TEQ_{DFP}-WHO_{98}/kg is based on measured serum concentrations from several populations in the mid 1990's.

Although measured concentrations should eliminate some of the uncertainties in estimates using intake rates and half-life assumptions, it is likely that these measured values represent a past history of higher exposure, and we must anticipate a continued downward trend to represent a "true" lifetime average concentration associated with current dose intake rates. Caution must be used when using body burden as a dose metric for species extrapolation when comparing short-term animal studies to steady-state human exposures. Under acute exposure conditions in the animals, the relationship between tissue concentrations and body burden may not be the same as under the steady-state conditions.

1.3.5. Mechanistic Dose Metrics

Several groups have proposed a variety of dose metrics based on mechanistic considerations, such as concentration of occupied AhR (Jusko, 1995), induced CYP1A2 (Andersen et al., 1997; Kohn et al., 1993) and reduced EGFR (Portier and Kohn, 1996). Although these dose metrics are intellectually appealing, it must be kept in mind that they are still hypothesized dose metrics and require further research to demonstrate their utility for cross-species extrapolations. In addition, these dose metrics are unlikely to be measured in sufficient human samples to be useful.

1.3.6. Summary

A variety of dose metrics have been proposed for estimating potential human health effects following exposure to dioxins. Many of them, such as tissue concentrations and the mechanistic dose metrics, have practical limitations that inhibit their use. Others, such as AUC, have limited utility for species extrapolations because of our limited understanding of the concept of physiological time. Some, such as AUC and administered dose, can be used to compare different human exposures, but are not necessarily suitable for cross-species extrapolations. Others, such as steady-state body burdens or blood concentrations, are useful for species extrapolations because they are directly related to tissue concentrations and can be estimated in both animals and humans. All of these dose metrics require more research to improve cancer and

noncancer risk prediction. This research could include efforts to quantify impacts of dose timing, peak concentrations, and AUC above a baseline.

The use of any of these dose metrics requires a number of assumptions, discussed above and in various chapters in Parts I and II. The choice of dose metric requires an understanding of the data available and their application in the intended use of the dose metric. Future research efforts could provide better guidance in choosing the dose metrics for dioxins and related chemicals. However, in the meantime, the use of steady-state body burdens can provide a reasonable description of dose for use in species extrapolations and risk assessments for many chronic effects and is clearly preferable to intake levels.

Table 1-1. The toxic equivalency factor (TEF) scheme for I-TEQ_{DF}[a]

Dioxin congener	TEF	Furan congener	TEF
2,3,7,8-TCDD	1.0	2,3,7,8-TCDF	0.1
1,2,3,7,8-PeCDD	0.5	1,2,3,7,8-PeCDF	0.05
1,2,3,4,7,8-HxCDD	0.1	2,3,4,7,8-PeCDF	0.5
1,2,3,6,7,8-HxCDD	0.1	1,2,3,4,7,8-HxCDF	0.1
1,2,3,7,8,9-HxCDD	0.1	1,2,3,6,7,8-HxCDF	0.1
1,2,3,4,6,7,8-HpCDD	0.01	1,2,3,7,8,9-HxCDF	0.1
1,2,3,4,6,7,8,9-OCDD	0.001	2,3,4,6,7,8-HxCDF	0.1
		1,2,3,4,6,7,8-HpCDF	0.01
		1,2,3,4,7,8,9-HpCDF	0.01
		1,2,3,4,6,7,8,9-OCDF	0.001

[a] Note that the scheme does not include dioxin-like PCBs. The nomenclature for this scheme is I-TEQ_{DF}, where "I" represents "International," TEQ represents the 2,3,7,8-TCDD toxic equivalence of the mixture, and the subscript DF indicates that only dioxins (D) and furans (F) are included in the TEF scheme.

Table 1-2. The toxic equivalency factor (TEF) scheme for TEQ_{DFP}-WHO_{94}[a]

Dioxin congener	TEF	Furan congener	TEF	Dioxin-like PCB	TEF
2,3,7,8-TCDD	1.0	2,3,7,8-TCDF	0.1	PCB-77	0.0005
1,2,3,7,8-PeCDD	0.5	1,2,3,7,8-PeCDF	0.05	PCB-126	0.1
1,2,3,4,7,8-HxCDD	0.1	2,3,4,7,8-PeCDF	0.5	PCB-169	0.01
1,2,3,6,7,8-HxCDD	0.1	1,2,3,4,7,8-HxCDF	0.1	PCB-105	0.0001
1,2,3,7,8,9-HxCDD	0.1	1,2,3,6,7,8-HxCDF	0.1	PCB-118	0.0001
1,2,3,4,6,7,8-HpCDD	0.01	1,2,3,7,8,9-HxCDF	0.1	PCB-123	0.0001
1,2,3,4,6,7,8,9-OCDD	0.001	2,3,4,6,7,8-HxCDF	0.1	PCB-156	0.0005
		1,2,3,4,6,7,8-HpCDF	0.01	PCB-157	0.0005
		1,2,3,4,7,8,9-HpCDF	0.01	PCB-167	0.00001
		1,2,3,4,,6,7,8,9-OCDF	0.001	PCB-114	0.0005
				PCB-170	0.0001
				PCB-180	0.00001
				PCB-189	0.0001

[a] The nomenclature for this TEF scheme is TEQ_{DFP}-WHO_{94}, where TEQ represents the 2,3,7,8-TCDD toxic equivalency of the mixture, and the subscript DFP indicates that dioxins (D), furans (F), and dioxin-like PCBs (P) are included in the TEF scheme. The subscript 94 following WHO displays the year changes were made to the TEF scheme.

Table 1-3. The toxic equivalency factor (TEF) scheme for TEQ_{DFP}-WHO_{98}[a]

Dioxin congener	TEF	Furan congener	TEF	Dioxin-like PCB	TEF
2,3,7,8-TCDD	1.0	2,3,7,8-TCDF	0.1	PCB-77	0.0001
1,2,3,7,8-PeCDD	1.0	1,2,3,7,8-PeCDF	0.05	PCB-81	0.0001
1,2,3,4,7,8-HxCDD	0.1	2,3,4,7,8-PeCDF	0.5	PCB-126	0.1
1,2,3,6,7,8-HxCDD	0.1	1,2,3,4,7,8-HxCDF	0.1	PCB-169	0.01
1,2,3,7,8,9-HxCDD	0.1	1,2,3,6,7,8-HxCDF	0.1	PCB-105	0.0001
1,2,3,4,6,7,8-HpCDD	0.01	1,2,3,7,8,9-HxCDF	0.1	PCB-118	0.0001
1,2,3,4,6,7,8,9-OCDD	0.0001	2,3,4,6,7,8-HxCDF	0.1	PCB-123	0.0001
		1,2,3,4,6,7,8-HpCDF	0.01	PCB-156	0.0005
		1,2,3,4,7,8,9-HpCDF	0.01	PCB-157	0.0005
		1,2,3,4,6,7,8,9-OCDF	0.0001	PCB-167	0.00001
				PCB-114	0.0005
				PCB-189	0.0001

[a] The nomenclature for this TEF scheme is TEQ_{DFP}-WHO_{98}, where TEQ represents the 2,3,7,8-TCDD toxic equivalency of the mixture, and the subscript DFP indicates that dioxins (D), furans (F), and dioxin-like PCBs (P) are included in the TEF scheme. The subscript 98 following WHO displays the year changes were made to the TEF scheme. Note that the changes to the TEFs since 1994 are as follows:

- for 1,2,3,7,8-PeCDD, the new WHO TEF is 1 and the I-TEF is 0.5;
- for OCDD, the new WHO TEF is 0.0001 and the I-TEF is 0.001;
- for OCDF, the new WHO TEF is 0.0001 and the I-TEF is 0.001;
- for PCB 77, the new TEF is 0.0001;
- the addition of PCB 81 (i.e., 3,4,4',5-TCB); and
- for the two di-ortho substituted HpCBs in the 1994 TEF scheme (i.e., PCBs 170 and 180), no TEFs have been assigned in the new WHO TEF scheme.

Table 1-4. The range of the in vivo relative potency estimates (REP) values for the major toxic equivalency contributors

Chemical	Number of in vivo endpoints	Range of REPs (mean ± std)	Number of endpoints from subchronic studies	Range of REPs (mean ± std)	TEF
1,2,3,7,8-PCDD	22	0.16–0.9 (0.5 ± 0.22)	16	0.19–0.9 (0.53 ± 0.24)	1
2,3,4,7,8-PCDF	40	0.018–4.0 (0.4 ± 0.7)	20	0.018–0.6 (0.20 ± 0.13)	0.5
1,2,3,6,7, 8-HxCDD	3	0.015–0.16	1	0.04	0.1
PCB 126	62	0.0024–0.98 (0.20 ± 0.20)	31	0.004–0.18 (0.13 ± 0.13)	0.1

TEF = toxic equivalency factor

Table 1-5. Comparison of administered dose and body burden in rats and humans[a]

	(A) Rat daily administered dose/body burden	(B) Human scaled administered dose/body burden[b]	(C) Human equivalent administered dose/body burden[c]	(A/B) Ratio of rat-to-human scaled dose	(A/C) Ratio of rat-to-human equivalent Dose
Dose (ng/kg/day)	1	0.27	0.0096	3.7	104
Body burden (ng/kg)	18	505	18	0.036	1

[a] This matrix compares the effects of different interspecies scaling factors between rats and humans. Column A indicates that a dose of 1 ng/kg/day to a rat leads to a steady-state body burden (BB) of 18 ng/kg, using the formula BB = half-life*dose*absorption fraction (0.5)/ln2. Columns B and C then use different interspecies scaling factors to convert the rat dose to a human equivalent dose. Column B uses body weight to the 3/4 power as the interspecies scaling factor to convert the rat dose of 1 ng/kg/day (from the column A dose row) to the equivalent human scaled dose of 0.27 ng/kg/day, which in turn corresponds to a human body burden of 505 ng/kg based on the human half-life of 7.1 years and f = 0.5 (used in this table for consistency). Column C uses body burden as the interspecies scaling factor to convert the rat body burden of 18 ng/kg (from column A body burden row) to the equivalent 18 ng/kg BB in humans, and then derives the human dose that would correspond with this body burden, i.e., 0.0096 ng/kg/day. The fifth column divides column A results by column B results, revealing that the $BW^{3/4}$ interspecies factor leads to a rat/human ratio of 3.7-fold. The last column divides column A by column C results, revealing that when body burden is used as the interspecies scaling factor the rat dose is over 100 times the equivalent human dose.

[b] Assumes administered dose scales across species as a function of $BW^{3/4}$

[c] Assumes administered dose scales across species as a function of equivalent body burdens

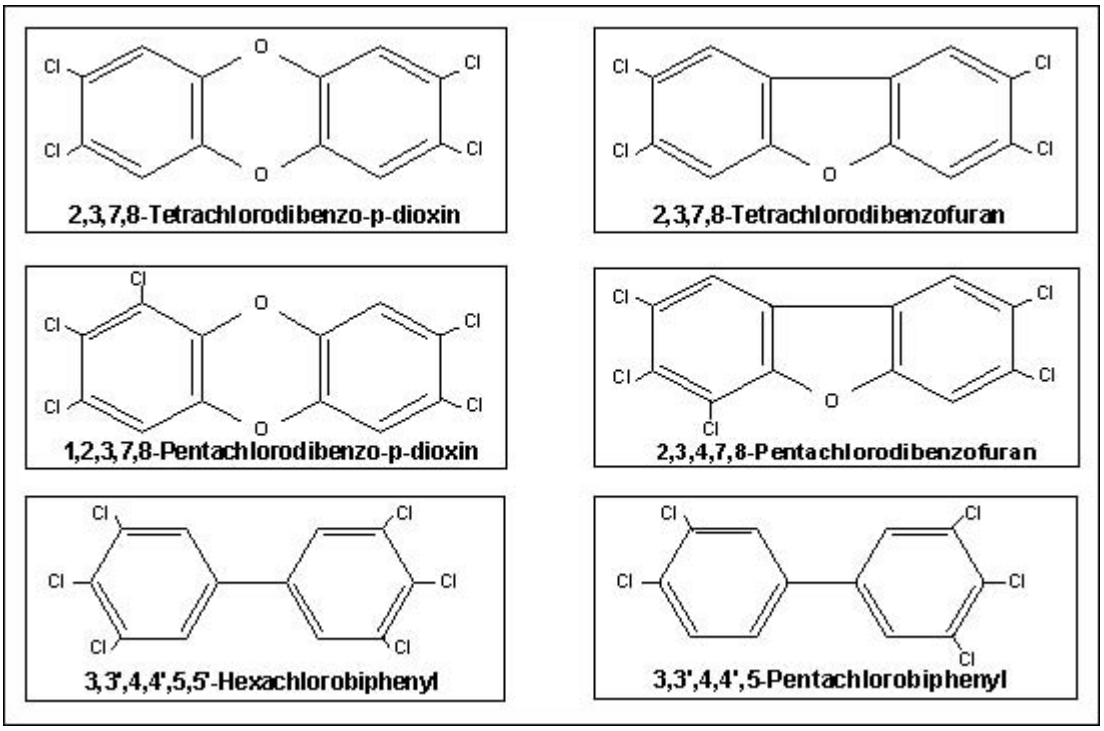


Figure 1-1. Chemical structure of 2,3,7,8-TCDD and related compounds.

2. EFFECTS SUMMARY

Since the identification in 1957 of 2,3,7,8-TCDD as a chloracnegen, more than 5000 publications have discussed its biological and toxicological properties. A large number of the effects of dioxin and related compounds have been discussed in detail throughout the chapters in Part II of this assessment. These discussions illustrate the wide range of effects produced by this class of compounds. The majority of effects have been identified in experimental animals; some have also been identified in exposed human populations. Although past EPA risk assessments have focused on cancer estimates based on extrapolation models as the major concern for dioxin and related compounds, more recent data suggest that noncancer effects may be occurring at or near human background steady-state body burden levels in animals and in humans. Evaluation of noncancer effects and their relationship to past and current body burdens and intake levels is an important feature of this reassessment. Direct comparisons between various noncancer effects and cancer in animals and humans and exposures of interest are presented in the form of *margins of exposure* (MOE).

Cross-sectional studies have been conducted to evaluate the prevalence or extent of disease in living 2,3,7,8-TCDD-exposed groups (Suskind and Hertzberg, 1984; Moses et al., 1984; Lathrop et al., 1984, 1987; Roegner et al., 1991; Grubbs et al., 1995; Sweeney et al., 1989; CDC Vietnam Experience Study, 1988; Webb et al., 1989; Ott and Zober, 1994). The limitations of the cross-sectional study design for evaluating hazard and risk are discussed in Part II, Chapter 7b, Section 7.11. Many of the earliest studies were unable to define exposure-outcome relationships owing to a variety of shortcomings, including small sample size, poor participation, short latency periods, selection of inappropriate controls, and the inability to quantify exposure to 2,3,7,8-TCDD or to identify confounding exposures.

Cohort and case-control studies have been used to investigate hypothesized increases in malignancies among the various 2,3,7,8-TCDD-exposed populations (Fingerhut et al., 1991a, b; Manz et al., 1991; Eriksson et al., 1990). In more recent analyses of occupational cohorts (Steenland et al., 1999; Ott and Zober, 1996; Flesch-Janys et al., 1998), cross-sectional studies of U.S. chemical workers (Sweeney et al., 1989), U.S. Air Force Ranch Hand personnel (Roegner et al., 1991; Grubbs et al., 1995), and Missouri residents (Webb et al., 1989), serum or adipose tissue levels of 2,3,7,8-TCDD were measured to evaluate 2,3,7,8-TCDD-associated effects in exposed populations. The ability to measure tissue or serum levels of 2,3,7,8-TCDD for all or a large sample of the subjects confirmed exposure to 2,3,7,8-TCDD and permitted the investigators to test hypothesized dose-response relationships.

A large number of effects of exposure to TCDD and related compounds have been documented in the scientific literature. Although many effects have been demonstrated in multiple species (see Table 2-1), other effects may be specific to the species in which they are measured and may have limited relevance to the human situation. Although the potential species-specific responses are an important consideration for characterizing potential hazard, all the observed effects of 2,3,7,8-TCDD illustrate the multiple sequelae that are possible when primary impacts are at the level of signal transduction and gene transcription. Even though not all observed effects may be characterized as "adverse" (i.e., some may be responses within the normal range or adaptive or compensatory and of unknown or neutral consequence), they represent a continuum of response expected from the fundamental changes in biology caused by exposure to dioxin-like compounds. As discussed in the following sections, the doses associated with this plethora of effects are best compared across species using a common measurement unit of steady-state body burden of 2,3,7,8-TCDD and other dioxin-like compounds, as opposed to the level or rate of exposure/intake.

The low end of the range of experimental lowest-observed-adverse-effect levels (LOAELs), no-observed-adverse-effect levels (NOAELs), and effective doses at the 1% response level (ED_{01}s) for critical endpoints from animal studies is compiled in Table 5-6 and Appendix A. These selected endpoints cover a spectrum from overt toxicity (e.g., fetal mortality, cancer), through developmental and reproductive toxicity endpoints, to enzyme induction as a marker of intracellular dioxin activity. Many of the studies report multiple statistically significant effects related to dioxin exposure. From these results, the values tabulated were selected on the basis of the lowest dose at which significant effects occurred—findings that were generally highlighted by the authors of the publication. In the event that multiple endpoints were elicited at the same dose, the effect considered of most consistency across studies and relevance to human risk assessment was selected (e.g., decreased sperm counts).

A variety of methods were employed to estimate body burdens corresponding to the LOAELs/NOAELs/ED_{01}s, including using measured body burden and lipid concentration data, absorption adjustments for single-dose studies, and first-order pharmacokinetic modeling estimates using absorbed dose and halflife. Additional details on study design, endpoint selection, and calculation of body burdens are included in Appendix A and can also be found in Sections 5.2 and 6.0 of this document and in other chapters of the dioxin reassessment. Human equivalent intakes for the body burden endpoints were calculated according to formulae discussed in Part II, Chapter 8 of this report and are displayed in order corresponding to the preceding three results columns in Table 5-6 and Appendix A. These comparisons result in the finding that, when animal data associated with effects at the low end of the range of experimental

observation (NOAELs/LOAELs/ED_{01}s) are compared to current average human body burdens of approximately 5 ng TEQ_{DFP}-WHO_{98}/kg—representing lifetime average intake values of approximately 3 pg TEQ_{DFP}-WHO_{98}/kg/day—or to current intake values of 1 pg TEQ_{DFP}-WHO_{98}/kg/day, relatively small MOEs are obtained. Similarly, some human noncancer effects (e.g., developmental delay, neurobehavioral outcomes, and impact on thyroid function in Dutch children) and cancer outcomes show comparatively small MOEs.

In the following sections which discuss these general effects, the focus is on developing an understanding of dioxin hazard and risk. This discussion is, by its nature, selective of findings that inform the risk assessment process. Readers are referred to the more comprehensive chapters for further discussion of the broader epidemiologic and toxicologic database.

2.1. BIOCHEMICAL RESPONSES (Cross-reference: Part II, Chapters 2, 3, and 8)

As described later in Section 3, mechanistic studies can reveal the biochemical pathways and types of biological events that contribute to adverse effects from exposure to dioxin-like compounds. For example, much evidence indicates that 2,3,7,8-TCDD acts via an intracellular protein, AhR, which is a ligand-dependent transcription factor that functions in partnership with a second protein (known as the AhR nuclear translocator, or Arnt) to alter gene expression. In addition, receptor binding may result in release of cytoplasmic proteins that, in turn, alter the expression or activity of cell-regulatory proteins (e.g., increases in Src activity). Therefore, from a mechanistic standpoint, TCDD's adverse effects appear likely to reflect alterations in gene expression or protein activity that occur at an inappropriate time and/or for an inappropriate length of time. Mechanistic studies also indicate that several other proteins (e.g. hif α, Rb, relA, src, sim, etc.) contribute to TCDD's gene-regulatory effects and that the response to 2,3,7,8-TCDD involves a relatively complex interplay between multiple genetic and environmental factors. This model is illustrated in Figure 2-1 (from Part II, Chapter 2). Comparative binding studies and other data suggest that biochemical events observed in response to TCDD exposure are also seen with other dioxin-like compounds in proportion to their TEFs.

Comparative data from animal and human cells and tissues suggest a strong qualitative similarity across species in response to dioxin-like chemicals. This further supports the applicability to humans of the generalized model of initial events in response to dioxin exposure. These biochemical and biological responses are sometimes considered adaptive or reflective of exposure to dioxin-like compounds. When they are seen within normal homeostatic limits, these biochemical changes are often not considered adverse in and of themselves. However, many of these changes are potentially on a continuum of dose-response relationships that leads to adverse responses and, considering the potential to shift population distributions in response, may be of

concern. Because of the distribution of responses and sensitivity within a population, it is possible that adaptive responses for some are frankly adverse for those at the tails of the distribution. For this reason, a balanced approach must be used when describing these events, recognizing that they may be adaptive or simply biomarkers of exposure to dioxin-like compounds, or they may represent early events in a pathway resulting in a risk of adverse effects in some humans.

If, as we can infer from the evidence, 2,3,7,8-TCDD and other dioxin-like compounds operate through these mechanisms, there are constraints on the possible models that can plausibly account for dioxin's biological effects and also on the assumptions used during the risk assessment process. For instance, the linear relationship expected between ligand concentration and receptor binding may or may not be reflective of dose-response relationships for downstream events requiring complex interactions of other regulatory proteins with the activated receptor. Puga et al. (2000a) have shown that interactions of TCDD with the AhR alters expression of over 300 genes in a single cell line at one time point and one dose. These data suggest that mechanisms of toxic action may be very complicated and that additional research will be necessary to further unravel the mechanistic relationships underpinning dioxin's toxicity.

Mechanistic knowledge of dioxin action may also be useful in other ways. For example, knowledge of genetic polymorphisms that influence 2,3,7,8-TCDD responsiveness may also allow the identification of individuals either refractory to or at particular risk from exposure to dioxin. In addition, knowledge of the biochemical pathways that are altered by dioxin-like compounds may help in the development of approaches to intervention or to drugs that can prevent dioxin's adverse effects.

As described in Part II, Chapter 2, biochemical and genetic analyses of the mechanisms by which dioxin modulates particular genes have revealed the outline of a novel regulatory system whereby a chemical signal can alter cellular regulatory processes. Future studies of dioxin action have the potential to provide additional insights into mechanisms of mammalian gene regulation that are of relatively broad interest. Additional perspectives on dioxin action can be found in several recent reviews (Birnbaum, 1994a, b; Schecter, 1994; Hankinson, 1995; Schmidt and Bradfield, 1996; Rowlands and Gustafsson, 1997; Gasiewicz, 1997; Hahn, 1998; Denison et al., 1998; Wilson and Safe, 1998; Schecter and Gasiewicz, 2003).

The ability of 2,3,7,8-TCDD and other dioxin-like compounds to modulate a number of biochemical parameters in a species-, tissue-, and temporal-specific manner is well recognized. Despite the ever-expanding list of these responses from the past 20 years and the elegant work on the molecular mechanisms mediating some of these, there still exists a considerable gap between our knowledge of individual biochemical changes and the degree to which they are related to the

more complex biological and toxicological endpoints elicited by these chemicals. A framework for considering these responses in a mode of action context is discussed later in this document.

TCDD-elicited activation of the AhR has been clearly shown to mediate altered transcription of a number of genes, including several oncogenes and those encoding growth factors, receptors, hormones, and drug-metabolizing enzymes. Table 2-2 provides an illustrative list of gene products whose regulation or activity is modulated by 2,3,7,8-TCDD. Although this list is not meant to be exhaustive, it demonstrates the range of potential dioxin impacts on pathways with potential to lead to adverse effects.

As discussed in Part II, Chapter 2, it is possible that the TCDD-elicited alteration of activity of these genes may occur through a variety of mechanisms. The transcription of some genes may be directly regulated by the activated AhR. Other alterations in gene expression may be secondary to the initial biochemical events directly regulated transcriptionally by the AhR. Some of the changes may also occur by post-transcriptional processes such as messenger ribonucleic acid (mRNA) stabilization or altered protein phosphorylation (Gaido et al., 1992; Matsumura, 1994). Nie et al. (2001) described cross-talk between Arnt-requiring pathways resulting in interactions between the AhR and the hypoxia signaling pathways. Thus, the molecular mechanisms by which many if not most of the biochemical processes discussed herein are altered by 2,3,7,8-TCDD treatment remain to be determined. Nevertheless, it is assumed, based on the cumulative evidence available, that all of these processes are mediated by the binding of 2,3,7,8-TCDD to the AhR. Although evidence has accumulated for the involvement of the AhR in many but not all of these processes, structure-activity relationships, genetic data, and reports from the use of biological models such as "knockout" mice that are lacking the AhR ($AhR^{-/-}$) are consistent with the involvement of the AhR as the initial step leading to these biochemical alterations. In fact, for every biochemical response that has been well studied, the data are consistent with the particular response being dependent on the AhR.

The dioxin-elicited induction of certain drug-metabolizing enzymes such as CYP1A1, CYP1A2, and CYP1B1 is clearly one of the most sensitive responses observed in a variety of different animal species, including humans, and it occurs at body burdens as low as 3–8 ng TCDD/kg in animals (see Part II, Chapter 8, Sections 8.3 and 8.4). These and other enzymes are responsible for the metabolism of a variety of exogenous and endogenous compounds. Several lines of experimental evidence suggest that these enzymes may be responsible for either enhancing or protecting against the toxic effects of a variety of agents, including known carcinogens as well as endogenous substrates such as hormones. These interactive effects are dependent on the compounds and the experimental system examined.

Several reports (Kadlubar et al., 1992; Esteller et al., 1997; Ambrosone et al., 1995; Kawajiri et al., 1993) provide evidence that human polymorphisms in CYPIA1 and CYPIA2 that result in higher levels of enzyme activity are associated with increased susceptibility to colorectal, endometrial, breast, and lung tumors. Also, exposure of AhR-deficient ("knockout") mice to benzo[a]pyene (BaP) results in no tumor response, suggesting a key role for the AhR—and perhaps CYPIA1 and CYPIA2—in BaP carcinogenesis (Dertinger et al., 1998; Shimizu et al., 2000). Modulation of these enzymes by dioxin may play a role in chemical carcinogenesis. However, the exact relationship between the induction of these enzymes and any toxic endpoint observed following dioxin exposure has not been clearly established.

In addition to what is known about the P450 isozymes (CYP1A1, CYP1A2, and CYP1B1), there exists some evidence from experimental animal data to indicate that the alteration of certain other biochemical events might have a more direct relationship to sensitive toxic responses observed following TCDD exposure. Some of these may be relevant to responses observed in humans, and further work in these areas is likely to lead to data that would assist in the risk characterization process. For example, changes in EGFR have been observed in tissues from dioxin-exposed animals and humans (see Part II, Chapter 3, Section 3.5, and Chapter 6, Section 6.5). EGF and its receptor possess diverse functions relevant to cell transformation and tumorigenesis, and changes in these functions may be related to a number of dioxin-induced responses, including neoplastic lesions, chloracne, and a variety of reproductive and developmental effects. Likewise, the known ability of TCDD to directly or indirectly alter the levels and/or activity of other growth factors and hormones, such as estrogen, thyroid hormone, testosterone, and gonadotropin-releasing hormone and their respective receptors as well as enzymes involved in the control of the cell cycle (Safe, 1995b), may affect growth patterns in cells/tissues, leading to adverse consequences. In fact, most of the effects that the dioxins produce at the cellular and tissue levels are due not to cell/tissue death but to altered growth patterns (Birnbaum, 1994b). Many of these alterations may occur at critical times in development and/or maturation and thus may be irreversible.

There does not yet exist a precise understanding of the relationships between the alteration of specific biochemical processes and particular toxic responses observed in either experimental animals or humans exposed to the dioxins. This is due predominantly to our incomplete understanding of the complex and coordinated molecular, biochemical, and cellular interactions that regulate tissue processes during development and under normal homeostatic conditions. A further understanding of these processes and how 2,3,7,8-TCDD may interfere

with them remains an important goal that would greatly assist in the risk characterization process. In particular, knowledge of the causal association of these responses coupled with dose-response relationships may lead to a better understanding of sensitivity to various exposure levels of the dioxin-like compounds. Nevertheless, it is important to recognize that many of the biochemical and biological changes observed are consistent with the notion that 2,3,7,8-TCDD is a powerful growth dysregulator. This hypothesis may play a considerable role in the risk characterization process by providing a focus on those processes, such as development, reproduction, immunity, and carcinogenesis, that are highly dependent on coordinated growth regulation.

2.2. ADVERSE EFFECTS IN HUMANS AND ANIMALS

2.2.1. Cancer (Cross-reference: Part II, Chapters 6, 7, and 8)

2.2.1.1. *Epidemiologic Studies*

Since the last formal EPA review in 1988 of the human database relating to the carcinogenicity of TCDD and related compounds, a number of new follow-up mortality studies have been completed. This body of information is described in Part II, Chapter 7a, Section 7.5, of this assessment, and summaries appear in an International Agency for Research on Cancer monograph (IARC, 1997), the Agency for Toxic Substances and Disease Registry (ATSDR) ToxProfile (ATSDR, 1999a), and the National Toxicology Program's report on carcinogens (NTP, 2001). Among the most important of these are the ones by Fingerhut et al. (1991a) and Steenland et al. (1999, 2001) from NIOSH of 5172 U.S. chemical manufacturing workers and the independent analyses by Aylward et al. (1996) and Salvan et al. (2001) and followup of the Dow sub-cohort by Bodner et al. (2003); a study of 2479 German workers involved in the production of phenoxy herbicides and chlorophenols by Becher et al. (1996, 1998) and by others in separate publications (Manz et al., 1991; Nagel et al., 1994; Flesch-Janys et al., 1995, 1998); a study of more than 2000 Dutch workers in two plants involved in the synthesis and formulation of phenoxy herbicides and chlorophenols (Bueno de Mesquita et al., 1993) and subsequent follow-up and expansion by Hooiveld et al., 1998); a smaller study by Zober et al. (1990) of 247 workers involved in a chemical accident cleanup and subsequent follow-up (Ott and Zober, 1996b); and an international study by Saracci et al. (1991) of more than 18,000 workers exposed to phenoxy herbicides and chlorophenols, with subsequent follow-up and expansion by Kogevinas et al. (1997). Recent reports also indicate increased cancer risks among the Seveso population (Bertazzi et al. 2001a, Warner et al. 2002).

Although uncertainty remains in interpreting these cohort results because not all potential confounders have been ruled out and coincident exposures to other carcinogens are likely (see Cole et al., 2003 for a critique), all provide support for an association between exposure to dioxin

and related compounds and increased cancer mortality. Strong inference regarding carcinogenic hazard often relies on the availability of studies with well-documented exposures. One of the strengths of these studies is that each has some exposure information that permits an assessment of dose response. Some of these data have, in fact, served as the basis for fitting the dose-response models in Part II, Chapter 8, Section 8.4.

In addition, during the development of its monograph on PCDDs/PCDFs (IARC, 1997), the IARC Working Group abstracted from the published literature data concerning the most highly exposed populations in the world. The group focused its attention on the most exposed subcohorts within cohorts with adequate latency. IARC suggests that if associations between exposure and risk are truly causal, they will become more apparent in these highly exposed subcohorts with adequate latency. Increased risk for all cancers combined and lung cancer mortality were consistent findings in the occupational cohort studies. Although the increase was generally low (20–50%), it was highest in the subcohorts with the presumed heaviest exposure. The results of the IARC Working Group's analysis regarding all cancer and lung cancer mortality in the recent studies are summarized in Table 2-3. Observed numbers of cases, standardized mortality ratios (SMR) and 95% confidence intervals (CI) are given for each of these two findings for each study.

In addition, the Working Group developed overall SMRs for the combined studies. The group state clearly that, although these total SMRs are low (1.4, 95% CI = 1.2–1.6 for all cancers and 1.4, 95% CI = 1.1–1.7 for lung cancer), these results are unlikely to be due to chance, nor can confounding by cigarette smoking likely account for the increase in lung cancer. Positive dose-response trends in the German studies and increased risk in the longer duration U.S. subcohort and the most heavily exposed Dutch workers support this view. In the opinion of these experts, increases of this magnitude in all cancers combined have rarely been found in occupational cohorts. These results are also supported by significantly increased mortality from lung and liver cancers subsequent to the Japanese rice oil poisoning accident where exposure to high levels of PCDFs and PCBs occurred (Kuratsune et al., 1988; Kuratsune, 1989).

Although smoking as a confounder cannot be totally eliminated as a potential explanation of the occupational studies results, analyses conducted to date (Fingerhut et al., 1991b; Ott and Zober, 1996b) suggest that smoking is not likely to explain the entire increase in lung cancer and may even suggest synergism between occupational exposure to dioxin and smoking. These analyses have not been deemed entirely satisfactory by some reviewers of the literature. The question of confounding exposures such as to asbestos and other chemicals in addition to smoking has not been entirely ruled out and must be considered as potentially adding to the observed increases. Although increases of cancer at other sites (e.g., non-Hodgkin's lymphoma,

soft tissue sarcoma, gastrointestinal cancer) have been reported (see Part II, Chapter 7a, Section 7.5), the data for an association with exposure to dioxin-like chemicals are less compelling due to the limited numbers of observed tumors at any specific site.

As discussed by IARC (McGregor et al., 1998) and Smith and Lopipero (2001), it is unusual for a cancer hazard characterization to focus on the "all cancers combined" category of epidemiological results, and continuing uncertainties regarding site-specific cancer increases following dioxin exposure remain a factor in concluding that the epidemiological information is limited. McGregor et al. (1998) note, however, that the predominant cancer promotion mechanism of action for dioxin will theoretically elicit pre-existing initiated cell lines. These promotional effects would be expected in multiple tissues, especially those most sensitive to the effects of dioxin. In epidemiological studies, there may not be a statistically increased tumor site(s), but rather a pattern of smaller increases that could vary across study populations because of differences in life histories, exposures, and pre-existing initiating events.

The cancer-promotion mechanism may also serve to accentuate existing tumor rate increases following other carcinogenic exposures, thereby acting in a synergistic manner. Timing of tumor induction may differ between a cancer promoter and initiator, where the effects of a promoter may not be monotonic with time, but rather may exhibit an earlier onset, harvesting effect, where the total cancer burden may not have changed but the onset has been accelerated. These timing issues are exacerbated by the pharmacokinetics of dioxin elimination, where initial peak body burdens during employment or after accidental exposures decline gradually after cessation of exposure.

Mathematically, a net carcinogenic effect in one or more organ sites must, by definition, increase the "all cancers combined" risk for the exposed population if the exposed and control groups are matched (i.e., they have the same background cancer rate absent the exposure). Thus, an increase in the all cancers category should be considered an expected result of a carcinogen exposure, not an unusual event. The statistical power of a study to detect such an effect is, however, the limiting factor in the presence of stochastic events and imperfect matching. This constraint is particularly applicable to rare tumor sites, but it also occurs for common tumor sites such as lung, colon, breast (♀), and prostate (♂) or for mechanistically linked sites (e.g., hormonally related breast, ovary, uterus), where substantial increases in site-specific relative risks are necessary to impact the all cancer category.

Ionizing radiation (a mutagenic carcinogen) provides an example where small increased relative risks at multiple sites lead to a significantly increased relative risk for "all nonleukemic cancers." In atomic bomb survivors, the relative risk for all nonleukemic cancers at 100 rads was 1.17 ($p<0.01$), comprised principally of small but statistically significant increases in stomach

(relative risk [RR] = 1.11), lung (1.33), breast (1.69), ovary (1.52), and bladder-kidney (1.55) cancers and nonstatistically significant increases in esophagus (1.23), liver (1.35), ovary (1.52), and multiple myeloma (1.51). Although the relative risk for leukemia was 3.95 ($p<0.01$), the excess cancer burden from nonleukemic sites in the exposed population was over twice that due to the leukemias (Hoel, 1987).

Some studies that are discussed in Part II, Chapter 7a, report small or no increased risk of cancer from exposure to 2,3,7,8-TCDD or its congeners. These studies generally suffer from one or more deficiencies that limit their ability to determine the carcinogenic hazard of dioxins. These deficiencies fall into the following categories: little statistical power to detect an effect of exposure because the measured exposures are lower than those seen in the studies cited above and are more similar to those of the comparison population; no measurements of internal exposure to 2,3,7,8-TCDD and potential for misclassification of exposure; and inadequate latency or follow-up.

The Ranch Hand study of U.S. Air Force personnel who sprayed the defoliant Agent Orange during the Vietnam War provides an illustrative example of statistical power constraints in the presence of low predicted relative risks. Statistical power is the ability of a study to detect a real difference between two groups at pre-defined levels of statistical significance (usually $p \leq 0.05$) and relative risk. Statistical power analysis based on the detailed dosimetry and health status data available for this cohort indicates insufficient statistical power to detect an elevated all-cancers risk at levels consistent with the occupational dose-response data. A predicted relative risk for all cancers combined can be estimated for the Ranch Hands by calculating the difference between their dose and that of the control group (mean background of 4.25 ppt TCDD in lipid) (Michalek et al., 1998) and then multiplying this dose increment by an estimated cancer risk slope factor for TCDD. The median AUC increment value for the overall Ranch Hand group is 468 ng TCDD/kg lipid * years, and for the high dioxin group the median is 2280 ng TCDD/kg lipid * years. Using the Becher et al. (1998) linear formula (RR = 1 + 0.000016 x AUC ng-TCDD/kg lipid * years, which equals ~ 3×10^{-3} risk/pg/kg/day) described in Section 5.3 and Table 5-2 of this document, the estimated all-cancers relative risk for the overall Ranch Hand cohort is approximately 1.01, and for the high-exposure group it is 1.04 as compared to the control population. Using formulae in Fleiss (1981) and Cohen (1977) and assuming two-sided testing at a significance level of 5%, the study has no power to detect 1 to 4% increases in relative risk. Data on the overall prevalence of cancer in the comparison group (18.9%) and sample sizes (all Ranch Hand 845 vs. 1224 controls; high category 241 vs. 1200 controls) used in the above analysis were obtained from the 1997 Ranch Hand morbidity report (http://www.brooks.af.mil/AFRL/HED/hedb/afhs/.html).

Recent suggestive cancer findings from the Ranch Hand database are consistent with these calculations, both in the magnitude of the risk ratios under review and in the constraints on statistical methods to detect such levels of incremental risk. Akhtar et al. (2003) provide results that suggest exposure to dioxin-contaminated herbicides may be associated with cancer, based on a statistically significant positive trend in "any site" cancer relative risk with exposure group, accompanied by a non-significant increase in the any site cancer standardized incidence ratio of 1.09 (Obs. 134, Exp 123.34, p=0.34).

In addition, one of the earliest reported associations between exposure to dioxin-like compounds in dioxin-contaminated phenoxy herbicides and increased cancer risk involved an increase in soft tissue sarcomas (Hardell and Sandstrom, 1979; Eriksson et al., 1981; Hardell and Eriksson, 1988; Eriksson et al., 1990). In this and in other recent evaluations of the epidemiologic database, many of the earlier epidemiological studies that suggested an association between dioxin exposure and soft tissue sarcoma have been criticized for a variety of reasons. Arguments regarding selection bias, lack of exposure or differential exposure misclassification, confounding, and chance in each individual study, which increases uncertainty around this association, have been presented in the scientific literature. Nonetheless, the incidence of soft tissue sarcoma is elevated, although not statistically so, in several of the most recent studies (Bertazzi et al., 1993, 1997, 1999; Fingerhut et al., 1991a; Hertzman et al., 1997; Kogevinas et al., 1997; Lampi et al., 1992; Lynge, 1998; Pesatori et al., 1999; Saracci et al., 1999; Vineis et al., 1986). It is probable that soft tissue sarcomas are not unlike other site-specific cancers whose risks from exposure to TCDD are difficult to define because of small numbers and lack of measures of internal exposure.

The accidental exposure of the population at Seveso, Italy, serves as an example of a more highly exposed group where, in previous assessments, latency was considered to be inadequate. Although Bertazzi and coworkers published results of cancer mortality after 10 and 15 years of latency, results are suggestive but not definitive regarding an association between exposure to TCDD and cancer deaths. Results of the analysis of 20 years of follow-up have recently been published (Bertazzi et al., 2001). This more recent follow-up of the same group of residents in zones A and B was completed after 20.5 years to December 31, 1996. The authors stated that their results support the evaluation of TCDD as a human carcinogen, especially with the increased estimates of relative risk for all cancer mortality and for several specific sites of cancer in the >15 year latency period. No soft tissue sarcomas were observed in zones A and B. However, less than one case would have been expected to occur by the end of the follow-up. In Zone A, where exposure was highest, the expectation of a soft tissue sarcoma was only 0.1. There was little power to detect a significant risk in that region.

In a commentary by Smith and Lopipero (2001) on this study, two "key" problems were identified. The "likely" exposure levels back-calculated to the time when the exposures occurred indicate that the weighted average for the two highest exposure zones in Seveso is only 136 ng/kg TCDD (lipid adjusted) versus a mean of 3600 ng/kg TCDD (lipid adjusted) in the combined U.S. industrial cohorts. This interpretation is consistent with the data in Figure 5-1 of this document. On this basis, one would not expect to find significant increases in all cancers combined based on extra risk estimates from the occupational cohorts. This situation is not unlike the one described above for the Ranch Hand cohort. However, in this case, associations with exposure to TCDD and cancer risk are being reported.

The other issue raised by these authors is the potential for smoking-related causes of disease to be confounders in this study. The relatively low dioxin exposure and the increase in major smoking-related causes of death raise questions regarding the attribution of these cancer effects to TCDD exposure. Other data are consistent with potential dioxin hazard in this exposed population, for example, the finding of increased diabetes mortality among women. Bertazzi (2001b) takes exception to these interpretations and argues against the perception of "low" exposure and smoking as a confounder. It is clear that the question of whether the Bertazzi (2001a) study contributes to the weight of evidence for carcinogenicity awaits further follow-up and improved exposure assessment.

In general, both past and more recent human studies have focused on males. Although males comprise all the case-control studies and the bulk of the cohort study analyses, animal and mechanism studies suggest that males and females might respond differently to TCDD. There are now, however, some limited data suggesting carcinogenic responses associated with dioxin exposure in females. The only report of a female cohort that had good TCDD exposure surrogate information was that of Manz et al. (1991), which found a borderline statistically significant increase in breast cancer. Although Saracci et al. (1991) did report reduced female breast and genital organ cancer mortality, the finding was based on few observed deaths and on chlorophenoxy herbicide rather than TCDD exposures. In the later update and expansion of this cohort, Kogevinas et al. (1997) provided evidence of a reversal of this deficit and reported a borderline significant excess risk of breast cancer in females.

Bertazzi et al. (1993, 1997, 1998) reported nonsignificant decreases in breast cancer and endometrial cancer in women living in geographical areas around Seveso that were contaminated by dioxin. Breast cancer rates in women who had been exposed as infants at the time of the Seveso explosion were increased. On the basis of 15 (1.5%) confirmed breast cancer cases in the Seveso Women's Health Study, a Cox proportional hazard ratio for breast cancer of 2.1 fold (95% CI 1.0 - 4.6) was reported for a ten-fold increase in serum TCDD levels (Warner et al.,

2002). Although Kogevinas et al. (1993) saw an increase in cancer incidence among female workers most likely exposed to TCDD, no increase in breast cancer was observed in their small cohort. In short, TCDD cancer experience for women may differ from that of men, but currently there are few data to adequately address this question.

Both laboratory animal data and mechanistic inferences suggest that males and females may respond differently to the carcinogenic effects of dioxin-like chemicals. Further data will be needed to address this question of differential response between sexes, especially to hormonally mediated tumors. In addition, studies by Brown et al. (1998) demonstrated that prenatal exposure of rats to 2,3,7,8-TCDD enhances their sensitivity as adults to chemical carcinogenesis. A mechanistic understanding of the impact of gestational dioxin exposure on mammary tissue development has been provided by the work of Fenton and coworkers (Fenton et al., 2002; Vorderstrasse et al., 2004). The experimental data in laboratory animals suggest that exposure to women or perinatal exposures may result in carcinogenic responses. The epidemiological data examining the association between exposure of adult women to dioxin and cancer is limited. No epidemiological data are available to address the question of the potential impact of exposure to dioxin-like compounds on childhood cancers or the effects of perinatal exposures on the development of cancers later in life. The epidemiological data to date have not adequately addressed these issues.

In summary, 2,3,7,8-TCDD and, by inference from more limited data, other dioxin-like compounds are described as potentially multisite carcinogens in the more highly exposed human populations—consisting primarily of adult males that have been studied. Although the epidemiologic data by themselves are not sufficient to infer a causal association between exposure to TCDD and other dioxin-like chemicals and increased cancer in humans (IARC, 1997; ATSDR, 1999a; DHHS, 2001), this "limited" epidemiologic database has been strengthened by emerging data that reflect further follow-up and better exposure metrics. Although uncertainty remains, the cancer findings in the epidemiologic literature are generally consistent with results from studies of multiple laboratory animal species, where dioxin-like compounds have clearly been identified as multisite carcinogens and tumor promoters.

2,3,7,8-TCDD has also been demonstrated to promote dose-dependent clonal expansion and neoplastic transformation in human epidermal keratinocytes immortalized by simian adenovirus SV40 exposure, leading to fixed alterations in regulatory gene expression (Yang et al., 1999) and squamous cell carcinoma when inoculated into athymic nude mice (Yang et al., 1992). These phenomena did not occur in the absence of SV40 virus induction or in control cell lines, including the immortalized cell culture.

Thus, the findings of increased risk at multiple sites in occupationally exposed humans appear to be plausible, given what is known about mechanisms of dioxin action and the fundamental level at which this class of compounds appears to act on gene expression and cellular regulation in target tissues. Although several studies found a positive trend in dose-response and have been the subject of empirical risk modeling (see Part II, Chapter 8, and Becher et al., 1998, and Steenland et al., 2001), the epidemiologic data alone provide little insight into the shape of the dose-response curve below the range of observation in these occupationally exposed populations. However, Mackie et al. (2003) suggest that there is no evidence of a dioxin cancer threshold from the epidemiology data. Steenland and Deddens (2003) also reported that the results of quantitative exposure-response analyses for low environmental levels based on the NIOSH cohort are consistent with the results from the Becher cohort and demonstrate that a doubling of background levels of exposure will increase lifetime risk of cancer death between 0.1 and 1%. The issue of the shape of the dose-response curve in occupational cohorts is further discussed in Section 5.2.1 of this document.

2.2.1.2. *Animal Carcinogenicity (Cross-reference, Part II: Chapters 6 and 8)*

An extensive database on the carcinogenicity of dioxin and related compounds in laboratory studies exists and is described in detail in Part II, Chapter 6. There is adequate evidence that 2,3,7,8-TCDD is a carcinogen in laboratory animals, based on long-term bioassays conducted in both sexes of several strains of rats and mice, hamsters, and fish (U.S. EPA, 1985; Huff et al., 1991; Zeise et al., 1990; IARC, 1997; DHHS, 2001). All the studies produced positive results, leading to conclusions that TCDD is a multi-site carcinogen that increases the incidence of tumors at sites distant from the site of treatment and at doses well below the maximum tolerated dose. Since this issue was last reviewed by the Agency, in 1988, TCDD has been shown to be a carcinogen in hamsters (Rao et al., 1988), which are relatively resistant to the lethal effects of TCDD. Other preliminary data have also shown TCDD to be a liver carcinogen in the small fish *Medaka* (Johnson et al., 1992).

In the past, limited attempts had been made to demonstrate the carcinogenicity of other dioxin-like compounds. A mixture of two isomers of hexachlorodibenzo-*p*-dioxin (HCDDs) produced liver tumors in both sexes of rats and mice when given by the gavage route (NTP, 1980), but not by the dermal route in Swiss mice (NTP, 1982a,b). Reports from Rozman (1999, 2000) and Rozman et al. (2000) demonstrated lung cancer in female rats given gavage exposures of 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin(HpCDD).

Recently, the National Toxicology Program (NTP, 2003 a-d) has conducted chronic bioassays to test the relative carcinogenic potency of four dioxin-like congeners (TCDD,

2,3,4,7,8-PeCDD, PCB 118, and PCB 126), both alone and in combination. In these studies, TCDD, PCB 126 and 2,3,4,7,8-PeCDF, were tested individually or in an equally potent mixture of all three chemicals in a 2-year bioassay in female Sprague-Dawley rats. The NTP study also included PCB 118, but the results and interpretation of this bioassay remain under review due to substantial contamination by PCB 126. Initial reports from the NTP study indicate that there is clear evidence of carcinogenicity for both TCDD and PCB 126. In these studies, both TCDD and PCB 126 exposures increases the incidence of cholangiocarcinoma of the liver, cystic keratinizing epithelioma of the lung, and gingival squamous cell carcinoma of the oral mucosa. Under the conditions of the 2-year study, there was some evidence of carcinogenic activity for the 2,3,4,7,8-PeCDF based on increased incidences of cholangiocarcinoma of the liver, cystic keratinizing epithelioma of the lung and gingival squamous cell carcinoma of the oral mucosa. The results from the mixture study also indicate clear evidence of carcinogenicity as evidenced by dose dependent increases in cholangiocarcinomas in the liver and cystic keratinizing epitheliomas of the lung. The data on the three individual chemicals and mixtures demonstrate consistent increases in the incidence of three tumor types. This evidence provides support that the carcinogenicity of dioxin-like chemicals is mediated through their interactions with the Ah receptor and that the TEF methodology may provide a useful tool in estimating the potential carcinogenic risks of dioxin-like chemicals.

TCDD is characterized as a nongenotoxic carcinogen because it is negative in most assays for DNA-damaging potential and is a potent "promoter" and a weak initiator or noninitiator in two-stage initiation-promotion (I-P) models for liver, skin, and lung. The liver response is characterized by increases in altered hepatocellular foci (AHF), which are considered to be preneoplastic lesions because increases in AHFs are associated with liver cancer in rodents. The results of the multiple I-P studies enumerated in Table 6-5 and in Part II, Chapter 6, Section 6.3, have been interpreted as showing that induction of AHFs by TCDD is dose-dependent (Maronpot et al., 1993; Teeguarden et al., 1999), exposure-duration dependent (Dragan et al., 1992; Teeguarden et al., 1999; Walker et al., 2000), and partially reversible after cessation of treatment (Dragan et al., 1992; Tritscher et al., 1995; Walker et al., 2000).

Other studies indicate that other dioxin-like compounds have the ability to induce AHFs. These studies showed that the compounds demonstrate a rank-order of potency for AHF induction that is similar to that for CYP1A1 (Flodstrom and Ahlborg, 1992; Waern et al., 1991; Schrenk et al., 1994). Non-ortho-substituted, dioxin-like PCBs have also induced the development of AHFs according to their potency to induce CYP1A1 (Hemming et al., 1995; van der Plas et al., 1999). It is interesting to note that liver I-P studies carried out in ovariectomized rats demonstrated the influence that the intact hormonal system has on AHF development. AHF

were significantly reduced in the livers of ovariectomized female rats (Graham et al., 1988; Lucier et al., 1991).

I-P studies on skin have demonstrated that TCDD is a potent tumor promoter in mouse skin as well as rat liver. Early studies demonstrated that TCDD is at least two orders of magnitude more potent than the "classic" promoter tetradecanoyl phorbol acetate (Poland et al., 1982), that TCDD skin tumor promotion is AhR dependent (Poland and Knutsen, 1982), that TCDD had weak or no initiating activity in the skin system (DiGiovanni et al., 1977), and that TCDD's induction of drug-metabolizing enzymes is associated with both metabolic activation and deactivation of initiating agents, as described by Lucier et al. (1979). More recent studies show that the skin tumor-promoting potencies of several dioxin-like compounds reflect relative AhR binding and pharmacokinetic parameters (Hebert et al., 1990).

Although few I-P studies have demonstrated lung tumors in rats or mice, the study by Clark et al. (1991) is particularly significant because of its use of ovariectomized animals. In contrast to liver tumor promotion, lung tumors were seen only in initiated (diethylnitrosamine [DEN]), TCDD-treated rats. No tumors were seen in DEN-only, TCDD-only, control, or DEN/TCDD intact rats. Liver tumors are ovary dependent, but ovaries appear to protect against TCDD-mediated tumor promotion in female rat lung. Perhaps the use of transgenic animal models will allow further understanding of the complex interaction of factors associated with carcinogenesis in rodents and, by extension, in humans. Several such systems are being evaluated (Eastin et al., 1998; van Birgelen et al., 1999; Dunson et al., 2000).

The tumor-promoting ability of a number of dioxin-like chemicals has been examined. As discussed in Part II, Chapter 6, Section 6, 1,2,3,7,8-PCDD; 1,2,3,4,6,7,8-HpCDD; 2,3,4,7,8-PCDF; 1,2,3,4,7,8-HCDF; PCB126; and PCB105 all promote the development of AHF within rodent liver, suggesting that they, like TCDD, are tumor promoters. (For a summary of positive tumor-promotion studies for PCDDs and PCDFs in rats, see Part II, Chapter 6, Table 6-5). In addition, complex mixtures of dioxins and furans and commercial PCB mixtures act as promoters of liver AHF. For the five principle dioxins, furans, and coplanar PCBs that comprise approximately 80% of the current, total dioxin/furan/PCB TEQ in human blood, all are positive in either rodent bioassays or rodent liver tumor-promotion studies or mouse skin tumor-promotion studies. Although the majority of dioxin-like congeners have not been tested for carcinogenicity in chronic rodent bioassays, these data suggest that it is likely that those individual congeners and mixtures of dioxin-like compounds that comprise the majority of the dioxin-like activity in human tissues are likely to be carcinogenic to rodents.

van den Berg et al. (2000) present a summary of the data (their Table 1) relied on by WHO's European Centre for Environment and Health (WHO-ECEH) and IPCS in their joint

consensus re-evaluation of the TEFs for PCDDs, PCDFs, and dioxin-like PCBs for mammals. These TEFs were derived using a tiered approach in which in vivo toxicity data were given more weight than in vitro data, toxicity more than biochemical endpoints, and chronic more than acute data. Table 2-4 summarizes the tumor incidence and promotion data that were cited in the development of these $TEFs_{DFP}$-WHO_{98}. The data presented are for those congeners that are principal contributors to the background body burden of dioxin TEQs in the United States (see Part I, Chapter 3). For 1,2,3,7,8-PeCDF and 2,3,4,7,8-PeCDF, the TEF was used to adjust the dose from the studies by Waern et al. (1991), and for PCB 126 similar dose adjustments are included from Hemming et al. (1995; their Fig. 4). For the comparison of TCDD to the HxCDDs, the primary TCDD data points from the Kociba et al. (1978) bioassay were graphed for both the original tumor count data and for the revised tumor counts from Goodman and Sauer (1992). This presentation of both the original and the revised tumor counts for TCDD reflects the contemporaneous performance and analysis of the HxCDD and TCDD bioassays and pathology and the recognition that the HxCDD pathology has not been re-analyzed.

Table 2-3 illustrates the comparability of the TCDD and other congener data sets based on TEFs. This analysis also demonstrates that the development of the TEFs for all of the congeners that contribute substantially to the background dioxin TEQ appropriately reflect either cancer bioassay or tumor promotion data. Furthermore, when one considers the impact of current TEF values on compounds that made up the majority of the TEQ prior to 1990, it is clear that more than 80% of the TEQ for either dioxins/furans or PCBs was made up of compounds for which the current TEF is supported by data on relative potencies which included tumor promotion or carcinogenic endpoints. This point is illustrated in Part II, Chapter 6, Table 6-10.

2.2.1.3. *Plausible Mode(s) of Carcinogenic Action*

Several potential mechanisms for TCDD carcinogenicity are discussed above and in Part II, Chapter 6, Section 6.4. These include oxidative stress, indirect DNA damage, endocrine disruption/growth dysregulation/altered signal transduction, and cell replication/apoptosis leading to tumor promotion. All of these mechanisms are biologically plausible as contributors to the carcinogenic process in humans, and none are mutually exclusive. Several biologically based models that encompass many of these activities are described in Part II, Chapter 8, Section 8.4. Further work is needed to elucidate a detailed mechanistic model for any particular carcinogenic response in animals or in humans; however, plausible modes of action with probable relevance to human carcinogenicity are discussed below.

TCDD is a potent tumor promoter in rat and mouse liver and in initiated human skin cells. In general terms, it is believed that cancer is likely due to the clonal expansion of damaged

cells that have a heritable genetic defect. Increased growth and accumulation of damage in critical genes ultimately aid in the progression into tumors. Consequently, promotion of carcinogenesis by TCDD may occur at several steps: (1) increased formation of initiated/susceptible cells through DNA mutation and/or increase rate of fixation of damaged DNA into the genome, (2) reduced loss of initiated cells through a suppression of apoptosis, (3) increase in growth rate and clonal expansion of initiated cells, and (4) accumulation of DNA damage in critical genes resulting in the progression of clonally expanded cell populations into tumors. Within this framework, it is hypothesized that TCDD may be acting as a tumor promoter through multiple mechanisms. Primarily, the activation of the AhR leads to alteration in genes that are involved in normal cell growth and differentiation pathways.

TCDD may contribute to the formation and accumulation of DNA damage via an indirect mechanism involving the production of reactive oxygen species. These reactive oxygen species may be formed as a result of autooxidation during futile metabolism of TCDD by the induction of CYP1 enzymes or via the CYP1-dependent production of estrogen metabolites capable of redox cycling. The clonal expansion of these damaged cells by TCDD and related chemicals is likely to occur through the altered expression and activity of a number of genes that regulate the cell-cycle. Activation of the AhR by TCDD results in altered expression or activity of the EGF receptor, retinoblastoma protein, TGF-beta, and many others. These proteins all regulate the cell cycle, and alterations of these proteins would alter cell growth properties.

The contribution of these two pathways in the carcinogenic actions of TCDD remains uncertain. However, Portier et al. (1996) have proposed a model in which the contribution of TCDD to the number of DNA damaged or initiated cells plays a significant role in its carcinogenic response. In contrast, Conolly and Andersen (1997) have proposed a tumor promotion model based on a negative selection mechanism in which the actions of TCDD are focused on its ability to alter cell growth properties. Descriptions of these models are provided in Part II, Chapter 8. Interestingly, the use of the model by Portier and colleagues leads to a result that is consistent with low-dose linearity, whereas the Andersen and Conolly model predicts highly nonlinear dose response relationships in the low-dose region. Presently, the available data do not allow for adequate discrimination between these two models.

TCDD causes a dose-related increase in thyroid follicular cell adenomas and carcinomas in rats and mice. One hypothesis for the induction of thyroid tumors involves the disruption of thyroid hormone homeostasis via the induction of the phase II enzymes UDP-glucuronosyltransferases (UGTs) (Hurley, 1998; Hill et al., 1998). Dioxin-like compounds induce the synthesis of UDP-glucuronosyltransferase-1 (UGT1) mRNA by an AhR-dependent transcriptional mechanism (Bock et al., 1998; Nebert et al., 1990). It is proposed that dioxin-like

chemicals increase the incidence of thyroid tumors through an extrathyroidal mechanism. Dioxin-like chemicals induce hepatic UGT, resulting in increased conjugation and elimination of thyroxine (T4) and leading to reduced serum T4 concentrations. T4 production is controlled by thyroid stimulating hormone (TSH), which is under negative and positive regulation from the hypothalamus, pituitary, and thyroid by thyrotrophin releasing hormone (TRH), TSH itself, thyroxine (T4), and triiodothyronine (T3). Consequently, the reduced serum T4 concentrations would lead to a decrease in the negative feedback inhibition on the pituitary gland. This would then lead to a rise in secreted TSH and stimulation of the thyroid. The persistent induction of UGT by dioxins and subsequent prolonged stimulation of the thyroid would result in thyroid follicular cell hyperplasia and hypertrophy of the thyroid, thereby increasing the risk of progression to neoplasia.

In support of this hypothesis, Kohn et al. (1996) modeled the effect of 2,3,7,8-TCDD on UGTs and thyroid hormones in female rats within the framework of a PBPK model. This mathematical model described release and uptake of thyroid hormones, metabolism, 2,3,7,8-TCDD induction of UGT1, regulation of TSH release from the pituitary by T4, and feedback on TRH and somatostatin, which inhibits TSH release. The model successfully reproduced the observed effects of 2,3,7,8-TCDD on serum T3, T4, and TSH and UGT1 mRNA and enzyme activity, suggesting that this is a plausible mechanism for an indirect role of 2,3,7,8-TCDD on the thyroid. This model is supported by the more recent experimental work of Schuur et al. (1997), which demonstrated the extrathyroidal effects of 2,3,7,8-TCDD on thyroid hormone turnover.

Although this discussion illustrates that there is no defined molecular mechanism leading to cancer in either liver or thyroid, it does demonstrate the concept of "mode of action" as defined in the Agency's proposed cancer guidelines (U.S. EPA, 1996, 1999, 2003). In each case, critical "key events" that correlate with carcinogenicity can be identified and measured, and these same events occur in both animals and humans. Although these relationships and linkages remain to be detailed, they form plausible, testable hypotheses whose acceptance by the scientific community is growing.

Despite this lack of a defined mechanism at the molecular level, there is a consensus that 2,3,7,8-TCDD and related compounds are receptor-mediated carcinogens in that (1) interaction with the AhR is a necessary early event; (2) 2,3,7,8-TCDD modifies a number of receptor and hormone systems involved in cell growth and differentiation, such as the EGFR and estrogen receptor; and (3) sex hormones exert a profound influence on the carcinogenic action of 2,3,7,8-TCDD.

2.2.1.4. *Other Data Related to Carcinogenesis*

Despite the relatively large number of bioassays on 2,3,7,8-TCDD, those by Kociba et al. (1978) and NTP (1982a), because of their multiple dose groups and wide dose range, continue to be the focus of dose-response modeling efforts and of additional review. Goodman and Sauer (1992) reported a re-evaluation of the female rat liver tumors in the Kociba study using the latest pathology criteria for such lesions. The review confirmed only approximately one-third of the tumors of the previous review (Squire, 1980). Although this finding did not change the determination of carcinogenic hazard—as 2,3,7,8-TCDD induced tumors in multiple sites in this study—it did have an effect on evaluation of dose-response and on estimates of risk at low doses. These issues are discussed in a later section of this document.

One of the more intriguing findings in the Kociba bioassay was reduced tumor incidences of the pituitary, uterus, mammary gland, pancreas, and adrenals in exposed female rats as compared to controls. Although this finding, coupled with evaluation of epidemiologic data, has led some authors to conclude that dioxin possesses "anticarcinogenic" activity (Kayajanian, 1997, 1999), it should be noted that in the Kociba study, the decreased incidence of tumors, with the exception of mammary gland tumors, is associated with significant weight loss in these rats. Examination of the data from NTP also demonstrates a significant decrease in these tumor types when there is a concomitant weight loss in the rodents, regardless of the chemical administered (Haseman and Johnson, 1996). It is also worth noting that the decrease in mammary tumors was only observed in one of seventeen rodent carcinogenesis studies, and was not observed in the recent NTP studies on TCDD, PCB 126, and 2,3,4,7,8-PeCDF (NTP, 2003 a-d).

As discussed in Section 3.2.3, under certain circumstances exposure to 2,3,7,8-TCDD may elicit beneficial effects. For example, 2,3,7,8-TCDD protects against the subsequent carcinogenic effects of polycyclic aromatic hydrocarbons (PAHs) in mouse skin, possibly reflecting induction of detoxifying enzymes (Cohen et al., 1979; DiGiovanni et al., 1980). In other situations, 2,3,7,8-TCDD-induced changes in estrogen metabolism may alter the growth of hormone-dependent tumor cells, producing a potential anticarcinogenic effect (Spink et al., 1990; Gierthy et al., 1993). While TCDD has been shown to inhibit the growth of certain breast cancer cell lines, Warner et al. (2002) have demonstrated an increase in breast cancer in highly exposed women from Seveso. Because the mechanism of the decreases in the tumor cells is unknown, extrapolation of these effects to humans is premature.

In considering overall risk, one must take into account factors such as the range of doses to target organs and hormonal state to obtain a complete picture of hazard and risk. Although exposure to dioxins may influence cancer response directly or indirectly and positively or negatively, it is unlikely that such data will be available to argue that dioxin exposure provides a

net benefit to human health. It is also important to note that the doses at which the incidence of certain tumors may decrease is in the same range at which adverse noncancer effects occur (see Appendix A).

2.2.1.5. *Cancer Hazard Characterization*

TCDD, CDDs, CDFs, and dioxin-like PCBs are a class of well-studied compounds whose human cancer potential is supported by a large database, including "limited" epidemiological support, unequivocal animal carcinogenesis, and biologic plausibility based on mode of action data. In 1985, EPA classified 2,3,7,8-TCDD and related compounds as "probable" human carcinogens, based on the available data. During the intervening years, the database relating to the carcinogenicity of dioxin and related compounds has grown and strengthened considerably. In addition, EPA guidance for carcinogen risk assessment has evolved (U.S. EPA, 1996, 1999, 2003). Under EPA's current approach, complex mixtures of dioxin and related compounds are considered "likely to be carcinogenic to humans," as are individual dioxin-like congeners other than TCDD. This descriptor is based primarily on the concept of toxic equivalency but also on the data available to support this characterization for individual congeners. Positive lifetime bioassays are available for a number of the principal congeners contributing to human TEQ body burden, specifically TCDD, 2,3,4,7,8-PeCDF, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, and PCB 126 (Kociba et al., 1978; NTP, 1980; NTP, 2003 a-d).

2,3,7,8-TCDD is best characterized as "carcinogenic to humans." This means that, based on the weight of all of the evidence (human, animal, mode of action), 2,3,7,8-TCDD meets the stringent criteria that allows EPA and the scientific community to accept a causal relationship between exposure and cancer hazard. The guidance (see EPA, 2003, section 2.6) suggests that "carcinogenic to humans" is an appropriate descriptor of carcinogenic potential when there is an absence of conclusive epidemiologic evidence to clearly establish a cause-and-effect relationship between human exposure and cancer but there is compelling carcinogenicity data in animals and mechanistic information in animals and humans demonstrating similar modes of carcinogenic action.

The "carcinogenic to humans" descriptor is suggested for 2,3,7,8-TCDD because *all* of the following conditions are met:

- Occupational epidemiologic studies all show an association between 2,3,7,8-TCDD exposure and increases in the all-cancers-combined category, in lung cancer, and perhaps in cancers at other sites, but the data are insufficient on their own to demonstrate a causal association.

- There is extensive carcinogenicity in both sexes of multiple species of animals at multiple sites.

- There is general agreement that the mode of 2,3,7,8-TCDD's carcinogenicity is AhR dependent and proceeds through modification of the action of a number of receptor and hormone systems involved in cell growth and differentiation, such as the EGFR and estrogen receptors.

- The human AhR and the rodent AhR are similar in structure and function and, once transformed, both bind to the same DNA response elements, designated DRE's.

- Human and rodent tissue and organ cultures respond to TCDD and related chemicals in a similar manner and at similar concentrations.

Other dioxin-like compounds are characterized as "likely to be carcinogenic to humans," primarily because of the lack of epidemiological evidence associated with their carcinogenicity, although there is a strong inference based on toxic equivalency that they would behave in humans as 2,3,7,8-TCDD does. Each of the congeners that contributes substantially to human body burden has been evaluated in vivo in cancer bioassays or tumor promotion assays. Each has a large database demonstrating AhR-mediated dioxin-like activities. Each has physico-chemical properties that contribute to their persistence. For each congener, the degree of certainty of carcinogenic hazard is dependent on the available congener-specific data and its consistency with the generalized mode of action that underpins toxic equivalency for 2,3,7,8-TCDD and related compounds. For the congeners most frequently encountered in human blood, milk, and adipose tissue, the database in support of 2,3,7,8-TCDD-like carcinogenic hazard is strong; those with weaker data supporting 2,3,7,8-TCDD-like carcinogenicity contribute relatively little to total TEQ.

On the basis of this logic, all complex environmental mixtures of 2,3,7,8-TCDD and dioxin-like compounds would be characterized as "likely" carcinogens, but the degree of certainty of the cancer hazard would be dependent on the major constituents of the mixture. For instance, the hazard potential, although still considered "likely," would be characterized differently for a mixture whose TEQ was dominated by octachlorodibenzo-*p*-dioxin as compared to one dominated by other PCDDs.

2.2.2. Reproductive and Developmental Effects

Several sections of this reassessment (Part II, Chapter 5 and Chapter 7b) have focused on the variety of effects that dioxin and dioxin-like agents can have on human reproductive health and development. The emphasis in each of these chapters has been on the discussion of the more recent reports of the impact of dioxin-like compounds on reproduction and development. These reports have been put into context with previous reviews of the literature applicable in risk assessment (Hatch, 1984; Sweeney, 1994; Kimmel, 1988) to develop a profile of the potential for dioxin and dioxin-like agents to cause reproductive or developmental toxicity, based on the available literature. An earlier version of the literature review and discussion contained in Part II, Chapter 5, has been previously published (Peterson et al., 1993).

The origin of concerns regarding a potential link between exposure to chlorinated dioxins and adverse developmental events can be traced to early animal studies reporting increased incidence of developmental abnormalities in rats and mice exposed early in gestation to 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (Courtney and Moore, 1971). 2,4,5-T is a herbicide that contains dioxin and related compounds as impurities. Its use was banned in the late 1970s, but exposure to human populations continued as a result of past production, use, and disposal.

2.2.2.1. *Human Effects*

The literature base with regard to potential human effects is detailed in Part II, Chapter 7b, Section 7.13. In general, there is limited epidemiological evidence to make a direct association between exposure to TCDD or other dioxin-like compounds and effects on human reproduction or development. One effect that may illustrate this relationship is the altered sex ratio (increased females) seen in the 6 years after the Seveso, Italy, accident (Mocarelli et al., 1996, 2000), and in a heavily exposed occupational cohort in Russia (Ryan et al., 2002). Particularly intriguing in these evaluations is the observation that exposure before and during puberty is linked to this sex ratio effect, and predominantly through the paternal side. Other sites have been examined for the effect of TCDD exposure on sex ratio with mixed results but with smaller numbers of offspring. Data on these sites are still preliminary, but effects similar to the Seveso findings are being reported. Continued evaluation of the Seveso population may provide other indications of impacts on reproduction and development but, for now, such data are limited and further research is needed.

Positive human data on developmental effects of dioxin-like compounds are limited to a few studies of populations exposed to a complex mixture of potentially toxic compounds (e.g., developmental studies from the Netherlands and effects of ingestion of contaminated rice oil in Japan [Yusho] and Taiwan [Yu-Cheng]). In the latter studies, however, all four manifestations

of developmental toxicity (reduced viability, structural alterations, growth retardation, and functional alterations) were observed to some degree following exposure to dioxin-like compounds as well as other agents. Data from the Dutch cohort of children exposed to PCBs and dioxin-like compounds (Huisman et al., 1995a, b; Koopman-Esseboom et al., 1994a-c; 1995a, b, 1996; Pluim et al., 1992, 1993, 1994; Weisglas-Kuperus et al., 1995; Patandin et al., 1998, 1999; ten Tusscher et al., 2003; Vreugdenhil et al., 2002) suggest impacts of background levels of dioxin and related compounds on neurobehavioral outcomes, thyroid function, immune function, and liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Although these effects cannot be attributed solely to dioxin and related compounds, several associations suggest that these are, in fact, likely to be AhR-mediated effects. Similarly, it is highly likely that the developmental effects in human infants exposed to a complex mixture of PCBs, PCDFs, and polychlorinated quaterphenyls (PCQs) in the Yusho and Yu-Cheng poisoning episodes may have been caused by the combined exposure to those PCB and PCDF congeners that are AhR agonists (Lü and Wong, 1984; Kuratsune, 1989; Rogan, 1989). However, it is not possible to determine the relative contributions of individual chemicals to the observed effects.

The incidents at Yusho and Yu-Cheng resulted in increased perinatal mortality and low birth weight in infants born to women who had been exposed. Rocker bottom heal was observed in Yusho infants, and functional abnormalities have been reported in Yu-Cheng children. Not all the effects that were seen are attributable only to dioxin-like compounds. The similarity of effects observed in human infants prenatally exposed to this complex mixture with those reported in adult monkeys exposed only to TCDD suggests that at least some of the effects in the Yusho and Yu-Cheng children are due to the TCDD-like congeners in the contaminated rice oil ingested by the mothers of these children. The similar responses include a clustering of effects in organs derived from the ectodermal germ layer, referred to as ectodermal dysplasia, including effects on the skin, nails, and Meibomian glands and developmental and psychomotor delay during developmental and cognitive tests (Chen et al., 1992). Some investigators believe that because some of the effects in the Yusho and Yu-Cheng cohorts do not correlate with TEQ, such effects could be exclusively due to nondioxin-like PCBs or to an interaction between the dioxins and the nondioxin-like congeners.

Of particular interest is the common developmental origin (ectodermal layer) of many of the organs and tissues that are affected in humans. An ectodermal dysplasia syndrome involving hyperpigmentation, deformation of the fingernails and toenails, conjunctivitis, gingival hyperplasia, and abnormalities of the teeth has been clearly associated with the Yusho and Yu-Cheng episodes, and in the non-human primate studies. Alaluusua et al. (1996, 1999)

investigated dioxin exposure and tooth development in Finnish children as a result of studies of dental effects in dioxin-exposed rats, mice, and nonhuman primates (Part II, Chapter 5, Section 5.2) and in PCB-exposed children (Rogan et al., 1988). The Finnish investigators examined enamel hypomineralization of permanent first molars in 6–7-year-old children. The length of time that infants breast-fed was not significantly associated with either mineralization changes or with TEQ levels in the breast milk. However, when the levels and length of breast-feeding were combined in an overall score, a statistically significant association was observed (r= 0.3, p=0.003, regression analysis). These data are discussed further in Part II, Chapter 7b, Section 7.13. Follow-up mechanistic studies on tooth development in TCDD sensitive and resistant rats revealed a relatively high dose impact on epithelial-mesenchymal interactions, particularly the mesenchymal odontocytes. This effect that was not associated with differential resistance to acute TCDD toxicity (Kiukkonen et al., 2002).

Other investigations into noncancer effects of human exposure to dioxin have provided human data on TCDD-induced changes in circulating reproductive hormones. This was one of the effects judged as having a positive relationship with exposure to TCDD in Part II, Chapter 7b, Section 7.13. Levels of reproductive hormones have been measured with respect to exposure to 2,3,7,8-TCDD in three cross-sectional medical studies. Testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were measured in trichlorophenol (TCP) and 2,4,5-T production workers from the NIOSH cohort (Egeland et al., 1994), in Army Vietnam veterans (CDC Vietnam Experience Study, 1988), and in Air Force Ranch Hands, who handled and/or sprayed Agent Orange during the Vietnam War (Roegner et al., 1991; Grubbs et al., 1995). A recent study also demonstrated an inverse correlation between TCDD levels and prolactin in 2,4,5,-T herbicide sprayers (Johnson et al., 2001). Alterations in breast development have been reported in young women, where a doubling of the serum dioxin concentration (CALUX assay) increased the odds of not having reached the adult stage of breast development by 2.3 fold (P<0.02) in the women (~17 yo) studied (Den Hond et al., 2002). Alterations in menstrual duration and flow have been reported in women exposed as premenarcheal girls 20 years previously as a result of the Seveso incident (Eskenazi et al., 2002a).

The risk of abnormally low testosterone was two to four times higher in exposed workers who had serum 2,3,7,8-TCDD levels above 20 ng/g than in unexposed referents (Egeland et al., 1994). In both the 1987 and 1992 examinations, mean testosterone concentrations were slightly but not significantly higher in Ranch Hands (Thomas et al., 1990; Grubbs et al., 1995). FSH and LH concentrations were no different between the exposed and comparison groups. No significant associations were found between Vietnam experience and altered reproductive

hormone levels (CDC Vietnam Experience Study, 1988). Only the NIOSH study (Egeland et al., 1994) found an association between serum 2,3,7,8-TCDD level and increases in serum LH.

The findings of the NIOSH and Ranch Hand studies are plausible, given the pharmacological and toxicological properties of 2,3,7,8-TCDD in animal models, which are discussed in Part II, Chapters 5 and 7. One plausible mechanism responsible for the effects of dioxins may involve their ability to influence hormone receptors. The AhR, to which 2,3,7,8-TCDD binds, and the hormone receptors are signaling pathways that regulate homoeostatic processes. These signaling pathways are integrated at the cellular level, and there is considerable "cross-talk" between these pathways. For example, studies suggest that 2,3,7,8-TCDD modulates the concentrations of numerous hormones and/or their receptors, including estrogen (Romkes and Safe, 1988; Romkes et al., 1987), progesterone (Romkes et al., 1987), glucocorticoid (Ryan et al., 1989), and thyroid hormones (Gorski and Rozman, 1987; Pavuk et al., 2003).

In summary, the results from both the NIOSH and the Ranch Hand studies are limited by the cross-sectional nature of the data and the type of clinical assessments conducted. However, the available data provide evidence that small alterations in human male reproductive hormone levels are associated with serum 2,3,7,8-TCDD.

2.2.2.2. *Experimental Animal Effects*

The extensive experimental animal database with respect to reproductive and developmental toxicity of dioxin and dioxin-related agents is discussed in Part II, Chapter 5. Dioxin exposure has been observed to result in both male and female reproductive effects as well as developmental effects. These latter effects are among the most responsive health endpoints to dioxin exposure (see Part II, Chapter 8, Section 8.3). In general, the prenatal and developing postnatal animal is more sensitive to the effects of dioxin than is the adult. In several instances (e.g., fetotoxicity in hamsters, rats, mice, and guinea pigs), the large species differences seen in acute toxicity are greatly reduced when developing animals are evaluated. Most of the data reviewed are from studies of six genera of laboratory animals. Although much of the data come from animals exposed only to TCDD, more recent studies of animals exposed to mixtures of PCDD/PCDF/ PCB congeners provide results that are consistent with the studies of TCDD alone.

2.2.2.2.1. ***Developmental toxicity.*** Dioxin exposure results in a wide variety of developmental effects; these are observed in three different vertebrate classes and in several species within each class. All four of the manifestations of developmental toxicity have been observed following

exposure to dioxin: reduced viability, structural alterations, growth retardation, and functional alterations. As summarized previously (Peterson et al., 1993), increased prenatal mortality (rat and monkey), functional alterations in learning (rat, mouse, and monkey) and sexual behavior (rat), and changes in the development of the reproductive system (rat, hamster, and mouse) occur at the lowest exposure levels tested (see also Part II, Chapter 8, Section 8.3).

Dioxin exposure has resulted in reduced prenatal or postnatal viability in virtually every species in which it has been tested. Previously, increased prenatal mortality appeared to be observed only at exposures that also resulted in maternal toxicity. However, the studies of Olson and McGarrigle (1990) in the hamster and Schantz and Bowman (1989) in the monkey suggested that this was not the case in all species. Although the data from these two studies were limited, prenatal death was observed in cases where no maternal toxicity was evident. In the rat, Peterson's laboratory (Bjerke et al., 1994a, b; Roman et al., 1995) reported increased prenatal death following a single exposure to TCDD during gestation that did not cause maternal toxicity, and Gray et al. (1995a) observed a decrease in postnatal survival under a similar exposure regimen. Although identifying the presence or absence of maternal toxicity may be instructive as to the specific origin of the reduced prenatal viability, it does not alter the fact that pre- and postnatal deaths were observed. In either case, the Agency considers these effects as being indicators of developmental toxicity in response to the exposure (U.S. EPA, 1991b).

Some of the most striking findings regarding dioxin exposure relate to the effects on the developing reproductive system in laboratory animals. Only a single, low-level exposure to TCDD during gestation is required to initiate these developmental alterations. Mably et al. (1992a-c) originally reported that a single exposure of the Holtzman maternal rat to as little as 0.064 μg/kg could alter normal sexual development in the male offspring. A dose of 0.064 μg/kg in these studies resulted in a maximal body burden in the maternal animal of 64 ng/kg during critical windows in development. More recently, these findings of altered normal sexual development have been further defined (Bjerke et al., 1994a, b; Gray et al., 1995a; Roman et al., 1995) and extended to female offspring and other strains (Faqi et al., 1998; Ohsako et al., 2001) and species (hamsters and mice) (Gray et al., 1995b; Theobald et al., 1997). In general, the findings of these later studies have produced qualitatively similar results that define a significant effect of dioxin on the developing reproductive system.

In the developing male rat, TCDD exposure during the prenatal and lactational periods results in delay of the onset of puberty, as measured by age at preputial separation. There is a reduction in testis weight, sperm parameters, and sex accessory gland weights. In the mature male exposed during the prenatal and lactational periods, there is an alteration of normal sexual behavior and reproductive function. Males exposed to TCDD during gestation are

demasculinized. Feminization of male sexual behavior and a reduction in the number of implants in females mated with exposed males have also been reported, although these effects have not been consistently found. These effects do not appear to be related to reductions in circulating androgens, which were shown in the most recent studies to be unaffected by TCDD. Most of these effects have occurred in a dose-related fashion, some at doses of 0.05 μg/kg and 0.064 μg/kg, the lowest doses tested (Mably et al., 1992c; Gray et al., 1997a).

In Part II, Chapter 8, ED_{01} values were estimated from the Mably et al. (1992a-c) and Gray et al. (1997a) reports. In these two studies more than 44 data sets were modeled, and 17 of these data sets had body burden ED_{01}s lower than 50 ng/kg. For the 12 endpoints in the Mably et al. studies that were modeled in Part II, Chapter 8, the median body burden ED_{01} estimate is 5.2 ng TCDD/kg. Although not modeled in Part II, Chapter 8, the data from Faqi et al. (1998) and Ohsaka et al. (2001) have LOAELs and NOAELs for developmental reproductive effects of TCDD in male rats ranging from body burdens of 12.5–200 ng TCDD/kg, which is consistent with the Mably et al. and Gray et al. studies.

In the developing female rat, Gray and Ostby (1995) demonstrated altered sexual differentiation in both the Long Evans and Holtzman strains. The effects observed depended on the timing of exposure. Exposure during early organogenesis altered the cyclicity, reduced ovarian weight, and shortened the reproductive lifespan. Exposure later in organogenesis resulted in slightly lowered ovarian weight, structural alterations of the genitalia, and a slight delay in puberty. However, cyclicity and fertility were not affected with the later exposure. The most sensitive dose-dependent effects of TCDD in the female rat were the structural alterations of the genitalia that occurred at 0.20 μg TCDD/kg administered to the dam (Gray et al., 1997b).

As described above, studies demonstrating adverse health effects from prenatal exposures often involved a single dose administered at a discrete time during pregnancy. The production of prenatal effects at a given dose appears to require exposure during critical times in fetal development. This concept is well supported by a recent report (Hurst et al., 2000) that demonstrated the same incidence of adverse effects in rat pups born to dams with a single exposure of 0.2 μg TCDD/kg body weight on gestation day 15 versus 1.0 μg TCDD/kg body weight on gestation day 8. Both of these experimental exposure paradigms resulted in the same fetal tissue concentrations and body burdens during the critical window of sensitivity. For example, exposure to 0.2 μg TCDD/kg on day 15 resulted in 13.2 pg TCDD/g fetal tissue on day16; exposure to 1.0 μg TCDD/kg on day 8 resulted in 15.3 pg TCDD/g fetus on day 16. This study demonstrates the appropriateness of the use of body burden to describe the effects of TCDD when comparing different exposure regimens. The uncertainties introduced when trying to compare studies with steady-state body burdens with single-dose studies may make it difficult

to determine a lowest effective dose. Application of pharmacokinetic models (described in Parts I and II) to estimate body burdens at the critical time of development is expected to be a sound method for relating chronic background exposures to the results obtained from single-dose studies.

Structural malformations, particularly cleft palate and hydronephrosis, occur in mice administered TCDD. The findings, although not representative of the most sensitive developmental endpoints, indicate that exposure during the critical period of organogenesis can affect the processes involved in normal tissue formation. The TCDD-sensitive events appear to require the AhR. Mouse strains that produce AhRs with relatively high affinity for TCDD respond to lower doses than do strains with relatively low-affinity receptors. Moreover, congeners that have a greater affinity for the AhR are more developmentally toxic than those that have a lower affinity. This is consistent with the rank ordering of toxic potency based on affinity for the receptor, as discussed in Part II, Chapter 9, Section 9.3. In addition, mice in which the Ah receptor has been knocked out do not develop cleft palate.

Recent work, not elaborated upon here, has demonstrated that developmental exposure of rodents to dioxin also permanently alters the development of the prostate in wild type but not AhR null mutant mice (Lin et al., 2003), and mammary development in rats and mice (Fenton et al, 2002; Vorderstrasse et al., 2003). The key role of the Ah receptor has also been demonstrated in the developing heart of AhR null mice (Lund et al., 2003).

2.2.2.2.2. ***Adult female reproductive toxicity.*** The primary effects of TCDD on female reproduction in animals appear to be decreased fertility, inability to maintain pregnancy for the full gestational period, and, in the rat, decreased litter size. In some studies of rats and of primates, signs of ovarian dysfunction such as anovulation and suppression of the estrous cycle have been reported (Kociba et al., 1976; Barsotti et al., 1979; Allen et al., 1979; Li et al., 1995a, b). Although the majority of reproductive effects are associated with high-dose exposures in experimental animals, the induction of endometriosis in primates occurs at body burdens near background human exposures. This effect is discussed further below.

2.2.2.2.3. ***Adult male reproductive toxicity.*** TCDD and related compounds decrease testis and accessory sex organ weights, cause abnormal testicular morphology, decrease spermatogenesis, and reduce fertility when given to adult animals in doses sufficient to reduce feed intake and/or body weight. In the testes of these different species, TCDD effects on spermatogenesis are characterized by loss of germ cells, the appearance of degenerating spermatocytes and mature spermatozoa within the lumens of seminiferous tubules, and a reduction in the number of tubules

containing mature spermatozoa (Allen and Lalich, 1962; Allen and Carstens, 1967; McConnell et al., 1978; Chahoud et al., 1989). This suppression of spermatogenesis is not a highly sensitive effect when TCDD is administered to postweanling animals, as an exposure of 1 μg/kg/day over a period of weeks appears to be required to produce these effects.

2.2.2.3. ***Other Data Related to Developmental and Reproductive Effects***

2.2.2.3.1. ***Endometriosis.*** The association of dioxin with endometriosis was first reported in a study of rhesus monkeys that had been exposed for 4 years to dioxin in their feed and then held for an additional 10 years (Rier et al., 1993). There was a dose-related increase in both the incidence and severity of endometriosis in the exposed monkeys as compared to controls. Follow-up on this group of monkeys revealed a clear association with total TEQ. A study in which rhesus monkeys were exposed to PCBs for up to 6 years failed to show any enhanced incidence of endometriosis (Arnold et al., 1996). However, many of these monkeys were no longer cycling, and the time may not have been adequate to develop the response. In the TCDD monkey study, it took 7 years before the first case of endometriosis was noted (Rier et al., 1993).

A recent study in Cynomolgus monkeys showed promotion of surgically induced endometriosis by TCDD within 1 year after surgery (Yang et al., 2000). Studies using rodent models for surgically induced endometriosis have also shown the ability of TCDD to promote lesions in a dose-related manner (Cummings et al., 1996, 1999; Johnson et al., 1997; Bruner-Tran et al., 1999). This response takes at least 2 months to be detected (Cummings et al., 1996, 1999; Johnson et al., 1997). Another study in mice that failed to detect dioxin promotion of surgically induced endometriosis held the mice for only 1 month, not long enough to detect a response (Yang et al., 1997). Prenatal exposure of mice also enhanced the sensitivity of the offspring to the promotion of surgically induced endometriosis by TCDD (Cummings et al., 1999).

The effects of TCDD in the murine model of endometriosis appear to be AhR-mediated, as demonstrated in a study in which AhR ligands were able to promote the lesions, whereas non-AhR ligands, including a nondioxin-like PCB, had no effect on surgically induced endometriosis (Johnson et al., 1997). Dioxin has also been shown to result in endometriosis with human endometrial tissue implanted in nude mice (Bruner-Tran et al., 1999).

Data on the relationship of dioxins to endometriosis in humans is intriguing, but preliminary. Studies in the early 1990s suggested that women who had higher levels of persistent organochlorines were at increased risk for endometriosis (Gerhard and Runnebaum, 1992). This was followed by the observation that Belgian women, who have the highest levels of dioxins in their background population, had higher incidences of endometriosis than those reported from

other populations (Koninckx et al., 1994). A study from Israel then demonstrated that there was a correlation between detectable TCDD in women who had surgically confirmed endometriosis in comparison to those who had no endometriosis (Mayani et al., 1997).

Recent studies from Belgium indicate that women with higher body burdens, based on serum TEQ determinations, are at greater risk for endometriosis (Pauwels et al., 1999). No association was seen with total PCBs in this study. A small study in the United States that did not involve surgically confirmed endometriosis saw no association between TCDD and endometriosis (Boyd et al., 1995). Likewise, a study in Canada saw no association between total PCBs and endometriosis (Lebel et al., 1998). The lack of an association with total PCBs is not surprising, because the rodent studies have indicated that this response is AhR-mediated (Johnson et al., 1997). The Seveso Women's Health Study reported "...a doubled, non-significant risk for endometriosis among women with serum TCDD levels of 100 ppt or higher, but no clear dose-response. Unavoidable disease misclassification in a population-based study may have led to an underestimate of the true risk of endometriosis"(Eskenazi et al., 2002b).

The animal results lend biological plausibility to the epidemiology findings (Birnbaum and Cummings, 2002). Endometriosis is not only an endocrine disorder, it is also associated with immune system alterations (Rier et al., 1995; Rier and Foster, 2002). Dioxins are known to be potent modulators of the animal immune system and to affect estrogen homeostasis. Further studies are clearly needed to provide additional support to this association of endometriosis and dioxins, as well as to demonstrate causality.

2.2.2.3.2. ***Androgenic deficiency.*** The effects of TCDD on the male reproductive system when exposure occurs in adulthood are believed to be due in part to an androgenic deficiency. This deficiency is characterized in adult rats by decreased plasma testosterone and 5α-dihydrotestosterone concentrations, unaltered plasma LH concentrations, and unchanged plasma clearance of androgens and LH (Moore et al., 1985, 1989; Mebus et al., 1987; Moore and Peterson, 1988; Bookstaff et al., 1990a). The cause of the androgenic deficiency was believed to be due to decreased testicular responsiveness to LH and increased pituitary responsiveness to feedback inhibition by androgens and estrogens (Moore et al., 1989, 1991; Bookstaff et al., 1990a, b; Kleeman et al., 1990). The single dose used in some of those earlier studies (15 µg TCDD/kg body weight) is now known to affect Leydig cells (Johnson et al., 1994).

2.2.2.4. *Developmental and Reproductive Effects Hazard Characterization*

There is limited direct evidence addressing the issues of how or at what levels humans will begin to respond to dioxin-like compounds with adverse impacts on development or reproductive function. The series of published Dutch studies suggest that pre- and early postnatal exposures to PCBs and other dioxin-like compounds may impact developmental milestones at levels at or near current average human background exposures. Although it is unclear whether these measured responses indicate a clearly adverse impact, if humans respond to TCDD similarly to animals in laboratory studies, there are indications that exposures at relatively low levels might cause developmental effects and at higher levels might cause reproductive effects. There is especially good evidence for effects on the fetus from prenatal exposure. The Yusho and Yu-Cheng poisoning incidents are clear demonstrations that dioxin-like compounds can produce a variety of mild to severe developmental effects in humans that resemble the effects of exposure to dioxins and dioxin-like compounds in animals.

Humans do not appear to be particularly sensitive or insensitive to effects of dioxin exposure in comparison to other animals. Therefore, it is reasonable to assume that human responsiveness would lie across the middle ranges of observed responses. This assumption still does not address the issues surrounding the potentially different responses that humans (or animals) might have to the more complex and variable environmental mixtures of dioxin-like compounds. One additional key point is that most of the epidemiology studies have focused on TCDD, and not the total TEQ. Eskenazi et al. (2004) have shown that background exposure to dioxins, furans and PCBs in the referent population (zone non-ABR) cohort at Seveso was substantial,with non-ABR residents having average serum 2,3,7,8-TCDD and TEQ levels of 20.2 ppt and 100.4 ppt, respectively. The exposure zone A median serum TCDD level was 272 ppt and zone B was 47 ppt. The authors suggest that previous Seveso studies "that considered only TCDD exposure, may have underestimated health effects due to total TEQ concentrations."

TCDD and related compounds have reproductive and developmental toxicity potential in a broad range of wildlife and domestic and laboratory animals. Many of the effects have been shown to be TCDD dose-related. The effects on perinatal viability and male reproductive development are among the most sensitive effects reported, occurring at a single prenatal exposure range of as little as 0.05–0.075 μg/kg, resulting in calculated fetal tissue concentrations of 3–4 ng/kg in the rat (Hurst et al., 2000). In these studies, effects were often observed at the lowest exposure level tested, thus a NOAEL has not been established for several of these endpoints. In general, the structure-activity results are consistent with an AhR-mediated mechanism for the developmental effects that are observed in the low-dose range. The structure-activity relationship in laboratory mammals appears to be similar to that for AhR binding. This

is especially the case with cleft palate in the mouse, but has also been seen with hydronephrosis in the mouse, and developmental reproductive effects in rats.

It is assumed that the responses observed in animal studies are indicative of the potential for reproductive and developmental toxicity in humans. This is an established assumption in the risk assessment process for developmental toxicity (U.S. EPA, 1991b). It is supported by the number of animal species and strains in which effects have been observed. The limited human data are consistent with an effect following exposure to TCDD or TCDD-like agents. In addition, the phylogenetic conservation of the structure and function of the AhR also increases our confidence that these effects may occur in humans.

There is extensive evidence in experimental animals (mice, rats, monkeys) that exposure to dioxin-like chemicals during development produces neurobehavioral effects. In fact, recent studies in rodents demonstrate effects on brain development (Zareba et al., 2002), attention (Markowski et al., 2002), and behavior (Hojo et al., 2002) at doses close to current human body burdens. The situation in humans is more complex. Studies in humans demonstrate associations between dioxin exposure and alterations in neurological development. These same studies often show similar associations between exposure to nondioxin-like PCBs and these same effects. On the basis of the human studies, it is possible that the alterations in neurological development are due to an interaction between the dioxins and the nondioxin-like PCBs. At present there are limited data that define the roles of the dioxins versus the nondioxin-like PCBs in these effects on neurological development.

In general, the structure-activity results on dioxin-like compounds are consistent with an AhR-mediated mechanism for many of the developmental effects that are observed. The structure-activity relationship in laboratory mammals appears to be similar to that for AhR binding. This is especially the case with teratogenesis in the mouse. However, a direct relationship with AhR binding has not yet been proven for those involving the developing nervous system.

2.2.3. Immunotoxicity

2.2.3.1. *Epidemiologic Findings*

The available epidemiologic studies on immunologic function in humans relative to exposure to 2,3,7,8-TCDD do not describe a consistent pattern of effects among the examined populations. Two studies of German workers in which one cohort was exposed to 2,3,7,8-TCDD (Ott et al., 1994), and the other to 2,3,7,8-tetrabrominated dioxin and furan (Zober et al., 1992), found dose-related increases of complements C3 or C4, whereas the Ranch Hands have continued to exhibit elevations in immunoglobulin A (IgA) (Roegner et al., 1991; Grubbs et al.,

1995). Other studies of groups with documented exposure to 2,3,7,8-TCDD have not examined complement components to any great extent or observed significant changes in IgA. Suggestions of immunological disturbances have been observed in a small group of exposed workers (Tonn et al., 1996) and in perinatally exposed children (ten Tusscher et al., 2003), providing support for a testable hypothesis to be evaluated in other exposed populations.

Comprehensive evaluation of immunologic status and function of the NIOSH (Halperin et al., 1998), Ranch Hand (Michalek et al., 1999b), and Hamburg chemical workers (Jung et al., 1998; Ernst et al., 1998) cohorts found no consistent differences between exposed and unexposed groups for lymphocyte subpopulations, response to mitogen stimulation, or rates of infection. However, recent data from the Seveso experience demonstrate subtle effects on immune function (Baccarelli et al., 2002).

More comprehensive evaluations of immunologic function with respect to exposure to 2,3,7,8-TCDD and related compounds are necessary to assess more definitively the relationships observed in nonhuman species. Longitudinal studies of the maturing human immune system may provide the greatest insight, particularly because animal studies have found significant results in immature animals, and human breast milk is a source of 2,3,7,8-TCDD and other related compounds. The studies of Dutch infants (ten Tusscher et al., 2003) described earlier provide an example of such a study design. Additional studies of highly exposed adults may also shed light on the effects of long-term chronic exposures through elevated body burdens. Therefore, there appears to be too little information to suggest definitively that 2,3,7,8-TCDD, at the levels observed, causes long-term adverse effects on the immune system in adult humans.

2.2.3.2. *Animal Findings*

Cumulative evidence from a number of studies indicates that the immune system of various animal species is a target for toxicity of TCDD and structurally related compounds, including other PCDDs, PCDFs, and PCBs. Both cell-mediated and humoral immune responses are suppressed following TCDD exposure, suggesting that there are multiple cellular targets within the immune system that are altered by TCDD. Evidence also suggests that the immune system is indirectly targeted by TCDD-induced changes in nonlymphoid tissues. TCDD exposure of experimental animals results in decreased host resistance following challenge with certain infectious agents, which likely result from TCDD-induced suppression of immunological functions.

The primary antibody response to the T cell-dependent antigen, sheep red blood cells (SRBCs), is the most sensitive immunological response that is consistently suppressed in mice exposed to TCDD and related compounds. The degree of immunosuppression is related to the

potency of the dioxin-like congeners. There is remarkable agreement among several different laboratories for the potency of a single acute dose of TCDD (i.e., suppression at a dose as low as 0.1 μg TCDD/kg with an average 50% immunosupressive dose [ID_{50}] value of approximately 0.7 μg TCDD/kg) to suppress this response in Ah-responsive mice. Results of studies that have compared the effects of acute exposure to individual PCDDs, PCDFs, and PCB congeners (which differ in their binding affinity for the AhR) on this response have provided critical evidence that certain dioxin-like congeners are also immunosuppressive. The degree of immunosuppression has been found to be related to potency of the dioxin-like congeners. Antibody responses to T cell-independent antigens such as trinitrophenyl-lipopolysaccharide and the cytotoxic T lymphocyte (CTL) response are also suppressed by a single acute exposure to TCDD, albeit at higher doses than those that suppress the SRBC response. Although a thorough and systematic evaluation of the immunotoxicity of TCDD-like congeners in different species and for different immunological endpoints has not been performed, it can be inferred from the available data that dioxin-like congeners are immunosuppressive.

Perinatal exposure of experimental animals to TCDD results in suppression of primarily T cell immune functions, with suppression persisting into adulthood. In mice, the effects on T cell functions appear to be related to the fact that perinatal TCDD exposure alters thymic precursor stem cells in the fetal liver and bone marrow and thymocyte differentiation in the thymus. These studies suggest that perinatal development is a critical and sensitive period for TCDD-induced immunotoxicity. Further efforts should be made to determine the consequences of perinatal exposure to TCDD and related compounds and mixtures on immune system integrity.

2.2.3.3. *Other Data Related to Immunologic Effects*

In addition to the TCDD-like congener results, studies using strains of mice that differ in the expression of the AhR have provided critical evidence to support a role for Ah-mediated immune suppression following exposure to dioxin-like compounds. Recent in vitro work also supports a role for Ah-mediated immune suppression. Other in vivo and in vitro data, however, suggest that non-Ah-mediated mechanisms may also play some role in immunotoxicity induced by dioxin-like compounds. However, more definitive evidence remains to be developed to support this latter view.

The immunosuppressive potency of individual dioxin-like compounds in mice is related to their structural similarity to TCDD. However, the immunotoxicity of TCDD and related congeners can be modified by co-exposure to nondioxin-like PCBs in simple binary or more complex mixtures, resulting in additive or antagonistic interactions. There is a need for the

generation of dose-response data of acute, subchronic, and chronic exposure to the individual congeners in a mixture and for the mixture itself in order to fully evaluate potential synergistic, additive, or antagonistic effects of environmentally relevant mixtures. A preliminary report demonstrating that the immunotoxicity of a food-like mixture of dioxins was well-predicted by the TEQ has been presented (Smialowicz et al., 1997).

Animal host resistance models that mimic human disease have been used to assess the effects of TCDD on altered host susceptibility. TCDD exposure increases susceptibility to challenge with bacteria, viruses, parasites, and tumors. Mortality is increased in TCDD-exposed mice challenged with certain bacteria. Increased parasitemia occurs in TCDD-exposed mice and rats challenged with parasitic infections. Low doses of TCDD also alter resistance to virus infections in rodents. Increased susceptibility to infectious agents is an important benchmark of immunosuppression; however, the role that TCDD plays in altering immune-mediated mechanisms important in murine resistance to infectious agents remains to be elucidated. Also, because little is known about the effects that dioxin-like congeners have on host resistance, more research is recommended in this area.

Studies in nonhuman primates exposed acutely, subchronically, or chronically to halogenated aromatic hydrocarbons (HAH) have revealed variable alterations in lymphocyte subpopulations, primarily T lymphocyte subsets. In three separate studies in which monkeys were exposed subchronically or chronically to PCBs, the antibody response to SRBC was consistently found to be suppressed. These results in nonhuman primates are important because they corroborate the extensive database of HAH-induced suppression of the antibody response to SRBC in mice and thereby provide credible evidence for immunosuppression by HAHs across species. In addition, these data indicate that the primary antibody response to this T cell-dependent antigen is the most consistent and sensitive indicator of HAH-induced immunosuppression.

The available database derived from well-controlled animal studies on TCDD immunotoxicity can be used for the establishment of NOELs. As the antibody response to SRBCs has been shown to be dose-dependently suppressed by TCDD and related dioxin-like compounds, this database is best suited for the development of dose-response modeling.

2.2.3.4. *Immunologic Effects Hazard Characterization*

Accidental or occupational exposure of humans to TCDD and/or related compounds variably affects a number of immunological parameters. Unfortunately, the evaluation of immune system integrity in humans exposed to dioxin-like compounds has provided data that are inconsistent across studies. The broad range of "normal" responses in humans due to the large

amount of variability inherent in such a heterogenous population, the limited number and sensitivity of tests performed, and poor exposure characterization of the cohorts in these studies compromise any conclusions about the ability of a given study to detect immune alterations. Consequently, there are insufficient clinical data from these studies to fully assess human sensitivity to TCDD exposure. Nevertheless, based on the results of the extensive animal work, the database is sufficient to indicate that immune effects could occur in the human population from exposure to TCDD and related compounds at some dose level. At present, it is EPA's scientific judgment that TCDD and related compounds should be regarded as nonspecific immunosuppressants and immunotoxicants until better data to inform this judgment are available.

It is interesting that a common thread in several human studies is the observed reduction in $CD4^+$ T helper cells, albeit generally within the "normal" range, in cohorts exposed to dioxin-like compounds. Even though these reductions may not translate into clinical effects, it is important to note that these cells play an important role in regulating immune responses and that their reduction in clinical diseases is associated with immunosuppression. It is also important to realize that those at the extremes of the population distribution may be at special risk of such alterations. Another important consideration is that a primary antibody response following immunization was not evaluated in any of the human studies. Because this immune parameter has been revealed to be the most sensitive in animal studies, it is recommended that TCDD and related compounds be judged immunosupressive and that this parameter be included in future studies of human populations exposed to TCDD and related compounds. It is also recommended that research focused on delineating the mechanism(s) underlying dioxin-induced immunotoxicity and immunosuppression continue.

2.2.4. Chloracne

Chloracne and associated dermatologic changes are widely recognized responses to TCDD and other dioxin-like compounds in humans. Along with the reproductive hormones discussed above and gamma glutamyl transferase (GGT) levels, which are discussed below, chloracne is one of the noncancer effects that has a strong positive association with exposure to TCDD in humans (see Part II, Chapter 7b, Section 7.13). Chloracne is a severe acne-like condition that develops within months of first exposure to high levels of dioxin and related compounds. For many individuals, the condition disappears after discontinuation of exposure, despite initial serum levels of dioxin in the thousands of parts per trillion; for others, it may remain for many years. The duration of persistent chloracne is on the order of 25 years, although

cases of chloracne persisting for more than 40 years have been noted (see Part II, Chapter 7b, Section 7.13).

In general, chloracne has been observed in most incidents where substantial dioxin exposure has occurred, particularly among TCP production workers and Seveso residents (see Part II, Chapter 7b). The amount of exposure necessary for development of chloracne has not been resolved, but studies suggest that high exposure (both high acute and long-term exposure) to 2,3,7,8-TCDD increases the likelihood of chloracne, as evidenced by chloracne in TCP production workers and Seveso residents who had documented high serum 2,3,7,8-TCDD levels (Beck et al., 1989; Fingerhut et al., 1991a; Mocarelli et al., 1991; Neuberger et al., 1991) or in individuals who had a work history with long duration of exposure to 2,3,7,8-TCDD-contaminated chemicals (Bond et al., 1989).

In earlier studies, chloracne was considered to be a "hallmark of dioxin intoxication" (Suskind, 1985). However, in only two studies were risk estimates calculated for chloracne. Both were studies of different cohorts of TCP production workers, one of which was employed in a West Virginia plant (Suskind and Hertzberg, 1984), the other in a plant in Michigan (Bond et al., 1989). Of the 203 West Virginia workers, 52.7% ($p<0.001$) were found to have clinical evidence of chloracne, and 86.3% reported a history of chloracne ($p<0.001$). None of the unexposed workers had clinical evidence or reported a history of chloracne. Among the Michigan workers, the relative risk for cases of chloracne was highest for individuals with the longest duration of exposure ($\geq$ 60 months; RR = 3.5, 95% CI = 2.3–5.1), those with the highest cumulative dose of TCDD (based on duration of assignment across and within 2,3,7,8-TCDD-contaminated areas in the plant) (RR = 8.0, 95% CI = 4.2–15.3), and those with the highest intensity of 2,3,7,8-TCDD exposure (RR = 71.5, 95% CI = 32.1–159.2).

Studies in multiple animal species have been effective in describing the relationship between 2,3,7,8-TCDD and chloracne, particularly in rhesus monkeys (McNulty, 1977; Allen et al., 1977; McConnell et al., 1978). Subsequent to exposure to 2,3,7,8-TCDD, monkeys developed chloracne and swelling of the meibomian glands, the modified sebaceous glands in the eyelid. The histologic changes in the meibomian glands are physiologically similar to those observed in human chloracne (Dunagin, 1984).

In summary, the evidence provided by the various studies convincingly supports what is already presumed—that chloracne is a common sequel of high levels of exposure to 2,3,7,8-TCDD and related compounds. More information is needed to determine the level and frequency of exposure to dioxin-like compounds needed to cause chloracne and whether personal susceptibility plays a role in the etiology. Finally, it is important to recall that the absence of chloracne does not imply lack of exposure (Mocarelli et al., 1991).

2.2.5. Diabetes

Diabetes mellitus is a heterogeneous disorder that is a consequence of alterations in the number or function of pancreatic beta cells responsible for insulin secretion and carbohydrate metabolism. Diabetes and fasting serum glucose levels were evaluated in more recent cross-sectional medical studies because of the apparently high prevalence of diabetes and abnormal glucose tolerance tests in one case report of 55 TCP workers (Pazderova-Vejlupkova et al., 1981). Recent epidemiology studies, as well as early case reports, have indicated a weak association between serum concentrations of dioxin and diabetes. This association was first noted in the early 1990s when a decrease in glucose tolerance was seen in the NIOSH cohort. This was followed by a report of an increase in diabetes in the Ranch Hand cohort (Michalek et al., 1999a; Longnecker and Michalek, 2000). An increase in diabetes in other occupational cohorts (Steenland et al., 1999; Vena et al., 1998) as well as in the Seveso population (Pesatori et al., 1998) has also been reported. There was not a significant increase in diabetes in the NIOSH mortality study, although 6 of the 10 most highly exposed workers did have diabetes (Calvert et al., 1999). However, mortality studies are limited in their ability to assess risk from diabetes mellitus because the prevalence of disease may not be available from death certificates.

A paper by Longnecker and Michalek (2000) found a pattern suggesting that low levels of dioxin may influence the prevalence of diabetes. However, these results did not show an exposure-response relationship. Because it is the only study of its type to have been published, additional population-based studies are warranted to validate its findings. A recent update of the Ranch Hand study shows a 47% excess of diabetes in the most heavily exposed group of veterans (Michalek et al., 1999a).

Most of the data suggest that the diabetes observed in the studies is Type II, or adult-onset diabetes, rather than insulin dependent, or Type I. Aging and obesity are the key risk factors for Type II diabetes. However, dioxins may shift the distribution of sensitivity, putting people at risk at younger ages or when they have less weight. Dioxin alters lipid metabolism in multiple species, including humans (Sweeney et al., 1997; Pohjanvirta and Tuomisto, 1994), and it also alters glucose uptake into both human and animal cells in culture (Enan and Matsumura, 1994; Olsen et al., 1994). Mechanistic studies have demonstrated that dioxin affects glucose transport (Enan and Matsumura, 1994), a property under the control of the hypoxia response pathway (Ouiddir et al., 1999). A key regulatory protein in this pathway is the partner of the AhR, Arnt (also known as HIF1-beta) (Gu et al., 2000; Taylor and Zhulin, 1999). Activation of the AhR by dioxin may compete with other pathways for Arnt, such as the hypoxia-inducible factor (HIF) pathway (Gradin et al., 1992). Dioxin has also been shown to downregulate the insulin growth factor receptor (Liu et al., 1992). These three issues—altered lipid metabolism, altered glucose

transport, and alterations in the insulin signaling pathway—all provide biological plausibility to the association of dioxins with diabetes.

A causal relationship between diabetes and dioxin has not been established, although both the toxicologic and epidemiological data are suggestive of a plausible association (Remillard and Bunce, 2002). Many questions have yet to be answered. For example, does diabetes alter the pharmacokinetics of dioxin? Diabetes is known to alter the metabolism of several drugs in humans (Matzke et al., 2000) and may also alter dioxin metabolism and kinetics. Because adult-onset diabetes is also associated with being overweight, and body composition has been shown to modify the apparent half-life of dioxin, could the rate of elimination of dioxins be lowered in people who have diabetes, causing them to have higher body burdens? This may be relevant to the background population, but it is hardly likely to be an explanation in highly exposed populations.

Key research needs are twofold. The first is to develop an animal model with which to study the association between dioxins and diabetes and glucose perturbation. Several rodent models for Type II diabetes exist and may be used. The second is to conduct population-based incidence studies that take into account dioxin levels as well as the many known factors associated with diabetes. Although diabetes may cause the underlying pathology leading to death, it is often not attributed as the cause of death and thus limits the utility of mortality studies.

2.2.6. Other Effects

2.2.6.1. *Elevated GGT*

As mentioned above, there appears to be a consistent pattern of increased GGT levels among individuals exposed to 2,3,7,8-TCDD-contaminated chemicals. Elevated levels of serum GGT were observed within a year after exposure in Seveso children (Caramaschi et al., 1981; Mocarelli et al., 1986) and 10 or more years after cessation of exposure among TCP and 2,4,5-T production workers (May, 1982; Martin, 1984; Moses et al., 1984; Calvert et al., 1992) and among Ranch Hands (Roegner et al., 1991; Grubbs et al., 1995). All of these groups had a high likelihood of substantial exposure to 2,3,7,8-TCDD. In addition, for those studies that evaluated dose-response relationships with 2,3,7,8-TCDD levels, the effect was observed only at the highest levels or categories of 2,3,7,8-TCDD and, in the NIOSH study, only in workers who reported drinking high levels of alcohol.

In contrast, although background levels of serum 2,3,7,8-TCDD suggested minimal exposure in Army Vietnam veterans, GGT was increased at borderline significance among Vietnam veterans as compared to non-Vietnam veterans (CDC Vietnam Experience Study,

1988). In addition, despite the increases observed in some studies of occupational cohorts, other studies of TCP production workers from West Virginia or Missouri residents measured but did not report elevations in GGT levels (Suskind and Hertzberg, 1984; Webb et al., 1989).

In clinical practice, GGT is often measured because it is elevated in almost all hepatobiliary diseases and is used as a marker for alcoholic intake (Guzelian, 1985). In individuals with hepatobiliary disease, elevations in GGT are usually accompanied by increases in other hepatic enzymes, for example, AST and ALT, and metabolites, for example, uro- and coproporphyrins. Significant increases in hepatic enzymes other than GGT and metabolic products were not observed in individuals whose GGT levels were elevated 10 or more years after exposure ended, suggesting that the effect may be GGT-specific. These data suggest that in the absence of increases in other hepatic enzymes, elevations in GGT are associated with exposure to 2,3,7,8-TCDD, particularly among individuals who were exposed to high levels.

The animal data with respect to 2,3,7,8-TCDD-related effects on GGT are sparse. Statistically significant changes in hepatic enzyme levels, particularly AST, ALT, and alkaline phosphatase, have been observed after exposure in rats and hamsters (Gasiewicz et al., 1980; Kociba et al., 1978; Olson et al., 1980). Only one study evaluated GGT levels (Kociba et al., 1978); moderate but statistically nonsignificant increases were noted in rats fed 0.10 μg/kg 2,3,7,8-TCDD daily for 2 years, and no increases were observed in control animals.

In summary, GGT is the only hepatic enzyme examined that was found in a number of studies to be chronically elevated in adults exposed to high levels of 2,3,7,8-TCDD. The consistency of the findings in a number of studies suggests that the elevation may reflect a true effect of exposure, but its clinical significance is unclear. Long-term pathological consequences of elevated GGT have not been illustrated by excess mortality from liver disorders or cancer or in excess morbidity in the available cross-sectional studies.

It must be recognized that the absence of an effect—for example, liver enzymes—in a cross-sectional study does not obviate the possibility that the enzyme levels may have increased concurrently with the exposure but declined after cessation. The apparently transient elevations in ALT levels among the Seveso children suggest that hepatic enzyme levels other than GGT may react in this manner to 2,3,7,8-TCDD exposure.

2.2.6.2. *Thyroid Function*

Many effects of 2,3,7,8-TCDD exposure in animals resemble signs of thyroid dysfunction or significant alterations of thyroid-related hormones. In the few human studies that have examined the relationship between 2,3,7,8-TCDD exposure and hormone concentrations in adults (CDC Vietnam Experience Study, 1988; Roegner et al., 1991; Grubbs et al., 1995;

Suskind and Hertzberg, 1984), the results are mostly equivocal. Cross-sectional analysis of the Ranch Hand cohort (Pavuk et al., 2003) found signs of elevated TSH means among the high TCDD exposure group in the 1985 and 1987 follow-ups, with an increasing trend across the decade 1982 - 1992, but no association with the occurrence of thyroid disease. Concentrations of thyroid binding globulin also appeared to be positively correlated with current levels of 2,3,7,8-TCDD in the BASF accident cohort (Ott et al., 1994). Little additional information on thyroid hormone levels has been reported for production workers and none for Seveso residents, two groups with documented high serum 2,3,7,8-TCDD levels.

Thyroid hormones play important roles in the developing nervous system in all vertebrate species, including humans—to the extent that all infants in the United States are tested for hypothyroidism shortly after birth. Several studies of nursing infants suggest that ingestion of breast milk with a higher dioxin TEQ may alter thyroid function (Pluim et al., 1993; Koopman-Esseboom et al., 1994c; Nagayama et al., 1997). These findings suggest a possible shift in the distribution of thyroid hormones, particularly T4, and point out the need for collection of longitudinal data to assess the potential for long-term effects associated with developmental exposures.

The exact processes that account for these observations in humans are unknown, but when put in perspective of animal responses, the following might apply: dioxin increases the metabolism and excretion of thyroid hormone, mainly T4, in the liver, and reduced T4 levels stimulate the pituitary to secrete more TSH, which enhances thyroid hormone production. Early in the disruption process, the body can overcompensate for the loss of T4, which may result in a small excess of circulating T4 to the increased TSH. In animals given higher doses of dioxin, the body is unable to maintain homeostasis, TSH levels remain elevated, and T4 levels decrease.

A plausible mode of action for thyroid effects is described in Section 2.2.1.3.

2.2.6.3. *Cardiovascular Disease*

Elevated cardiovascular disease has been noted in several occupational cohort studies (Steenland et al., 1999; Sweeney et al., 1997; Flesch-Janys et al., 1995) and in the Seveso (Pesatori et al., 1998) and the rice oil poisoning studies. This appears to be associated with ischemic heart disease and in some cases with hypertension. Recent data from the Ranch Hand study indicate that dioxin may be a possible risk factor for the development of essential hypertension (Grubbs et al., 1995). Elevated blood lipids have also been seen in several cohorts. The association of dioxins with heart disease in humans has biological plausibility, given the data in animals. First is the key role of hypoxia in heart disease and the potential for involvement of the activated AhR in blocking an hypoxic response (Gradin et al., 1996; Gu et al., 2000). Dioxin

has been shown to perturb lipid metabolism in multiple laboratory species (Pohjanvirta and Tuomisto, 1994). The heart—in fact the entire vascular system—is a clear target for the adverse effects of dioxin in fish and birds (Hornung et al., 1999; Cheung et al., 1981). Recent studies have demonstrated that the heart is also a target in mammals (Lund et al., 2003; NTP 2003a). In mammals, dioxin has been shown to disturb heart rhythms at high doses in guinea pigs (Gupta et al., 1973; Pohjanvirta and Tuomisto, 1994).

2.2.6.4. *Oxidative Stress*

Several investigators have hypothesized that some of the adverse effects of dioxin and related compounds may be associated with oxidative stress. Induction of CYP1A isoforms has been shown to be associated with oxidative DNA damage (Park et al., 1996). Altered metabolism of endogenous molecules such as estradiol can lead to the formation of quinones and redox cycling. This has been hypothesized to play a role in the enhanced sensitivity of female rats to dioxin-induced liver tumors (Tritscher et al., 1996). Lipid peroxidation, enhanced DNA single-strand breaks, and decreased membrane fluidity have been observed in liver as well as in extrahepatic tissues following exposure to high doses of TCDD (Stohs, 1990). A dose- and time-dependent increase in superoxide anion in peritoneal macrophages following exposure to TCDD (Alsharif et al., 1994). A recent report that low-dose (0.15 ng TCDD/kg/day) subchronic exposure can lead to oxidative changes in several tissues in mice (Slezak et al., 2000) suggests that this mechanism or mode of toxicity deserves further attention.

Table 2-1. Effects of TCDD and related compounds in different animal species

Effect	Humans	Monkey	Guinea pig	Rat	Mouse	Hamster	Cow	Rabbit	Chicken	Fish	Avian wildlife	Marine mammals	Mink
Presence of AhR	+	+	0	+	+	+	+	+	+	+	+	+	+
Binding of TCDD: AhR complex to the DRE (enhancer)	+		+	+	+	+	+	+	+	+			
Enzyme induction	+	+	+	+	+	+		+	+	+	+	+	+
Acute lethality	0	+	+	+	+	+	+	+	+	+	+	+	+
Wasting syndrome	+	+	+	+	+	+	+	+		+	+	+	+
Teratogenesis/fetal toxicity, mortality	+/-	+	+	+	+	+		+	+	+	+	+	+
Endocrine effects	+/-	+		+	+					+	+	+	+
Immunotoxicity	+/-	+	+	+	+	+	+		+	+		+	
Carcinogenicity	+/-			+	+	+				+			
Neurotoxicity	+	+		+	+				+				
Chloracnegenic effects	+	+			+		+	+		+			
Porphyria	+	0	0	+	+	0			+				
Hepatotoxicity	+	+	+/-	+	+	+/-	+	+	+	+	+	+	+
Edema		+	0	0	+	+			+	+			
Testicular atrophy		+	+	+	+								
Bone marrow hypoplasia		+	+		+/-				+				
Teeth	+	+		+									

+ = observed.
+/- = observed to limited extent, or +/- results.
0 = not observed.
Blank cells = no data.

Table 2-2. Some biochemical responses to TCDD

CYP1A1	Human chorionic gonadotrophin
CYP1A2	Interleukin-1beta
CYP1B1	Gastrin
GST Ya	TNF alpha
GST Yb	TGF-beta
GST Yc	EGF
UDP glucuronyl transferase	Fibrinogen
QR quinone reductase/ Nmo	Plastin
Aldehyde dehydrogenase	EGFR
Ornithine decarboxylase	c-erbA related hormone receptor
Malic enzyme	Estrogen receptor
Phospholipase A2	25Dx-putative progesterone receptor
60kDa microsomal esterase	MDR-1 multidrug resistance
Aminolevulinic acid synthetase	Aryl hydrocarbon binding protein
Choline kinase	c-fos
EctoATPase	c-jun
Prostaglandin synthetase -2 (COX-2)	Cystatin-like protein
Plasminogen activator inhibitor-2	MHC-Q1
Urokinase plasminogen activator	Protein kinase C
Nedd-4-like ubiquitin protein ligase	pp60 c-src protein kinase
PEPC kinase	p21 ras
Terminal transferase	p27/Kip1
Testosterone 7alpha hydroxylase	bcl-2

Note: This list is not a comprehensive list of all responses known to be affected by TCDD.

Source: Sutter et al., 1992; Lai et al., 1996.

Table 2-3. Summary of the combined cohort and selected industrial cohort studies with high exposure levels, as described by IARC (1997)[a]

Reference	All cancers			Lung cancer		
	Observed	SMR	95% CI	Observed	SMR	95% CI
International cohort						
Kogevinas et al. (1997)[b]	394	1.2	1.1–1.3	127	1.2	1.0–1.4
Industrial populations (high-exposure subcohorts)						
Fingerhut et al. (1991a)[c] (USA)	114	1.5	1.2–1.8	40	1.4	1.0–1.9
Becher et al. (1996)[d] (Germany)	105	[1.3]	[1.0–1.5]	33	[1.4]	[1.0–2.0]
Hooiveld et al. (1996)[e] (Netherlands)	51	1.5	1.1–1.9	14	1	0.5–1.7
Ott and Zober (1996b)[f] (BASF accident)	18	1.9	1.1–3.0	7	2.4	1.0–5.0
TOTAL	[288]	[1.4]	[1.2–1.6]	[94]	[1.4]	[1.1–1.7]
p value	<0.001			<0.01		

[a] Adapted from IARC; Table 38 (1997); non-Hodgkin's lymphoma, soft-tissue sarcoma, and gastrointestinal results not shown. TOTALs were calculated by the IARC Working Group.

[b] Men and woman > 20 years since first exposure. These data include the cohorts of Fingerhut et al. (1991a,b), Becher et al. (1996), Hooiveld et al. (1996a), the original IARC cohort (Saracci et al., 1991), and other cohorts.

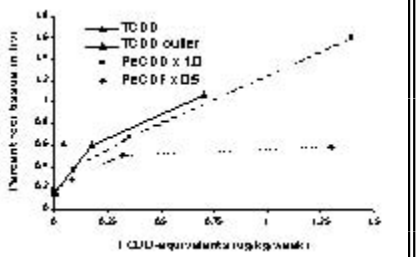
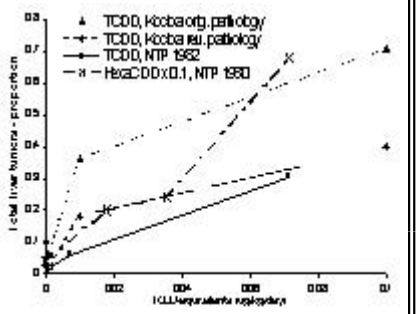
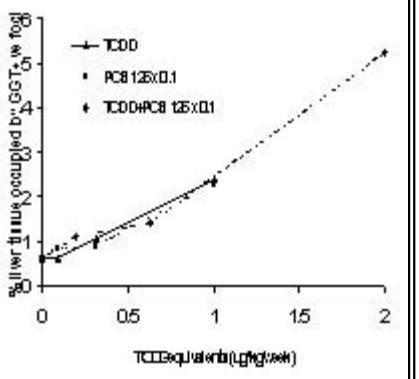
[c] Men ≥ 20 years latency and ≥ 1 year exposure.

[d] Men, cohorts I and II, summed (Boehringer-Ingelheim, Bayer-Uerdingen cohorts).

[e] Men and women, Factory A.

[f] Men, chloracne subgroup, ≥ 20 years latency. Data presented for lung cancer are all respiratory tract cancers combined.

Table 2-4. Tumor incidence and promotion data cited for the TEF-WHO$_{98}$ for principal congeners

Congener	TEF-WHO$_{98}$ tumor incidence/promotion citation[a]	TEF-WHO$_{98}$	% of adipose TEQ$_{DFP}$-WHO$_{98}$ tissue conc.[b]	Dose-response graphs: dose adjusted to reflect TEF multiplier
2,3,7,8-TCDD	TEF Standard	1	8	
1,2,3,7,8-PeCDD	Waern et al. (1991)	1	15	
2,3,4,7,8-PeCDF	Waern et al. (1991)	0.5	7	
1,2,3,6,7,8-HxCDD	NTP (1980); 1,2,3,6,7,8-HxCDD/ 1,2,3,7,8,9-HxCDD; 1:2 mixture; long-term bioassays, Osborne-Mendel rats in NTP studies, Sprague-Dawley rats in Kociba et al. (1978)	0.1	10	
1,2,3,7,8,9-HxCDD		0.1	2	
PCB 126	Hemming et al. (1995)	0.1	33	

[a] van den Berg et al., 2000. Hexa-CDD referenced to previous TEF reviews.
[b] See Part II, Chapter 4, Tables 4-46, 4-47

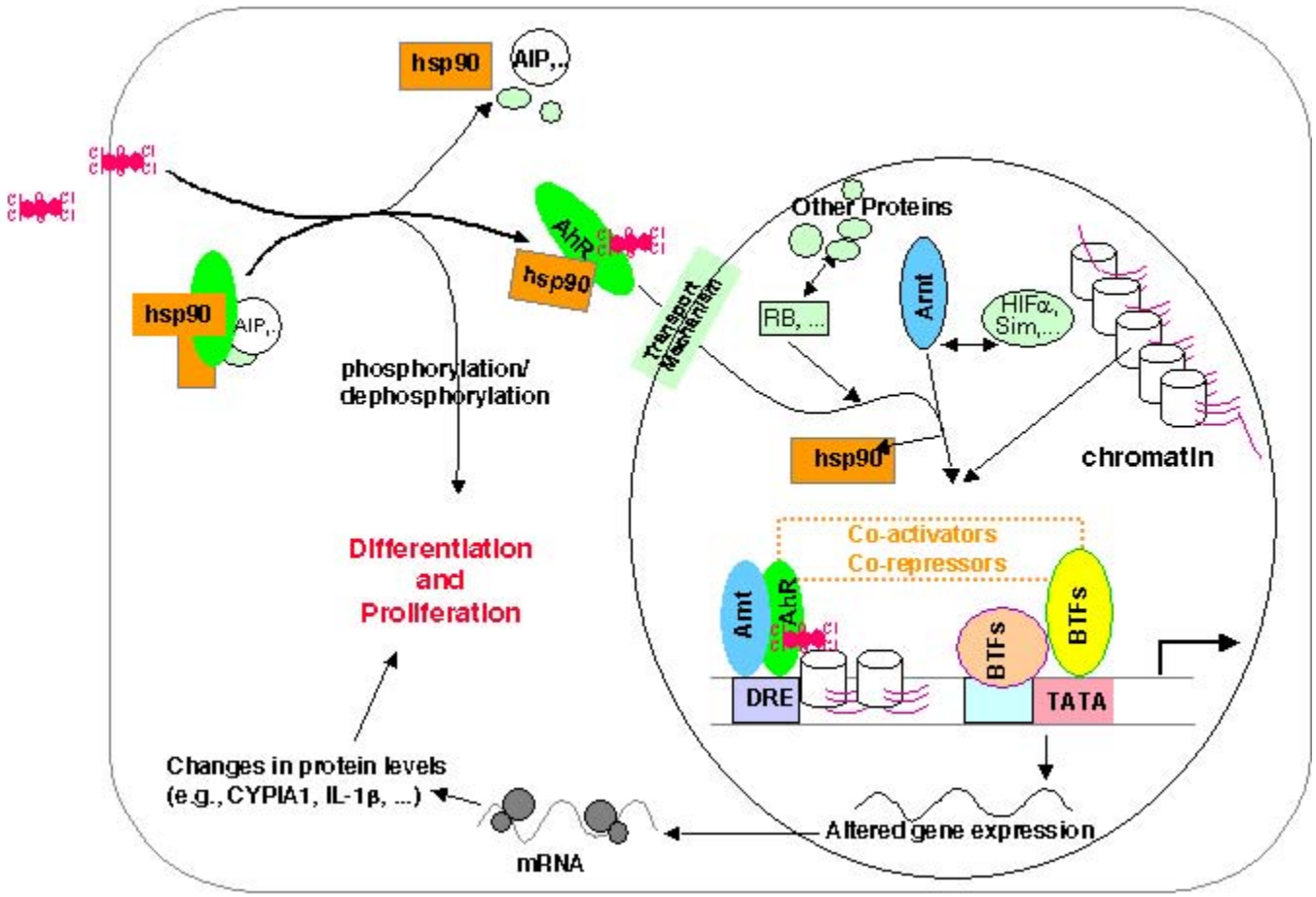


Figure 2-1. Cellular mechanism for AhR action. TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; AhR, aryl hydrocarbon receptor; AIP, associated immunophilin-like protein; hsp90, 90 kilodalton heat shock protein; p, sites of phosphorylization; Arnt, AhR nuclear translocator protein; RB, retinoblastoma protein; NF-kB, nuclear transcription factor; HIF, hypoxia inducible factor; DRE, dioxin-responsive element; BTFs, basal transcription factors; TATA, DNA recognition sequence.

3. MECHANISMS AND MODE OF DIOXIN ACTION

Mechanistic studies can reveal the biochemical pathways and types of biological and molecular events that contribute to dioxin's adverse effects (See Part II, Chapter 2, for a detailed discussion). For example, much evidence indicates that TCDD acts via an intracellular protein (the AhR), which functions as a ligand-dependent transcription factor in partnership with a second protein (Arnt). Therefore, from a mechanistic standpoint, TCDD's adverse effects appear likely to reflect alterations in gene expression that occur at an inappropriate time and/or for an inappropriately long time. Mechanistic studies also indicate that several other proteins contribute to TCDD's gene regulatory effects and that the response to TCDD probably involves a relatively complex interplay between multiple genetic and environmental factors. If TCDD operates through such a mechanism, as all evidence indicates, then there are certain constraints on the possible models that can plausibly account for TCDD's biological effects and, therefore, on the assumptions used during the risk assessment process (e.g., Poland, 1996; Limbird and Taylor, 1998).

Mechanistic knowledge of dioxin action may also be useful in other ways. For example, a further understanding of the ligand specificity and structure of the AhR will likely assist in the identification of other chemicals to which humans are exposed that may add to, synergize, or block the toxicity of TCDD. Knowledge of genetic polymorphisms that influence TCDD responsiveness may also allow the identification of individuals at greater risk from exposure to dioxin. In addition, knowledge of the biochemical pathways that are altered by TCDD may help identify novel targets for the development of drugs that can antagonize dioxin's adverse effects.

As described below, biochemical and genetic analyses of the mechanisms by which dioxin may modulate particular genes have revealed the outline of a novel regulatory system whereby a chemical signal can alter cellular regulatory processes. Future studies of dioxin action have the potential to provide additional insights into mechanisms of mammalian gene regulation that are of a broader interest. Additional perspectives on dioxin action can be found in several reviews (Birnbaum, 1994a, b; Schecter, 1994; Hankinson, 1995; Schmidt and Bradfield, 1996; Gasiewicz, 1997; Rowlands and Gustafsson, 1997; Denison et al., 1998; Hahn, 1998; Wilson and Safe, 1998; Schecter and Gasiewicz, 2003; Matsumura, 2003; Carlson and Perdew, 2002).

Knowledge of the mode(s) of action by which the broad class of chemicals known as dioxins act may facilitate the risk assessment process by contributing to the weight of the evidence for hazard characterization and by imposing bounds on the models used to describe possible responses of humans resulting from exposure to mixtures of these chemicals (see

Sections 2 and 5 of this document). The relatively extensive database on TCDD, as well as the more limited database on related compounds, has been reviewed, with emphasis on the role of the specific cellular receptor for TCDD and related compounds—the AhR—in the postulated mode(s) of action. This discussion focuses on summarizing the elements of the mode(s) of dioxin action that are relevant for understanding and characterizing dioxin risk for humans. These elements include:

- Similarities between humans and other animals with regard to receptor structure and function;

- The relationship between receptor binding and toxic effects; and

- The extent to which the purported mechanism(s) or mode(s) of action might contribute to the diversity of biological responses seen in animals and, to some extent, in humans.

In addition, this section identifies important and relevant knowledge gaps and uncertainties in the understanding of the mechanism(s) of dioxin action and indicates how these may affect the approach to risk characterization.

3.1. MODE VERSUS MECHANISM OF ACTION

In the context of revising its carcinogen risk assessment guidelines, EPA has proposed giving greater emphasis to use of all of the data in hazard characterization, dose-response characterization, exposure characterization, and risk characterization (U.S. EPA, 1996, 1999, 2003). One aid to the use of more information in risk assessment has been the definition of mode versus mechanism of action. Mechanism of action is defined as the detailed molecular description of key events in the induction of cancer or other health endpoints. Mode of action refers to the description of key events and processes, starting with interaction of an agent with the cell through functional and anatomical changes, resulting in cancer or other health endpoints.

Despite a desire to construct detailed biologically based toxicokinetic and toxicodynamic models to reduce uncertainty in characterizing risk, few examples have emerged. Use of a mode of action approach recognizes that, although all of the details may not have been worked out, prevailing scientific thought supports moving forward using a hypothesized mode of action supported by data. This approach is consistent with advice offered by the National Academy of

Sciences' National Research Council in its report entitled *Science and Judgment in Risk Assessment* (NAS/NRC, 1994).

Mode of action discussions help to provide answers to the questions: How does the chemical produce its effect? Are there mechanistic data to support this hypothesis? Have other modes of action been considered and rejected? In order to demonstrate that a particular mode of action is operative, it is generally necessary to outline the hypothesized sequence of events leading to effects, to identify key events that can be measured, to outline the information that is available to support the hypothesis, and to discuss those data that are inconsistent with the hypothesis or support an alternative hypothesis. Following this, the information is weighed to determine whether there is a causal relationship between key precursor events associated with the mode of action and cancer or other toxicological endpoint in animals, and ultimately whether this inference can be extended to humans.

3.2. GENERALIZED MODEL FOR DIOXIN ACTION

Dioxin and related compounds are generally recognized to be receptor-mediated toxicants. The generalized model has evolved over the years to appear as illustrated in Table 3-1 and Figure 2-1.

3.2.1. The Receptor Concept

One of the fundamental concepts that influences our approach to risk assessment of dioxin and related compounds is the receptor concept. The idea that a drug, hormone, neurotransmitter, or other chemical produces a physiological response by interacting with a specific cellular target molecule, that is, a "receptor," evolved from several observations. First, many chemicals elicit responses that are restricted to specific tissues. This observation implies that the responsive tissue (e.g., the adrenal cortex) contains a "receptive" component whose presence is required for the physiologic effect (e.g., cortisol secretion). Second, many chemicals are quite potent. For example, picomolar to nanomolar concentrations of numerous hormones and growth factors elicit biological effects. This observation suggests that the target cell contains a site(s) to which the particular chemical binds with high affinity. Third, stereoisomers of some chemicals (e.g., catecholamines, opioids) differ by orders of magnitude in their ability to produce the same biological response. This observation indicates that the molecular shape of the chemical strongly influences its biological activity. This, in turn, implies that the binding site on or in the target cell also has a specific, three-dimensional configuration. Together, these types of observations support the prediction that the biological responses to some chemicals involve

stereospecific, high-affinity binding of the chemicals to specific receptor sites located on or in the target cell. Many of these characteristics have been noted for TCDD and related compounds.

The availability of compounds of high specific radioactivity has permitted quantitative analyses of their binding to cellular components in vitro. To qualify as a potential receptor, a binding site for a given chemical must satisfy several criteria: (1) the binding site must be saturable, that is, the number of binding sites per cell should be limited; (2) the binding should be reversible; (3) the binding affinity measured in vitro should be consistent with the potency of the chemical observed in vivo; (4) if the biological response exhibits stereospecificity, so should the in vitro binding; (5) for a series of structurally related chemicals, the rank order for binding affinity should correlate with the rank order for biological potency; and (6) tissues that respond to the chemical should contain binding sites with the appropriate properties.

The binding of a chemical ("ligand") to its specific receptor is assumed to obey the law of mass action; that is, it is a bimolecular, reversible interaction. The concentration of the liganded, or occupied, receptor [RL] is a function of both the ligand concentration [L] and the receptor concentration [R] as shown in equation 3-1:

$$[L] + [R] \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [RL] \qquad (3\text{-}1)$$

Inherent in this relationship is the fact that the fractional occupancy (i.e., $[RL]/[R_t]$) is a function of ligand concentration [L] and the apparent equilibrium dissociation constant K_D, which is a measure of the binding affinity of the ligand for the receptor, that is, $[RL]/[R_t] = [L]/(K_D + [L])$, where $K_D = [L]\,[R_t]/[LR] = k_2/k_1$. Therefore, the relationship between receptor occupancy and ligand concentration is hyperbolic. At low ligand concentrations (where $[L] << K_D$), a small increase in [L] produces an approximately linear increase in fractional receptor occupancy. At high ligand concentration (where $[L] >> K_D$), the fractional occupancy of the receptor is already very close to 1, that is, almost all receptor sites are occupied. Therefore, a small increase in [L] is likely to produce only a slight increase in receptor occupancy. These issues are discussed in regard to TCDD binding to the AhR and dose-response in Part II, Chapter 8.

Ligand binding constitutes only one aspect of the receptor concept. By definition, a receptor mediates a response, and the functional consequences of the ligand-receptor binding represent an essential aspect of the receptor concept. Receptor theory attempts to quantitatively relate ligand binding to biological responses. The classic "occupancy" model of Clark (1933)

postulated that (1) the magnitude of the biological response is directly proportional to the fraction of receptors occupied, and (2) the response is maximal when all receptors are occupied. However, analyses of numerous receptor-mediated effects indicate that the relationship between receptor occupancy and biological effect is not as straightforward as Clark envisioned.

In certain cases, no response occurs even when there is some receptor occupancy. This suggests that there may be a threshold phenomenon that reflects the biological "inertia" of the response (Ariens et al., 1960). In other cases, a maximal response occurs well before all receptors are occupied, a phenomenon that reflects receptor "reserve" (Stephenson, 1956). Therefore, one cannot simply assume that the relationship between fractional receptor occupancy and biological response is linear. Furthermore, for a ligand (such as TCDD) that elicits multiple receptor-mediated effects, one cannot assume that the binding-response relationship for a simple effect (such as enzyme induction) will necessarily be identical to that for a different and more complex effect (such as cancer).

The cascades of events leading to different complex responses (e.g., altered immune response to pathogens or development of cancer) are likely to be different, and other rate-limiting events likely influence the final biological outcome, resulting in different dose-response curves. Thus, even though ligand binding to the same receptor is the initial event leading to a spectrum of biological responses, ligand-binding data may not always mimic the dose-effect relationship observed for particular responses.

Another level of complexity is added when one considers different chemical ligands that bind to the same receptor. Relative potencies are determined by two properties of the ligand: affinity for the receptor and capacity to confer a particular response in the receptor (e.g., a particular conformational change), also called efficacy (Stephenson, 1956). Ligands with different affinities and the same degree of efficacy would be expected to produce parallel dose-response curves with the same maximal response within a particular model system. However, ligands of the same affinity with different efficacies may result in dose-response curves that are not parallel or that differ in maximal response. These issues relate particularly to Ah receptor ligands that are not "dioxins," where different efficacies or an inability to elicit the suite of dioxin-like responses compound differences in binding affinity for the Ah receptor. This complicates the use of the toxic equivalency approach, particularly for extrapolation purposes beyond the closely related congener groups. As described previously, this argues strongly for the use of all available information in setting TEFs and highlights the important role that scientific judgment plays in addressing uncertainty in the face of incomplete mechanistic understanding.

3.2.2. A Framework to Evaluate Mode of Action

In its revised proposed guidelines for carcinogen risk assessment (U.S. EPA, 1999, 2003), EPA recommends the use of a structured approach to evaluating mode of action. This approach is similar to and builds upon an approach developed within the WHO/IPCS Harmonization Project (WHO, 2000). Fundamentally, the approach uses a modification of the "Hill Criteria" (Hill, 1965), which have been used in the field of epidemiology for many years to examine causality between associations of exposures and effects. The framework calls for a summary description of the postulated mode of action, followed by the identification of key events that are thought to be part of the mode of action. These key events are then evaluated as to strength, consistency, and specificity of association with the endpoint under discussion. Dose-response relationships between the precursor key events are evaluated and temporal relationships are examined to be sure that "precursor" events actually precede the induction of the endpoint. Finally, biological plausibility and coherence of the data with the biology are examined and discussed. All of these "criteria" are evaluated and conclusions are drawn with regard to postulated mode of action.

In the case of dioxin and related compounds, elements of such an approach are found for a number of effects, including cancer, in Part II. Application of the framework to dioxin and related compounds may now proceed in a step-wise fashion to evaluate the association between the chemical or complex mixture and clearly adverse effects. The approach can be applied sequentially to early events, for example, receptor binding and intermediate events such as enzyme induction or endocrine impacts. Additional data will be required to extend the framework to most effects, but several have data that would support a framework analysis, a number of which are discussed below.

3.2.3. Mechanistic Information and Mode of Action—Implications for Risk Assessment

A substantial body of evidence from investigations using experimental animals indicates that the AhR mediates the biological effects of TCDD. The key role of the AhR in the effects of dioxin and related compounds is substantiated by four lines of research: (1) structure/activity relationships, (2) responsive versus nonresponsive mouse strains, (3) mutant cell lines, and (4) the development of transgenic mice in which the gene for the AhR has been "knocked out" (Birnbaum, 1994a; Fernandez-Salguero et al., 1996; Lahvis and Bradfield, 1998). Dioxin appears not to cause effects in the AhR knockout mouse (Fernandez-Salguero et al., 1996; Lahvis and Bradfield, 1998; Peters et al., 1999).

It is clear that the AhR is necessary, but not sufficient, for essentially all of the well-studied responses to dioxin. The AhR functions as a ligand-activated transcription factor, controlling the expression of specific genes via interaction with defined nucleotide sequences in the promoter regions. In order to control transcription, the TCDD-AhR complex interacts with another protein, Arnt, to bind to the dioxin response element. This complex is also bound by other nuclear coactivators and/or corepressors to bind to the transcriptional complex and initiate transcription (Gu et al., 2000). However, Arnt has many other partners that control hypoxia response, neuronal differentiation, morphological branching, etc. (Gu et al., 2000).

It is possible that there are other mechanisms that impact how dioxin initiates its toxic effects, apart from its direct transcriptional activation of drug metabolizing genes. It may be that the adverse effects of dioxin may result from competition of the ligand-activated AhR with other Arnt partners (Gradin et al., 1996). The AhR, Arnt, and Arnt partners are all members of the Per-Arnt-Sim (PAS) family of basic helix-loop-helix proteins that function as nuclear regulatory proteins (Gu et al., 2000). The PAS proteins are highly conserved, with homologous proteins being present in prokaryotes. They play key roles in circadian rhythms and development. The embryolethality of Arnt knockout mice, as well as the reduced fertility and viability of the AhR knockout mice (Abbott et al., 1999), point to a key role of these proteins in normal physiology.

Another potential mechanism by which TCDD can cause effects involves the protein/protein interactions of the AhR. When not bound to a ligand, the AhR exists in a multimeric protein complex that involves two molecules of heat shock protein 90 as well as other proteins, including AIP/XAP2/ara9, ara3, ara6, src, rel, and Rb (Carver et al., 1998; Enan and Matsumura, 1996; Puga et al., 2000b). AIP/XAP2/ara9 is a 37 kilodalton protein that is related to known immunophilins and is involved in the control of signal transduction processes. C-src has been shown to be associated with the AhR in several tissues and is a tyrosine kinase (Enan and Matsumura, 1996). Dioxin has been shown to cause a rapid increase in phosphorylation upon exposure. Recent studies have shown that rel, which is a key component of the NF-kappaB complex that controls apoptosis, binds to the AhR complex (Tian et al., 1999; Puga et al., 2000c). Similarly, several investigators have demonstrated an association between the AhR and the retinoblastoma protein; this has been shown to affect cell cycling (Puga et al., 2000b).

Thus, the AhR may act as a negative regulator of key regulator molecules involved in phosphorylation, cell cycling, and apoptosis in its unliganded state. Upon binding of TCDD, these other proteins are now able to exert their effects. In addition, dioxin may act by competing for Arnt, thus blocking key roles of other PAS regulatory proteins. Both of these mechanisms for the effects of dioxin are in addition to the direct role of the ligand-bound form of the receptor in

control of transcription via the well-studied mechanism of binding to a dioxin-response element in DNA.

Although studies using human tissues are much less extensive, it appears reasonable to assume that dioxin's mode of action to produce effects in humans includes receptor-mediated key events. Studies using human organs and cells in culture are consistent with this hypothesis. A receptor-based mode of action would predict that, except in cases where the concentration of TCDD is already high (i.e., [TCDD]~K_D), incremental exposure to TCDD will lead to some increase in the fraction of AhRs occupied. However, it cannot be assumed that an increase in receptor occupancy will necessarily elicit a proportional increase in all biological response(s), because numerous molecular events (e.g., cofactors, other transcription factors, genes) that contribute to the biological endpoint are integrated into the overall response. That is, the final biological response should be considered as an integration of a series of dose-response curves, with each curve dependent on the molecular dosimetry for each particular step.

Dose-response relationships that will be specific for each endpoint must be considered when using mathematical models to estimate the risk associated with exposure to TCDD. It remains a challenge to develop models that incorporate all the complexities associated with each biological response. Furthermore, the parameters for each mathematical model may apply only to a single biological response within a given tissue and species.

Given TCDD's widespread distribution, its persistence, and its accumulation within the food chain, it is likely that most humans are exposed to some level of dioxin; thus, the population at potential risk is large and genetically heterogeneous. By analogy with the findings in inbred mice, polymorphisms in the AhR probably exist in humans. Therefore, a concentration of TCDD that elicits a particular response in one individual may not do so in another. For example, studies of humans exposed to dioxin following an industrial accident at Seveso, Italy, failed to reveal a simple and direct relationship between blood TCDD levels and the development of chloracne (Mocarelli et al., 1991). These differences in responsiveness to TCDD may reflect genetic variation either in the AhR or in some other component of the dioxin-responsive pathway. Therefore, analyses of human polymorphisms in the AhR and Arnt genes have the potential to identify genotypes associated with higher (or lower) sensitivities to dioxin-related effects. Such molecular genetic information may be useful in the future for accurately predicting the health risks posed by dioxin to humans.

Complex responses (such as cancer) probably involve multiple events and multiple genes. For example, a homozygous recessive mutation at the *hr* (hairless) locus is required for TCDD's action as a chloracnegen and tumor promoter in mouse skin (Poland et al., 1982). Thus, the *hr*

locus influences the susceptibility of a particular tissue (in this case, skin) to a specific effect of dioxin (tumor promotion). An analogous relationship may exist for the effects of TCDD in other tissues. For example, TCDD may produce porphyria cutanea tarda only in individuals who have inherited uroporphyrinogen decarboxylase deficiency (Doss et al., 1984). Such findings suggest that, for some adverse effects of TCDD, the population at risk may be limited to individuals who have a particular genetic predisposition.

Other factors can influence an organism's susceptibility to TCDD. For example, female rats are more prone to TCDD-induced liver neoplasms than are males; this phenomenon is related to the hormonal status of the animals (Lucier et al., 1991). In addition, hydrocortisone and TCDD synergize in producing cleft palate in mice (Abbott et al., 1992). Retinoic acid and TCDD produce a similar synergistic teratogenic effect (Couture et al., 1990). These findings indicate that, in some cases, TCDD acts in combination with hormones or other chemicals to produce adverse effects. Such phenomena might also occur in humans. If so, the difficulty in assessing risk is increased, given the diversity among humans in hormonal status, lifestyle (e.g., smoking, diet), and chemical exposure.

Dioxin's action as a tumor promoter and developmental toxicant presumably reflects its ability to alter cell proliferation and differentiation processes. There are several plausible mechanisms by which this could occur. First, TCDD might activate a gene (or genes) that is directly involved in tissue proliferation. Second, TCDD-induced changes in hormone metabolism may lead to tissue proliferation (or lack thereof) and altered differentiation secondary to altered secretion of a trophic hormone. Third, TCDD-induced changes in the expression of growth factor or hormone receptors may alter the sensitivity of a tissue to proliferative stimuli. Fourth, TCDD-induced toxicity may lead to cell death, followed by regenerative proliferation. These mechanisms likely differ among tissues and period of development, and they may be modulated by different genetic and environmental factors.

The parallels between animal and human data relating to dioxin's tumor-promotion potential can assist in informing determinations of human risk, recognizing that the complexity of these intracellular processes limits our current mechanistic understanding. Using a weight-of-evidence approach, the Agency considers the cancer promotion data from in vitro and in vivo animal studies to be relevant and informative to humans. Although the specific mechanism(s) by which dioxin causes cancer remains to be established (as, indeed, for cancer in general), the intracellular factors and mechanistic pathways involved in dioxin's cancer-promotion mode of action all have parallels between animals and humans. No qualitative differences have been

reported to indicate that humans should be considered fundamentally different from the multiple animal species in which bioassays have demonstrated dioxin-induced neoplasia. Notably:

- the intracellular molecular protein, DNA, and RNA factors and mechanisms postulated in dioxin cancer promotion are common to animals and humans, reflecting intracellular functions that have been preserved phylogenetically over millions of years. These factors include the AhR, Arnt heterodimerization, cellular growth and differentiation functions, dioxin responsive elements, DNA transcription mechanisms, and oxidative enzyme induction; and,

- similar dioxin-induced toxic outcomes are evident between animals and humans across a variety of endpoints, progressing from enzyme induction, altered intracellular regulatory proteins, dermal lesions, and liver function and porphyria through to in vitro neoplastic cell promotion and clonal expansion following viral or chemical induction (in addition to the epidemiological cancer results following occupational exposures).

As detailed in Part II, Chapter 2 (mechanism of action), the mode of action parallels between humans and animals can be traced through dioxin's impacts at the subcellular level, as follows:

AhR binding: The AhR has been phylogenetically retained over hundreds of millions of years of evolution in humans and animals (Hahn, 1998) and is highly expressed in developing tissues (Abbott et al., 1995), pointing to a fundamental role in cellular growth, differentiation and/or endogenous/xenobiotic metabolism. Species-specific AhR molecular structures reveal them to be members of a family of transcription-activating proteins that exhibit a basic helix-loop-helix (bHLH) DNA binding motif, PAS domain for dimerization and ligand binding, and a C-terminal transactivation domain related to transcription induction and associated with a variety of toxic endpoints.

Notable similarities exist in the AhR across animal taxa, particularly at the bHLH and PAS sites (Fujii-Kuriyama et al., 1995), with human AhR being structurally most closely related to that of the guinea pig (75% base homology) and other sensitive animal strains (Korkalainen et al., 2001). Dioxin-resistant strains of rats and hamsters exhibit mutations in the AhR and/or increased homology differences, particularly in the C-terminal transactivation domain and Q-rich

subdomain (Korkalainen et al., 2001). Human AhR binding affinities vary ~20-fold (Kd ~ 0.3–38.8 nM) (Okey et al. 1997), encompassing the range from sensitive C57BL/6 mice (0.27 nM) to relatively resistant DBA/2 mice (1.5 nM) (Ema et al., 1994). Evidence suggests that within species, the AhR binding affinity correlates with biochemical effects and toxicity (Birnbaum et al., 1990, Poland and Glover, 1980), whereas between species, relative AhR binding affinities do not determine dioxin sensitivity because multiple downstream events intercede (DeVito and Birnbaum, 1995). Differences in conformational changes in the AhR following ligand binding are also likely to impact toxicity (Henry and Gasiewicz, 2003).

TCDD-AhR binding to Arnt: Following ligand binding, the TCDD-Arnt complex translocates to the nucleus, where it heterodimerizes (joins) with the bHLH-PAS transcription partner protein, Arnt. Arnt has been phylogenetically retained over evolutionary time in both humans and animals in several related forms and is essential for fetal survival. Arnt molecular weights vary across species from 85 kDa for the mouse, 87 kDa for humans, and 88 kDa for the rat (Pohjanvirta et al., 1999). The Arnt protein also dimerizes with other receptor/transcription pathways in the cell nucleus, indicating its importance and fundamental role in regulating DNA transcription (Schmidt and Bradfield, 1996; Zaher et al., 1998; Ge and Elferink, 1998; Tian et al., 1999).

Cross-talk among intracellular regulatory proteins: As noted, cancer is inherently a loss of the regulation of normal cell growth, differentiation, and death (apoptosis) that is locked into the genetic coding through clonal expansion. Central to the control of cell cycling and programmed cell death are numerous regulatory proteins (e.g., EGF, HIF-1α, TNF-α, TGF-β_1, NF-κB, RB), whose functional roles, although being rapidly elucidated, remain uncertain. These regulatory proteins are expressed in humans and animals and can be impacted by dioxin exposure, as in the role of EGF in dioxin-induced cleft palate in mice (Bryant et al., 2001). The Arnt protein is a common co-transcription factor for many bHLH-PAS regulatory proteins in addition to its role in the TCDD-AhR transcription pathway. The potential exists, therefore, for prolonged, inappropriate TCDD-AhR induction to impact multiple Arnt-related functions in the nucleus, thereby altering other regulatory pathways.

Competition for the Arnt protein has been demonstrated regarding the hypoxia inducible factor 1 (HIF-1α) pathway following dioxin administration and Arnt cross-talk (Gradin et al., 1996; Nie et al., 2001). In addition, dioxin-induced clonal expansion in human and animal cell cultures has resulted in fixed changes to the intranuclear expression of plasminogen activation

inhibitor (PAI-2), tumor necrosis factor alpha (TNF-α), and transforming growth factor β_1 (TGF-β_1), although it remains to be determined whether these changes were cause or effect of the dioxin-promoted clonal expansion (Yang et al., 1999).

Dioxin response elements (DREs): In the well-studied pathway of cytochrome mixed function oxidase induction (e.g., CYP1A1, 1A2), the ligand-AhR-Arnt heterodimer binds 1:1 to DREs upstream of the DNA gene battery transcription site (Denison et al., 1989). This mechanism is common to the mouse (six DREs) (Lusska et al. 1993), the rat (three DREs), and humans (two DREs) (Swanson and Bradfield, 1993), and is based on the 3'A-CGCAC5' DNA sequence. Subsequent to DRE binding, the C-terminal transactivation domain of the AhR alters histone proteins and causes unwinding of the chromatin, exposing the dioxin promoter and aryl hydrocarbon hydroxylase (AHH) gene battery to constitutively expressed DNA transcription proteins (Whitlock et al., 1996).

Enzyme induction: At least seven enzyme genes, and likely more, are included in the AhR-Arnt induced gene battery: three oxidative P450 cytochromes (CYP1A1, 1A2 ,1B1) and four non-P450 enzymes responsive to reactive oxygenated metabolites and oxidative stress (for example, a quinone oxidoreductase, aldehyde dehydrogenase, glucuronosyltransferase, and glutathione transferase [Nebert et al., 2000; Zhang et al., 1998]). These enzymes are expressed in humans and animals. Similar EC_{50}s were reported for CYP1A1 induction in lymphocytes in mice (1.3 nM) and humans (1.8nM) (Clark et al., 1992). However, substantial interspecies differences have been noted between cultured human and mouse embryonic palatal cells regarding CYP1A1 induction and morphological effects. Paralleling a ~200-fold lower sensitivity for morphological and cellular effects on embryonic palatal tissue, human cell cultures expressed ~350-fold fewer receptors and exhibited ~1500-fold lower dioxin-induced CYP1A1 m-RNA induction than mice (Abbott et al., 1999). Notably, though, effects on human and rat embryonic palatal shelf tissue occur at similar in vitro concentrations as compared to the much higher sensitivity shown in mice, suggesting that mice may exhibit a particular sensitivity to effects on palatal differentiation (Abbott and Birnbaum, 1990, 1991; Couture et al., 1990).

For CYP1A2 there is a ~40-fold variability in protein and enzyme activity levels in the human population (Eaton et al., 1995; Nebert et al., 1996). The importance of CYP1A2 to dioxin toxicity in rodents has been demonstrated in knockout mice, where dioxin-induced porphyrin changes did not occur in the absence of CYP1A2, and hepatic toxicity was substantially reduced

(Smith et al., 2001). This is likely due to the lack of hepatic sequestration in the absence of CYP1A2 (Diliberto et al., 1999).

Recent human epidemiological data have reported long-term hepatic enzyme and porphyrin ratio changes many years after industrial dioxin exposure (Neuberger et al., 1999). The prolonged up-regulation of mixed-function oxidase (MFO) enzymes has been postulated to impact the carcinogenic potential of xenobiotics that are metabolically activated, such as the PAHs. Indeed, carcinogenicity from PAHs is absent in AhR-knockout mice, presumably from lack of induction of the mixed-function oxidases. In a related mechanistic postulate, emphasis has been placed on the existence of both MFOs (CYP1A1, 1A2) and detoxifying/scavenging phase II transferase enzymes in the dioxin-induced gene battery, suggesting an evolutionary mechanism that creates reactive oxidative products through the MFOs (possibly as a result of endogenous ligand metabolism) yet provides a protective mechanism for mitigating the resulting oxidative stress through the phase II transferase enzymes. Abnormal regulation of this mechanism could cause oxidative stress that is related both to DNA damage and cell cycling/apoptosis regulation (Nebert et al., 2000).

Toxic effects and clonal proliferation: A spectrum of toxic effects has been demonstrated in both animals and humans following dioxin exposure, including developmental impacts, hormonal changes, skin lesions, and liver damage (DeVito et al., 1995). Dioxin has also been demonstrated to promote neoplastic changes and clonal expansion in human and animal cell cultures following viral induction. Exposure of normal human keratinocytes in vitro leads to accelerated differentiation, increased cell proliferation, and decreased senescence in differentiating cells (Ray and Swanson, 2003). These changes were accompanied by decreased levels of a number of cell regulatory proteins, including p53, supporting the concept that dioxin may exert its tumor promoting effects, in part, through this mechanism.

In Yang et al. (1992), human epidermal keratinocytes immortalized by adenovirus 12 - simian virus 40 exposure (SV40) underwent neoplastic transformation after 2 weeks of dioxin exposure in vitro at $\geq$ 0.1 nM, exhibiting increased saturation density, colony formation on soft agar, and squamous cell carcinoma when inoculated into athymic nude mice. These phenomena did not occur in the absence of SV40 virus induction or in control cell lines, including the immortalized cell culture. Both the neoplastic cell transformation and AHH induction in the untransformed cells were dose dependent. Follow-up analyses demonstrated alterations in growth regulatory gene expression (PAI-2, TNF-α, and TGF-β_1) that became fixed in the genome following successive division in TCDD-damaged cells (Yang et al., 1999).

Conversely, under certain circumstances, exposure to TCDD may elicit beneficial effects in selected tissue or cells. For example, TCDD protects against the subsequent carcinogenic effects of PAHs in mouse skin, possibly reflecting induction of detoxifying enzymes (Cohen et al., 1979; DiGiovanni et al., 1980). In other situations, TCDD-induced changes in estrogen metabolism may alter the growth of hormone-dependent tumor cells, producing a potential anticarcinogenic effect (Spink et al., 1990; Gierthy et al., 1993). However, several recent studies in mice indicate that the AhR has an important role in the genetic damage and carcinogenesis caused by components in tobacco smoke, such as BaP, through its ability to regulate CYP1A1 gene induction (Dertinger et al., 1998; Shimizu et al., 2000). TCDD's biological effects likely reflect a complicated interplay between genetic and environmental factors. These issues complicate the risk assessment process for dioxin.

Thus, it is clear that the robust database on mode(s) of dioxin action related to biochemical effects and to clearly adverse effects supports an understanding of dioxin's impact on biological and cellular processes. This database is among the best available for xenobiotic chemicals. The short-comings described above will stimulate additional research to further elucidate details in this understanding of the impact of dioxins, but they should not detract from the recognition that, among the data available to aid hazard characterization and risk assessment, these are remarkably consistent and useful findings.

Table 3-1. Early molecular events in response to dioxin[a]

Diffusion into the cell
Binding to the AhR protein
Impacts on cytoplasmic phosphorylation
Dissociation from hsp90
Active translocation from cytoplasm to nucleus
Association with Arnt protein
Competition for Arnt with other nuclear cofactors
Conversion of liganded receptor to the DNA-binding form
Binding of liganded receptor heteromer to enhancer DNA
Enhancer activation
Altered DNA configuration
Histone modification
Recruitment of additional proteins
Nucleosome disruption
Increased accessibility of transcriptional promoter
Binding of transcription factors to promoter
Enhanced mRNA and protein synthesis

[a] These events are discussed in detail in Part II, Chapter 2.

4. EXPOSURE CHARACTERIZATION

This section summarizes key findings developed in the exposure portion of the Agency's dioxin reassessment. These findings are developed in the companion document entitled *Part I: Estimating Exposure to Dioxin-Like Compounds*, which is divided into three volumes: (1) Sources of Dioxin in the United States, (2) Properties, Environmental Levels, and Background Exposures, and (3) Site-Specific Assessment Procedures. Readers are encouraged to examine the more detailed companion document for further information on the topics covered here and to see complete literature citations. The characterization discussion provides cross-references to help readers find the relevant portions of the companion document.

This discussion is organized as follows: (1) sources, (2) fate, (3) environmental media and food concentrations, (4) background exposures, (5) potentially highly exposed populations, and (6) trends. The key findings are presented in italics.

4.1. SOURCES (Cross-reference: Part I, Volume 1: Sources of Dioxin-Like Compounds in the United States)

CDD/CDFs have never been intentionally produced other than on a laboratory-scale basis for use in scientific analysis. Rather, they have been generated as unintended by-products in trace quantities in various combustion, industrial, and biological processes. PCBs, on the other hand, were commercially produced in large quantities, but they are no longer commercially produced in the United States. EPA has classified sources of dioxin-like compounds into five broad categories:

1. *Combustion Sources.* CDD/CDFs are formed in most combustion systems, which can include waste incineration (such as municipal solid waste, sewage sludge, medical waste, and hazardous wastes), burning of various fuels (such as coal, wood, and petroleum products), other high temperature sources (such as cement kilns), and poorly or uncontrolled combustion sources (such as forest fires, building fires, and open burning of wastes). Some evidence exists that very small amounts of dioxin-like PCBs are produced during combustion, but they appear to be a small fraction of the total TEQs emitted.

2. *Metals Smelting, Refining, and Processing Sources.* CDD/CDFs can be formed during various types of primary and secondary metals operations, including iron ore sintering, steel production, and scrap metal recovery.

3. *Chemical Manufacturing.* CDD/CDFs can be formed as by-products from the manufacture of chlorine-bleached wood pulp, chlorinated phenols (e.g., pentachlorophenol, or PCP), PCBs, phenoxy herbicides (e.g., 2,4,5-T), and chlorinated aliphatic compounds (e.g., ethylene dichloride).

4. *Biological and Photochemical Processes.* Recent studies suggest that CDD/CDFs can be formed under certain environmental conditions (e.g., composting) from the action of microorganisms on chlorinated phenolic compounds. Similarly, CDD/CDFs have been reported to be formed during photolysis of highly chlorinated phenols.

5. *Reservoir Sources.* Reservoirs are materials or places that contain previously formed CDD/CDFs or dioxin-like PCBs and have the potential for redistribution and circulation of these compounds into the environment. Potential reservoirs include soils, sediments, biota, water, and some anthropogenic materials. Reservoirs become sources when they have releases to the circulating environment.

The development of national estimates of annual environmental releases to air, water, and land is complicated by the fact that only a few facilities in most industrial sectors have been evaluated for CDD/CDF emissions. Thus, an extrapolation is needed to estimate national emissions. The extrapolation method involves deriving an estimate of emissions per unit of activity (i.e., an emission factor) at the tested facilities and multiplying this by the total activity level in the untested facilities.

In order to convey the level of uncertainty in both the measure of activity and the emission factor, EPA developed a qualitative confidence rating scheme. The confidence rating scheme, presented in Table 4-1, uses qualitative criteria to assign a high, medium, or low confidence rating to the emission factor and activity level for those source categories for which emission estimates can be reliably quantified. The overall "confidence rating" assigned to a quantified emission estimate was determined by the confidence ratings assigned to the corresponding "activity level" and "emission factor." If the lowest rating assigned to either the activity level or the emission factor terms is "high," then the category rating assigned to the

emission estimate is high (also referred to as "A"). If the lowest rating assigned to either the activity level or emission factor terms is "medium," then the category rating assigned to the emission estimate is medium (also referred to as "B"). If the lowest rating assigned to either the activity level or emission factor terms is "low," then the category rating assigned to the emission estimate is low (also referred to as "C").

For many source categories, either the emission factor information or the activity level information were inadequate to support development of reliable quantitative release estimates for one or more media. For some of these source categories, sufficient information was available to make preliminary estimates of environmental releases of CDD/CDFs or dioxin-like PCBs; however, the confidence in the activity level estimates or emission factor estimates was so low that the estimates cannot be included in the sum of quantified emissions from sources with confidence ratings of A, B, or C. These estimates were given an overall confidence class rating of D. For other sources, some information exists suggesting that they may release dioxin-like compounds; however, the available data were judged to be insufficient for developing any quantitative emission estimate. These estimates were given an overall confidence class rating of E.

4.1.1. Inventory of Releases

This dioxin reassessment has produced an "inventory" of sources of environmental releases of dioxin-like compounds for the United States (Table 4-2). The inventory was developed by considering all sources identified in the published technical and scientific literature and by the incorporation of results from numerous individual emissions test reports of individual industrial and combustion source facilities. In order to be representative of the United States, data generated from U.S. sources of information were always given first priority for developing emission estimates. Data from other countries were used for making estimates in only a few source categories where foreign technologies were judged similar to those found in the United States and the U.S. data were judged to be inadequate. The inventory is limited to sources whose releases can be reliably quantified (i.e., those with confidence ratings of A, B, or C, as defined above). As discussed below, this document does provide preliminary estimates of releases from Class D sources, but they are presented separately from the inventory.

The inventory presents the environmental releases in terms of two reference years: 1987 and 1995. The year 1987 was selected primarily because little empirical data existed for making source-specific emission estimates prior to this time; 1995 represents the latest year that could reasonably be addressed within the timetable for producing the rest of this document. EPA

expects to conduct periodic revisions and updates to the source inventory in the future to track changes in environmental releases over time.

Figure 4-1 displays the emission estimates to air for sources included in the inventory and shows how the emission factors and activity levels were combined to generate emission estimates. Figure 4-2 compares the annual mean I-TEQ emission estimates to air for the two reference years (1987 and 1995).

The following conclusions are made for sources of dioxin-like compounds included in the inventory:

- *EPA's best estimates of releases of CDD/CDFs to air, water, and land from reasonably quantifiable sources were approximately 3300 g TEQ_{DF}-WHO_{98} (3000 g I-TEQ) in 1995 and 14,000 g TEQ_{DF}-WHO_{98} (12,800 g I-TEQ) in 1987.* This finding is derived directly from Table 4-2.

- *The inventory indicates that, between 1987 and 1995, there was approximately a 76% decrease in total environmental releases of CDDs/CDFs from known sources in the United States.* EPA is currently evaluating source releases for the year 2000. Preliminary indications support the observation of a continued reduction in total environmental releases from 1995 levels. The inventory updated for the year 2000 will undergo scientific peer review.

- *The environmental releases of CDD/CDFs in the United States occur from a wide variety of sources, but they are dominated by releases to the air from combustion sources.* The current (1995) inventory indicates that emissions from combustion sources are more than an order of magnitude greater than emissions from the sum of emissions from all other categories. Approximately 70% of all quantifiable environmental releases were contributed by air emissions from just three source categories in 1995: municipal waste incinerators (representing 38% of total environmental releases); backyard burning of refuse in barrels (19%); and medical waste incinerators (14%).

- *The decrease in estimated releases of CDD/CDFs between 1987 and 1995 (approximately 76%) was due primarily to reductions in air emissions from municipal and medical waste incinerators, and further reductions are anticipated.*

For both categories, these emission reductions have occurred from a combination of improved combustion and emission controls and from the closing of a number of facilities. EPA's regulatory programs estimate that full compliance with recently promulgated regulations should result in further reductions in emissions from the 1995 levels of more than 1800 I-TEQ. These reductions will occur in the following source types: municipal waste combustors, medical waste incinerators, and various facilities that burn hazardous waste (see Part I, Volume 1, for further details about these reductions). No federal regulations are in place or currently under development for limiting dioxin emissions from backyard burning of refuse in barrels. A number of states have general restrictions on the practice of backyard trash burning.

- *Insufficient data are available to comprehensively estimate point source releases of dioxin-like compounds to water.* Sound estimates of releases to water are available only for chlorine bleached pulp and paper mills (356 g I-TEQ_{DF} or TEQ_{DF}-WHO_{98} for 1987 and 20 g I-TEQ_{DF} or TEQ_{DF}-WHO_{98} for 1995) and the manufacture of ethylene dichloride (EDC)/vinyl chloride monomer (VCM) (< 1 g I-TEQ_{DF} or TEQ_{DF}-WHO_{98} in 1995). Other releases to water bodies that cannot be quantified on the basis of existing data include effluents from publicly owned treatment works (POTW) and most industrial/commercial sources. EPA's Office of Water estimates that when full compliance with limitations on effluent discharges of CDD/CDF from chlorine bleached pulp and paper mills is achieved, annual emissions will be reduced to 5 g I-TEQ_{DF} or TEQ_{DF}-WHO_{98}.

- *Based on the available information, the inventory includes only a limited set of activities that result in direct environmental releases to land.* Total releases to land quantified in the national inventory are estimated at 110 g TEQ_{DF}-WHO_{98} in 1995 and are principally from municipal wastewater treatment sludge (76.6 g) and the use of 2,4-D (28.9 g). Not included in the inventory's definition of an environmental release is the disposal of sludge and ashes into approved landfills.

- *Significant amounts of dioxin-like compounds produced annually are not considered environmental releases and, therefore, are not included in the national inventory.* Examples include dioxin-like compounds generated internal to a process but destroyed before release, waste streams that are disposed of in approved landfills and

are therefore outside the definition of annual environmental releases, and products that contain dioxin-like compounds but for which environmental releases, if any, cannot be estimated.

The procedures and results of the U.S. inventory may have underestimated releases from contemporary sources. A number of investigators have suggested that national inventories may underestimate emissions because of the possibility of unknown sources. This claim has been supported with mass balance analyses that suggest that deposition exceeds emissions (Rappe, 1991; Harrad and Jones, 1992; Bruzy and Hites, 1995); however, the uncertainty, in both the emissions and deposition estimates for the United States prevents the use of this approach for reliably evaluating the issue.

A variety of other arguments indicate that the inventory could underestimate emissions of dioxin-like compounds:

- A number of sources lacked sufficient data to include in the inventory but there were limited evidence indicating that these sources can emit CDD/CDFs. These sources are listed in Tables 4-3 and 4-4 and include various components of the metals industries, such as electric arc furnaces and foundries and uncontrolled or minimally controlled combustion practices (e.g., accidental fires at landfills).

- The possibility remains that truly unknown sources exist. Many of the sources that are well-accepted today were discovered only in the past 10 years. For example, CDD/CDFs were found unexpectedly in the wastewater effluent from bleached pulp and paper mills in the mid 1980s. Ore sintering is now listed as one of the leading sources of CDD/CDF emissions in Germany, but it was not recognized as a source until the early 1990s.

4.1.2. General Source Observations

For any given time period, releases from both contemporary formation sources and reservoir sources determine the overall amount of the dioxin-like compounds that are being released to the open and circulating environment. Because existing information is incomplete with regard to quantifying contributions from contemporary and reservoir sources, it is not currently possible to estimate the total magnitude of release for dioxin-like compounds from all

sources into the U.S. environment. For example, in terms of 1995 releases from reasonably quantifiable sources, this document estimates releases of 3300 g TEQ_{DF}-WHO_{98} (3000 g I-TEQ_{DF}) for contemporary formation sources and 2900 g I-TEQ_{DF} or TEQ_{DF}-WHO_{98} for reservoir sources.

In addition, there remain a number of unquantifiable and poorly quantified sources. No quantitative release estimates can be made for agricultural burning or for most CDD/CDF reservoirs or for any dioxin-like PCB reservoirs. The preliminary 1995 estimate of releases from poorly characterized contemporary formation sources is 1400 g I-TEQ_{DF} or TEQ_{DF}-WHO_{98}. The preliminary release estimates for contemporary formation sources and reservoir sources are presented in Table 4-2. Table 4-3 lists all the sources that have been reported to release dioxin-like compounds but cannot be characterized on even a preliminary basis.

Additional observations and conclusions about all sources of dioxin-like compounds are summarized below:

- *The contribution of dioxin-like compounds to waterways from nonpoint source reservoirs is likely to be greater than the contribution from point sources.* Current data are only sufficient to support preliminary estimates of nonpoint source contributions of dioxin-like compounds to water (i.e., from urban storm water runoff and rural soil erosion). These estimates suggest that, on a nationwide basis, total nonpoint releases are significantly larger than point source releases.

- *Current emissions of CDD/CDFs to the U.S. environment result principally from anthropogenic activities.* Evidence that supports this finding includes matches in time of rise of environmental levels with time when general industrial activity began rising rapidly (see trend discussion in Part I, Volume 2, Chapter 6), the lack of any identified large natural sources, and observations of higher CDD/CDF body burdens in industrialized versus less industrialized countries (see discussion on human tissue levels in Part I, Volume 2, Chapter 4).

- *Although chlorine is an essential component for the formation of CDD/CDFs in combustion systems, the empirical evidence indicates that for commercial-scale incinerators, chlorine levels in feed are not the dominant controlling factor for rates of CDD/CDF stack emissions.* Important factors that can affect the rate of CDD/CDF formation include the overall combustion efficiency, post-combustion flue gas

temperatures and residence times, and the availability of surface catalytic sites to support CDD/CDF synthesis. Data from bench-, pilot- and commercial-scale combustors indicate that CDD/CDF formation can occur by a number of mechanisms. Some of these data, primarily from laboratory and pilot-scale combustors, have shown direct correlation between chlorine content in fuels and rates of CDD/CDF formation. Other data, primarily from commercial-scale combustors, show little relation between availability of chlorine in feeds and rates of CDD/CDF formation.

- The conclusion that chlorine in feed is not a strong determinant of CDD/CDF emissions applies to the overall population of commercial-scale combustors. For any individual commercial-scale combustor, circumstances may exist in which changes in chlorine content of feed could affect CDD/CDF emissions. For uncontrolled combustion, such as open burning of household waste, the chlorine content of the waste may play a more significant role in rates of CDD/CDF formation and release than is observed at commercial-scale combustors. The full discussion on this issue is presented in Part I, Volume 1, Chapter 2.

- *Dioxins are present in some ball clays, but insufficient data are available to estimate whether environmental releases occur during mining and use.* Recent studies in the United States and Europe have measured dioxins (principally CDDs) in some ball clays and other related clays. As discussed in Part I, Volume 1, Chapter 13, it is likely that the dioxin present in ball clay is of a natural origin. Ball clay is principally used in the manufacture of ceramics, which involves firing the clay in high-temperature kilns. This activity may cause some portion of the CDDs contained in the clay to be released into the air, but emission tests have not yet been conducted that would allow characterizing these releases.

- *Data are available to estimate the amounts of CDD/CDFs contained in only a limited number of commercial products.* No systematic survey has been conducted to determine levels of dioxin-like compounds in commercial products. The available data do, however, allow estimates to be made of the amounts of dioxin-like compounds in bleached pulp (40 g I-TEQ_{DF} or TEQ_{DF}-WHO_{98} in 1995), POTW sludge used in fertilizers (3.5 g I-TEQ_{DF} or 2.6 g TEQ_{DF}-WHO_{98} in 1995), pentachlorophenol-treated wood (8400 g I-TEQ_{DF} or 4800 g TEQ_{DF}-WHO_{98} in 1995),

dioxazine dyes and pigments (< 1 g I-TEQ_{DF} or TEQ_{DF}-WHO_{98} in 1995), and 2,4-D (18.4 g I-TEQ_{DF} or 28.9 g TEQ_{DF}-WHO_{98} in 1995).

- *No significant release of newly formed dioxin-like PCBs is occurring in the United States.* Unlike CDD/CDFs, PCBs were intentionally manufactured in the United States in large quantities from 1929 until production ceased in 1977. Although it has been demonstrated that small quantities of coplanar PCBs can be produced during waste combustion, no strong evidence exists that the dioxin-like PCBs make a significant contribution to TEQ releases during combustion. The occurrences of dioxin-like PCBs in the U.S. environment most likely reflect past releases associated with PCB production, use, and disposal. Further support for this finding is based on observations of reductions since the 1980s in PCBs in Great Lakes sediment and other areas.

- *It is unlikely that the emission rates of CDD/CDFs from known sources correlate proportionally with general population exposures.* Although the inventory shows the relative contribution of various sources to total emissions, it cannot be assumed that these sources make the same relative contributions to human exposure. It is quite possible that the major sources of dioxin in food (see the discussion in Part I, Volume 2, Chapter 2, indicating that diet is the dominant exposure pathway for humans) may not be those sources that represent the largest fractions of current total emissions in the United States. It is important to consider the geographic locations of sources relative to the areas from which much of the beef, pork, milk, and fish come. That is, many of the agricultural areas that produce dietary animal fats are not located near or directly downwind of the major sources of dioxin and related compounds.

- *The contribution of reservoir sources to human exposure may be significant.* Several factors support this finding:
  1. Because the magnitude of releases from current sources of newly formed PCBs are most likely negligible, human exposure to the dioxin-like PCBs is thought to be derived almost completely from reservoir sources. Key pathways involve releases from both soils and sediments to both aquatic and terrestrial food chains. As discussed in Part I, Volume 2, Chapter 4, one-third of general population

TEQ_{DFP} exposure is due to PCBs. Thus, at least one-third of the overall risk from dioxin-like compounds comes from reservoir sources.

2. CDD/CDF releases from soil via soil erosion and runoff to waterways may be significant. These releases appear to be greater than releases to water from the primary sources included in the inventory. CDD/CDFs in waterways can bioaccumulate in fish, leading to human exposure via their consumption. As discussed in Part I, Volume 2, Chapter 4, fish consumption makes up about one-fifth of the total general population CDD/CDF TEQ exposure. This suggests that a significant portion of the CDD/CDF TEQ exposure could be due to releases from the soil reservoir. It is not known, however, how much of the soil erosion and runoff represents recently deposited CDD/CDFs from primary sources or longer-term accumulation. Much of the eroded soil comes from tilled agricultural lands, which would include a mix of CDD/CDFs from various deposition times. The age of CDD/CDFs in urban runoff is less clear.

3. Potentially, soil reservoirs could have vapor and particulate releases that deposit on plants and enter the terrestrial food chain. The magnitude of this contribution, however, is unknown.

Collectively, these three factors suggest that reservoirs are a significant source of current background TEQ exposure, perhaps contributing half or more of the total.

4.2. ENVIRONMENTAL FATE (Cross-reference: Part I, Volume 2, Chapter 2)

The estimates of environmental releases are presented above in terms of TEQs. This is done for convenience in presenting summary information and to facilitate comparisons across sources. For purposes of environmental fate modeling, however, it is important to use the individual CDD/CDF and PCB congeners values rather than TEQs because the physical/chemical properties of individual dioxin congeners vary and will behave differently in the environment. For example, the relative mix of congeners released from a stack cannot be assumed to remain constant during transport through the atmosphere and deposition to various media. The full congener-specific release rates for most sources are given in an electronic database that is available as a companion to this document (U.S. EPA, 1998) Database of Sources of Environmental Releases of Dioxin-Like Compounds in the United States. EPA/600/P-98/002Ab.

In Part I, Volume 3, site-specific procedures are provided for estimating the impact of emissions on local populations, and this section emphasizes that congener specific emission values should be used in modeling their environmental fate. Finally, it is important to recognize that this document does not use source release estimates to generate background population intake/risk estimates; rather, these estimates are derived primarily from food levels and consumption rates.

Dioxin-like compounds are widely distributed in the environment as a result of a number of physical and biological processes. The dioxin-like compounds are essentially insoluble in water, they are generally classified as semivolatile, and they tend to bioaccumulate in animals. Some evidence has shown that these compounds can degrade in the environment, but in general they are considered to be very persistent and relatively immobile in soils and sediments. These compounds are transported through the atmosphere as vapors or attached to airborne particulates and can be deposited on soils, plants, or other surfaces (by wet or dry deposition). The dioxin-like compounds enter water bodies primarily via direct deposition from the atmosphere or by surface runoff and erosion. From soils, these compounds can reenter the atmosphere as either resuspended soil particles or vapors. In water, they can be resuspended into the water column from sediments, they can be volatilized out of the surface waters into the atmosphere, or, they can become buried in deeper sediments. Immobile sediments appear to serve as permanent sinks for the dioxin-like compounds. Although anthropogenic materials (such as PCP) are not always considered an environmental compartment, dioxin-like compounds are also found in such materials, and from there they have the potential to be released into the broader environment.

Atmospheric transport and deposition of the dioxin-like compounds are a primary means of their dispersal throughout the environment. The dioxin-like compounds have been measured in wet and dry deposition in most locations, including remote areas. Numerous studies have shown that they are commonly found in soils throughout the world. Industrialized countries tend to show similar elevated concentrations in soil, and detectable levels have been found in nonindustrialized countries. The only satisfactory explanation available for this distribution is air transport and deposition. Finally, by analogy these compounds would be expected to behave similarly to other compounds that have similar properties, and this postulated mechanism of global distribution is becoming widely accepted for a variety of persistent organic compounds.

The two primary pathways for the dioxin-like compounds to enter the ecological food chains and human diet are air-to-plant-to-animal and water/sediment-to-fish. Vegetation receives these compounds via atmospheric deposition in the vapor and particle phases. The compounds are retained on plant surfaces and bioaccumulated in the fatty tissues of animals that

feed on these plants. Vapor phase transfers onto vegetation have been experimentally shown to dominate the air-to-plant pathway for the dioxin-like compounds, particularly for the lower chlorinated congeners. In the aquatic food chain, dioxins enter water systems via direct discharge or deposition and runoff from watersheds. Fish accumulate these compounds through their direct contact with water, suspended particles, and bottom sediments and through their consumption of aquatic organisms.

Although these two pathways are thought to normally dominate contribution to the commercial food supply, others can also be important. Elevated dioxin levels in cattle resulting from animal contact with PCP-treated wood have been documented by the U.S. Department of Agriculture. Animal feed contamination episodes have led to elevations of dioxins in poultry in the United States, milk in Germany, and meat/dairy products in Belgium (see Part I, Volume 2, Chapter 5).

4.3. ENVIRONMENTAL MEDIA AND FOOD CONCENTRATIONS (Cross-reference: Part I, Volume 2, Chapter 3)

Background levels of dioxin-like compounds in various environmental media, including food, are presented in Table 4-4 in terms of means, variability, and sample sizes used to support the estimates. Estimates for background levels of dioxin-like compounds in environmental media are based on a variety of studies conducted at different locations in North America. Of the studies available for this compilation, only those conducted in locations representing "background" were selected. The amount and representativeness of the data vary, but in general they were derived from studies that were not designed to estimate national background means. The environmental media concentrations were similar to those in studies from Western Europe. These data are the best available for comparisons with site-specific values. Because of the limited number of locations examined, it is not known whether these estimates adequately capture the full national variability. As new data are collected, these ranges are likely to be expanded and refined. The limited data on dioxin-like PCBs in environmental media are summarized in Part I, Volume 2, Chapter 3.

Estimates for levels of dioxin-like compounds in food are based on data from a variety of studies conducted in North America. Beef, pork, and poultry estimates were derived from statistically based national surveys. Milk estimates were derived from a survey of a nationwide milk sampling network. Dairy estimates were derived from milk fat concentrations, coupled with appropriate assumptions for the amount of milk fat in dairy products. The background egg concentrations were based on an analysis of 15 egg samples collected from retail stores in eight

states (CA, OH, GA, NY, PA, OR, MN, WS; two samples per state except one in OR), where each sample was a composite of 24 individual eggs (i.e., 15 samples represented 360 eggs). The fish data, as discussed below, were derived from multiple studies, with samples collected both directly from water bodies and from retail outlets. All fish concentrations were expressed on the basis of fresh weight in edible tissue. As with other environmental media, food levels found in the United States were similar to levels found in Europe.

The procedure to evaluate background fish exposures emphasizes the use of both species-specific consumption rates and species-specific concentrations. EPA's national bioaccumulation study (U.S. EPA, 1992b) provides some species-specific information on freshwater/estuarine fish caught in the wild at various locations in the United States. Additional species-specific data on store-bought fish are available from studies conducted by the U.S. Food and Drug Administration (FDA) during the mid to latter 1990s (Jensen and Bolger, 2000; Jensen et al., 2000). An important aspect of the FDA studies is that they include data on store-bought catfish, tuna, shellfish, and salmon, which are some of the most highly consumed species. Accordingly, the data used to characterize CDD/CDF fish levels are much improved over previous estimates, with more than 300 individual samples and good representation of the most highly consumed species. However, the levels of dioxins in fish remain more uncertain than those in the other foods.

The compilation of data from different studies still lacks the geographic coverage and statistical power of the other food surveys. The EPA and FDA studies did not address dioxin-like PCBs; rather, these are based on a much smaller data set derived from the open literature. Also, the estimates of dioxin intake resulting from fish consumption do not include consumption of fish oils. Currently, insufficient data are available to support estimates of dioxin intake from direct fish oil consumption.

The general population dioxin intake calculations used in this document are a function of both consumption rate and dioxin concentration in food. The concentration data used in this document were measured in raw foods; therefore, if cooking significantly alters the dioxin concentration in consumed portions it must be accounted for in estimating dioxin intake.

This issue has been examined in a number of studies that measured the effects of cooking on the levels of CDDs, CDFs, and PCBs in foods (see Part I, Volume 2, Chapter 3). These studies have a range of results, depending on food type and cooking method. Most of the cooking experiments suggested that cooking reduces the total amount of dioxins in food but causes relatively little change in its concentration.

Although some cooking experiments have shown increases and others have shown decreases in dioxin concentrations, the relative prevalence of these impacts have not been

established. Therefore, given that most experiments show little change and others show change in both directions, the most reasonable assumption that can be made from the existing data is that dioxin concentration in uncooked food is a reasonable surrogate for dioxin concentration in cooked food. Although cooking in general does not reduce dioxin concentration in food, some specific food preparation practices can be adopted that can reduce dioxin intake by significantly reducing overall animal fat consumption. For example, carefully trimming fat from meat, removing skin from chicken and fish, and avoiding cooking in animal fats should reduce both animal fat and dioxin intake.

Some evidence from Europe suggests that during the 1990s a decline occurred in concentrations of dioxins and furans in food products, particularly dairy products (see Part I, Volume 2, Chapter 6). For example, the United Kingdom's Ministry of Agriculture, Fisheries, and Food collected milk samples in 1990 and again from similar locations in 1995. In 1990, the I-TEQ_{DF} ranged from 1.1 to 3.3 ppt, whereas the 1995 I-TEQ_{DF} ranged from 0.7 to 1.4. In Germany, a sampling of 120 dairy products in 1994 found I-TEQ_{DF} concentrations that were 25% lower than those in a similar sampling program in 1990. Liem et al. (2000) reports on a European cooperative study coordinated by the National Institute of Public Health and the Environment in the Netherlands and the Swedish National Food Administration. Ten countries supplied data on food concentrations, food consumption patterns, and other data used to evaluate exposure to dioxins in Europe. Some of the data suggested reductions in concentrations over time, but the available information was insufficient to draw general conclusions.

No systematic study of temporal trends in dioxin levels in food has been conducted in the United States. Although not statistically based, one U.S. study examined dioxin levels in 14 preserved food samples from various decades in the 20th century (Winters et al., 1998). It was found that meat samples of the 1950s through the 1970s had concentrations that were two-three times higher for the CDD/CDF TEQs and about 10 times higher for the PCB TEQs, as compared to current meat concentrations.

The food data and associated exposure estimates presented here reflect a mid-1990's time frame. New studies underway now or recently completed could be used in future updates to this report to make exposure estimates for a new reference year, such as 2000. The following studies on dioxin levels in food were not completed in time to be included in this document and should be considered in future updates:

- The milk levels used in Tables 4-4 and 4-6 are based on a study by Lorber et al. (1998) where milk samples were collected in 1996. A very similar milk survey was

conducted by Schaum et al. (2003) involving the collection and analysis of TEQ_{DFP} in cow milk samples from 45 dairy plants in July of 2000 and again in January 2001. This study reported TEQ_{DFP} levels in whole milk which were about half the levels found by Lorber et al. (1998). Follow-up work by Schuda et al. (2004), which addressed CDD/Fs only, allowed estimation of 2000/2001 TEQ_{DF} milk levels on a lipid basis. This approach showed similar TEQ_{DF} levels in milk lipid, or perhaps a slight decrease, when comparing CDD/F TEQs in the two sampling times (0.71 pg TEQDF/g lipid in 2000/2001 compared to 0.82 pg TEQDF/g lipid in 1996).

- USDA is currently conducting a nationwide survey of dioxin levels in beef, pork and poultry. Samples were collected in 2002 and 2003 and data analysis is now underway. The survey design and data analysis are structured in a similar way to the earlier USDA surveys used in this report and should allow for trend analysis.

- The Institute of Medicine of the National Academies published a review of dioxin levels in foods in 2003 (Institute of Medicine of the National Academies, 2003). This document presents policy options for reducing dietary exposure to dioxins in food and related research recommendations. Appendix B of the Institute of Medicine's report summarizes FDA's Total Diet Survey of dioxin levels in food collected in 2001. A wide variety of foods were sampled including dairy products, eggs, meats, fish, fruits, vegetables and fats/oils.

The food consumption rates used here are based primarily on USDA's 1994-1996 Continuing Survey of Food Intakes by Individuals. As new USDA survey data come available, these should be incorporated into future updates of this report.

4.4. BACKGROUND EXPOSURES (Cross-reference: Part I, Volume 2, Chapter 4)

4.4.1. Tissue Levels

The average CDD/CDF/PCB tissue level for the general adult U.S. population appears to be declining, and the best estimate of current (late 1990s) levels is 25 ppt (TEQ_{DFP}-WHO_{98}, lipid basis).

The tissue samples collected in North America in the late 1980s and early 1990s showed an average TEQ_{DFP}-WHO_{98} level of about 55 pg/g lipid. This finding is supported by a number of studies—all conducted in North America—that measured dioxin levels in adipose, blood, and

human milk. However, the number of participants in most of these studies was relatively small and they were not statistically selected in ways that ensure their representativeness of the general U.S. adult population. One study, the 1987 National Human Adipose Tissue Survey, involved more than 800 individuals and provided broad geographic coverage, but it did not address coplanar PCBs. Similar tissue levels of these compounds have been measured in Europe and Japan during similar time periods.

Because dioxin levels in the environment have been declining since the 1970s (see the trends discussion in Part I, Volume 2, Chapter 6), it is reasonable to expect that levels in food, human intake, and, ultimately, human tissue have also declined over this period. The changes in tissue levels are likely to lag the decline seen in environmental levels, and the changes in tissue levels cannot be assumed to occur proportionally with declines in environmental levels.

CDC (2000) summarizes levels of CDDs, CDFs, and PCBs in human blood collected between 1995 to 1997 from 316 U.S. residents (ages 20–70 years). The individuals sampled had no known exposures to dioxin other than normal background. Although the samples in this data set were not collected in a manner that can be considered statistically representative of the national population and they lack wide geographic coverage, they are judged to provide a better indication of current tissue levels in the United States than the earlier data.

PCBs 105, 118, and 156 are missing from the blood data for the comparison populations reported by CDC (2000). These congeners account for 62% of the total PCB TEQ estimated in the early 1990s. Assuming that the missing congeners from the CDC study data contribute in the same proportion to the total PCB TEQ as in earlier data, they would increase the estimate of current body burdens by another 3.3 pg TEQ/g lipid, for a total PCB TEQ of 5.3 pg/g lipid and a total of 25.4 pg TEQ_{DFP}-WHO_{98}/g lipid (i.e., the TEQ_{DF}-WHO_{98} concentration was 20.1 pg/g lipid, and the TEQ_{P}-WHO_{98} concentration was estimated at 5.3 pg/g lipid). A summary of the CDC (2000) data is shown in Table 4-5.

A portion of the CDC blood data were plotted as a function of age. This plot, shown in Figure 4-3, indicates that blood levels generally increase with age, as does the variability in blood levels.

The calculation of a current tissue level of 25.4 pg/g lipid TEQ_{DFP}-WHO_{98} is further supported by the observation that this mean tissue level is consistent with the best estimate of current adult intake, 66 pg TEQ_{DFP}-WHO_{98}/d. Using this intake in a one-compartment, steady-state pharmacokinetic model yields a tissue level estimate of about 11.3 pg TEQ_{DFP}/g lipid (assumes TEQ_{DFP} has an effective half-life of 7.1 years, 80% of ingested dioxin is absorbed into the body, and lipid weight is 25% of the adult assumed body weight of 70 kg, or 17.5 kg).

Because intake rates appear to have declined in recent years, and steady-state is not likely to have been achieved, it is reasonable to observe higher measured tissue levels, such as the 25.4 pg TEQ/g lipid, than those predicted by the model.

Characterizing national background levels of dioxins in tissues is uncertain because the current data cannot be considered statistically representative of the general population. It is also complicated by the fact that tissue levels are a function of both age and birth year. Because intake levels have varied over time, the accumulation of dioxins in a person who turned 50 years old in 1990 is different than that in a person who turned 50 in 2000. As discussed in Part I, Volume 2, Chapter 6, exposure to dioxin-like compounds peaked during the 1960s, with declining exposures since then. Therefore, a person born in 1910 will see a rise in body levels that peaks at 50 to 70 years old. At the other end of the spectrum, a person born in 1970 will experience a higher body concentration very early in life, with declining levels in later years.

A pharmacokinetic (PK) modeling framework was developed to study trends in population body burdens of CDDs/CDFs throughout the 20th century and into the 21st century (Lorber, 2002). It was assumed that individuals within a population were exposed to doses rising from 0.50 pg WHO_{98}-TEQ_{DF}/kg-day during the 1940s to about 6.5 pg WHO_{98}-TEQ_{DF}/kg-day by the late 1960s, down to 1.0 pg WHO_{98}-TEQ_{DF}/kg-day by 1980, and finally to 0.50 pg WHO_{98}-TEQ_{DF}/kg-day by 2000, remaining constant at that level into the 21st century. It was found that a modeled population tissue level distribution will vary, depending on the year the modeled population is sampled. The results of this analysis are presented in Figure 4-4, which shows modeled population tissue level distributions for four years. An "age trend" is seen in the figure for modeled populations sampled in 1985 and 1995, as was seen in the CDC monitoring study of actual blood measurements of WHO_{98}-TEQ_{DFP} (see Fig. 4-3). Figure 4-4 also suggests that this age trend will disappear in the 21st century and that the CDD/CDF tissue level will drop below 10 ppt TEQ_{DF}-WHO_{98} lipid basis by 2030.

Monitoring studies which are currently underway should help determine whether the decline in body burdens has been continuing into the 21st century, as suggested by modeling. Results from the National Health and Nutrition Examination Survey of 1999-2000 (NHANES 1999-2000) were recently made available (CDC, 2003). NHANES 1999-2000 included data on dioxin-like compounds in the blood of 1921 sampled individuals, aged 12 and higher, and sampled from numerous locations around the country. These compounds included the 17 dioxin and furan congeners, as well as PCB congeners 126, 77, 169, and 81.

The current estimate of background body burden is based on 6 different studies totaling 316 individuals around the country which measured concentrations of these compounds in

populations characterized as "background" (CDC, 2000). Often these populations were selected the "background" population for studies which targeted other potentially exposed populations. The dates of these surveys, as noted above, were from about 1995 to 1997. In addition to being more recent, the NHANES 1999-2000 sampled population was much larger, but perhaps most importantly, NHANES was statistically designed to be representative of U.S. background after several years of data collection while the merged population from the 6 studies was not.

However, the amount of blood serum available for individual measurements in NHANES 1999-2000 was too small to be able to detect and characterize current levels of dioxin like compounds in the population. A large majority of the measurements were nondetects. For this reason, an effort is underway to pool remaining, available individual samples from NHANES and measure them for dioxin-like compounds, which would provide an updated measure of average concentrations of these compounds in the blood of U.S. citizens (ages 12 and greater, circa 1999-2000, and with all other delimiters relevant to the pooled samples, of course).

4.4.2. Intake Estimates

Adult daily intakes of CDD/CDFs and dioxin-like PCBs are estimated to average 43 and 23 pg TEQ_{DFP}-WHO_{98}/day, respectively, for a total intake of 66 pg/day TEQ_{DFP}-WHO_{98}. Daily intake is estimated by combining exposure media concentrations (food, soil, and air) with contact rates (ingestion, inhalation). Table 4-6 summarizes the media concentrations, contact rates, and resulting intake estimates.

The intake estimate is supported by an extensive database on food consumption rates and estimates of dioxin-like compounds in food (as discussed above). PK modeling provides further support for the intake estimates. Applying a simple steady-state PK model to an adult average blood level of 25 ppt TEQ_{DFP}-WHO_{98} (on a lipid basis) yields a daily intake of 146 pg TEQ_{DFP}-WHO_{98}/day (assumes TEQ_{DFP} has an effective half-life of 7.1 years, 80% of ingested dioxin is absorbed into the body, and lipid weight is 25% of the adult assumed body weight of 70 kg, or 17.5 kg). This PK-modeled CDD/CDF/PCB intake estimate is about 2.2 times higher than the direct intake estimate of 66 pg TEQ_{DFP}-WHO_{98}/day. This difference is to be expected with this application of a simple steady-state PK model to current average adipose tissue concentrations. Current adult tissue levels reflect intakes from past exposure levels, which are thought to be higher than current levels (Lorber, 2002; also in Part I, Volume 2, Chapter 6). Because the direction and magnitude of the difference in intake estimates between the two approaches are understood, the PK-derived value is judged supportive of the pathway-derived estimate. It

should be recognized, however, that the pathway-derived value will underestimate exposure if it has failed to capture all the significant exposure pathways.

4.4.3. Variability in Intake Levels

CDD/CDF and dioxin-like PCB intakes for the general population may extend to levels at least three times higher than the mean. Variability in general population exposure is primarily the result of the differences in dietary choices that individuals make. These are differences in both quantity and types of food consumed. An increased background exposure can result from either a diet that favors consumption of foods high in dioxin content or a diet that is disproportionately high in overall consumption of animal fats.

The best data available to determine the variability of total fat consumption come from several analyses of the Bogalusa Heart Study (Cresanta et al., 1988; Nicklas et al., 1993, 1995, Nicklas, 1995; Frank et al., 1986). These data show that the 95th percentile of total fat consumption is about twice the mean and the 99th percentile is approximately three times the mean. For a diet that has a broad distribution of animal fats (as does the typical U.S. diet), this same distribution can be assumed for dioxin intake.

Although body burden data cannot be assumed to be perfectly representative of current intakes (because they reflect past exposures as well as current ones), they also provide some support for this finding, based on the observation that the 95th percentile blood level in the CDC (2000) study was almost twice the mean level.

Intakes of CDDs/CDFs and dioxin-like PCBs are more than three times higher for a young child than for an adult, on a body-weight basis. This figure is based on combining age-specific food consumption rate and average food concentrations, as was done above for adult intake estimates (see Table 4-7).

Only 4 of the 17 toxic CDD/CDF congeners and 1 of the 11 toxic PCBs account for most of the toxicity in human tissue concentrations: 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 1,2,3,6,7,8-HxCDD, and 2,3,4,7,8-PCDF and PCB 126. This finding is derived directly from the data described earlier on human tissue levels and is supported by intake estimations that indicate that these congeners are also the primary contributors to dietary dose. These five compounds make up about 80% of the total TEQ_{DFP}-WHO_{98} tissue level.

4.5. POTENTIALLY HIGHLY EXPOSED POPULATIONS OR DEVELOPMENTAL STAGES (Cross-reference: Part I, Volume 2, Chapter 5)

As discussed earlier, background exposures to dioxin-like compounds may extend to levels at least three times higher than the mean. This upper range is assumed to result from the normal variability of diet and human behaviors. Exposures from local elevated sources or exposures resulting from unique diets would be in addition to this background variability. Such elevated exposures may occur in small segments of the population, such as individuals living near discrete local sources. Nursing infants represent a special case: for a limited portion of their lives, these individuals may have elevated exposures on a body-weight basis when compared with nonnursing infants and adults.

Dioxin contamination incidents involving the commercial food supply have occurred in the United States and in other countries. For example, in the United States, contaminated ball clay was used as an anticaking agent in soybean meal, which resulted in elevated dioxin levels in some poultry and catfish. This incident, which occurred in 1998, involved a small faction of the national poultry production, and the use of contaminated ball clay has since been eliminated. Elevated dioxin levels have also been observed in a few beef and dairy animals, where the contamination was associated with contact with pentachlorophenol-treated wood. Evidence of this kind of elevated exposure was not detected in the national beef survey. Consequently, its occurrence is likely to be low, but it has not been determined.

These incidents may have led to small increases in dioxin exposure to the general population. However, it is unlikely that they have led to disproportionate exposures to populations living near where they occurred because in the United States meat and dairy products are highly distributed on a national scale. If contamination events were to occur in foods that are predominantly distributed on a local or regional scale, then such events could lead to more highly exposed local populations (see Part I, Volume 2, Chapter 5).

Elevated exposures associated with the workplace or with industrial accidents have also been documented. U.S. workers in certain segments of the chemical industry had elevated levels of TCDD exposure, with some tissue measurements in the thousands of part per trillion TCDD. There is no clear evidence that elevated exposures are currently occurring among U.S. workers. Documented examples of past exposures for other groups include certain Air Force personnel exposed to Agent Orange during the Vietnam War and people exposed as a result of industrial accidents in Europe and Asia.

Consumption of breast milk by nursing infants leads to higher levels of exposure and higher body burdens of dioxins during early years of life as compared with those of nonnursing infants (Part I, Volume 2, Chapter 5).

Kreuzer et al. (1997) and Abraham et al. (1994, 1995, 1998, 2000) compared dioxin levels in infants who were breast-fed with those who were formula-fed. All the studies showed elevations in the concentrations of dioxins in the breast-fed infants. Collectively, these studies included more than 100 infants, and they found that blood levels in infants aged 4-12 months were generally higher than 20 pg TEQ_{DF}-WHO_{98}/g lipid in nursing infants and lower than 5 pg TEQ_{DF}-WHO_{98}/g lipid in formula fed infants. Limited data suggest a similar difference for dioxin-like PCBs. Abraham et al. (1995) reported that at 11 months a breast-fed infant had a concentration of 31.4 pg TEQ_{P}-WHO_{98}/g lipid, compared to 2.5 pg TEQ_{P}-WHO_{98}/g lipid for the formula-fed infant.

U.S. dioxin intakes from nursing were calculated using time-dependent values for breast milk concentrations, consumption rates, and body weights. These calculations estimated an intake immediately after birth of 242 pg TEQ_{DFP}-WHO_{98}/kg/day. This level dropped to 18 pg TEQ_{DFP}-WHO_{98}/kg/day after 12 months of nursing. The average intake over 1-year of nursing was calculated to be 87 pg TEQ_{DFP}-WHO_{98}/kg/day. The cumulative intake for a 1 year nursing scenario represented about 13% of the total lifetime cumulative intake (see Lorber and Phillips, 2002, and Part I, Volume 2, Chapter 5, for details on these calculations).

CDC (1997) reported that in 1995, 55% of all babies experienced some breast-feeding, with about half of those breast-feeding beyond 5 months. The average duration of breast-feeding was 28.7 weeks. In a policy statement, the American Academy of Pediatrics (1997) stated that exclusive breast feeding provides ideal nutrition and is sufficient to support optimal growth and development for 6 months after birth. It recommended that breast-feeding continue for at least 12 months and thereafter for as long as mutually desired.

To better evaluate the impact of nursing on infants, changes in body burden were calculated using a one-compartment, first-order pharmacokinetic model (Lorber and Phillips, 2002). First, the model was validated using data from Abraham et al. (1998). Dioxin and furan concentrations for six mother/infant pairs were provided, including two breast milk measurements while the mother was feeding her infant and a blood measurement for the infant at about 1 year. These mothers' milk concentrations were used as the independent source term for the model, and the infant blood concentrations served as dependent model prediction. Other required parameters included the infant's body weight and lipid fraction over time (assigned average male and female infant values), absorption fraction (assigned a constant value of 0.80),

and, most importantly, an assumption of a rapid dissipation rate of TEQs in the infant (half-life < 1 year) during the early months of life. This dissipation rate was developed by Kreuzer et al. (1997), and it contrasts the more typical 7-year half-life found in adults for TCDD.

The average observed infant concentration was 24 pg TEQ_{DF}-WHO_{98}/g lipid, compared to a predicted concentration of 26 pg TEQ_{DF}-WHO_{98}/g lipid. The observed high and low concentrations were 5 and 44 pg TEQ_{DF}-WHO_{98}/g lipid, compared to predicted high and low concentrations in these infants of 10 and 36 pg TEQ_{DF}-WHO_{98}/g lipid. When the model was rerun at a higher TEQ dissipation rate of 7 years, the average predicted concentration rose to 39 pg TEQ_{DF}-WHO_{98}/g lipid. This demonstrated the appropriateness and importance of the assignment of a rapid dissipation rate of TEQs in infants.

This framework was used to evaluate various nursing scenarios: formula only and 6 weeks, 6 months, 1 year, and 2 years nursing. These scenarios reasonably capture the range of current nursing practices. This modeling effort required using the intake assumptions described earlier—242 pg TEQ_{DFP}-WHO_{98}/kg/day at birth and an average of 87 pg TEQ_{DFP}-WHO_{98}/kg/day over a year of breast-feeding—and other parameters noted above including the fraction of the oral dose that is absorbed into the body, changes in body weight over time, and changes in body fat fraction over time. For the infant, the half-life was less than 1 year, and during adulthood the half-life increased as the fraction of body fat increased. The longer half-life during the later years of life was based on a model presented in Michalek et al. (1996). The complete set of input values is listed in Lorber and Phillips (2002) as well as in Part I, Volume 2, Chapter 5.

The modeling results in terms of changes in lipid concentrations and body burdens as a function of age are shown in Figure 4-5**.** Some key observations include:

- For the 6-month, 1-year, and 2-year nursing scenarios, lipid concentrations peaked at around 9 weeks at 44 ppt TEQ_{DFP}-WHO_{98}. For the formula-fed infants they peaked at less than 10 ppt after the first year.

- In all four scenarios, the lipid concentrations merged at about 10 years of age at a concentration of about 13 ppt TEQ_{DFP}-WHO_{98}. Lipid and body burdens declined slightly from age 10 to about age 20 and then rose gradually through adulthood. This rise was due to the increase in half-life with age. At age 70, the modeled lipid and body burden concentrations were 13 ppt TEQ_{DFP}-WHO_{98} lipid and 5 ppt TEQ_{DFP}-WHO_{98} whole body weight.

- Breast-feeding leads to higher total lifetime exposures to TEQs as compared to formula feeding. Using an AUC approach, 70-year cumulative lifetime exposures were evaluated. The results suggest that breast-feeding added between 3% (for the 6-week breast-feeding scenario) and 18% (for the 2-year scenario) more accumulated exposure to TEQs as compared to formula-feeding.

The above analysis indicates that the average annual infant intake resulting from 1 year of nursing, 87 pg TEQ_{DFP}-WHO_{98}/kg/day, significantly exceeds the currently estimated adult intake of 1 pg TEQ_{DFP}-WHO_{98}/kg/day. The impact of nursing on infant body burdens, however, is much less, that is, infant body burdens will not exceed adult body burdens by 87 times. Rather, the modeling suggests that peak infant body burdens are only about two times the current adult body burdens (44 vs. 25 pg TEQ_{DFP}-WHO_{98}/g lipid). The reduced impact on body burden levels in nursing infants (relative to the intake) is due to the rapidly expanding infant body weight and lipid volume, and the faster elimination rate in infants. Body burden levels in nursing infants should decline in the future if, as discussed earlier, general population exposures decline.

Consumption of fish, meat, or dairy products containing elevated levels of dioxins and dioxin-like PCBs can lead to elevated exposures in comparison with the general population. The above discussion identified the general population distribution as extending up to roughly three times the mean. Most people will have exposures within this range even if they have unusual diets in terms of meat and dairy products. This is because (1) most people eat food from multiple sources, which tends to average out the contamination levels, and (2) meat and dairy products have similar dioxin levels, so substitution of one type of meat for another should not have a great impact on total exposure. Clearly, elevated exposures are possible in unusual situations, such as when an individual consumes large quantities of meat or dairy products that have significantly increased dioxin levels.

Elevated exposures resulting from fish consumption can occur in different situations. Concentrations in freshwater fish are significantly greater than in meat and dairy products; therefore, individuals who consume large quantities of freshwater fish at background contamination levels may have intakes higher than the general population distribution. A simple scenario was devised to evaluate this hypothesis. Through a review of the literature, EPA (U.S. EPA, 1997) concluded that a range of consumption of 59 to 170 g/day describes subsistence fish consumption behavior. These consumption rates were adopted to characterize the range of exposures in this scenario. Further, it is assumed that freshwater fish is the primary source of protein, that is, no meat or eggs are consumed. Assuming that all other exposure pathways stay

the same and using background exposure media concentrations, adult daily intake in this subsistence fisher scenario is calculated to range from 2.2 to 5.7 pg TEQ_{DFP}-WHO_{98}/kg-day. These intakes are about two to six times higher than the adult general population mean daily intake of 0.93 pg TEQ_{DFP}-WHO_{98}/kg-day. If subsistence fishers obtain their fish from areas where the concentration of dioxin-like chemicals in the fish is elevated, their exposure could be higher. Although this scenario appears reasonable, no clearly supportive data could be found to confirm that such highly exposed subpopulations exist in the United States.

One study that measured dioxin-like compounds in the blood of sport fishers in the Great Lakes area showed elevations over mean background but within the range of normal variability. However, another study that measured 90 PCB congeners (seven of which were dioxin-like PCBs, although PCB 126 was not measured) in the blood of sport fishers who consume high amounts of fish caught from Lake Michigan (> 26 pounds of sport fish per year) did find significant elevations of PCBs in their blood as compared to a control population (individuals consuming < 6 pounds of sport fish per year). The average total concentration of PCBs in the blood of the sport fishers was more than three times higher than that of the control population. Similarly, elevated levels of coplanar PCBs have been measured in the blood of fishers on the north shore of the Gulf of the St. Lawrence River who consume large amounts of seafood. Elevated CDD/CDF levels in human blood have been measured in Baltic fishermen. For further details on these studies see Part I, Volume 2, Chapter 5.

High exposures to dioxin-like compounds as a result of consuming meat and dairy products would most likely occur in situations where individuals consume large quantities of these foods and the level of these compounds is elevated. Most people eat meat and dairy products from multiple sources, and even if large quantities are consumed they are not likely to have unusually high exposures. Individuals who raise their own livestock for basic subsistence have the potential for higher exposures if local levels of dioxin-like compounds are high. One study in the United States showed elevated levels in chicken eggs near a contaminated soil site. European studies at several sites have shown elevated CDD/CDF levels in milk and other animal products near combustion sources, and some of these studies have also documented elevations in the levels of dioxin-like compounds in blood from families who consume their own home products.

Table 4-1. Confidence rating scheme

Confidence category	Confidence rating	Activity level estimate	Emission factor estimate
Categories/media for which emissions can be reasonably quantified			
A	High	Derived from comprehensive survey	Derived from comprehensive survey
B	Medium	Based on estimates of average plant activity level and number of plants or limited survey	Derived from testing at a limited but reasonable number of facilities believed to be representative of source category
C	Low	Based on data judged possibly nonrepresentative.	Derived from testing at only a few, possibly nonrepresentative facilities or from similar source categories
Categories/media for which emissions cannot be reasonably quantified			
D	Preliminary estimate	Based on extremely limited data, judged to be clearly nonrepresentative.	Based on extremely limited data, judged to be clearly nonrepresentative.
E	Not quantified	No data.	(1) Argument based on theory but no data (2) Data indicating dioxin formation but not in a form that allows developing an emission factor

Table 4-2. Inventory of environmental releases (grams/year) of TEQ_{DF}-WHO_{98} in the United States

Emission source category	Confidence rating[a] reference year 1995				Confidence rating[a] reference year 1987		
	A	B	C	D	A	B	C
Releases (g TEQ/yr) to air							
Waste incineration							
Municipal waste incineration		1250				8877	
Hazardous waste incineration		5.8				5	
Boilers/industrial furnaces			0.39				0.78
Medical waste/pathological incineration			488				2590
Crematoria			9.1[b]				5.5[b]
Sewage sludge incineration		14.8				6.1	
Tire combustion			0.11				0.11
Pulp and paper mill sludge incinerators[c]							
Power/energy generation							
Vehicle fuel combustion							
- leaded[d]			2				37.5
- unleaded			5.6				3.6
- diesel			33.5				27.8
Wood combustion							
- residential			62.8[b]				89.6[b]
- industrial		27.6				26.4	
Coal combustion							
- utility boilers		60.1				50.8	
- residential				30			
- commercial/Industrial				40			
Oil combustion							
- industrial/utility			10.7				17.8
- residential				6			
Other high temperature sources							
Cement kilns (hazardous waste burning)			156.1				117.8
Lightweight aggregate kilns burning hazardous waste			3.3[b]				2.4[b]
Cement kilns (nonhazardous waste burning)			17.8				13.7
Petroleum refining catalyst regeneration			2.21				2.24

Table 4-2. Inventory of environmental releases (grams/year) of TEQ_{DF}-WHO_{98} in the United States (continued)

Emission source category	Confidence rating[a] reference year 1995				Confidence rating[a] reference year 1987		
	A	B	C	D	A	B	C
Releases (g TEQ/yr) to air (continued)							
Other high temperature sources (continued)							
Cigarette combustion			0.8				1
Carbon reactivation furnaces			0.08[b]				0.06[b]
Kraft recovery boilers		2.3				2	
Combustion of landfill gas				7			
Biogas combustion				< 1			
Minimally controlled or uncontrolled combustion[e]							
Backyard barrel burning[f]			628				604
Landfill fires				1000			
Accidental fires (structural)				< 20			
Accidental fires (vehicles)				30			
Forest and brush fires				200			
Metallurgical processes							
Ferrous metal smelting/refining							
- sintering plants		28					32.7
- electric arc furnaces				40			
- foundries				20			
Nonferrous metal smelting/refining							
- primary copper		< 0.5[b]				< 0.5[b]	
- secondary aluminum			29.1				16.3
- secondary copper			271				983
- secondary lead		1.72				1.29	
- primary magnesium				15			
Coke production				7			
Drum and barrel reclamation			0.08				0.08
Chemical manufacturing/processing sources							
Ethylene dichloride/vinyl chloride		11.2[b]					
TOTAL RELEASES TO AIR[g]	3125				13515		

Table 4-2. Inventory of environmental releases (grams/year) of TEQ_{DF}-WHO_{98} in the United States (continued)

Emission source category	Confidence rating[a] reference year 1995 A	B	C	D	Confidence rating[a] reference year 1987 A	B	C
Releases (g TEQ/yr) to water							
Chemical manufacturing/ processing sources Bleached chemical wood pulp and paper mills	19.5				356		
POTW (municipal) wastewater				10			
Ethylene dichloride/vinyl chloride		0.43[b]					
Reservoir sources Urban runoff to surface water				190			
Rural soil erosion to surface water				2700			
TOTAL RELEASES TO WATER[g]	19.93				356		
Releases (g TEQ/yr) to land							
Chemical manufacturing/ processing sources Bleached chemical wood pulp and paper mill sludge	1.4				14.1		
Ethylene dichloride/vinyl chloride		0.73[b]					
Municipal wastewater treatment sludge	76.6				76.6		
Commercially marketed sewage sludge	2.6				2.6		
2,4-Dichlorophenoxy acetic acid	28.9				33.4		
TOTAL RELEASES TO LAND[g]	110.23				126.7		
OVERALL RELEASES (g/yr) TO THE OPEN AND CIRCULATING ENVIRONMENT	**3255 (SUM OF COLUMNS A, B, C)**				**13,998 (SUM OF COLUMNS A, B, C)**		

[a] The most reliable estimates of environmental releases are those sources within Categories A, B, and C, which are defined as:

A = Characterization of the Source Category judged to be **Adequate for Quantitative Estimation** with **High Confidence** in the **Emission Factor** and **High Confidence** in **Activity Level**.

Table 4-2. Inventory of environmental releases (grams/year) of TEQ_{DF}-WHO_{98} in the United States (continued)

B = Characterization of the Source Category judged to be **Adequate for Quantitative Estimation** with **Medium Confidence** in the **Emission Factor** and at least **Medium Confidence** in **Activity Level**.

C = Characterization of the Source Category judged to be **Adequate for Quantitative Estimation** with **Low Confidence** in either the **Emission Factor** and/or the **Activity Level**.

D = **Preliminary Indication** of the Potential Magnitude of I-TEQ_{DF} Emissions from "Unquantified" (i.e., Category D) Sources in Reference Year 1995. **Based on extremely limited data, judged to be clearly nonrepresentative**.

[b] Congener-specific emissions data were not available; the I-TEQ estimate was used as a surrogate for the TEQ_{DF}-WHO_{98} emissions estimate.

[c] Included within estimate for Wood Combustion - industrial.

[d] Leaded fuel production and the manufacture of motor vehicle engines requiring leaded fuel for highway use have been prohibited in the United States. (See Section 4.1 for details.)

[e] This refers to conventional pollutant control, not dioxin emissions control. Very few of the sources listed in this inventory control specifically for CDD/CDF emissions.

[f] This term refers to the burning of residential waste in barrels.

[g] TOTAL reflects only the total of the estimates made in this report.

Table 4-3. Sources that are currently unquantifiable (Category E)[a]

Category	Unquantified sources
Combustion sources	Uncontrolled combustion of PCBs Agricultural burning
Metal smelting and refining	Primary aluminum Primary nickel
Chemical manufacturing	Mono- to tetrachlorophenols Pentachlorophenol Chlorobenzenes Chlorobiphenyls (leaks/spills) Dioxazine dyes and pigments 2,4-Dichlorophenoxy acetic acid Tall oil-based liquid soaps
Biological and photochemical processes	Composting
Reservoir sources	Air Sediments Water Biota PCP-treated wood

[a] There exist no or insufficient data characterizing environmental releases from these sources. Therefore, it is currently not possible to arrive at an estimate of annual environmental releases.

Table 4-4. Summary of North American CDD/CDF and PCB TEQ-WHO$_{98}$ levels in environmental media and food[a]

Media	CDD/CDFs[b]	PCBs[b]
Urban soil, ppt	n= 270 9.3 ± 10.2 Range = 2–21	n = 99 2.3
Rural soil, ppt	n = 354 2.7 Range = 0.11–5.7	n = 62 0.59
Sediment, ppt	n=11 5.3 ± 5.8 Range = <1–20	n = 11 0.53 ± 0.69
Urban air, pg/m^3	n=106 0.12 ± 0.094 Range = 0.03–0.2	n=53 0.0009
Rural air, pg/m^3	n=60 0.013 Range = 0.004–0.02	n=53 0.00071
Freshwater fish and shellfish, ppt[c]	n=222 1.0 (NA[d])	n = 1 composite of 10 samples plus 6 composites 1.2[e] (NA[d])
Marine fish and shellfish, ppt[c]	n=158 0.26 (NA[d])	n = 1 composite of 13 samples plus 5 composites 0.25 (NA[d])
Water, ppq	n=236 0.00056 ± 0.00079 (NA[d])	NA[d]
Milk, ppt (Note: each composite for CDD/F/PCB comprised of 40+ U.S. regional samples)	n=8 composites 0.018[e]	n = 8 composites 0.0088[e]
Dairy, ppt[f]	n = 8 composites 0.12[e]	n = 8 composites 0.058[e]
Eggs, ppt (Note: each composite for CDD/F data comprised of 24 eggs)	n=15 composites 0.081[e]	n = 18 plus 6 composites 0.10[e] (NA[d])

Table 4-4. Summary of North American CDD/CDF and PCB TEQ-WHO_{98} levels in environmental media and food (continued)

Media	CDD/CDFs[b]	PCBs[b]
Beef ppt	n=63 0.18 ± 0.11 Range = 0.11–0.95	n = 63 0.084
Pork, ppt	n=78 0.28 ± 0.28 Range = 0.15–1.8	n = 78 0.012
Poultry, ppt	n=78 0.068 ± 0.070 Range = 0.03–0.43	n = 78 0.026
Vegetable fats, ppt	n=30 0.056 ± 0.24[g] (NA[d])	n = 5 composites 0.037[e]

[a] Whole-weight basis; concentrations provided in parenthesis for food products are calculated at ND = 0.
[b] Values are the arithmetic mean TEQs and standard deviations. Nondetects were set to one-half the limit of detection, except for soil and CDD/CDFs in vegetable fats for which nondetects were set to zero.
[c] The TEQ_{df} fish concentrations reported here are species-specific ingestion rate weighted averages.
[d] NA = not available; congener-specific PCB data and data to calculate TEQ concentrations at ND = 0 are limited.
[e] Standard deviations could not be calculated due to limitations associated with the data (i.e., composite analyses).
[f] TEQ calculated by setting nondetects to zero.
[g] Dairy concentration calculated from milk lipid concentrations and then assuming a fat fraction for dairy.

Table 4-5. Background serum levels in the United States 1995–1997

Value	TEQ_{DFP}-WHO_{98} (pg/g lipid)	2,3,7,8-TCDD (pg/g lipid)
Median	18.7	1.9
Mean	22.1[a]	2.1
95th Percentile	38.8	4.2

[a] After adjusting to account for missing PCBs, the mean is 25.4 pg/g lipid.

Source: CDC, 2000

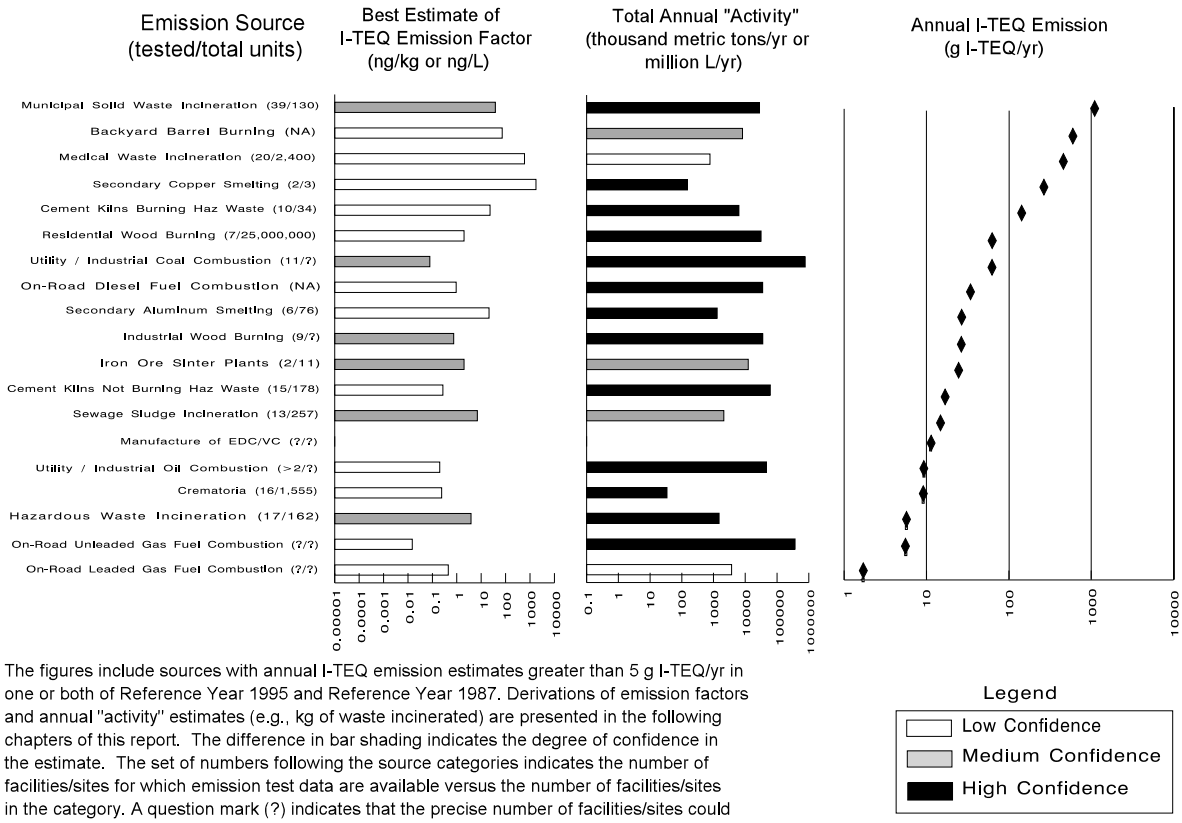
Table 4-6. Adult contact rates and background intakes of dioxin-like compounds

Exposure route	Contact rate	Dioxins and furans		Dioxin-like PCBS		Total
		Concentration TEQ_{DF}-WHO_{98}	Intake (pg TEQ_{DF}-WHO_{98}/kg-d)	Concentration TEQ_{P}-WHO_{98}	Intake (pg TEQ_{P}-WHO_{98}/kg-d)	Intake (pg TEQ_{DFP}-WHO_{98} /kg-d)
Soil ingestion	50 mg/d	9.3 pg/g	0.0066	2.3 ppt	0.0016	0.0082
Soil dermal	12 g/d	9.3 pg/g	0.0016	2.3 ppt	0.00039	0.002
Freshwater fish and shellfish[a]	5.9 g/d	1.0 pg/g	0.084	1.2 pg/g	0.1	0.18
Marine fish and shellfish[a]	9.6 g/d	0.26 pg/g	0.036	0.25 pg/g	0.034	0.07
Inhalation	13.3 m^3/d	0.12 pg/m^3	0.023	NA	NA	0.023
Milk	175 g/d	0.018 pg/g	0.045	0.0088 pg/g	0.022	0.067
Dairy	55 g/d	0.12 pg/g	0.094	0.058 pg/g	0.046	0.14
Eggs	0.24 g/kg-d	0.081 pg/g	0.019	0.10 pg/g	0.024	0.043
Beef	0.67 g/kg-d	0.18 pg/g	0.13	0.084 pg/g	0.06	0.19
Pork	0.22 g/kg-d	0.28 pg/g	0.062	0.012 pg/g	0.0026	0.065
Poultry	0.5 g/kg-d	0.068 pg/g	0.034	0.026 pg/g	0.013	0.047
Other meats	0.35 g/kg-d	0.18 ppt	0.062	0.041 pg/g	0.014	0.076
Vegetable fat	17 g/d	0.056 pg/g	0.014	0.037 pg/g	0.009	0.023
Water	1.4 L/d	0.0005 pg/L	0.000011	NA	NA	0.000011
Total			**0.61 (43 pg/d)**		**0.33 (23 pg/d)**	**0.94 (66 pg/d)**

[a] The TEQ_{df} fish concentrations reported here are species-specific ingestion rate weighted averages.

Table 4-7. Variability in average daily toxic equivalent (TEQ) intake as a function of age

Age range	Intake, mass basis pg TEQ_{DFP}-WHO_{98}/d	Intake, body weight basis pg TEQ_{DFP}-WHO_{98}/kg-d
1–5 years	50	3.3
6–11 years	54	1.8
12–19 years	61	1.1
Adult	66	0.9



The figures include sources with annual I-TEQ emission estimates greater than 5 g I-TEQ/yr in one or both of Reference Year 1995 and Reference Year 1987. Derivations of emission factors and annual "activity" estimates (e.g., kg of waste incinerated) are presented in the following chapters of this report. The difference in bar shading indicates the degree of confidence in the estimate. The set of numbers following the source categories indicates the number of facilities/sites for which emission test data are available versus the number of facilities/sites in the category. A question mark (?) indicates that the precise number of facilities/sites could not be estimated.

Figure 4-1. Estimated CDD/CDF I-TEQ emissions to air from combustion sources in the United States, 1995.

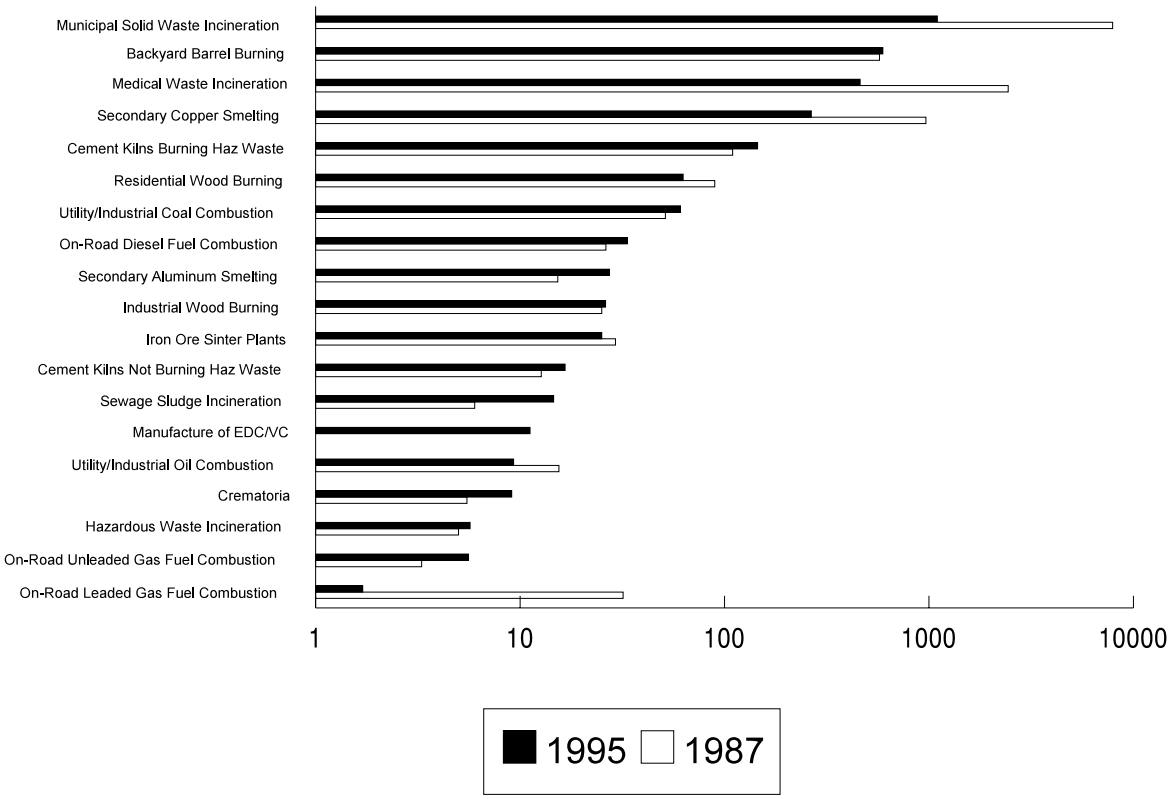


Figure 4-2. Comparison of estimates of annual I-TEQ emissions to air (grams I-TEQ/yr) for reference years 1987 and 1995.

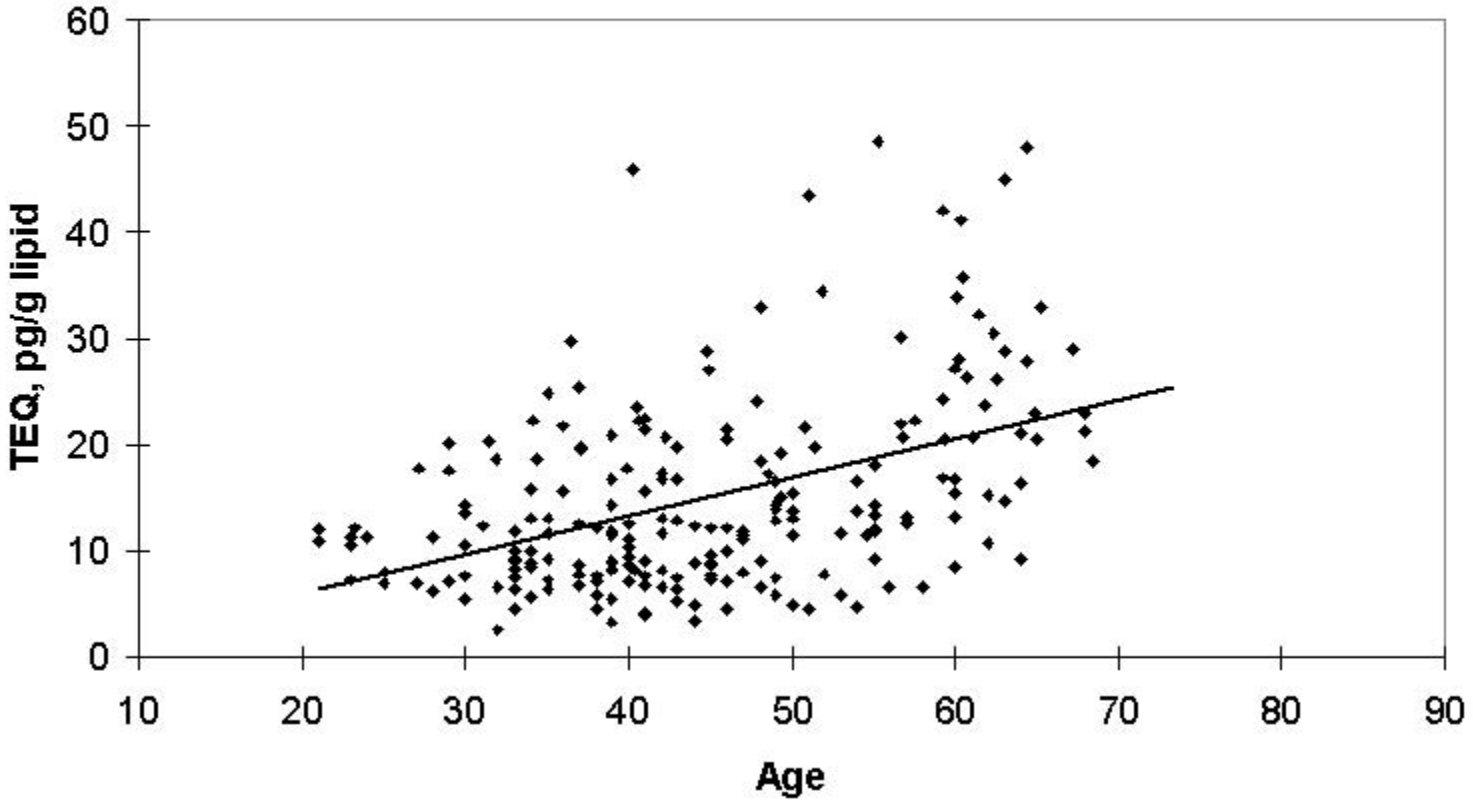


Figure 4-3. Blood levels (I-TEQ for CDD/CDF + WHO_{94}) versus age of a subset of participants in the CDC (2000).

Source: ATSDR, 1999b

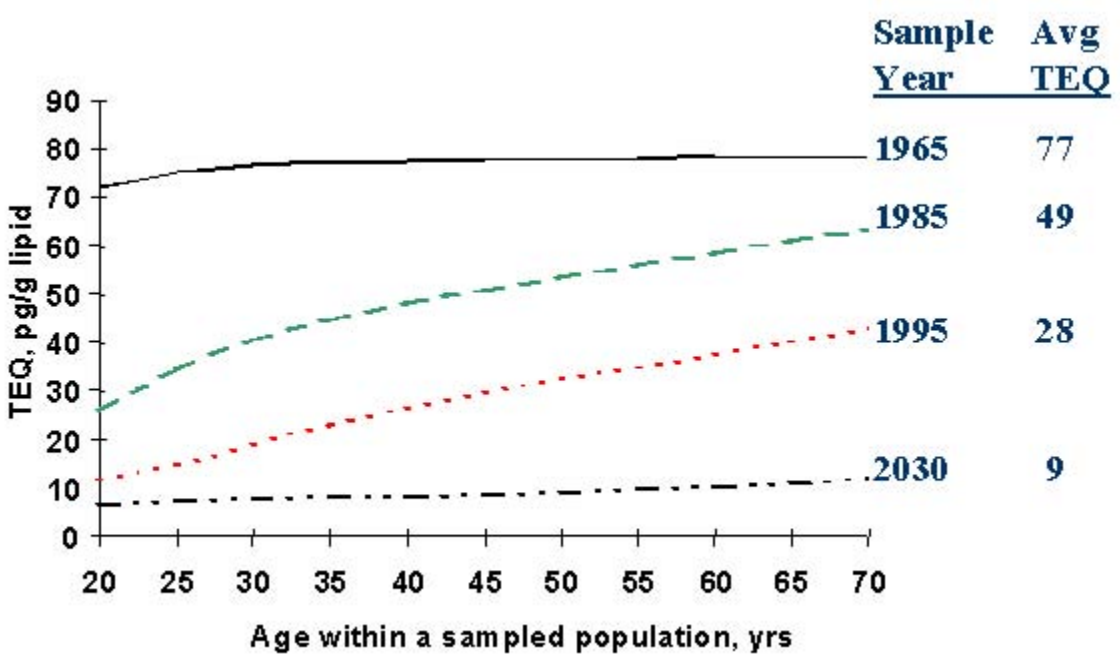
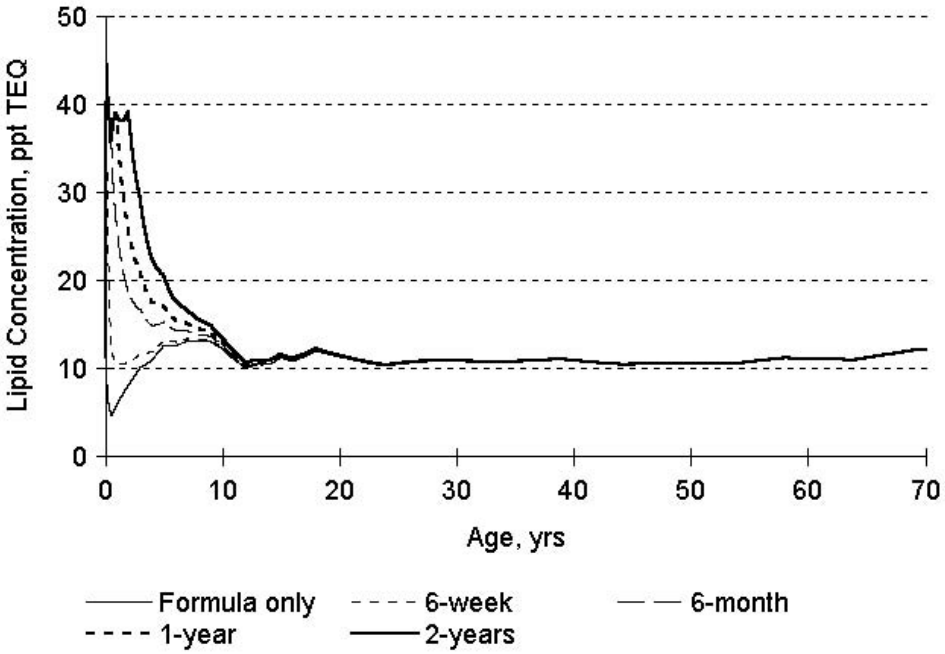


Figure 4-4. Predicted distributions and average TEQ_{DF} - WHO_{98} concentrations within an adult population for four years: 1965, 1985, 1995, and 2030. (CDD/CDFs only, not PCBs).

Source: Adapted from Lorber, 2002

(A)



(B)

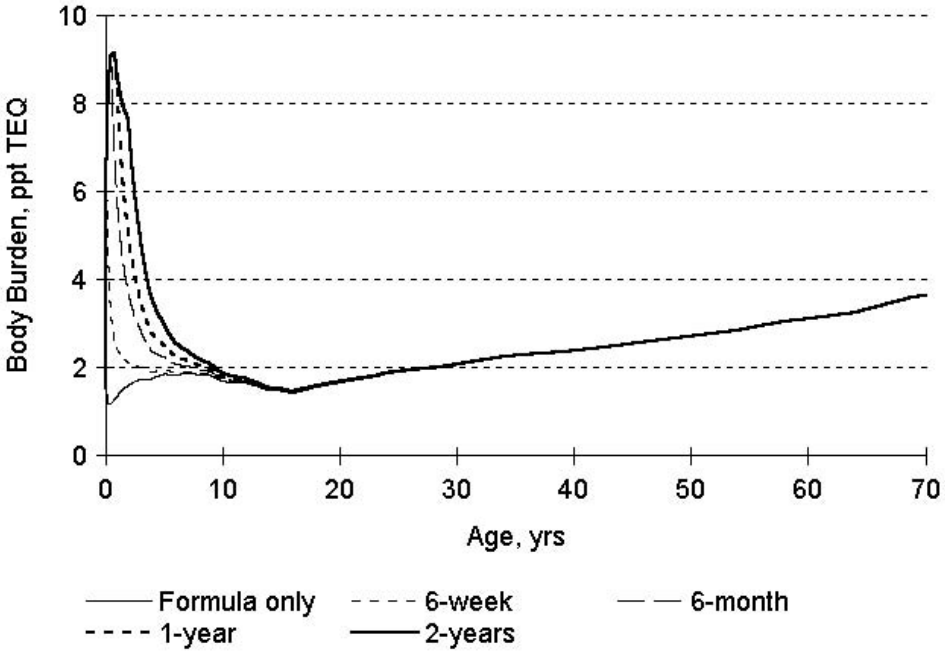


Figure 4-5. Demonstration of the model for evaluating impacts on lipid concentrations (A) and body burdens (B) of infants resulting from various nursing scenarios during a lifetime.

5. DOSE-RESPONSE CHARACTERIZATION

Previous sections of this integrated summary focused on characterizing the hazards of and exposure to dioxin-like compounds. In order to bring these issues together and provide an adequate characterization of risk, the relationships of exposure to dose and, ultimately, to response must be evaluated. Key questions to be asked include: (1) What can be said about the shape of the dose-response function in the observable range and what does this imply about dose-response in the range of environmental exposures? and (2) What is a reasonable limit (critical dose or point of departure [POD]) at the lower end of the observable range and what risk is associated with this exposure? In addition, one can address the issue of extrapolation beyond the range of the data in light of the answers to the above questions. Although extrapolation of risks beyond the range of observation in animals and/or humans is an inherently uncertain enterprise, it is recognized as an essential component of the risk assessment process (NAS/NRC, 1983). The level of uncertainty is dependent on the nature (amount and scope) of the available data and on the validity of the models that have been used to characterize dose-response. These form the bases for scientific inference regarding individual or population risk beyond the range of current observation (NAS/NRC, 1983, 1994).

Dose-response analysis can be implemented in a variety of ways in risk assessment, depending on the extent and quality of the available data. At the basic level, dose-response information comes from a comparison of doses or levels at which there are no observed adverse effects with those at which the lowest adverse effect is observed. Such an analysis can be enhanced through the application of mathematical models to interpolate between empirically measured data points (plus incorporating their statistical variability), with the option for extrapolation below these data points subject to model shape assumptions when going beyond the range of known data. One such form of modeling is the benchmark dose (BMD) analysis, where a mathematical model is used to calculate the dose necessary to elicit a predetermined response rate (e.g., an effective dose [ED] for a 1% response: ED_{01}). Ultimately, the development and use of physiologically-based pharmacokinetic PBPK models and biologically-based dose response models goes beyond the mathematical replication of data points by linking the model to relevant and measurable biological parameters in the species of interest, and potentially between species (Kim et al., 2002).

These dose-response concepts are developed in Part II, Chapter 8, where the body of literature concerning dose-response relationships for TCDD is presented. Among other things, this chapter addresses the important concept of selecting an appropriate metric for cross-species

scaling of dose and presents the results of empirical modeling for many of the available data sets on TCDD exposures in humans and in animals. Although not all human observations or animal experiments on TCDD are amenable to this level of dose-response modeling, more than 200 data sets were evaluated for shape, leading to an effective dose value expressed as a percent response being presented for each endpoint being evaluated.

The analysis of dose-response relationships for TCDD, considered within the context of toxic equivalency, mechanism of action, and background human exposures, helps elucidate the common ground and the boundaries of the science and science policy components inherent in this risk characterization for the broader family of dioxin-like compounds. For instance, the dose-response relationships provide a basis to infer a POD for extrapolation for cancer and noncancer risk for a complex mixture of dioxin-like congeners given the assumption of toxic equivalency as discussed in Part II, Chapter 9, Section 9.6. Similarly, these relationships provide insight into the shape of the dose-response at the POD, which can help inform choices for extrapolation models for both TCDD and total TEQ. Dose-response modeling also provides a perspective on the relationship between the level at which effects are seen in experimental systems or epidemiologic studies and background exposures and body burdens for dioxin and related compounds.

In evaluating the dose-response relationships for TCDD as a basis for assessing this family of compounds, both empirical dose-response modeling approaches and mode of action based approaches have been developed and applied (see Part II, Chapter 8, Section 8.3 and 8.4; Portier et al., 1996; Kim et al., 2003). Empirical models have advantages and disadvantages relative to more ambitious mechanism-based models. Empirical models provide a simple mathematical model that adequately describes the pattern of response for a particular data set; they can also provide the means for hypothesis testing and interpolation between data points. In addition, they can provide qualitative insights into underlying mechanisms. However, the major disadvantage of empirical models is their inability to quantitatively link data sets in a mechanistically meaningful manner. On the other hand, mechanism-based modeling can be a powerful tool for understanding and combining information on complex biological systems. Use of a truly mechanism-based approach can, in theory, enable more reliable and scientifically sound extrapolations to lower doses and between species. However, any scientific uncertainty about the mechanisms that the models describe is inevitably reflected in uncertainty about the predictions of the models.

PBPK models have been validated in the observable response range for numerous compounds in both animals and humans. The development of PBPK models for disposition of

TCDD in animals has proceeded through multiple levels of refinement, with newer models showing increasing levels of complexity by incorporating data for disposition of TCDD and its molecular actions with the AhR and other proteins, as well as numerous physiological parameters (Part II, Chapter 1). These models have provided insights into key determinants of TCDD disposition in treated animals. Development of such models continues and the current generation of dioxin PBPK models are being submitted for publication (DeVito et al., personal communication). Pharmacokinetic models have been extended to generate predictions for early biochemical consequences of tissue dosimetry of TCDD, such as induction of CYP1A1, and are being developed to address the impacts of enzyme induction (e.g., CYP1A2) on TCDD storage and half-life. It is anticipated that these enhanced PBPK models will improve the understanding of early phase human distributional and half-life kinetic data. However, extension of these models to more complex responses is more uncertain at this time, particularly regarding selection of the appropriate tissue metric to link to the effect(s) under consideration. Differences in interpretation of the mechanism of action embodied in these pharmacodynamic models lead to varying estimates of dose-dependent behavior for similar responses. The shape of the dose-response curves governing extrapolation to low doses are determined by these hypotheses and assumptions.

At this time, the knowledge of the mechanism of action of dioxin, receptor theory, and the available dose-response data do not firmly establish a scientific basis for replacing a linear procedure for estimating cancer potency. Consideration of this same information indicates that the use of different procedures to estimate the risk of exposure for cancer and noncancer endpoints may not be appropriate. Both the cancer and noncancer effects of dioxin appear to result from qualitatively similar modes of action. Initial steps in the process of toxicity are the same, and many early events appear to be shared. Thus, the inherent potential for low dose significance of either type of effect (cancer or noncancer) should be considered equal and evaluated accordingly. In the observable range around 1% excess response, the quantitative differences are relatively small. Below this response, the different mechanisms can diverge rapidly. The use of predicted biochemical responses as dose metrics for toxic responses is considered a potentially useful application of these models. However, greater understanding of the linkages between these biochemical effects and toxic responses is needed to reduce the potentially large uncertainty associated with these predictions.

5.1. DOSE METRIC(S)

One of the most difficult issues in risk assessment is determining the dose metric to use for animal-to-human extrapolations. An appropriate animal-to-human extrapolation of tissue dose is required to provide significant insight into differences in sensitivity among species. As noted in Section 1.3, the most appropriate dose metric should reflect both the magnitude and frequency of exposure, and it should be clearly related to the toxic endpoint of concern by a well-defined mechanism. However, this is often difficult, because human exposures with observable responses may be very different from highly controlled exposures in animal experiments. In addition, comparable exposures may be followed by very different pharmacokinetics (absorption, distribution, metabolism and/or elimination) in animals and humans. Finally, the sequelae of exposure in the form of a variety of responses related to age, organ, and species sensitivity complicate the choice of a common dose metric. Despite these complexities, relatively simple default approaches, including body surface or body weight scaling of daily exposures, have often been recommended (U.S. EPA, 1992a, 1996; ATSDR, 1999).

As discussed in Section 1.3, dose can be expressed in a number of ways. For TCDD and other dioxin-like compounds, attention has focused on the consideration of dose expressed as daily intake (ng/kg/day), body burden (ng/kg), or AUC (DeVito et al., 1995; Aylward et al., 1996). The concept of physiological time (lifetime of an animal) complicates the extrapolation, as the appropriate scaling factor is uncertain for toxic endpoints. Because body burden incorporates differences between species in TCDD half-life (these differences are large between rodent species and humans [see Part II, Chapter 8, Table 8.2]), this dose metric appears to be the most practical for many effects of this class of compounds (DeVito et al., 1995).

Average lifetime body burden is best suited for steady-state conditions, with difficulties arising when this dose metric is applied to the evaluation of acute exposures, such as those occurring in the 1976 accidental exposure in Seveso, Italy (Bertazzi and di Domenico, 1994). In cases such as this one, increased body burden associated with the acute exposure event is expected to decline (half-life for TCDD is approximately 7 years) until it begins to approach a steady-state level associated with the much smaller daily background intake. In general, daily excursions in human exposure are relatively small and have minor impact on average body burden. Instead, PBPK models suggest that human body burdens increase over time and begin to approach steady-state after approximately 25 years with typical background doses. Occupational exposures represent the middle ground where daily excursions during the working years can significantly exceed daily background intakes for a number of years, resulting in elevated body burdens.

The relationship between occupational exposures and body burden and between body burden and AUC are demonstrated in Figure 5-1. This figure graphs two hypothetical body burden scenarios during the 70-year lifespan of an individual. The first is a continuation to 70 years of age of the background body burden scenario discussed—with caveats and assumptions— in Part I, Volume 3, Chapter 5. In this scenario, an infant is breast-fed for 6 months by a mother who has a background dioxin body burden level and is subsequently exposed to the average current level of dioxin in the food supply (1 pg/kg/day). This background scenario leads to a 70 year lifetime area under the curve (AUC) of 184 ng/kg*Y, equivalent to a lifetime average body burden (LABB) of 2.6 ng/kg (~184/70 years).

In the second scenario, the same individual incurs an additional occupational exposure between 20 and 30 years of age of 100 pg/kg/day—100 times background—which then ceases. The buildup of dioxin body burden is evident in the peak level and shark fin appearance. AUC in this occupational scenario is 3911 ng/kg*Y, and LABB is 55.9 ng/kg. Note that in the occupational scenario the AUC and LABB are only 21 times background.

Table 5-1 and Figure 5-2 summarize literature on average levels of dioxin TEQs in the background human population and peak levels in commonly cited epidemiological cohorts. Table 5-1 collates data on tissue lipid levels (ppt lipid adjusted) in populations, principally from serum, and tabulates either current levels for the background population or back-calculated peak levels for the exposed cohorts. Figure 5-2 graphs the estimated range and central tendency of the total TEQ_{DFP} body burden (ng/kg whole body), combining the range of measured 2,3,7,8-TCDD values with the estimate of the background non-2,3,7,8-TCDD TEQ level from the U.S. population in the late 1980s/early 1990s. TEQ levels are calculated for PCDD, PCDF, and PCBs, based on TEQ_{DFP}-WHO_{98} values, and assume a constant 25% body fat ratio when converting from serum lipid ppt to ng/kg body burden. Total TEQ values for the Hamburg cohort women were calculated by the authors, but did not include a dioxin-like PCB contribution. Seveso values reported by Needham et al. (1999) are based on stored serum samples from subjects undergoing medical examinations contemporaneous with the exposure and were not back-calculated. Additional information consistent with Figure 5-2 has recently been published (Eskenazi et al., 2004) that demonstrate similar Seveso Zones A and B initial levels, with an important further measurement of background 2,3,7,8-TCDD (20.2 ppt serum lipid) and other congener TEQ contributions (80.2 ppt) in the unexposed background population (non-ABR women) in this time period.

As discussed earlier, using background total body burden (TEQ_{DFP}-WHO_{98}) as a point of comparison, these often-termed "highly exposed" populations have peak body burdens that are

relatively close to general population backgrounds at the time. When compared with background body burdens of the late 1980s, many of the median values and some of the mean values fall within a range of one order of magnitude (factor of 10) and all fall within a range of two orders of magnitude (factor of 100). General population backgrounds at the time are likely to have been higher than present background body burdens.

One uncertainty in comparing peak body burdens is the use of a first-order elimination rate with an overall half-life of 7.1 years. Recent evidence suggests that the elimination of TCDD may be dependent on the level of exposure, in addition to an early distributional or sequestration phase. Populations with high exposures may have half-lives significantly less than 7.1 years. Relatively rapid early elimination was noted in two highly exposed Austrian women (initial half-lives of ~1.5 and 2.9 years; Geusau et al., 2002). Supportive data are also available through an analysis of the Seveso populations (Michalek et al., 2002). In this analysis, a period of fast elimination within the first 0.27 years after the exposure in Seveso was observed, followed by a period of slower elimination between 3 and 16.35 years from exposure. The mean TCDD half-life in the first 0.27 years after exposure in the Seveso cohort was 0.34 years in males (n=6) and 0.43 years in females (n=10). From 3 years onward in the Seveso cohort, the half-life in males was 6.9 years (n=9) and 9.6 years in females (n=13). For Ranch Handers, the half-life was 7.5 years (n=97) between 9 and 33 years after exposure. This analysis indicates that dioxin body burdens and elimination kinetics may be more complex at higher doses than represented by a single first-order half-life, including issues of tissue distribution and dose-dependent elimination. This is consistent with the limited data available in rodents that also indicates a dose-dependent elimination.

There are a number of physiologically-based pharmacokinetic models of TCDD in both experimental animals and humans. Several of the rodent models assume that the elimination rate of TCDD is a constant (Wang et al., 1997; 2000; Emond et al., 2004). One model by Anderson et al. (1993) has a dose dependent doubling of the elimination rate which is dependent upon Ah receptor occupancy. Kohn et al. (1993; 1996) has the elimination rate increasing in proportion to body weight and includes an increased elimination of TCDD from the liver at high doses due to hepatocyte cell death. The Carrier et al. (1995a, b) model describes a dose-dependent elimination of TCDD and other dioxins due to a dose-dependent hepatic sequestration of these chemicals. While these models use different approaches, they all provide reasonable fits to the available experimental data.

Attempts to develop pharmacokinetic models for TCDD in humans have also resulted in a variety of mathematical descriptions of the elimination rate. Maruyama et al. (2002, 2003)

have assumed that the elimination rate is constant. Van der Molen et al. (1998; 2000) multiply a constant elimination rate by the ratio of liver fat/body fat. This results in an overall change in the elimination of TCDD based on body composition and body weight. Gentry et al. (2003) and Clewell et al. (2004) describe the elimination of TCDD in proportion to hepatic CYP1A2 expression. Aylward et al. (2004) modified the Carrier et al. (1995a, b) model to include an elimination of dioxins directly into the large intestine based on lipid partitioning. This model provided reasonable fits to data from Seveso patients as well as three Austrian patients. Finally, Michalek et al. (2002) used a classical pharmacokinetic approach to describe the Seveso data. This work suggests that there is an early distribution phase that results in a rapid loss of TCDD from the blood (half-life of 0.37 years) followed by a prolonged terminal elimination phase (half-life approximately 6.9 years).

Hence, there are a number of pharmacokinetic models available that describe the absorption, distribution and elimination of TCDD in animals and humans. While these models provide reasonable fits to the available data, they employ a wide range of descriptions of the elimination of TCDD. Some assume first order elimination, while others assume dose-dependent pharmacokinetics. Others suggest that body composition significantly influences the elimination of dioxins. Presently, it is difficult to determine which of these model structures provides the most accurate description of the pharmacokinetics of TCDD and other dioxins.

Advances in understanding the dose-dependency of the pharmacokinetics of TCDD and related chemicals will improve our ability to describe the relationship between exposure, dose and response. The development of more accurate models may affect both exposure group assignment in epidemiology studies and the calculation of dose-response curves, although the magnitude and direction of these postulated impacts remains to be quantified. Estimates of back-calculated doses are important because the ability to detect effects in epidemiologic studies is dependent on a sufficient difference between control and exposed populations. Using published first-order back-calculation procedures, the relatively small difference (< 10–100-fold) in body burden between exposed and controls in the dioxin epidemiology studies makes exposure characterization in the studies a particularly serious issue. This point also strengthens the importance of measured blood or tissue levels in the epidemiologic analyses, despite the uncertainties associated with calculations extending the distribution of measured values to the entire cohort and assumptions involved in back-calculations.

As a bounding exercise on the impact of half-lives on back-extrapolated exposure estimates, EPA has compared the impacts of varying half-life values on back-calculated peak and AUC results. This scenario is constructed by calculating the peak body burden 20 years prior to a

terminal level for various half-lives versus a 7.1 year fixed half-life, assuming first order kinetics ($C_t = C_0e^{-rt}$). A constant dosing regimen is then constructed to simulate an occupational exposure that would achieve these same peak body burdens following 10 years exposure, maintaining the same half-life as in the 20 year follow-up. For each half-life value, a different dose level is necessary and was mathematically derived to reach the required peak level after ten years occupational exposure.

In this occupational scenario, peak and AUC ratios ($AUC_{variable\ half\text{-}life}/AUC_{7.1years}$) varied in a non-linear manner depending on the input half-life. Half-life values of 4 years and longer had low, single digit numerical impacts on the peak and AUC ratios compared to the 7.1 year half-life results (e.g., at a 4 year half-life, the ratio for the peak value = 4.6, the AUC ratio = 3.8; at a 5 year half-life, the ratio for peak = 2.3, AUC = 2). At half-lives below 4 years, peak and AUC ratios rose dramatically to approximately 1 and 2 orders of magnitude for 3 and 2 year half-lives, respectively. The terminal body burden did not influence the ratio because the mathematical function remained constant. More complex PBPK models, where half-life varies with body burden, are under development and will be more influenced by the terminal body burden for each individual. This bounding exercise suggests that impacts on back-calculated peak and AUC values may become significant if the models predict prolonged periods with half-lives of less than 4 years.

5.1.1. Calculations of Effective Dose

Comparisons across multiple endpoints, multiple species, and multiple experimental protocols are too complicated to be made on the basis of the full dose-response curve. As discussed above, comparisons of this sort can be made by either choosing a given exposure and comparing the responses or choosing a particular response level and comparing the associated exposures. In the analyses contained in Chapter 8, Section 8.3, and elsewhere in the reassessment, emphasis is placed on comparing responses using estimated exposures associated with a given level of excess response or risk. To avoid large extrapolations, this common level of excess risk was chosen such that for most studies the estimated exposure is in or near the range of the exposures seen in the studies being compared, with extra weight given to the human data. A common metric for comparison is the effective dose, which is the dose resulting in an excess response over background in the studied population. This excess response rate can be calculated as a fraction of the minimum to maximum response (e.g., 1% increase in risk). Alternatively, for continuous data the dose can be calculated as the amount necessary to move an additional percentage of distribution of the response past a predetermined "effect" level. EPA

has suggested this approach in calculating BMDs (Allen et al., 1994) and in its proposed approaches to quantifying cancer risk (U.S. EPA, 1996, 1999, 2003).

Although effective dose evaluation at the 10% response level (ED_{10} or lower bound on ED_{10} [LED_{10}]) is somewhat the norm, given the power of most chronic toxicology studies to detect an effect, this level is actually higher than those typically observed in the exposed groups in studies of TCDD impacts on humans. To illustrate, lung cancer mortality has a background lifetime risk of approximately 4% (smokers and nonsmokers combined), so that even a relative risk of 2.0 (two times the background lifetime risk) represents approximately a 4%, or 4 in 100, increased lifetime risk (see Chapter 8 for a comprehensive elaboration of formulae). On the basis of this observation, and recognizing that many of the TCDD-induced endpoints studied in the laboratory include 1% effect levels in the experimental range, Chapter 8 presents effective doses of 1%, or $ED_{01,}$ and 10%, or ED_{10}, values.

The use of effective dose values below 10% is consistent with the Agency's guidance on the use of mode of action in assessing risk, as described in the proposed carcinogen risk assessment guidelines (U.S. EPA, 1996, 1999, 2003) and in the evaluation framework discussed in Section 3.3, in that the observed range for many "key events" for TCDD extends down to or near the 1% response level. Determining the dose at which key events for dioxin toxicity begin to be seen in a heterogeneous human population provides important information for decisions regarding risk and safety.

5.2. EMPIRICAL MODELING OF INDIVIDUAL DATA SETS

As described in Chapter 8, Section 8.3, empirical models have advantages and disadvantages relative to more ambitious mechanism-based models. Empirical models provide a simple mathematical model that adequately describes the pattern of response for a particular data set and that can also provide the means for hypothesis testing and interpolation between data points. In addition, they can provide qualitative insights into underlying mechanisms. However, the major disadvantage is their inability to quantitatively link data sets in a mechanistically meaningful manner.

Data available for a number of biochemical and toxicological effects of TCDD and for its mechanism of action indicate that there is good qualitative concordance between responses in laboratory animals and humans (see Table 2-1). In addition, as described below, human data on exposure and cancer response appear to be qualitatively consistent with animal-based risk estimates derived from carcinogenicity bioassays. These and other data presented throughout this reassessment would suggest that animal models are generally an appropriate basis for estimating

human responses to dioxin-like compounds. Nevertheless, there are clearly differences in exposures and responses between animals and humans, and recognition of these is essential when using animal data to estimate human risk. The level of confidence in any prediction of human risk depends on the degree to which the prediction is based on an accurate description of these interspecies extrapolation factors. See Chapter 8, Section 8.3, for a further discussion of this point.

Almost all dioxin research data are consistent with the hypothesis that the binding of TCDD to the AhR is the first step in a series of biochemical, cellular, and tissue changes that ultimately lead to toxic responses observed in both experimental animals and humans (see Part II, Chapter 2, Section 2.3). Therefore, an analysis of dose-response data and models should use, whenever possible, information on the quantitative relationships among ligand (i.e., TCDD) concentration, receptor occupancy, and biological response. However, it is clear that multiple dose-response relationships are possible when considering ligand receptor-mediated events. For example, dose-response relationships for relatively simple responses, such as enzyme induction, may not accurately predict dose-response relationships for complex responses such as developmental effects and cancer.

Cell- or tissue-specific factors may determine the quantitative relationship between receptor occupancy and the ultimate response. Indeed, for TCDD there are much experimental data from studies using animal and human tissues to indicate that this is the case. This serves as a note of caution, as empirical data on TCDD are interpreted in the broader context of complex exposures to mixtures of dioxin-like compounds as well as to nondioxin-like toxicants.

As for other chemical mechanisms where high biological potency is directed through the specific and high-affinity interaction between chemical and critical cellular target, the supposition of a response threshold for receptor-mediated effects is a subject for scientific debate. The basis of this controversy has been summarized by Sewall and Lucier (1995).

Based on classic receptor theory, the occupancy assumption states that the magnitude of biological response is proportional to the occupancy of receptors by drug molecules. The "typical" dose-response curve for such a receptor-mediated response is sigmoidal when plotted on a semilog graph or hyperbolic if plotted on an arithmetic plot. Implicit in this relationship is low-dose linearity (0–10% fractional response) through the origin. Although the law of mass action predicts that a single molecule of ligand can interact with a receptor, thereby inducing a response, it is also widely held that there must be some dose that is so low that receptor occupancy is trivial and, thus, no perceptible response is obtainable.

Therefore, the same receptor occupancy assumption of the classic receptor theory is interpreted by different parties as support for and against the existence of a threshold. It has been stated that the occupancy assumption cannot be accepted or rejected on experimental or theoretical grounds (Goldstein et al., 1974). To determine the relevance of receptor interaction for TCDD-mediated responses, one must consider (1) alternatives as well as limitations of the occupancy theory, (2) molecular factors contributing to measured endpoints, (3) limitations of experimental methods, (4) contribution of measured effect to a relevant biological/toxic endpoint, and (5) background exposure.

Throughout this reassessment, each of these considerations has been explored within the current context of the understanding of the mechanism of action of TCDD, of the methods for analysis of dose-response for cancer and noncancer endpoints, and of the available data sets of TCDD dose and effect for several rodent species, as well as humans who were occupationally exposed to TCDD at levels exceeding the exposure of the general population.

5.2.1. Cancer

As discussed in Section 2.2.1.4, TCDD is characterized as carcinogenic to humans when using a weight-of-evidence approach, and is a carcinogen in all species and strains of laboratory animals tested. The epidemiological database for TCDD, described in detail in Part II, Chapter 7a, suggests that exposure may be associated with increases in all cancers combined and respiratory cancer and with the possibility of elevated risks at other sites. Although there are sufficient data in animal cancer studies to model dose-response for a number of tumor sites, as with many chemicals it is generally difficult to find human data with sufficient information to model dose-response relationships. For TCDD, three studies of human occupational exposure have sufficient information to perform a quantitative dose-response analysis: Becher et al. (1998) (the Hamburg cohort); Ott and Zober (1996) (the BASF cohort); and Steenland et al. (2001) (the NIOSH cohort).

The all-cancer mortality ED_{01}/LED_{01} results from these three studies are detailed in Part II, Chapter 8, Section 8.3, and tabulated and graphed in Table 5-2, along with the bioassay results for liver cancer in female Sprague-Dawley rats (Kociba et al., 1978). Table 5-2 includes only the results and mathematical formulae that were published by the primary authors in the peer-reviewed literature. These calculations and formulae were chosen because they are based on the full primary data set and not on secondary analyses using summary results. In order to graph results for the occupational cohort studies, the central points for data ranges were requested from,

and kindly provided by, the authors (Drs. Steenland, Zober and Becher) and are included in the table.

Slightly different approaches are used for modeling cancer in humans than are used for modeling in animal studies. The modeling approach used in the analysis of the human epidemiology data for all cancers combined and lung cancer involves applying the estimated human body burden-to-cancer response and estimating parameters in a mathematical risk model for each data set. For the three occupational cohort studies, exposure subgroups were defined by the authors using measured and then back-extrapolated TCDD levels in a subset of workers to inform exposure calculations for the remainder of the cohort. None of the studies sampled TCDD blood serum levels for more than a fraction of its cohort, and these samples were generally taken decades after the last known exposure. In each study, serum fat or body fat levels of TCDD were back-calculated using a first-order model. The assumed half-life of TCDD used in the model varied from study to study.

Steenland et al. and Becher et al. used the measured and back-extrapolated TCDD concentrations to refine and quantitate job exposure matrices, which were then used to estimate dioxin cumulative dose for each member of their entire cohort. Ott and Zober (1996a) used regression procedures with data on time spent at various occupational tasks to estimate TCDD levels for all members of the cohort. The cohorts were then divided into exposure groups on the basis of the estimated TCDD levels. As noted, central measures of the ranges from the primary data were provided to the Agency by the authors, removing the need to estimate this parameter from the upper and lower range points in the literature.

Risk outcomes in these cohorts were expressed as standardized mortality ratios (SMRs) or rate ratios. SMRs are calculated by comparing the cancer rates in the subcohorts to the age- and gender-matched general community in that time period. SMR results are usually expressed as a ratio, with SMR = 100 set as the community, or expected, cancer death rate. Rate ratios are calculated from within cohort data using the lowest exposed group as the control value for both dose and risk. Although the lowest exposed group is defined to have a risk equal to unity (rate ratio = 1), this low group may not, in fact, have an SMR equal to the general community (it could be either lower or higher).

The three occupational cohort studies provide best fit dose-response models within the range of their data. These models and the resulting formulae allow for the calculation of ED_{01}/LED_{01} values, from which a linear extrapolation can be performed, consistent with the EPA's draft cancer guidelines. There are several assumptions and uncertainties involved in modeling these data, including extrapolation of dosage (both in back-calculation and in

elimination kinetics), the type of extrapolation model employed, and whether the origin point should be fixed (i.e., SMR = 100) or allowed to float.

Based on the model formulae using the full data set as provided in the primary literature (Steenland et al., 2001; Ott and Zober, 1996; Becher et al., 1998; detailed in Chapter 8), the calculated ED_{01} central estimates for all cancers combined range from 1.4 to 62 ng TCDD/kg LABB (Table 5-3). The lower bounds on these doses (based on a modeled 95% CI) range from 0.71 ng TCDD/kg to 30.5 ng TCDD/kg (not available for models published by Becher et al., 1998, due to the absence of statistical parameter measures). A parallel measure of unit excess risk per one part per trillion TCDD body burden above background (assumed 5 ppt) is also tabulated. These values are strongly dependent on the study chosen and the model used, and it must be recognized that the risks posed to some members of the population from TCDD may be zero, depending on the model chosen to extrapolate results below the range of observation. Male and female values do not match because of differences in the input variable of background lifetime all-cancer mortality risk.

Analysis of model results indicates that the power model applied to the Steenland et al. (2001) data leads to unreasonably high risks at low exposure levels, based on calculations of the attributable risk that this model would predict from background dioxin levels in the general population. This result is due to the very steep slope of this power curve at low environmental levels. The steep dose-response curve also makes the power model very sensitive to the background dose that is incorporated into the calculations and the location of the calculation point on the dose-response curve. Exclusion of the Steenland et al. power model reduces the ED_{01} range to 6–62 ng TCDD/kg LABB and the LED_{01} range to 11.5–31 ng TCDD/kg LABB (lower confidence values were unavailable for the Becher et al. 1998 data). For the purposes of this assessment, the piecewise linear formula published by Steenland et al. (2001) is the preferred model from this data set.

These epidemiologically derived ED_{01} values are summarized in Table 5-4 (additional details in Part II, Chapter 8), along with the resulting cancer slope factors. The results of the Kociba et al. (1978) cancer bioassay are also included in Table 5-4 for comparison purposes, using the Goodman and Sauer (1992) revision to the liver tumor pathology results. Dose-response modeling for this bioassay used the EPA Benchmark Dose software and multistage model to calculate the ED_{01}/LED_{01}. The similarity between the cancer bioassay ED_{01} results in rodents (Kociba et al. 1978) and the human epidemiology results is noteworthy when the exposure metric is based on lifetime average body burden (LABB). LABB is calculated as the AUC divided by lifetime years, and it equilibrates tissue doses across species.

The epidemiological data and dose-response models have stimulated considerable contemporary interest and statistical analysis, particularly the option of performing a pooled or meta-analysis on the entire occupational cohort data set. In reviewing this literature, care should be taken to note which published analyses form the basis for the statistical tests, the recent provision of data-derived central dose estimates for the ranges given in the literature (courtesy of the primary authors), and the availability of more detailed primary dose-response literature (Steenland et al., 2001; Becher et al., 1998), which supercede studies used previously (Aylward et al., 1996; Flesch-Janys et al., 1998). For instance, the dose-response pattern for the NIOSH cohort summary data, as published by Aylward et al. (1996), demonstrates a different high dose point from the more recent and detailed analysis of the full dataset, as published by Steenland et al. (2001).

Starr (2001, 2003) reviewed meta-analysis data and results that were included in the external review draft of the EPA dioxin reassessment, and the analysis performed by Crump et al. (2003; see below). The draft EPA meta-analysis was based on summary results published by Aylward et al. (1996; NIOSH), Ott and Zober (1996; BASF), and Flesch-Janys et al. (1998). Exposure range midpoints were either obtained from the original publication (Aylward et al., 1996) or were based on a log-normal fit to the data ranges to estimate the midpoint (for Ott and Zober, 1996; Flesch-Janys et al., 1998). On the basis of these earlier data sets and the application of a linear model, Starr concluded that the assumption of a fixed origin at an SMR = 100 should be rejected on statistical grounds. Although a significantly increased cancer risk was evident in these cohorts, the overall results using an unconstrained linear model (not fixed to the SMR = 100 point) were concluded to be consistent with the null hypothesis of no dose-response relationship between TCDD and the cancer rate.

In a subsequent dioxin meta-analysis performed as part of the Joint European Commission on Food Additives, Crump et al. (2003) performed similar and expanded statistical analyses on a more recent data set using data-derived central estimates of exposure levels for Ott and Zober (1996; Hamburg cohort) and from Steenland et al. (2001; NIOSH cohort). Fitting a linear model to the data again indicated that the baseline SMR = 100 assumption could be rejected, based on statistical tests.

Goodness of fit trend tests for this linear model were statistically significant both with the background SMR set equal to 100 and with the background SMR estimated (p=0.01). A further series of trend tests were performed by successively removing the highest cumulative exposure to determine the lowest exposure for which there remained statistically significant evidence for an effect. This progressive analysis of the data was considered by Crump et al. to provide a more

robust test for trends than a linear goodness of fit test. The analysis demonstrated an increase in total cancer at cumulative TEQ serum levels that would result from a lifetime average intake of 7 pg TEQ/kg body weight/day (assuming 50% uptake, $t_{1/2}$ 7.6 years, 25% body fat), with no trend for increase at 6 pg/kg/day.

The pooled analysis of the Ott and Zober (1996), Flesch-Janys et al. (1998), and Steenland et al. (2001) data yielded ED_{01} estimates of 51 ng/kg body burden (baseline SMR fixed at 100) and 91 ng/kg body burden (baseline SMR estimated), corresponding to ED_{01} daily intake estimates of 25 and 45 (95% CI = 21–324) pg/kg/day, respectively, above current background TCDD-TEQ for all cancers combined (calculated using the half-life and absorption assumptions in Crump et al.). These results are consistent with the range of ED_{01}s in Part II, Chapter 8, and Tables 5-3 and 5-4. On the basis of their results and comparison to other published analyses, Crump et al. (2003) concluded that they could not see a clear choice between their ED_{01} estimate of 45 pg/kg/day and the Steenland et al. (2001) estimate of 7.7 pg/kg/day, citing advantages to each study.

The choice of model is central to the above statistical analyses of the individual studies and the meta-analysis. The epidemiological data are not sufficient to mandate the selection of any particular model shape. The published literature includes power, linear, piecewise linear, and multiplicative models (see Table 5-2). The EPA's draft carcinogen risk assessment guidelines (U.S. EPA, 1999) propose applying a standard curve-fitting procedure within the range of the data (e.g., Benchmark Dose software), recognizing that more elaborate models will be appropriate for more complex information and that, ultimately, biologically based pharmacokinetic models would be preferred.

The curve-fitting procedure is used to determine a POD, generally at the 10% response level, but where more sensitive data are available, a lower point for linear extrapolation can be used to improve the assessment (e.g., 1% response for dioxin, ED_{01}). Extrapolation from the POD to lower doses is conducted using a straight line drawn from the POD to the origin—zero incremental dose, zero incremental response—to give a probability of extra risk. The linear default is selected on the basis of the agent's mode of action when the linear model cannot be rejected and there is insufficient evidence to support an assumption of nonlinearity. Additional important uncertainties in the human epidemiological data are discussed in Part II, Chapter 8, Section 8.3, and include the representativeness and precision of the dose estimates that were used, the choice of half-life and whether it is dose dependent, and potential interactions between TCDD and smoking or other toxicants.

For the animal data, both empirical and mechanistic models have been applied to examine cancer dose-response. Portier et al. (1984) used a simple multistage model of carcinogenesis with up to two mutation stages affected by exposure to model the five tumor types observed to be increased in the 2-year feed study by Kociba et al. (1978) (Sprague-Dawley rats) and the eight tumor types observed to be increased in the 2-year gavage cancer study conducted by NTP (1982a) (Osborne-Mendel rats and $B6C3F_1$ mice). The findings from this analysis, which examined cancer dose-response within the range of observation, are presented in Part II, Chapter 8, Table 8.3., which is reproduced with slight modifications as Table 5-5. All but one of the estimated ED_{01}s are above the lowest dose used in the experiment (approximately 1 ng TCDD/kg/day in both studies) and are thus interpolations rather than extrapolations. The exception, liver cancer in female rats from the Kociba study, is very near the lowest dose used in this study and is only a small extrapolation (from 1 ng TCDD/kg/day to 0.77 ng TCDD/kg/day). Steady-state body burden calculations were also used to derive doses for comparison across species. Absorption was assumed to be 50% for the Kociba et al. (feed experiment) and 100% for the NTP study (gavage experiment).

The shapes of the dose-response curves as determined by Portier et al. (1984) are also presented in Table 5-5. The predominant shape of the dose-response curve in the experimental region for these animal cancer results is linear. This does not imply that a nonlinear model such as the quadratic or cubic—or for that matter a "J-shaped" model—would not fit these data. In fact, it is unlikely that in any one case a linear model or a quadratic model could be rejected statistically. These studies had only three experimental dose groups; hence, these shape calculations are not based on sufficient doses to guarantee a consistent estimate, and they should be viewed with caution.

The ED_{01} steady-state body burdens range from a low value of 14 ng/kg, based on the linear model associated with liver tumors in female rats, to as high as 1190 ng/kg, based on a cubic model associated with thyroid follicular cell adenomas in female rats. Lower bounds on the steady-state body burdens in the animals range from 10 ng TCDD/kg to 224 ng/kg. The corresponding estimates of daily intake level at the ED_{01} obtained from an empirical linear model range from 0.77 to 43 ng TCDD/kg body weight/day, depending on the tumor site, species, and sex of the animals investigated. Lower confidence bounds on the estimates of daily intake level at the ED_{01} in the animals range from 0.57 to 14 ng TCDD/kg body weight/day.

In addition, using a mechanistic approach to modeling, Portier and Kohn (1996) combined the biochemical response model by Kohn et al. (1993) with a single initiated-phenotype two-stage model of carcinogenesis to estimate liver tumor incidence in female

Sprague-Dawley rats from the 2-year cancer bioassay by Kociba et al. (1978). By way of comparison, the ED_{01} estimate obtained from this linear mechanistic model was 0.15 ng TCDD/kg body weight/day, based on intake, which is equivalent to 2.7 ng TCDD/kg steady-state body burden. No lower bound on this modeled estimate of steady-state body burden was provided.

As discussed in Part II, Chapter 8, Section 8.2, the use of different dose metrics can lead to widely diverse conclusions. For example, the ED_{01} intake for the animal tumor sites presented above ranges from less than 1 to tens of ng/kg/day, and the lowest dose with an increased tumorigenic response (thyroid tumors) in a rat is 1.4 ng TCDD/kg/day (NTP, 1982a). The daily intake of dioxins in humans is estimated at approximately 1 pg TEQ/kg/day. This implies that humans are exposed to doses 1400 times lower than the lowest tumorigenic daily dose in rat thyroid. However, 1.4 ng TCDD/kg/d in the rat leads to a steady-state body burden of approximately 25 ng TCDD/kg, assuming a half-life of TCDD of 25 days and absorption from feed of 50%[2]. If the body burden of dioxins in humans is approximately 20 ng TEQ/kg lipid, or 5 ng TEQ/kg body weight (assuming about 25% of body weight is lipid), "average" humans are exposed to about five times less TCDD than the minimal carcinogenic dose for the rat. The difference between these two estimates is entirely due to the approximately 100-fold difference in the half-life of TCDD in humans and rats. At least for this comparison, if cancer is a function of average levels in the body, the most appropriate metric for comparison is the average or steady-state body burden, because this accounts for the large differences in animal and human half-lives.

Comparisons of human and animal ED_{01}s from Part II, Chapter 8, Section 8.3, for cancer response on a body burden basis show similar potential for the carcinogenic effects of TCDD. In humans, cancer ED_{01}s ranged from approximately 6 ng/kg to 62 ng/kg (excluding the Steenland et al., 2001, power model). This is similar to the empirical modeling estimates from the animal studies, which ranged from 14 ng/kg to 1190 ng/kg (most estimates were in the range of 14 to 500 ng/kg). The lower bounds on the human body burdens at the ED_{01}s (based on a modeled 95% CI) ranged from 11.5 ng TCDD/kg to 31 ng TCDD/kg (again, the lower values that would have resulted from the Becher et al., 1998, analysis could not be included because error bounds on the models were unavailable). Lower bounds on the steady-state body burdens in the animals ranged from 10 ng TCDD/kg to 224 ng/kg. The estimate for the single mechanism-based model presented earlier (2.7 ng/kg) is below the lower end of the human ED_{01} estimates.

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[2]Steady-state body burden (ng/kg) = (daily dose (ng/kg/day) * (half-life)/Ln(2)) (f), where f is the fraction absorbed from the exposure route (unitless) and half-life is the half-life in days.

Using human and animal cancer ED_{01}s, their lower bound estimates, and the value of 2.7 ng TCDD/kg from the single mechanism-based model, slope factors and comparable risk estimates for a human background body burden of approximately 5 ng TEQ/kg (20 ng TEQ/kg lipid) can be calculated using the following equations:

Slope factor (per pg TEQ/kgBW/day) = risk at ED_{01} / intake (pg TEQ/kgBW/day) associated with human equivalent steady-state body burden at $ED_{01,}$ where:

Risk at ED_{01} = 0.01; and

$$\text{Intake (pg TEQ/kg BW/day)} = \left[\frac{\text{body burden at } ED_{01} \text{ (ng TEQ/kg)} * \text{Ln(2)}}{\text{half-life (days)} \times f}\right] * 1000 \text{ (pg/ng)} \quad (5\text{-}1)$$

half-life = 2593 days in humans and 25 days in rats (see Table 8.1 in Part II, Chapter 8)

f = fraction of dose absorbed; assumed to be 0.8 (80%)

and

Upper bound on excess risk at human background body burden = (human background body burden (ng/kg))(risk at ED_{01})/lower bound on human equivalent steady-state body burden (ng/kg) at ED_{01}, where: (5-2)

Risk at ED_{01} = 0.01

Use of these approaches reflects methodologies being developed within the context of the revised draft carcinogen risk assessment guidelines (U.S. EPA, 1999, 2003). Under these draft guidelines (EPA, 2003, section 5.4), risk estimates may be based on linear extrapolation or nonlinear hazard quotients, depending on the mode of action, accompanied by a statement on the extent of extrapolation generally expressed as the margin of exposure (MOE = POD/exposure). The formulae used in this quantitative linear analysis for dioxin are approximate for a number of the cancer slope factors derived from human data in Table 5.4 because of the calculation of risk for 1pg TCDD/kg body weight/day above background, the use of lifetable analysis to derive the expected cancer rates, and the changing gradient of the dose-response curves as body burden increases, especially for the power formulae. As discussed below, these methods can be compared to previous approaches using the linearized multistage (LMS) procedure to determine whether the chosen approach has significantly changed the estimation of slope. The estimates of ED_{01}/LED_{01} represent the human-equivalent body burden for 1% excess cancer risk based on exposure to TCDD and are assumed for purposes of this analysis to be equal for TCDD

equivalents (total TEQ). This assumption is based on the toxic equivalency concept discussed throughout this report and in detail in Part II, Chapter 9. All cancer slope factors can be compared to the Agency's previous slope factor of 1.6×10^{-4} per pg TCDD/kg body weight/day, which is equivalent to 1.6×10^{5} per mg TCDD/kg body weight/day (U.S. EPA, 1985).

5.2.1.1. *Estimates of Slope Factors and Risk at Current Background Body Burdens Based on Human Data*

Traditionally, EPA has relied on central estimates of risk from epidemiological studies rather than on upper bound estimates, which can exhibit substantial statistical spread in these results. This practice developed because epidemiological data were most often from high-end occupational exposures—as with the principal dioxin literature—where the data were likely to provide upper estimates of cancer slope and where all excess cancer increases were attributed to the single exposure of interest, amidst a variety of other potential carcinogenic exposures. For the analyses conducted herein, the Agency has presented both central (e.g., ED_{01}) and upper bound (e.g., LED_{01}) estimates where these are available.

The estimates of slope factors (risk per pg TCDD/kg body weight/day) calculated from the human ED_{01}s presented in Part II, Chapter 8, Table 8.3.1, range from 5.1 x 10^{-3} if the ED_{01} for all cancer deaths in the Hamburg cohort is used to 0.57×10^{-3} if the ED_{01} for all cancer deaths in the smaller BASF cohort is used. All of the other slope factors for all cancer deaths in the three cohorts fall within this range (Table 5-4). The meta-analysis by Crump et al. (2003) leads to similar results, with the reported ED_{01} of 46 ng/kg (95% lower bound = 31 ng/kg) BB, resulting in a cancer slope factor of 0.65 (95% upper confidence limit = 0.97) x 10^{-3} risk per pg TCDD/kgBW/day (adopting the EPA assumptions of baseline SMR = 100, halflife = 7.1 years, 80% absorption; alternatively, adopting a floating SMR results in a CSF = 0.37 (0.69) x 10^{-3}).

There is no compelling reason to choose one slope factor over the next from among those calculated, given that each study had particular strengths and weaknesses (See Part II, Chapter 7a). The results cluster around a cancer slope factor of 10^{-3} risk/pgTCDD/kg body weight/day above background, which represents EPA's most current upper bound slope factor for estimating human cancer risk based on human data. By inference, this risk value could also apply to total TEQ intake. As described in Section 4.4.2, current intakes in the United States are approximately 1 pg TEQ_{DFP}-WHO_{98}/kg body weight/day, and body burdens are approximately 5 ng TEQ_{DFP}-WHO_{98}/kg body weight (which equates to a serum level of approximately 20 pg/g lipid). Uncertainties associated with these estimates from human studies are discussed in Part II, Chapter 8, Section 8.3, and in Becher et al. (1998).

These estimates compare well with the published estimates of cancer slope and risk for the Hamburg and NIOSH cohorts by Becher et al. (1998) and Steenland et al. (2001), respectively. The risk estimates by Becher et al. were derived from data on TCDD exposure to male workers with a 0 or 10-year latency. These estimates range from 1.3×10^{-3} to 5.6×10^{-3} per pg TCDD/kg body weight/day, and were calculated using German background cancer rates. The fraction of dioxin assumed absorbed is not stated by Becher et al. but, presumably, if the absorption fraction was set at 100%, this would contribute to the slight differences to the EPA values in Table 5.5. The Steenland et al. calculations were performed for either no lag or a 15-year lag. The authors calculated a lifetime all cancer excess risk above background of between 5 x 10^{-4} (piecewise linear) to 9.4 x 10^{-3} (power model) per pgTCDD/kg/day. The Steenland et al. results are lower than those presented in Table 5-4 because the authors assumed 50% absorption and a lower additional dose (i.e., incorporating a two-fold doubling of dose over background into the Steenland et al. results reproduces their calculations).

In both analyses, all excess cancers are attributed to TCDD exposure, despite significant levels of other dioxin-like compounds in blood measurements. Notable, though, is the Becher et al. determination of a very similar slope coefficient for total TEQ and TCDD, based on their measured data, which is consistent with the TEF methodology. The results from Steenland et al. are more consistent with a reduced cancer slope factor when based on TEQ. Although risk estimates using TCDD alone in these cohorts might suggest an overestimate of risk because dose is underestimated, no evidence for this has emerged from the analysis because TCDD dominates the total TEQ in these occupational cohorts.

5.2.1.2. *Estimates of Slope Factors and Risk at Current Background Body Burdens Based on Animal Data*

Upper bound slope factors (per pg TCDD/kg body weight/day) for human cancer risk calculated from lower bounds on ED_{01}s (LED_{01}s) for the animal cancers presented in Table 5-5 range from 3×10^{-3} to 0.1×10^{-3}, that is, from 19 times greater than the previous upper bound estimate on cancer slope (1.6×10^{-4} [U.S. EPA, 1985]) to less than 50% of this value. The highest slope factor is derived from the same study as the 1985 estimate; that is, the slope factor derived from the female liver cancer in the Kociba et al. (1978) study continues to give the highest slope factor.

5.2.1.2.1. ***Reconciling the Portier (1984) and EPA (1985) slope estimates.*** In attempting these comparisons, two issues became apparent. First, the body burden and the intake at the ED_{01} from Portier et al. (1984) does not result in the same slope factor as EPA's (U.S. EPA, 1985). Despite the use of the same study results, a slope factor of 1.8×10^{-5} per pg TCDD/kg body weight/day results when using the LMS approach in Portier et al. (1984), which is approximately a factor of 10 lower than EPA's estimate of the slope (U.S. EPA, 1985). The differences are attributable to the aims of the respective calculations at the time. Portier et al. calculated "virtually safe doses" assuming that rodent and human doses scaled on a mg/kg basis, and they used the original tumor counts from the study. EPA, on the other hand, used $(\text{body weight})^{2/3}$ to arrive at a human equivalent dose and the pathology results from a reread of the original Kociba study (U.S. EPA, 1980). In addition, EPA adjusted tumor counts for early mortality in the study. The factor to adjust for $(\text{body weight})^{2/3}$ scaling in the rat is 5.8. The correction for early mortality can be accounted for with a factor of 1.6 (this is the ratio of the intake values at the ED_{01} with and without the early mortality correction). If the Portier et al. slope factor (1.8×10^{-5} per pg TCDD/kg body weight/day) is multiplied by these two factors, a slope of 1.7×10^{-4} per pg TCDD/kg body weight/day is calculated. This is essentially equivalent to the EPA estimate of 1.6×10^{-4} per pg TCDD/kg body weight/day. Reconciling these issues is important to ensuring appropriate comparisons of slope factor estimates.

5.2.1.2.2. ***Calculating a revised estimate of cancer slope from Kociba et al. (1978)***. Of greater consideration is the calculation of slope factor estimates using current methods of analysis that recognize the importance of the dose metric and the differences in half-life of dioxins in the bodies of laboratory animals and humans (see Part II, Chapter 8, Section 8.2, for detailed discussion). The major difference between the approaches used to calculate risks in the mid-1980s (Portier et al., 1984; U.S. EPA, 1985) and the current approach is the use of body burden as the dose metric for animal-to-human dose equivalence. The decision to use body burden accounts for the approximately 100-fold difference between half-lives of TCDD in humans and rats (2593 days vs. 25 days [see Part II, Chapter 8, Table 8.1]).

The use of equation 5-1 results in an estimated body burden at the LED_{01} of 6.1 ng TEQ/kg, derived from the EPA (U.S. EPA, 1985) Kociba et al. tumor counts. This compares favorably with the Portier estimate of 10 ng TEQ/kg found in Table 5-5. The difference is entirely accounted for by the early deaths adjustment by EPA. Use of these body burdens at the LED_{01} results in slope factor estimates of 3.3×10^{-3} per pg TCDD/kg body weight/day and 4.9×10^{-3} per pg TCDD/kg body weight/day for the Portier at al. (1984) (10 ng/kg) and the newly

derived body burden (6.1 ng/kg), respectively. Again, the difference is due solely to the adjustment for early mortality, which EPA considers a better estimate of upper bound lifetime risk than the unadjusted estimate. EPA's revised slope factor (4.9×10^{-3} per pg TCDD/kg body weight/day) would be 31 times greater than the slope factor from 1985.

However, a second issue with the modeling of the Kociba et al. data relates to the use of the appropriate tumor counts. As mentioned in Section 2.2, Goodman and Sauer (1992) reported a second re-evaluation of the female rat liver tumors in the Kociba et al. study using the latest pathology criteria for such lesions. Results of this review are discussed in more detail in Part II, Chapter 6, Section 6.2. The review confirmed only approximately one-third of the tumors seen in the previous review (U.S. EPA, 1980). Although this finding did not change the determination of carcinogenic hazard, because TCDD induced tumors in multiple sites in this study, it does have an effect on evaluation of dose-response and on estimates of risk. Because neither the original EPA slope factor estimate (U.S. EPA, 1985) nor that of Portier et al. (1984) reflect this reread, it is important to factor these results into the estimate of the ED_{01} and slope factor.

Using the LMS procedure used by EPA in 1985 and the tumor counts as reported in Part II, Chapter 6, Table 6.2, the revised slope factor is reduced by approximately 3.6-fold to yield a slope factor of 4.4×10^{-5} per pg TCDD/kg body weight/day. However, because the original estimates used a $(\text{body weight})^{2/3}$ scaling, an adjustment must also be made to remove this interspecies scaling factor in order to obtain a correct result when comparing with body burden as the interspecies metric. When dose is adjusted and equation 5-1 is used, an LED_{01} of 22.2 ng TEQ/kg and a slope factor of 1.4×10^{-3} per pg TCDD/kg body weight/day are derived. This represents EPA's most current upper bound slope factor for estimating human cancer risk based on animal data. It is 8.7 times larger than the slope factor calculated in U.S. EPA (1985). This number reflects the increase in slope factor based on the use of the body burden dose metric (31 times greater) and the Goodman and Sauer (1992) pathology (3.6 times less). These results can also be obtained using EPA's Benchmark Dose software and entering adjusted tumor counts and dose data to obtain a $BMDL_{01}$ from which an LED_{01} body burden of 22 ng/kg can be derived (see Tables 5-2, 5-4).

5.2.1.3. *Estimates of Slope Factors and Risk at Current Background Body Burdens Based on a Mechanistic Model*

As discussed above, Portier and Kohn (1996) combined the biochemical response model of Kohn et al. (1993) with a single initiated-phenotype two-stage model of carcinogenesis to estimate liver tumor incidence in female Sprague-Dawley rats from the Kociba et al. (1978)

bioassay. The model is described in more detail in Part II, Chapter 8, Section 8.4. This model adequately fit the tumor data, although it overestimated the observed tumor response at the lowest dose in the Kociba et al. study. The shape of the dose-response curve was approximately linear, and the estimated ED_{01} value for this model was 1.3 ng/kg/day. The corresponding body burden giving a 1% increased effect was 2.7 ng/kg.

The model authors believe that the use of CYP1A2 as a dose metric for the first mutation rate is consistent with its role as the major TCDD-inducible estradiol hydrolase in liver and with its hypothesized role in the production of estrogen metabolites leading to increased oxidative DNA damage and increased mutation (Yager and Liehr, 1996; Hayes et al., 1996; Dannan et al., 1986; Roy et al., 1992). Although no lower bound estimate of the ED_{01} is calculated, a maximum likelihood estimate of the slope factor of 7.1×10^{-3} per pg TCDD/kgBW/day can be calculated. This estimate represents an example of the type of modeling based on key events in a mode of action for carcinogenesis that is consistent with the future directions in dose-response modeling described in EPA's revised proposed cancer risk assessment guidelines (U.S. EPA, 1999). Although a number of uncertainties remain regarding structure and parameters of the model, the slope estimate is consistent with those derived from humans and animals. More details on this model can be found in Part II, Chapter 8, Section 8.4.

An alternative mechanistic model has been proposed (Conolly and Andersen, 1997). This model was developed for focal lesion growth, based on two types of initiated cells and applying the negative selection mechanism for hepatic tumor promotion proposed by Jirtle et al. (Jirtle and Meyer, 1991; Jirtle et al., 1991). In this model, even though the two types of initiated cells express the same biochemical marker, they respond differently to promotional stimulation in the liver. The model presumes that a promotional stimulus to the liver is countered by mito-inhibitory signals generated by the liver to constrain proliferation. One set of mutated cells is sensitive to this mito-inhibition, whereas the other set of mutated cells is insensitive and responds only to the promotional stimulus. The result is that, under increasing doses of the promoter, one group of focal lesions is decreasing in size—and hence, number of cells—whereas the other group is increasing in size.

The Conolly and Andersen model is different from the Portier and Kohn (1996) model in that it can result in U-shaped dose-response curves for the total number and mean size of observable focal lesions without using U-shaped parametric forms for the mutation rates or the birth rates. Conolly and Andersen did not apply their model to cancer risk estimation. Presently, there are insufficient experimental data to support or refute the use of either the Portier and Kohn or the Conolly and Andersen model.

5.2.2. Noncancer Endpoints

The analysis of noncancer endpoints following dioxin exposure uses the same dose metrics as for the preceding cancer analysis, although with increased emphasis on LOAELs and NOAELs. Summarized here are noncancer results based on the 200+ ED_{01} calculations performed in Part II, Chapter 8, combined with a tabulation (Table 5-6; Appendix A) of the lower range of measured, empirical, LOAEL/NOAEL results. Noncancer endpoints following dioxin exposure present similar—lower for some effects—PODs as compared to cancer ED_{01}s, with many of the PODs falling in a range of ~10–50 ng/kg BB and lower still for subclinical endpoints.

Before presenting these results, consideration should be give to a number of difficulties and uncertainties associated with comparing the same or different endpoints across species, such as differences in sensitivity of endpoints, times of exposure, exposure routes, and species and strains; the use of multiple or single doses; and variability between studies even for the same response. The estimated ED_{01}s may be influenced by experimental design, suggesting that caution should be used when comparing values from different designs. Caution should also be used when comparing studies that extrapolate ED_{01}s outside the experimental range. Furthermore, it may be difficult to compare values across endpoints. For example, the human health risk for a 1% change of body weight may not be equivalent to a 1% change in enzyme activity. Similarly, a 1% change in response in a population for a dichotomous endpoint is different from a 1% change in a continuous endpoint, where the upper bound of possible values may be very large, leading to a proportional increase in what constitutes the 1% effect level. Finally, background exposures are often not considered in these calculations simply because they were not known.

Part II, Chapter 8, presents estimated ED_{01}s for more than 200 data sets. These data sets were categorized by exposure regimen (single exposure vs. multiple exposures), effect (biochemical, hepatic, tissue, immune, and endocrine) and developmental stage (adult vs. developmental). The Hill model was fit to a majority of the data sets. This model not only provides estimates of the ED_{01}, it also provides insight into the shape of the dose-response curve in the form of a shape parameter. The shape parameter, or the Hill coefficient, can be used to determine whether the dose-response curve is linear or threshold-like. An analysis of the shape parameters for the different response categories implies that many dose-response curves are consistent with linearity over the range of doses tested. This analysis does not imply that the curves would be linear outside this range of doses, but it does inform the choices for

extrapolation. This is particularly true when body burdens or exposures at the lower end of the observed range are close to body burdens or exposures of interest for humans, which is the case with dioxin-like chemicals and biochemical effects.

Several general trends were observed and discussed in Part II, Chapter 8, relating to the ED_{01} results. The lowest ED_{01}s tended to be for biochemical effects, followed by hepatic responses, immune responses, and responses in tissue weight. However, there was a wide range of ED_{01}s within each category. For example, in the immune category, there was a range of almost six orders of magnitude in the ED_{01} estimates. In addition, some of the lowest ED_{01} estimates were for changes in immune function in adult mice, with ED_{01}s ranging from 2 to 25 ng TCDD/kg. Overall shape parameter data suggest that biochemical responses to TCDD are more likely to be linear within the experimental dose range. The more complex responses are more likely to assume a nonlinear shape. Nonetheless, a large number (> 40%) of the more complex responses have shape parameters that are more consistent with linearity than with nonlinearity.

Table 5-6 summarizes the range of experimental LOAEL, NOAEL, and ED_{01} values for critical endpoints from animal studies. The published data supporting these values are presented in Appendix A. These endpoints were chosen because they are considered adverse (e.g., developmental or reproductive toxicity) or are on the critical path for cancer and noncancer effects. In addition, these effects were chosen because the body burdens at which the effects occur are approximately 50 ng/kg or lower. The use of ED_{01}s and NOAELs and/or LOAELs in this analysis provides a "point of departure" for a discussion of margins of exposure for a variety of health endpoints. No one endpoint has been chosen as the "critical effect," as is often done in RfD calculations. For the effects listed in Table 5-6 and Appendix A, the MOE is approximately 10 or less. In some cases, particularly for ED_{01} values for the developmental toxicities of TCDD in rats (Mably et al., 1992a-c; Gray et al., 1997a, b; Faqi et al., 1998; Markowski et al., 2001), the MOE is less than 1. These estimates of the MOE assume a background human body burden of 5 ng TEQ/kg body weight.

Results from the analysis of ED_{01}s and an examination of LOAELs in additional studies suggest that noncancer effects can occur at body burden levels in animals equal to or less than body burdens calculated for tumor induction in animals. This is especially true when considering biochemical changes that may be on the critical path for both noncancer and cancer effects, such as enzyme induction or impacts on growth factors or their receptors. Although human noncancer effects were not modeled in Part II, Chapter 8, the observation of effects in the Dutch studies (discussed in Section 2.2.2 in this document) suggest that subtle but important noncancer human

effects may be occurring at body burden levels equivalent to those derived for many biochemical—and some clearly adverse—effects in animals.

5.3. MODE-OF-ACTION–BASED-DOSE-RESPONSE MODELING

As described in Part II, Chapter 8, Section 8.3, mechanism-based modeling can be a powerful tool for understanding and combining information on complex biological systems. Use of a truly mechanism-based approach can, in theory, enable reliable and scientifically sound extrapolations to lower doses and between species. However, any scientific uncertainty about the mechanisms that the models describe is inevitably reflected in uncertainty about the predictions of the models. The assumptions and uncertainties involved in the mechanistic modeling described in Chapter 8 are discussed at length in that chapter and in cited publications.

The development and continued refinement of PBPK models of the tissue dosimetry of dioxin has provided important information concerning the relationships between administered dose and dose-to-tissue compartments (Part II, Chapter 8, Section 8.2). Aspects of these models have been validated in the observable response range for multiple tissue compartments, species, and class of chemical. These models will continue to provide important new information for future revisions of this health assessment document. Such information will likely include improved estimates of tissue dose for liver and other organs where toxicity has been observed, improved estimates of tissue dose(s) in humans, and improved estimates of tissue dose for dioxin-related compounds.

In this reassessment, the development of biologically based dose-response models for dioxin and related compounds has led to considerable and valuable insights regarding both mechanisms of dioxin action and dose-response relationships for dioxin effects. These efforts, described in some detail in Part II, Chapter 8, Section 8.3, have provided additional perspectives on traditional methods such as the linearized multistage procedure for estimating cancer potency or the uncertainty factor approach for estimating levels below which noncancer effects are unlikely to occur. These methods have also provided a biologically based rationale for what had been primarily statistical approaches. The development of models like those in Chapter 8 allows for an iterative process of data development, hypothesis testing, and model development.

5.4. SUMMARY OF DOSE-RESPONSE CHARACTERIZATION

All humans tested contained detectable body burdens of TCDD and other dioxin-like compounds that are likely to act through the same mode of action. The receptor modeling theory outlined in Chapter 8 indicates that xenobiotics that operate through receptor binding

mechanisms, such as dioxin, will follow a linear dose-response binding in the 1–10% receptor occupancy region. This theoretical basis suggests—and this is supported by empirical findings—that the proximal biochemical and transcription reactions for dioxins, such as effects on DNA transcription and enzyme induction, may also follow linear dose-response kinetics. More distal toxic effects could be linear or sublinear/threshold depending on (1) the toxic mechanism, (2) location on the dose-response curve, and (3) interactions with other processes such as intracellular protein binding and co-factor induction/repression.

Empirical data provide dose-response shape information down to approximately the 1% effect level for many toxic endpoints. Many examples of adverse effects experienced at these low levels have too much data variability to clearly distinguish on a statistical basis (goodness of fit) between dose-response curve options and whether dose-response follows linear, supra/sublinear, power curve, or threshold kinetics. Toxic effects seen only at higher doses are presumably more likely to result from multiple cellular perturbations and are thus less likely to follow linear relationships.

Empirical dose-response data from cancer studies—both human epidemiological and bioassays—do not provide consistent or compelling information supportive of either threshold or supralinear models (see Tables 2-3 and 5-2) and are insufficient to move from EPA's default linear extrapolation policy in the proposed carcinogen risk assessment guidelines (U.S. EPA, 1996, 1999, 2003). This policy indicates that, for cancer dose-response, the data are to be modeled within the observed range and a POD calculated from which a linear extrapolation to the origin is generated. For noncancer endpoints, EPA proposes using an MOE approach, rather than an RfD approach, due to the inability to determine levels that are likely to be without appreciable effects of lifetime exposure to the population (including susceptible subpopulations) for all adverse effects, particularly given the current level of background exposure and human body burdens. Data on background levels of dioxins, furans and coplanar PCBs (see Part I, Volume 3, and Section 4.4 in this document) indicate that current levels in humans are already substantially along the dose-response curve. Thus, theoretical issues regarding increases from zero body burden levels are moot, and assessments must consider both background and additional increments of dose to this background level.

MOEs between population levels and the empirically observed (not modeled) 1% effect levels for a number of biochemical/toxic endpoints are on the order of less than 1 to 2 orders of magnitude. Thus, the extrapolation between observed effects and background levels is not large, with any increments to background further advancing along the dose-response curve through or toward the observed range. This further reduces the level of uncertainty when evaluating the

significance of MOEs. It is possible that any additional exposure above current background body burdens will be additive to ongoing responses. The magnitude of the additional response will be a function of the toxic equivalency of the incremental exposure. This observation, the relatively small MOE for "key events" potentially on the pathway to cancer and noncancer effects, and the high percentage of observed linear responses suggest that a proportional model should be used when extrapolating beyond the range of the experimental data. Short of extrapolating linearly over one to two orders of magnitude to estimate risk probabilistically for cancer and noncancer effects in the face of the uncertainties described above, a simple MOE approach may be useful to decision makers when discussing risk management goals. However, this decision would have to be based on a policy choice, because this analysis does not strongly support either approach.

Because human data for cancer dose-response analysis were available and because of a strong desire to stay within the range of responses estimated by these data, the risk chosen for determining a POD was the 1% excess risk. Doses and exposures associated with this risk (the ED_{01}s) were estimated from the available data using both mechanistic and empirical models. Comparisons were made on the basis of body burdens to account for differences in half-life across the numerous species studied.

In humans, restricting the analysis to log-linear models resulted in cancer ED_{01}s ranging from 6.0 ng/kg to 62 ng/kg. These were similar to the estimates from empirical modeling of the animal studies, which ranged from 14 ng/kg to 1190 ng/kg (most estimates were in the range of 14 to 500 ng/kg), and 2.7 ng/kg for the single mechanism-based model. Lower bounds on these ED_{01} estimates were used to calculate upper bound slope factors and risk estimates for average background body burdens.

Table 5-4 summarizes the ED_{01}/LED_{01} and slope factor calculations for the occupational cohort and bioassay studies. The slope factor calculations are performed by linearly extrapolating the ED/LED_{01} values to the background response rates, consistent with procedures outlined in the draft proposed guidelines for carcinogen risk assessment (U.S. EPA, 1996, 1999, 2003). A slope factor estimate of approximately 1×10^{-3} per pg TCDD/kg body weight/day represents EPA's most current upper bound slope factor for estimating human cancer risk based on human data. A slope factor of 1.4×10^{-3} per pg TCDD/kg body weight/day represents EPA's most current upper bound slope factor for estimating human cancer risk based on animal data. Details on the specific procedures and calculations are provided in the footnotes. Additional details on the study characteristics and dose-response data and graphs are available in Section 5.2 and Table 5-2. The Agency, although fully recognizing the range and the public health-conservative nature of the slope factors that make up the range, suggests the use of 1×10^{-3} per

pg TEQ/kg body weight/day as an estimator of upper bound cancer risk for both background intakes and incremental intakes above background.

Upper bound slope factors allow the calculation of the high end (greater than 95%) of the probability of cancer risk in the population. This means that there is a greater than 95% chance that cancer risks will be less than the upper bound. Use of the ED_{01} rather than the LED_{01} to provide more likely estimates based on the available epidemiological and animal cancer data result in slope factors and risk estimates that are within a factor of 2 from the upper bound estimates. Even though there may be individuals in the population who might experience a higher cancer risk on the basis of genetic factors or other determinants of cancer risk not accounted for in epidemiologic data or animal studies, the vast majority of the population is expected to have less risk per unit of exposure, and some may have zero risk. On the basis of these slope factor estimates (per pg TEQ/kg body weight/day), upper bound cancer risk at average current background body burdens (5 ng TEQ/kg body weight) exceed 10^{-3} (1 in 1000). Current background body burdens reflect higher average intakes from the past (approximately 3 pg TEQ/kg body weight/day). For a very small percentage of the population (< 1%), estimated upper bound risks may be two to three times higher than this upper bound, based on average intake, if their individual cancer risk slope is represented by the upper bound estimate and they are among the most highly exposed (among the top 5%), based on dietary intake of dioxin and related compounds.

Estimates for noncancer endpoints show greater variability. In general, when compared on a body burden basis, the noncancer endpoints displayed lower ED_{01}s and NOAELs and/or LOAELs for short-term exposures versus longer-term exposures and for simple biochemical endpoints versus more complex endpoints such as tissue weight changes or toxicity. A number of significant, adverse, noncancer responses occurred at LOAEL/NOAEL/ED_{01}s of < 10–50 ng/kg, levels that are similar to the ED_{01}s estimated for cancer effects (see Tables 5-4, 5-6 and Appendix A). The mechanism-based models for noncancer endpoints gave a lower range of ED_{01}s (0.17 to 105 ng/kg) when compared to the broader noncancer data set. Although most of these estimates were based on a single model, the estimate from a different model—the hepatic zonal induction model—gave an ED_{01} for CYP1A2 induction of 51 ng/kg and, hence, was within the same range.

Although highly variable, these estimates suggest that any choice of body burden of more than 100 ng/kg as a POD would likely yield > 1% excess risk for some endpoint in humans, including those with clear clinical significance. Also, choosing a POD of less than 1 ng/kg would likely be an extrapolation below the range of these data. Any choice in the middle range

of 1 to 100 ng/kg would be supported by the analyses, although the data provide the greatest support in the range of 10 to 50 ng/kg. This range of body burdens should also provide a useful point of comparison when evaluating impacts of risk management on average body burdens in the general population or on estimates of impact of incremental exposures above background on individual body burdens at various ages.

Table 5-1. Peak serum dioxin levels in the background population and epidemiological cohorts

Cohort	No.	Total TEQ (ppt lipid)			2,3,7,8-TCDD (ppt lipid)	PCBs	Non-2,3,7,8-TCDD TEQ (ppt lipid)	Comment
		Lower	Central Tendency	Upper	Central Tendency	Mean TEQ	Central Tendency	
CDC comparison population, USA 1995–1997; CDC (2000)	316	2[a]	25.4 mean[b]	50[a]	2.1 mean 1.9 median (95% UCL = 4.2)	5.3 (est.)[b]	23.3 mean	TEQ_{DFP}-WHO_{98}; serum; missing PCBs 105, 118, 156 estimated
Background, Dioxin Assessment, USA ~1990s	pooled results	30	52.8 mean 55 median	70	5.2 mean SD ~1.32[c]	18.8 mean 20 median	47.6 mean	TEQ_{DFP}-WHO_{98}; serum, adipose, breast milk[d]
Back-calculated								
Ranch Hand, low; Ketchum et al. (1999)	276				52.3 median (range 27–94)			serum
Ranch Hand, high; Ketchum et al. (1999)	283				195.7 median (range 94–3,290)			serum
Hamburg cohort, women; Flesch-Janys et al. (1999)	$65_{2,3,7,8}$ 64_{TEQ}	19.3	811.2 mean[e] 172.8 median[e]	6789.1	506.8 mean 125.8 median (range 2.4–6397.4)		304.4 mean[e]	I-TEQs, dioxin and furan TEQ only; serum
NIOSH, Fingerhut et al. (1991b), NTIS	253				2,000 mean (range[f] 2–32,000)			serum
BASF, severe chloracne; Ott et al. (1993)	56				1008 geom. mean (range[g] 20–13360)			serum

Table 5-1. Peak serum dioxin levels in the background population and epidemiological cohorts (continued)

Cohort	No.	Total TEQ (ppt lipid)			2,3,7,8-TCDD (ppt lipid)	PCBs	Non-2,3,7,8-TCDD TEQ (ppt lipid)	Comment
		Lower	Central Tendency	Upper	Central Tendency	Mean TEQ	Central Tendency	
BASF, moderate chloracne; Ott et al. (1993)	59				420.8 geom. mean (range[g] 2.72–4915)			serum
BASF, no chloracne; Ott et al. (1993)	139				38.4 geom. mean (range[g] 2.72–2981)			serum
Seveso Zone A; Landi et al. (1998)	7				230 geom. mean 325.9 median (range 41.2–399.7)			serum
Seveso Zone A, medical; Needham et al. (1999)[h]	296				381–489 median (range 1.5–56,000)			Samples taken 1976, not back-calculated; serum; using ½ DL
Seveso Zone B; Landi et al. (1998)	51				47.5 geom. mean 52.5 median (range 5.3–273)			serum
Seveso Zone B, medical; Needham et al. (1999)[h]	80				87–147 median (range 1.8–725)			Samples taken 1976, not back-calculated; serum; using ½ DL
Seveso Zone R, medical; Needham et al. (1999)[h]	48				15–89 median (range 1–545)			Samples taken 1976; not back-calculated; serum; using ½ DL
Seveso NonABR; Landi et al. (1998)	52				4.9 geom. mean 5.5 median (range 1.0–18.1)			serum

Table 5-1. Peak serum dioxin levels in the background population and epidemiological cohorts (continued)

Cohort	No.	Total TEQ (ppt lipid)			2,3,7,8-TCDD (ppt lipid)	PCBs	Non-2,3,7,8-TCDD TEQ (ppt lipid)	Comment
		Lower	Central Tendency	Upper	Central Tendency	Mean TEQ	Central Tendency	
Dutch Accident; Hooiveld et al. (1996)	14				1841.8 arith. mean 1433.8 geom. mean (range 301–3683)			serum
Dutch Main Production; Hooiveld et al. (1996)	5				608.2 arith. mean 285.9 geom. mean (range 17–1160)			serum

[a] Estimated from ATSDR (1999b) Calcasieu comparison population graph.
[b] CDC data scaled upward to adjust for missing data on PCB congeners 105, 118 and 156 by matching to PCB congener ratios measured in the early 1990s.
[c] SD approximated from unweighted estimate.
[d] Weighted average levels for the subset of serum lipid TEQs were 4.54 ng/kg for 2,3,7,8-TCDD and 55.4 ng/kg for total TEQ (PCB contribution not adjusted for missing congeners).
[e] PCDD- and PCDF-derived TEQ only, using I-TEFs.
[f] Lower interval on current level.
[g] Range estimated from exponential log distribution graph.
[h] Ranges for median values for Seveso result from age groupings in original publication (Needham et al., 1999; Tables 1, 2, 5)

Table 5-2. Published cancer epidemiology and bioassay data and dose-response formulae

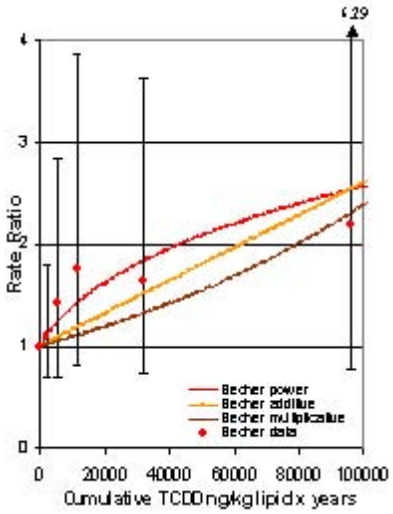
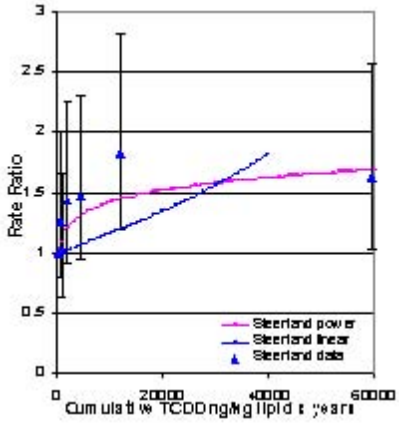
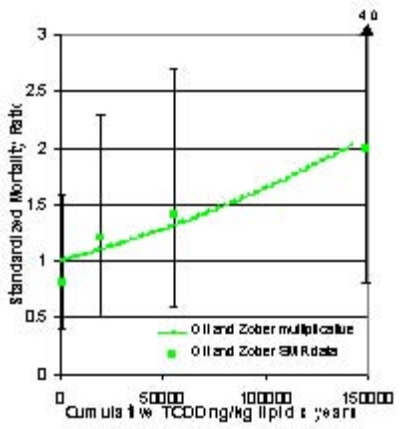
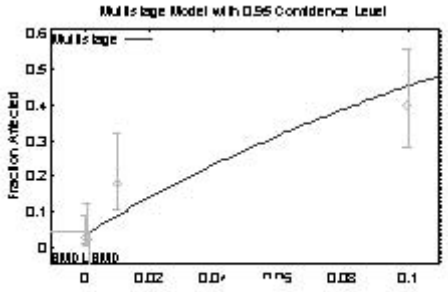
Study	Exposure groups	Central estimate of range (ng/kg fat x years)[a]	All cancer deaths observed (latency)	
Hamburg cohort, Becher et al. (1998)	0–1 1–4 4–8 8–16 16–64 64+ µg/kg fat*Years n = 1189 male; measured = 275; cancer deaths = 124	0 2000 5657 11314 32000 96000 Harmonic mean, 1.5 x upper limit	1.00 RR 1.12 (0-yr lag)[b] 1.42 P trend = 0.03 1.77 1.63 2.19 Power: p=0.026 RR = (0.00017x+1)^0.326 Additive: p=0.031 RR = 1+0.000016 x Multiplicative: p=0.043 RR = exp(0.00000869 x)	
NIOSH cohort, Steenland et al. (2001)	<335 335–<520 520–<1212 1212–<2896 2896–<7568 7568–<20455 >20455 ppt lipid *Years n = 3538 male; measured = 199; cancer deaths = 256	260 402 853 1895 4420 12125 59838 Median	1.00 RR 1.26 (15-yr lag) 1.02 1.43 1.46 1.82 1.62 Power: p=0.003 RR = (x/background)^0.097 Piecewise linear, <40000:[c] RR = exp(0.000015 x)	
BASF cohort, Ott and Zober (1996)	<0.1 0.1–0.99 1.0–1.99 2.0+ µg/kg bw. peak; n = 243 male; measured = 138; cancer deaths = 31	598 19407 55057 148800 Arithmetic mean	0.80 SMR 1.2 (0-yr lag) 1.4 2.0 Conditional risk ratio = 1.22 (95% CI 1.00–1.50)[d] RR = exp(0.00000503 x)	

Table 5-2. Published cancer epidemiology and bioassay data and dose-response formulae (continued)

Study	Exposure groups	Central estimate of range (ng/kg fat x years)[a]	All cancer deaths observed (latency)	
S-D Rats, Kociba et al. (1978); Goodman and Sauer (1992) pathology	0 0.001 0.01 0.1 µg/kg/day	0 540 1700 8100 ng/kg lipid, not AUC	2/86 Tumors 1/50 9/50 18/45	

[a] Central estimates provided courtesy of Drs. Steenland, Zober, and Becher.
[b] RR data provided only for the zero-lag analysis in Becher et al. (1998)
[c] Coefficient for the piecewise linear model (0.000015) provided by Dr. Steenland. The initial slope in the piecewise regression is applicable only to 40,000 ng/kg lipid years.
[d] Slope factor calculated from the conditional risk ratio, CR=1.22; see Chapter 8

Table 5-3. All cancer risk in humans through age 75[a]

Study	Model and Sex	ED_{01}	95% CI (lower, upper)	Unit excess risk for 1 ppt body burden above background
Steenland et al. (2001)	power male	1.38	0.71, 8.95	0.0079 (0.0027, 0.0132)
	power female	1.84	0.92, 14.9	0.0064 (0.0022, 0.0107)
	piecewise linear male	18.6	11.5, 48.3	0.00052 (0.00020, 0.00084)
	piecewise linear female	23.1	14.3, 59.8	0.00042 (0.00016, 0.00067)
Becher et al. (1998)	power-male	5.971		0.0018
	power-female	7.58		0.0014
	additive-male	18.22		0.00055
	additive-female	22.75		0.00044
	multiplicative-male	32.16		0.0003
	multiplicative-female	39.82		0.00024
Ott and Zober (1996)	multiplicative-male	50.9	25.0, ∞	0.00019 (0, 0.00039)
	multiplicative-female	62.1	30.5, ∞	0.00015 (0, 0.00032)

[a] Units are constant body burden in ng/kg not adjusted for lipid: see Part III, Chapter 8, Table 8-2, for details.

Table 5-4. Summary of all site cancer ED_{01} and slope factor calculations

Study	ED_{01} (LED_{01}) (ng/kg)	Cancer slope factor for 1 pg/kg/day above background[a] (UCL)
Hamburg cohort, Becher et al. (1998), power	6	5.1 E-3
Hamburg cohort, Becher et al. (1998), additive	18.2	1.6 E-3
Hamburg cohort, Becher et al. (1998), multiplicative	32.2	0.89 E-3
NIOSH cohort, Steenland et al. (2001), piecewise linear[b]	18.6 (11.5)	1.5 E-3 (2.5 E-3)
BASF cohort, from Ott and Zober (1996), multiplicative	50.9 (25.0)	0.57 E-3 (1.2 E-3)
Sprague-Dawley rats, Kociba et al.(1978); Goodman and Sauer (1992), pathology	31.9 (22)[c] BMD dose 38 (27.5) BMD adipose	0.97 E-3 (1.4 E-3) 0.8 E-3 (1.1 E-3)

[a] Assumes 25% of body weight is lipid; 80% of dioxin dose is absorbed from the normal diet in humans; the TCDD half-life is 7.1 years in humans. Background all cancer mortality rate calculated through lifetable analysis to 75 years. Summary results are for male all cancer risk, because the male lifetime (to 75 years) all cancer risk is greater than for females, leading to correspondingly higher cancer slope factors. As detailed in Part III, Chapter 8, $RelRisk_{(ED01)} = 0.99 + 0.01/Risk_{(0\ dose)}$. Based on the manner in which the dose-response data were calculated using Cox Regression rate ratio analyses, risks are given as cancer slope factors for 1 pg/kg/day above background, assumed 5 ppt TCDD in lipid.

[b] Steenland et al. (2001) power model results are not included, as this formula predicts unreasonably high attributable risks at background dioxin levels in the community due to the steep slope of the power curve formula at very low levels.

[c] Modeled using U.S. EPA Benchmark Dose Software, version 1.2, with either dose or adipose concentration as the metric. Absorption from food pellets in animals is assumed to be 50%. BMD = 0.00176849 ug/kg/day. BMDL = 0.00122517 ug/kg/day. Therefore, rat LED_{01} = 1.2251 x 25 x 0.5/ln2 = 22 ng/kg; human equivalent LED_{01} = 22 x ln2 x 1000/2593/0.8 = 7.38 pg/kg/day; slope factor = 0.01/7.38 = 1.4 E-3 risk/pg/kg/day.

Table 5-5. Doses yielding 1% excess risk (95% lower confidence bound) based upon 2-year animal carcinogenicity studies using simple multistage (Portier et al., 1984) models[a]

Tumor	Shape	ED_{01}	
		Animal intake for 1% excess risk in ng/kg/day (95% lower confidence bound)	Steady-state body burden in ng/kg at ED_{01} (95% lower confidence bound)
Liver cancer in female rats (Kociba)	Linear	0.77 (0.57)	14 (10)
Squamous cell carcinoma of the tongue in male rats (Kociba)	Linear	14.1 (5.9)	254 (106)
Squamous cell carcinoma of the nasal turbinates or hard palate in male rats (Kociba)	Cubic	41.4 (1.2)	746 (22)
Squamous cell carcinoma of the lung in female rats (Kociba)	Cubic	40.4 (2.7)	730 (48)
Squamous cell carcinoma of the nasal turbinates or hard palate in female rats (Kociba)	Linear	5.0 (2.0)	90 (36)
Thyroid follicular cell adenoma in male rats (NTP)	Linear	4.0 (2.1)	144 (76)
Thyroid follicular cell adenoma in female rats (NTP)	Cubic	33.0 (3.1)	1190 (112)
Liver adenomas and carcinomas in female rats (NTP)	Quadratic	13.0 (1.7)	469 (61)
Liver adenomas and carcinomas in male mice (NTP)	Linear	1.3 (0.86)	20.6 (13.6)
Liver adenomas and carcinomas in female mice (NTP)	Linear	15.1 (7.8)	239 (124)
Thyroid follicular cell adenomas and carcinomas in female mice (NTP)	Linear	30.1 (14.0)	478 (222)
Subcutaneous tissue sarcomas in female mice (NTP)	Lin-Cubic	43.2 (14.1)	686 (224)
Leukemias and lymphomas in female mice (NTP)	Linear	10.0 (5.4)	159 (86)

[a] Reprinted with slight modifications from Part II, Chapter 8, Table 8.3.2.

Table 5-6. Body burdens for critical endpoints in animals with human equivalent daily intake

Animal	Endpoint	Study	Estimated body burden (ng/kg)			Human equiv.[a] intakes (pg/kg/day)
			LOAEL	NOAEL	ED_{01}	
Rats	Cancer	Kociba et al. (1978)	180	18	32	60; 6; 11
Rhesus monkeys	Fetal mortality	Bowman et al. (1989)	90	21	NC	30; 7
	Developmental neurotoxicity	Schantz et al. (1992)	21	–	NC	7
	Endometriosis	Rier et al. (1993)	21	–	NC	7
Rats	Reproductive tox. (multigenerational)	Murray et al. (1979)	180	18	NC	60; 6
Rats	Developmental/ reproductive toxicity	Mably et al. (1992)	38	–	0.34	13; 0.1
		Gray et al. (1997)	30	–	0.08	10; 0.03
		Faqi et al. (1998)	25	–	0.6	8; 0.2
		Ohsako et al. (2001)	30	8	NC	10; 3
Rats	Developmental immunotoxicity	Gehrs and Smialowicz (1999)	60	–	NC	20
Rats	Developmental Neurotoxicity	Markowski et al. (2001)	108	36[b]	0.7	36; 12; 0.2
Mice	Immunological effects (adult)	Burleson et al. (1996)	6	3	NC	2; 1
		Smialowicz et al. (1994)	300	–	2.9	100; 1
		Narasimhan et al. (1994)	100	50[b]	1.5	33; 17; 0.5
		Vecchi et al. (1983)	1200	–	7	401; 2
Rats	Thyroid effects	Sewall et al. (1995)	76	22	26	25; 7; 8
Mice	CYP1A1/1A2 enzyme induction	DeVito et al. (1994)	24	–	22	8; 7
		Diliberto et al. (2001)	2.8	–	67	0.9; 22
		Vogel et al. (1997)	5.1	0.51	0.003	1.6; 0.16; 0.001
		Narasimhan et al (1994)	25	10	3	8; 3; 2; 1
Rats	CYP1A1/1A2 enzyme induction	van Birgelen et al. (1995)	243	–	19	81; 6
		Schrenk et al. (1994)	72	–	26	24; 9
		Sewall et al. (1995)	8	2	3.5	3; 0.7; 1
		Walker et al. (1999)	76	–	59	25; 20

Table 5-6. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

[a] Human equivalent intakes were estimated according to the following equation: daily intake (pg/kg/day) = (body burden (ng/kg)*Ln2*1000)/(t½*absorption) where t½ = 2593 days and absorption fraction = 0.8 (Poiger and Schlatter 1986; see Section II). Corresponding human equivalent intake values are arranged in sequence from the previous three columns.

[b] NOAEL values are based on the highest individual dose group in which there are no statistically significant changes. Statistically significant dose response trends plus apparent declines are also evident at all dose levels—20 and 60 ng/kg orally—in all fixed-ratio test groups in Markowski et al. (2001) and in the 50 ng/kg dose group in Narasimhan et al. (1994).

- - = no NOAEL value, as effects seen in the lowest dose group in the study.
NC = Not calculated due to insufficient dose response information (less than three doses and a control) or due to presentation of the data in graphical form without tabulation of mean and variance estimates.
Note: This table is reproduced in Appendix A with explanatory details of study design, results, and calculation procedures, formulae, and assumptions.

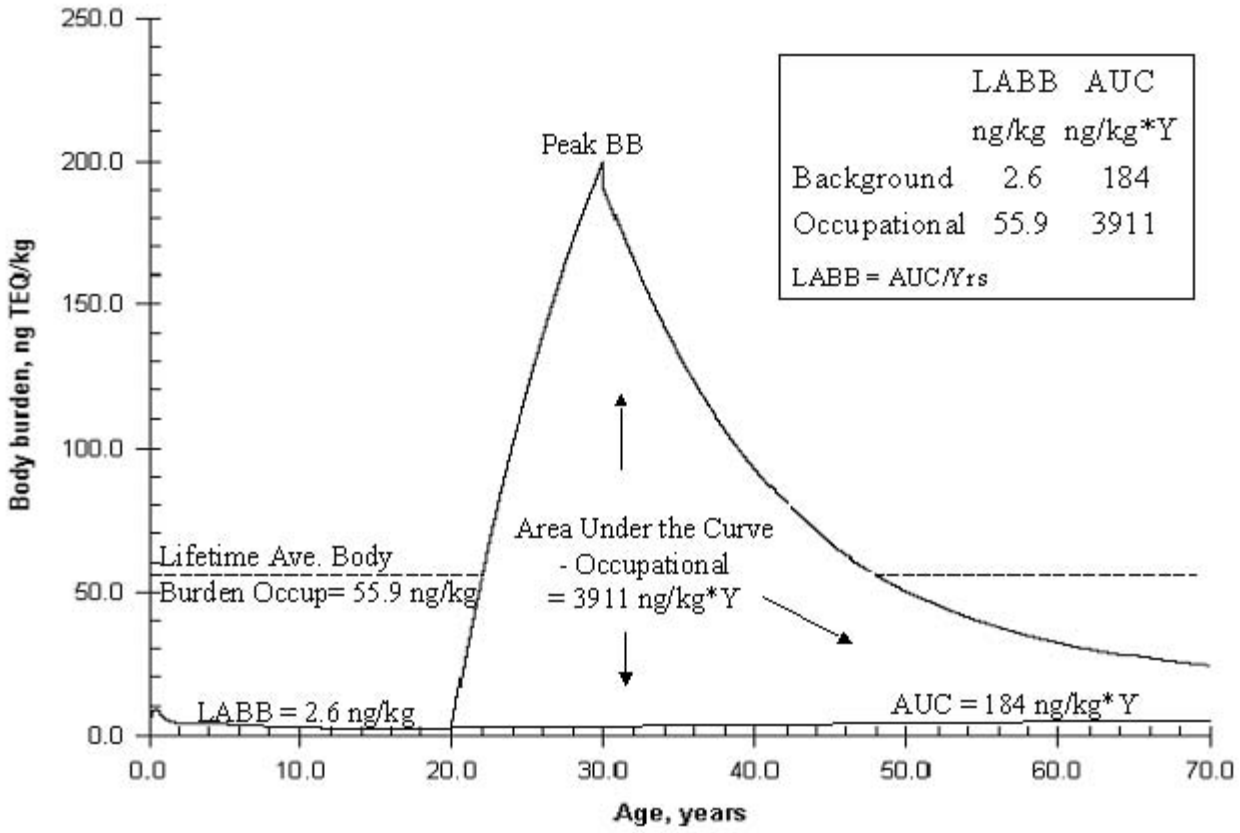


Figure 5-1. Comparison of lifetime average body burden and area under the curve in hypothetical background and occupational scenarios.

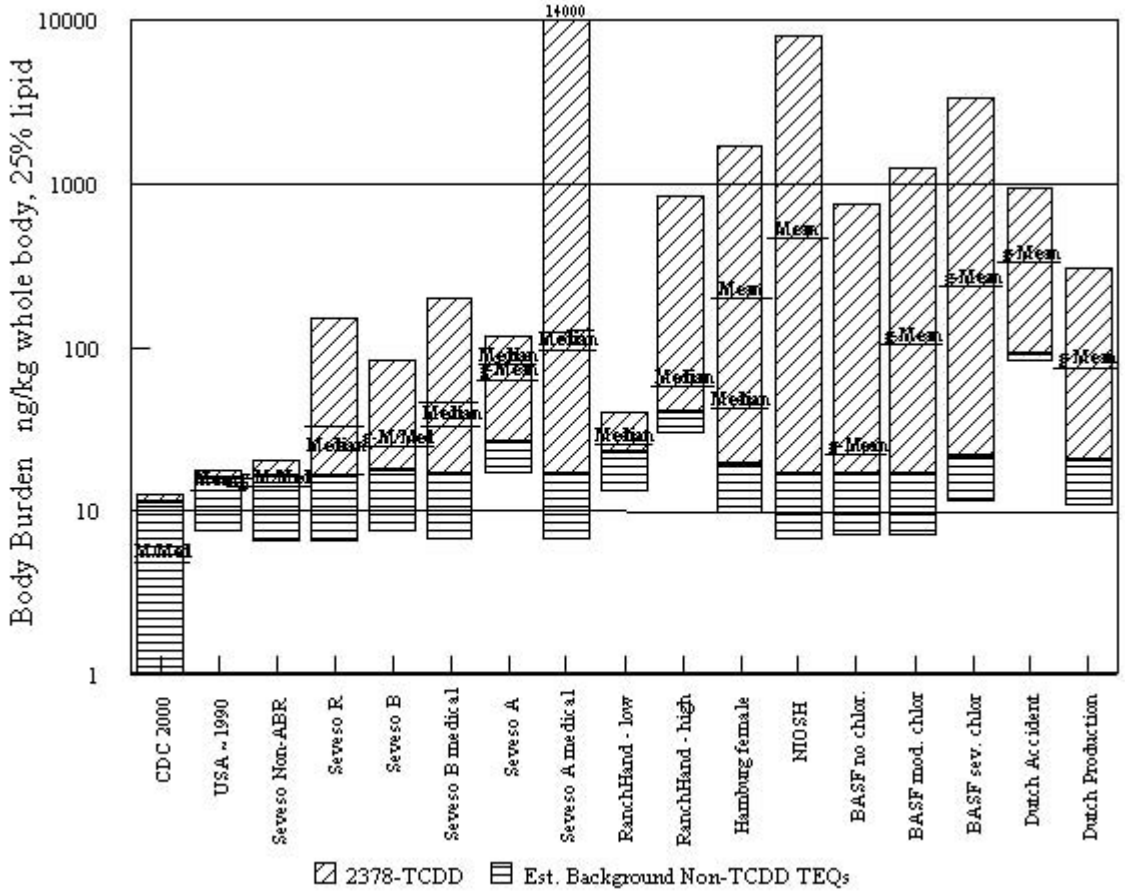


Figure 5-2. Peak dioxin body burden levels in background populations and epidemiological cohorts (back-calculated) (See Table 5-1). For the background U.S. populations (CDC; USA ~1990s), the bars represent the range of total TEQ measured in the population. The lower shaded portion represents the variability from non-2,3,7,8-TCDD-derived TEQs, the upper shaded portion the variability in the 2,3,7,8-TCDD. Note that the respective bar sizes do not represent the total non-2,3,7,8-TCDD TEQ or 2,3,7,8-TCDD contributions, because a portion of each of these contributions is contained within the region between the x-axis and bottom of the bar, namely the minimum estimated body burden. For each of the back-calculated epidemiological cohort exposures, the bar was estimated on the basis of the combination of two distributions: the 2,3,7,8-TCDD levels measured in the respective cohort plus the estimated range of background non-2,3,7,8-TCDD-derived TEQs from the U.S. population. The lower estimate is the combination of the lower 2,3,7,8-TCDD and lower non-2,3,7,8-TCDD TEQ contributions; the shading junction represents the variability in background U.S. population non-2,3,7,8-TCDD levels that have been added to this bar; the mean/median/geometric mean indicators represent the addition of the measured 2,3,7,8-TCDD central estimate with the mean background U.S. population non-2,3,7,8-TCDD TEQ level (~47.6 ppt lipid, 11.9 ng/kg body burden at 25% body fat); and the upper limit is the combination of the upper 2,3,7,8-TCDD and upper non-2,3,7,8-TCDD TEQs.

6. RISK CHARACTERIZATION

Characterizing risks from dioxin and related compounds requires the integration of complex data sets and the use of science-based inferences regarding hazard, mode of action, dose response, and exposure. It also requires consideration of incremental exposures in the context of an existing background exposure that, for the majority of the population, is independent of local sources and dominated by exposure through the food supply. Finally, this characterization must consider risks to special populations and developmental stages (subsistence fishers, children, etc.) as well as to the general population. It is important that this characterization convey the current understanding of the scientific community regarding these issues, highlight uncertainties in this understanding, and specify where assumptions have been used or inferences made in the absence of data. Although characterization of risk is inherently a scientific exercise, it must by nature go beyond empirical observations and draw conclusions in untested areas. In some cases, these conclusions are, in fact, untestable, given the current capabilities in analytical chemistry, toxicology, and epidemiology. This situation should not detract from one's confidence in the conclusions of a well-structured and well-documented characterization of risk, but it should serve to confirm the importance of considering risk assessment as an iterative process that benefits from evolving methods and data collection and is subject to change as the knowledge base improves.

Dioxin and related compounds can produce a wide variety of effects in animals and may produce many of the same effects in humans.

There is adequate evidence, based on all the available information, as discussed in Parts I and II of this Reassessment and in this Integrated Summary, to support the inference that the potential exists for humans to respond with a broad spectrum of effects from exposure to dioxin and related compounds, depending on the magnitude and duration of exposure. This inference is based on the similarities in receptor and receptor binding and their sequellae observed in animals and in humans. Effects will likely range from detection of biochemical changes at or near background levels of exposure to detection of adverse effects with increasing severity as body burdens increase above background levels. Data presented in Part II, Chapter 8, and illustrated in Table 5-6 and Appendix A support this general conclusion.

Enzyme induction, changes in hormone levels, and indicators of altered cellular function seen in humans and laboratory animals represent effects of unknown clinical significance but that may be early indicators of toxic response. Induction of activating/metabolizing enzymes at or

near background levels, for instance, may be adaptive and, in some cases, beneficial, or it may be considered adverse. Induction may lead to more rapid metabolism and elimination of potentially toxic compounds, or it may lead to increases in reactive intermediates and may potentiate toxic effects. Examples of both of these situations are available in the published literature, and events of this type formed the basis for a biologically based model discussed in Part III, Section 5.

Subtle effects, such as the impacts on neurobehavioral and developmental outcomes in laboratory animals and humans, the thyroid function and immune system alterations seen in the Dutch children exposed to background levels of dioxin and related compounds, or the changes in circulating reproductive hormones in men exposed to TCDD, illustrate the types of responses that support the finding of subtle yet arguably adverse effects at or near background body burdens. Clearly adverse effects, including, perhaps, cancer, may not be detectable until exposures contribute to body burdens that exceed current background by one or two orders of magnitude (10 or 100 times). MOEs in this range are considerably less than those typically seen for environmental contaminants of toxicologic concern, particularly when the health endpoint is cancer, as observed in epidemiologic studies.

Clear mechanistic relationships between biochemical and cellular changes seen at or near background body burden levels and production of adverse effects detectable at higher levels remain uncertain, but modes of action consistent with available data have been discussed in several chapters in Part II. Information on these mechanistic relationships and modes of action is useful in hazard characterization, and data are accumulating to suggest refined mode of action hypotheses for further testing.

It is well known that individual species vary in their sensitivity to any particular dioxin effect. Laboratory rodents (typically strains of rats and mice) are not necessarily the most sensitive responders for several well-studied effects. However, the evidence available to date indicates that humans most likely fall in the middle rather than at either extreme of the range of sensitivity for individual effects among animals. In other words, evaluation of the available data suggests that humans, in general, are neither extremely sensitive nor insensitive to the individual effects of dioxin-like compounds.

Human data provide direct or indirect support for evaluation of likely effect levels for several of the endpoints observed in laboratory studies (e.g., cancer and neurobehavioral and endocrine endpoints), although the influence of variability among humans remains difficult to assess. Discussions have highlighted certain prominent, biologically significant effects of TCDD and related compounds. In TCDD-exposed men, subtle changes in biochemistry and physiology, such as enzyme induction, altered levels of circulating reproductive hormones, or reduced

glucose tolerance and, perhaps, diabetes, have been detected in a limited number of epidemiologic studies.

These findings, coupled with the knowledge derived from animal experiments, suggest the potential for adverse impacts on human metabolism and developmental and/or reproductive biology and, perhaps, other effects in the range of current human exposures. These biochemical, cellular, and organ-level endpoints have been shown to be affected by TCDD, but specific data on these endpoints do not generally exist for other congeners. Despite this lack of congener-specific data, there is reason to infer that these effects may occur for all dioxin-like compounds, based on the concept of toxic equivalency.

In this document, dioxin and related compounds are characterized as developmental, reproductive, immunological, endocrinological, and carcinogenic hazards. The deduction that humans are likely to respond with noncancer effects from exposure to dioxin-like compounds is based on the finding that these compounds impact cellular regulation at a fundamental level and on the demonstration of adverse effects among a broad range of species. For example, because developmental toxicity following exposure to TCDD-like congeners occurs in fish, amphibians, reptiles, birds, and mammals, it is likely to occur at some level in humans.

It is not currently possible to state exactly how or at what levels individuals will respond with specific adverse impacts on development or reproductive function, but the analyses of the Dutch cohort data and laboratory animal studies suggest that some effects may occur at or near background levels. Fortunately, there have been few human cohorts identified with TCDD exposures high enough to raise body burdens significantly over background levels (see Table 5-1 and Figure 5-2 in this document), and when these cohorts were examined, relatively few clinically significant effects were detected. However, the power of these studies to detect these effects remains an issue. The lack of sufficient exposure gradients and adequate human information and the focus of most currently available epidemiologic studies on occupationally TCDD-exposed adult males make it difficult to evaluate the inference that noncancer effects associated with exposure to dioxin-like compounds may be occurring in the broader human population. It is important to note, however, that when exposures to very high levels of dioxin-like compounds have been studied—such as in the Yusho and Yu-Cheng cohorts—a spectrum of adverse effects have been detected in men, women, and children. Many of these effects are similar to what has been observed not only in small laboratory animals, but in wildlife and in nonhuman primates.

Some have argued that in the absence of better human data, deducing that a spectrum of noncancer effects will occur in humans overstates the science; however, most of the scientists

involved as authors and reviewers in the reassessment have indicated that such inference is reasonable, given the weight of evidence from available data. As presented, this logical conclusion represents a testable hypothesis that may be evaluated by further data collection. EPA, its federal colleagues, and others in the general scientific community are continuing to fill critical data gaps, which will reduce our uncertainty regarding both hazard and risk characterization for dioxin and related compounds. However, as discussed by EPA's SAB (U.S. EPA, 2001b) "neither knowledge breakthroughs nor fully developed techniques for producing more unbiased risk assessments can be expected to be available in the near future."

Dioxin and related compounds are structurally related and elicit their effects through a common mode of action.

The scientific community has identified and described a series of common biological steps that are necessary for most, if not all, of the observed effects of dioxin and related compounds in vertebrates, including humans. Binding of dioxin-like compounds to a cellular protein called the aryl hydrocarbon receptor (AhR) represents the first step in a series of events attributable to exposure to dioxin-like compounds, including biochemical, cellular, and tissue-level changes in normal biological processes. Binding to the AhR appears to be necessary for all well-studied effects of dioxin, but it is not sufficient in and of itself to elicit these responses.

There remains some uncertainty as to whether every dioxin response is AhR-mediated. Some data from the use of sensitive biological tools, such as AhR-deficient ($AhR^{-/-}$) mice, suggest a small residual of effects from exposure to TCDD, and, thus, we cannot rule out receptor-independent alternative pathways. However, these reported non-AhR-mediated responses occur in animals at doses that are orders of magnitude higher than current human exposures and require much higher doses than other AhR-mediated effects in animals. Thus, these putative non-AhR-mediated mechanisms are unlikely to impact any of the assumptions made in this reassessment.

Exposure of animals—and in some cases humans—to chemicals whose structure and AhR binding characteristics are similar to those of 2,3,7,8-TCDD can elicit similar effects. In the past 5 years, significant data have accumulated that support the concept of toxic equivalence, a concept that is at the heart of risk assessment for the complex mixtures of dioxin and related compounds encountered in the environment. These data have been analyzed and summarized in Part II, Chapter 9. This chapter was added to EPA's dioxin reassessment to address questions raised by the SAB in 1995. The SAB suggested that, because the TEQ approach was a critical component of risk assessment for dioxin and related compounds, the Agency should be explicit

in its description of the history and application of the process and go beyond reliance on the Agency's published reference documents on the subject (U.S. EPA, 1987, 1989a).

The analyses in Parts II and III of this document demonstrate that, although variability in the data underpinning the scientific judgments regarding toxic equivalency exists, when data are restricted to longer exposure and in vivo data, the empirical analysis strongly supports the judgment of experts in setting TEF values. This is particularly true for the use of TEFs for assessing the animal cancer endpoint but will likely apply even more strongly to noncancer effects as additional congener-specific data are collected. A focus on the five congeners that make up greater than 80% of human body burden on a TEQ basis reveals rather robust data sets, which form the basis for assigned TEFs. This focus reduces the impact of the uncertainties in TEFs assigned to less-studied congeners. In its recent review (U.S. EPA, 2001b), EPA's SAB agreed that the general framework for calculating TEFs and applying them to obtain a TEQ is well described in Part II, Chapter 9. The Board recognized that uncertainties remained regarding toxicities of joint exposures that are not dominated by well-studied congeners, and recommended further development of the TEF methodology (e.g., development of probability density functions around experimental results to assist future expert judgment in reviewing and revising TEFs) (see Finley et al., 2003).

EPA and the international scientific community have adopted toxic equivalency of dioxin and related compounds as prudent science policy.

Dioxin and related compounds always exist in nature as complex mixtures. As discussed in the exposure document, these complex mixtures can be characterized through analytic methods to determine concentrations of individual congeners. Dioxin and related compounds can be quantified and biological activity of the mixture can be estimated using relative potency values and an assumption of dose additivity. Such an approach has evolved over time to form the basis for the use of TEQ in risk assessment for this group of compounds. Although such an approach is dependent on critical assumptions and scientific judgment, it has been characterized by the SAB as a "useful, interim" way to deal with the complex mixture problem, and it has been accepted by numerous countries and several international organizations. Alternative approaches, including the assumption that all congeners carry the toxic equivalency of 2,3,7,8-TCDD or that all congeners other than 2,3,7,8-TCDD can be ignored, have been rejected as inadequate for risk assessment purposes.

Significant additional literature is now available on the subject of toxic equivalency of dioxin and related compounds, as summarized (through 2000) in Part II, Chapter 9. An

international evaluation of all of the available data (van den Berg et al., 1998) reaffirmed the TEQ approach and provided the scientific community with the latest values for TEFs for PCDDs, PCDFs, and dioxin-like PCBs. Consequently, we can infer with greater confidence that humans will respond to the cumulative exposure of AhR-mediated chemicals. This reassessment recommends that the WHO_{98} TEF scheme be used to assign toxic equivalency to complex environmental mixtures for assessment and regulatory purposes. Further research is needed to address remaining uncertainties inherent in the current approach, in particular those regarding the impact of actual exposures compared to measured body burdens of highly persistent congeners and the continuing debate regarding the role of other Ah-agonists in the diet on the toxicity of dioxin-like compounds. WHO has suggested that the TEQ scheme be reevaluated on a periodic basis and that TEFs and their application to risk assessment be reanalyzed to account for emerging scientific information. EPA supports this suggestion and intends to participate in future re-evaluations.

Complex mixtures of dioxin and related compounds are highly potent, "likely" carcinogens.

A weight-of-evidence evaluation suggests that mixtures of dioxin and related compounds (CDDs, CDFs, and dioxin-like PCBs) are strong cancer promoters and weak direct or indirect initiators and that they are likely to present a cancer hazard to humans. Because dioxin and related compounds always occur in the environment and in humans as complex mixtures of individual congeners, it is appropriate that the characterization apply to the mixture. According to the Agency's revised proposed guidelines for carcinogen risk assessment, the descriptor "likely to be carcinogenic to human" is appropriate when the available tumor effects and other key data are adequate to demonstrate carcinogenic potential to humans (U.S. EPA, 1999, 2003) yet are not sufficient to infer a cause-and-effect relationship.

"Adequate data" are recognized to span a wide range. Even though the database from cancer epidemiologic studies remains a point of scientific discussion, it is the view of this reassessment that this body of evidence is supported by the laboratory data that indicate that TCDD increases cancer mortality of several types. Although not all confounders were ruled out in any one study, positive associations between surrogates of dioxin exposure, either length of occupational exposure or proximity to a known source combined with some information based on measured blood levels, and cancer have been reported.

These epidemiologic data strongly suggest a role for dioxin exposure to contribute to a carcinogenic response but are not sufficient to confirm a causal relationship between exposure to

dioxin and increased cancer incidence. Available human studies alone cannot demonstrate whether a cause-and-effect relationship between dioxin exposure and increased incidence of cancer exists. Therefore, evaluation of cancer hazard in humans must include an evaluation of all of the available animal and in vitro data as well as the data from exposed human populations.

The data for complex mixtures of dioxin and related compounds represent a case that, according to discussions in the draft guidelines, would approach the strong-evidence end of the adequate data spectrum. Epidemiologic observations of an association between exposure and cancer responses (TCDD); unequivocal positive responses in both sexes, multiple species, multiple sites, and different routes in lifetime bioassays or initiation-promotion protocols or other shorter-term in vivo systems such as transgenic models (TCDD plus numerous PCDDs, PCDFs, dioxin-like PCBs); and mechanistic or mode-of action data that are assumed to be relevant to human carcinogenicity, including, for instance, initiation-promotion studies (PCDDs, PCDFs, dioxin-like PCBs) all support the description of complex mixtures of dioxin and related compounds as likely to be human carcinogens. On the basis of these observations, complex environmental mixtures of TCDD and dioxin-like compounds should be characterized as "likely" carcinogens, with the degree of certainty of the characterization being dependent on the constituents of the mixture, when known. For instance, the hazard potential, although "likely," would be characterized differently for a mixture whose TEQ was dominated by octaCDD as compared with one dominated by pentaCDF.

As discussed in Section 2.2.1.5, under EPA's current approach for carcinogen risk assessment, individual congeners can also be characterized as to carcinogenic hazard. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is best characterized as "carcinogenic to humans." This means that, on the basis of the weight of all of the evidence (human, animal, mode of action), TCDD meets the criteria that allow EPA and the scientific community to accept a causal relationship between TCDD exposure and cancer hazard. The guidance suggests that "carcinogenic to humans" is an appropriate descriptor of human carcinogenic potential when there is an absence of conclusive epidemiologic evidence to clearly establish a cause-and-effect relationship between human exposure and cancer but there is compelling evidence of carcinogenicity in animals and mechanistic information in animals and humans demonstrating similar modes of carcinogenic action. The "carcinogenic to humans" descriptor is suggested for TCDD because all of the following conditions are met:

- There is strong and consistent evidence from occupational epidemiologic studies for an association between TCDD exposure and increases in cancer at all sites, in lung cancer

and, perhaps, at other sites, but the data are insufficient on their own to support a causal association. This point was discussed in detail by the International Agency for Research on Cancer (IARC, 1997).

- There is extensive carcinogenicity in both sexes of multiple species at multiple sites.

- There is general agreement that the mode of TCDD's carcinogenicity is as an AhR-dependent promoter and proceeds through gene expression and/or a modification of the action of a number of receptor and hormone systems involved in cell growth and differentiation, such as the epidermal growth factor receptor and the estrogen receptor.

- The human AhR and the rodent AhR are similar in structure and function and, once activated, both bind to the same DNA response elements, designated DREs.

- Human and rodent tissue and organ cultures respond to TCDD and related chemicals in a similar manner and at similar concentrations. TCDD has the ability to transform immortalized human and rodent cells that then have demonstrable tumorigenicity.

Other individual dioxin-like compounds are characterized as "likely to be carcinogenic to humans" primarily because of the lack of epidemiological evidence associated with their carcinogenicity, although the inference based on toxic equivalency is strong that they would behave in humans as TCDD does. Other factors, such as the available congener-specific chronic bioassays, also support this characterization. For each congener, the degree of certainty is dependent on the available congener-specific data and their consistency with the generalized mode of action that underpins toxic equivalency for TCDD and related compounds.

Although uncertainties remain regarding quantitative estimates of upper-bound cancer risk from dioxin and related compounds, efforts of this reassessment to bring more data into the evaluation of cancer potency have resulted in evaluation of the slope of the dose-response curve at the low end of the observed range (using the LED_{01}) using a simple proportional (linear) model and a calculation of both upper-bound risk and MOE based on human equivalent background exposures and associated body burdens. Evaluation of shape parameters (used to estimate degree of linearity or nonlinearity of dose-response within the range of observation) for biochemical effects that can be hypothesized as key events in a generalized dioxin mode-of-action model do

not argue for significant departures from linearity below a calculated ED_{01}, extending down to at least one to two orders of magnitude lower exposure.

Risk estimates for intakes associated with background body burdens or incremental exposures based on this slope factor represent a plausible upper bound on risk, based on the evaluation of animal and human data. The slope factors, based on the most sensitive cancer responses calculated by authors of peer-reviewed publications and presented in Part II, Chapter 8, and Section 5 for both animals and humans, fall in a range of approximately 0.6×10^{-3} to 5×10^{-3} per pg TEQ/kg body weight/day.

The ranges of estimates of upper-bound cancer potency calculated from the human and animal data overlap. The range above is bounded on the upper end by the estimate of slope from the Hamburg cohort epidemiology study and on the lower end by the estimates from the Ott and Zober epidemiology study, with the NIOSH piece-wise linear epidemiology model and the reanalyzed Kociba rat study falling intermediate in this range. Consequently, the Agency, although fully recognizing this range and the public health-conservative nature of the slope factors that make up the range, suggests the use of 1×10^{-3} per pg TEQ/kg body weight/day as an estimator of upper-bound cancer risk for both background intakes and incremental intakes above background.

This decision reflects the weight given to the individual estimates from the human studies and the comparability of the revised estimate from the animal data. A recently published meta-analysis (Crump, 2003) is consistent with this estimate. In addition, this decision reflects the judgment that, because ED_{01} estimates require little extrapolation from the range of observation and current body burdens are within a factor of 10 of the ED_{01} estimates, use of a linear model is both consistent with the data and unlikely to require more than an order of magnitude extrapolation. This bounding on extrapolation would apply to both estimates of risk at current background exposures and to additional increments above current background. Application of upper-bound slope factors allows the calculation of a high-end bounding estimate of the probability of cancer risk in the population. This means that there is greater than a 95% chance that "true" population cancer risks will be less than the upper-bound estimate.

Use of the human ED_{01}s rather than the LED_{01}s to provide more likely upper-bound estimates based on the available epidemiological data is a matter of EPA science policy and compares well with upper-bound animal cancer data. Use of either ED_{01} or LED_{01} results in slope factors and risk estimates that are within a factor of 2; well within the inherent uncertainty of these estimates. Although there may be individuals within a population who may experience a higher cancer risk on the basis of genetic factors or other determinants of cancer risk not

accounted for in epidemiologic data or animal studies, the vast majority of the population is expected to have less risk per unit of exposure than the bounding estimate would suggest, and some may have zero risk.

On the basis of these slope factor estimates (per pg TEQ/kg body weight/day), upper-bound risks at average current background body burdens (5 ng TEQ/kg body weight) that result from historical average intakes of approximately 3 pg TEQ/kg body weight/day may exceed 10^{-3} (1 in a 1000). A very small percentage of the population (< 1%) has estimated risks that are a few times higher than an upper bound based on average intake if their individual cancer risk slope is represented by the upper bound estimate and they are among the most highly exposed (among the top 5%), based on dietary intake of dioxin and related compounds. This estimate of the range of upper-bound risk for the general population has increased by approximately an order of magnitude from the estimate described at background exposure levels in EPA's earlier draft of this reassessment (10^{-4}–10^{-3}) (U.S. EPA, 1994). This has occurred because, despite the fact that average intakes and body burdens are going down, estimates of upper-bound risk per unit dose have gone up by a factor of approximately 6 over the Agency's 1985 estimate and the range of exposure through the diet has been characterized.

EPA's approach to the development of an upper-bound estimate on cancer risk is consistent with its own past practices described above and with FDA's approach. In its recent report (U.S. EPA, 2001b), the SAB agreed that the treatment of the range of upper-bound risks obtained for the general population in this assessment is consistent with past EPA practice. FDA's past estimates of a risk-specific dose associated with a one-in-a-million risk (0.057 pg/kg body weight/day) (FDA 1990) have been based on animal data and have differed from EPA's only in minor ways regarding tumor counts and in the approach to cross-species scaling. In 1992, while EPA's reassessment was underway, FDA's risk-specific dose was adopted by the U.S. Public Health Service's Committee to Coordinate Environmental Health and Related Programs (CCEHRP) as the risk-specific dose for TEQ. In 1998, ATSDR used this risk-specific dose as a line of support for its policy guideline on dioxin and dioxin-like compounds in soil.

WHO and a number of individual countries have taken a different science-policy approach and have treated dioxins as nongenotoxic carcinogens and assumed that a safety factor approach, based on noncancer effects observed at lower doses than cancer in animals, would be adequate to account for concerns for both cancer and noncancer effects. This approach assumes that there is a virtual threshold for cancer effects above those for many noncancer effects. This position has been reiterated as recently as June 2001 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The differences between EPA (plus a number of other U.S. federal

agencies) and these international organizations in their approach to assessing potential cancer risk reflect differences in science policy.

Despite EPA's use of the epidemiology data to describe an upper bound on cancer risk, the peer panels who met to review earlier drafts of the cancer epidemiology chapter suggested that the epidemiology data alone were not adequate to support the characterization of dioxin and related compounds as "known" human carcinogens but that the results from the human studies were largely consistent with observations from laboratory studies of dioxin-induced cancer and, therefore, should be weighed in the assessment. Other scientists, including those who attended the peer panel meetings, felt either more or less strongly about the weight of evidence from cancer epidemiology studies, representing the range of opinions that still exists on the interpretation of these studies. Similar opinions were expressed in the comments documented in the SAB's reports in 1995 and in 2001 (U.S. EPA, 1995, 2001b).

In its reevaluation of the cancer hazard of dioxin and related compounds, IARC (1997) found that whereas the epidemiologic database for 2,3,7,8-TCDD was still "limited," the overall weight of the evidence provided by human, animal and mechanistic data was sufficient to characterize 2,3,7,8-TCDD as a Category 1 "known" human carcinogen. Other related members of the class of dioxin-like compounds were considered to have "inadequate" epidemiologic data to factor into hazard categorization. A similar classification of 2,3,7,8-TCDD as a "known" carcinogen has been published within the context of the Department of Health and Human Services' report on carcinogens (NTP, 2001). Here, too, the characterization is based on the weight of the human, animal, and mode of action information in humans and animals.

Therefore, given that 2,3,7,8-TCDD is contained in complex mixtures of dioxin and related compounds and that the TEQ approach has been adopted as a reasonable approach to assessing risks of these complex mixtures, it is also reasonable to apply estimates of upper-bound cancer potency derived from epidemiology studies where 2,3,7,8-TCDD was associated with excess cancer risk to complex mixtures of dioxin and related compounds.

The current evidence suggests that both receptor binding and most early biochemical events such as enzyme induction demonstrate linearity of dose-response within the range of observation. The mechanistic relationship of these early events to the complex process of carcinogenesis remains uncertain, although modes of dioxin action have been proposed. If these findings imply low-dose linearity in biologically based cancer models under development, then the probability of cancer risk may also be linearly related to exposure to TCDD. Until the mechanistic relationship between early cellular responses and the parameters in biologically

based cancer models is better understood, the shape of the dose-response curve for cancer below the range of observation can be inferred only with uncertainty.

Initial attempts to construct a biologically based model for certain dioxin effects as described in this reassessment will need to be continued and expanded to accommodate more of the available biology and to apply to a broader range of potential health effects associated with exposure to dioxin-like compounds. Associations between exposure to dioxin and certain types of cancer have been noted in occupational cohorts with average body burdens of TCDD approximately one to three orders of magnitude (10 to 1000 times) higher than average TCDD body burdens in the general population. In terms of TEQ, the average body burden in these occupational cohorts level is within one to two orders of magnitude (10 to 100 times) of average background body burdens in the general population (see Table 5-1 and Figure 5-2). Thus, there is no need for large-scale, low-dose extrapolations when applying models based on curve-fitting empirical data in order to evaluate background intakes and body burdens, and there are few if any data to suggest large departures from linearity in this somewhat narrow window between the lower end of the range of observation and the range of general population background exposures. Nonetheless, the relationship of apparent increases in cancer mortality in these worker populations to calculations of general population risk remains a source of uncertainty.

Use of a "margin of exposure" approach to evaluate risk for noncancer and cancer endpoints.

The likelihood that noncancer effects may be occurring in the human population at environmental exposure levels has received increased attention in recent years and is a major focus of this reassessment. This likelihood is often evaluated using an MOE approach. An MOE is calculated by dividing a "point of departure" at the low end of the range of observation in human or animal studies (the human-equivalent LOAEL, NOAEL, BMD, or effective dose [EDxx]) by the comparable surrogate of human exposure at the level of interest. It differs from a reference dose (RfD), which establishes a level of exposure below which the Agency considers it unlikely that any adverse effects will occur. The Agency has used the MOE approach for a number of years in its noncancer assessment of the safety of pesticides. The MOE concept has also been incorporated into the *Draft Final Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2003) as an alternative approach to dose-response analysis if the shape of the dose-response curve is uncertain. These draft cancer guidelines recommend differing approaches and default assumptions for linear versus nonlinear cancer data, where linear data can be approximated through the cancer slope factor and nonlinear data through an RfD and Hazard

Index approach. For both linear and nonlinear approaches to cancer characterization, the Agency recommends a statement of the extent of extrapolation of risk estimates from observed data to exposure levels of interest and its implications for certainty or uncertainty in quantifying risk. The extent of this extrapolation can be expressed as a *margin of exposure* (MOE).

As the exposure of interest approaches the range of observation of effects and MOEs get smaller, reaching any conclusion regarding the certainty of no harm is much more difficult and relies heavily on scientific judgment regarding the adequacy of the available data. In order for a decision relying on the MOE to be adequately protective of health, information is provided to allow the decisionmaker, to the extent information allows, to take into account the nature of the effect at the POD; the shape and slope of the dose-response curve; the adequacy of the overall database to assess human hazard; interindividual variability in the human population with regard to exposure, metabolism, and toxic response; and other factors. Background exposures should be factored into the calculation. Considering MOEs based on estimates of incremental exposure alone divided by the human exposure of interest is not considered to give an accurate portrayal of the implications of that exposure unless background exposures are insignificant.

One of the difficulties in assessing the potential health risk of exposure to dioxins is that background exposures are often a significant component of total exposure when based on TEQ. The average levels of background intake and current average body burdens of dioxin-like compounds in terms of TEQs in the general population (1 pg TEQ/kg body weight/day and 5 ng TEQ/kg body weight, respectively) are within a factor of 10 of human-equivalent levels associated with NOELS, LOAELs, or ED_{01} values derived from studies in laboratory animals exposed to TCDD or TCDD equivalents for both cancer and noncancer toxic effects (see Table 5-6 and Appendix A). Therefore, in many cases, the MOE compared to background using these toxic endpoints is a factor of 10 or less. These estimates and others are presented and discussed in Part II, Chapter 8.

As discussed in Chapter 8, these data, although variable, suggest that choosing a human-equivalent body burden associated with an ED_{01} value above 100 ng/kg as a point of departure would likely yield a greater than 1% excess risk for some toxicity endpoint in humans. Also, choosing a POD below 1 ng/kg would likely be an extrapolation below the range of these data. Given the nature of the data and the range of uncertainty around individual data sets, any choice for a 1% effect point of departure in the middle range of 1 ng/kg to 100 ng/kg would be supported by the analyses, although the data provide the greatest support for defining a point of departure consistent with principles of safety assessment in the range of 10 ng/kg to 50 ng/kg. This range also includes body burdens consistent with the empirically derived NOAELs and

LOAELs for many of the effects that have traditionally been used as a POD for safety assessment by WHO, JECFA, and ATSDR.

Although somewhat dependent on experimental design or the model chosen to derive the ED_{01}, NOAEL, and LOAEL values, this range provides a perspective on the nature and variety of effects that have been evaluated within approximately an order of magnitude, from biochemical markers of exposure to more clearly adverse effects in animals. This range of body burdens should also provide a useful point of comparison when evaluating impacts of risk management on average body burdens in the general population or on estimates of impact of incremental exposures above background on the range of individual body burdens at various ages.

Because of the relatively high background levels as compared to effect levels, the Agency is not recommending the derivation of a reference dose (RfD) for dioxin and related compounds. Although RfDs are often useful because they represent a health risk goal below which there is likely to be no appreciable risk of noncancer effects over a lifetime of exposure, their primary use by the Agency is to evaluate increments of exposure from specific sources when background exposures are low. Any RfD that the Agency would recommend using a traditional approach for setting an RfD using uncertainty factors to account for limitations of knowledge is likely to be below—perhaps significantly below (by a factor of 10 or more)—current background intakes and body burdens. Because exceeding the RfD is not a statement of risk, comparing an incremental exposure to an RfD when the RfD has already been exceeded by average background exposures has little value for evaluating possible risk management options. In addition, the calculation of an RfD (with its traditional focus on a single "critical" effect) distracts from the large array of effects associated with similar body burdens of dioxin.

The Agency's SAB, in its comments on an earlier draft of this document, remarked that there might be value in calculating an RfD, despite a recognition of these concerns. The RfD could be used for purposes of comparison with other chemical-specific RfDs, to ensure that proper emphasis was given to noncancer effects and to set a goal for future exposure reductions. These comments notwithstanding, the Agency feels that all of these ends can be accomplished without the establishment of an RfD.

As discussed earlier, a range of values has been presented that indicates that dioxin and related compounds can produce effects, some of which are indicative of a biological response to dioxin exposure and some of which are arguably adverse, at or near current background body burdens or intake levels. Several of the studies within this range could logically be chosen as the "critical" effect upon which an RfD could be set. No one effect provides the obvious choice, as evidenced by approaches taken by WHO, JECFA and ATSDR, all of which chose different

effects upon which to base their tolerable or minimal risk levels. A range of ED_{01}s has been described in Chapter 8 and a summary of NOAELs, LOAELs, and ED_{01}s for low-dose effects is presented in Table 5-6 and Appendix A.

Depending on the choice of the endpoint, a composite uncertainty factor would need to be determined in order to set an RfD. This composite uncertainty factor should account for, at a minimum, pharmacodynamic aspects of cross-species scaling (traditionally, a factor of 3)—because pharmacokinetic factors are assumed to be accounted for by cross-species scaling on the basis of body burden—and interindividual human variability (traditionally, a factor of 10). In addition, selection of a LOAEL within the range would suggest an additional factor of uncertainty as large as 10. Recently published results also indicate neurobehavioral impacts on adult rats exposed perinatally at levels that yield body burden ED_{01}s below current average human body burdens and as low as the lowest noncancer effects previously evaluated (Markowski et al., 2001). In addition, many of the developmental reproductive effects observed in rats (Mably et al., 1992a-c) have ED_{01} values less than current background exposures. These results suggest that there may be additional database needs regarding risks to children. The above considerations would traditionally yield a composite uncertainty factor in the range of 30 to 100 or more.

Coupled with the relatively narrow range of possible "critical" effects discussed above, the range of plausible composite uncertainty factors make the selection of any particular value as the Agency's RfD more difficult than usual and probably unnecessary, particularly in light of the fact that any value that the Agency might choose using traditional approaches would be below current background body burden or intake levels.

When evaluating incremental exposures associated with specific sources, knowing the increment relative to background may help in understanding the impact of the incremental exposure. For instance, it would be misleading to focus on only the incremental exposure in evaluating the potential impact on human health when a relatively large background body burden of dioxin already exists in the exposed population. In these circumstances, the incremental exposure needs to be evaluated in the context of these background levels to aid in determining whether these incremental exposures have regulatory significance. This approach would parallel the Agency's approach to evaluating lead exposures. Other parallel science and management issues between dioxin-like compounds and lead are under discussion within the Agency. Providing guidance on the how to judge the significance of incremental increases to background using the MOE approach is beyond the science scope of the reassessment and will have to be addressed elsewhere by EPA. However, it is clear, in light of relatively high background

exposures, that the MOE approach is more useful than an RfD for characterizing dioxin noncancer risks.

Other national and international bodies have chosen to define "safe" or "tolerable" levels for dioxin and related compounds (e.g., WHO, 1998; ATSDR, 1999a; SCF, 2000). These estimates cluster within a factor of 4 of current average intake levels, although estimates in the past have spanned many orders of magnitude. Some commenters on earlier drafts of this reassessment have suggested that EPA's approach is inconsistent with these efforts and overly "conservative." Two distinctions can help in understanding these apparent differences. First, in its reassessment, EPA has not tried to establish a tolerable or acceptable level of risk. Rather, it has tried to provide a science-based description of hazard and potential risk without making a policy judgment of acceptability. Second, whether one is providing a risk descriptor or an acceptable risk determination, a number of judgments need to be made as one moves from experimental observation to conclusion. Apparently subtle differences in these judgments can result in significantly different conclusions. These differences in judgment fall into three major areas: (1) the original focus on cancer rather than noncancer effects as the primary endpoint of regulatory concern and the assumption by some that all nongenotoxic compounds have thresholds below which cancer risk is minimal or nonexistent; (2) the use of intake as the cross-species dose metric despite the large difference in half-life in animals versus humans (for TCDD, for instance, the difference between rats and humans is over a factor of 100); and (3) the size of the "safety" factor or "uncertainty" factors used to derive a "safe or "tolerable" level.

The latter factor is currently the most widely divergent. More recent assessments have taken noncancer endpoints into account and have applied a range of uncertainty factors. For instance, ATSDR (1999a) set a minimal risk level (MRL), which is defined similarly to EPA's RfD, for dioxin and related compounds of 1.0 pg TEQ/kg body weight/day. The ATSDR assessment is based on the results of Schantz et al. (1992), a study that is included in Table 5-6 and Appendix A. ATSDR used intake as the interspecies dose metric and a composite uncertainty factor of 90, accounting for intraindividual human variability (10), a minimal LOAEL/NOAEL (3), and residual pharmacodynamic differences (3).

Hypothetically, had ATSDR relied on the TCDD body burdens measured during this series of rhesus monkey experiments (see Bowman et al., 1989) and had all other factors been equal, the MRL would likely have been determined to be in the range of 0.07 pg TEQ/kg body weight/day (see Table 5-6 and Appendix A), or more than 10 times lower than the existing ATSDR MRL and current average intake levels. The ATSDR assessment, however, selects a

single "critical" effect from among a number of choices and uses "traditional" uncertainty factors, but it uses intake rather than body burden as the dose metric.

Several recent assessments have recognized the value of body burden rather than daily intake as the preferred dose metric. WHO (1998) has set a tolerable daily intake (TDI) of 1–4 pg TEQ/kg body weight/day using a range of effects and body burden and has indicated that, although current exposures in that range are "tolerable" (a decision taking into account risk management in addition to traditional hazard assessment), efforts should be made to ultimately reduce intake levels to the lower end of the range and perhaps further. Findings in this reassessment and comments made by the SAB (U.S. EPA, 2001b) are consistent with this recommendation. The WHO assessment relied on an evaluation of the most sensitive effects that are considered adverse (hormonal, reproductive, and developmental effects) and were seen at low doses in animal studies (rats and monkeys). Body burden was used as a dose metric, and a composite uncertainty of 10 was recommended to account for a number of factors, including the use of a LOAEL rather than a NOAEL, differences in animal-to-human susceptibility, and differences in half-lives of elimination for the different components of the TEQ mixture.

In May 2001, the European Commission Scientific Committee on Food (SCF, 2000) established a tolerable weekly intake of 14 pg TEQ/kg body weight/week (equivalent to a TDI of 2 pg TEQ/kg body weight/day), based on several new studies, which are also now included in EPA's range of low-dose effects, and on a composite uncertainty factor of 9.6. This factor accounts for interindividual variability in toxicokinetics (a factor of 3.2) and marginal effects close to a NOAEL (a factor of 3). The committee concluded that no uncertainty factor needed to be applied for differences in toxicodynamics between experimental animals and humans and for interindividual variation among humans. In June 2001, WHO JECFA determined a provisional tolerable monthly intake (PTMI) of 70 pg TEQ/kg body weight/month (equivalent to 2.33 pg TEQ/kg body weight/day), based on an approach similar to that used by the SCF. The same two studies and safety factors of 3.2 or 9.6 were used, but two models were used to extrapolate the maternal body burden at the NOEL/LOEL of the studies. The committee chose the PTMI as the mid-point of the range of values from its analysis.

It should be clear from the discussion above that there is a consensus that sensitive animal responses falling within a relatively narrow range of body burdens can be used as a POD for regulatory guidance, but the choice of individual studies varies. The EPA assessment is the only one to bound the full range of effects (from arguably adaptive and questionably adverse to arguably adverse to clearly adverse) observed through the application of a uniform modeling approach, as well as through evaluating experimental LOAELs and NOAELs. There is also an

emerging consensus that body burden should often be used as a cross-species dose metric. This has implications for ATSDR's current MRL derivation. Finally, there is no consensus on the size or nature of uncertainty factors to be applied. Traditional approaches that might be applied by EPA or that have been applied by ATSDR would likely require additional information to support the choice or removal of uncertainty factors as performed by WHO, SCF, and JECFA. In particular, the focus on accounting for residual toxicodynamic differences in cross-species scaling and interindividual variability in the general population to account for sensitive individuals, including children, would suggest larger uncertainty factors than have been proposed by these groups if EPA were to set an RfD.

The choice of any composite uncertainty factor greater than 10 applied to effect levels based on body burden in any of the analyses described above would result in TDIs or MRLs below current background intakes. The use of uncertainty factors in the range of 30 to 100 or more, as traditionally used by EPA, would result in values even further below some current background body burdens or intake levels than the values presented by other organizations. Given the range of choices for a POD, the range of potential composite uncertainty factors and the uninformative nature of an RfD below current background levels, the Agency has chosen to continue to focus on MOE analyses and to not establish an RfD for dioxin and related compounds.

Children's risk from exposure to dioxin and related compounds may be increased, but more data are needed to fully address this issue.

The issue of children's risk from exposure to dioxin-like compounds has been addressed in a number of sections throughout this reassessment. Data suggest a sensitivity of response in both humans and animals during the developmental period, both prenatal and postnatal. However, these data are limited. Because evaluation of the impacts of early exposures on both children's health and health later in life is important for a complete characterization of risk, collection of additional data should be a high priority in order to reduce uncertainties in future risk assessments.

Data from the Dutch cohort of children exposed to PCBs and dioxin-like compounds suggest subtle impacts on neurobehavioral outcomes, thyroid function, and immune system alterations from prenatal—and perhaps postnatal—exposure to 1980s background levels of dioxin and related compounds. Although these effects cannot be attributed solely to dioxin and related compounds, several associations suggest that these effects are, in fact, likely to be Ah-mediated. An investigation of background dioxin exposure and tooth development was done in

Finnish children as a result of studies of dental effects in dioxin-exposed rats, mice, and nonhuman primates and in PCB-exposed children. The Finnish investigators examined enamel hypomineralization of permanent first molars in 6- and 7-year-old children. The length of time that infants breast fed was not significantly associated with either mineralization changes or with TEQ levels in the breast milk. However, when the levels and length of breast feeding were combined in an overall score, a statistically significant association was observed.

In addition, effects have been seen in cases where significantly elevated exposure occurred. The incidents at Yusho and Yu-Cheng resulted in increased perinatal mortality and low birth weight in infants born to women who had been exposed. Rocker bottom heal was observed in Yusho infants, and functional abnormalities have been reported in Yu-Cheng children. The similarity of effects observed in human infants prenatally exposed to the complex mixture in Yusho and Yu-Cheng and those reported in adult monkeys exposed perinatally to only TCDD suggests that at least some of the effects on children are due to the TCDD-like congeners in the contaminated rice oil ingested by the mothers of these children. The similar responses include a clustering of effects in organs derived from the ectodermal germ layer, referred to as ectodermal dysplasia, including effects on the skin, nails, and Meibomian glands, and developmental and psychomotor delay during developmental and cognitive tests.

Some investigators believe that because all of the effects in the Yusho and Yu-Cheng cohorts do not correlate with TEQ, some of the effects are due exclusively to nondioxin-like PCBs or to a combination of all the congeners. In addition, on the basis of these data, the extent of the association between overt maternal toxicity and embryo/fetal toxicity in humans is still not clear. Further studies in the offspring as well as follow-up of the Seveso incident may shed further light on this issue. In addition to the chloracne and acute responses to TCDD exposure seen in Seveso children, elevated levels of serum GGT have been observed within a year after exposure in some of the more highly exposed Seveso children. Long-term pathologic consequences of elevated GGT have not been illustrated by excess mortality from liver disorders or cancer or in excess morbidity, but further follow-up is needed. It must be recognized that the absence of an effect thus far does not obviate the possibility that the enzyme levels increased concurrently with the exposure but declined after cessation. The apparently transient elevations in ALT levels among the Seveso children suggest that hepatic enzyme levels other than GGT may react in this manner to TCDD exposure. Recent studies in Seveso have also demonstrated an altered sex ratio in the second generation (Mocarelli et al., 2000).

Impacts on thyroid hormones provide an example of an effect of elevated postnatal exposure to dioxin and related compounds. Several studies of nursing infants suggest that

ingestion of breast milk that has a higher dioxin TEQ may alter thyroid function. Thyroid hormones play important roles in the developing nervous system of all vertebrate species, including humans. In the United States, all infants are tested for hypothyroidism shortly after birth. Results from the studies mentioned above suggest a possible shift in the population distribution of thyroid hormone levels, particularly T4, and point out the need for collection of longitudinal data to assess the potential for long-term effects associated with developmental exposures.

A large number of studies in animals, including studies of single congeners and exposures to complex mixtures, have addressed the question of effects of dioxin-like chemicals after in utero or lactational exposure. However, the vast majority of the data are derived from studies of 2,3,7,8-TCDD, single congeners (e.g., PCB 77), or commercial mixtures of PCBs. Exposure patterns have included single doses to the dams as well as dosing on multiple days during gestation beginning as early as the first day of gestation. These studies are discussed in detail in Part II, Chapter 5. The observed toxic effects include developmental toxicity, neurobehavioral and neurochemical alterations, endocrine effects, and developmental immunotoxicity. For instance, results of this body of work suggest that 2,3,7,8-TCDD clearly has the potential to produce alterations in male reproductive function (rats, mice, hamsters), male sexual behavior (rats), and female genitalia (rats, hamsters) after prenatal exposure. In addition, impacts on neuromotor and cognitive behavior as well as on development of the immune system have been indicated in a number of studies.

No epidemiological data and limited animal data are available to address the question of the potential impact of exposure to dioxin-like compounds on childhood cancers or on cancers of later life. The direct impacts of increased early postnatal exposure on the carcinogenic process may be small, noting the limited impact of nursing on total body burden (see the discussion of breast milk exposures and body burdens below), the assumption that cancer risk is a function of average lifetime body burden, and the possibility that, because dioxin is a potent cancer promoter rather than a direct initiator of the cancer process, exposures later in life might be more important than those received earlier. However, recent studies of Brown et al. (1998) suggest that prenatal exposure of rats to dioxin and related compounds may indirectly enhance their sensitivity as adults to chemical carcinogenesis from other chemical carcinogens. Further work is needed to evaluate this issue.

Fetuses, infants, and children are exposed to dioxins through several routes. The fetus is exposed in utero to levels of dioxin and related compounds that reflect the body burden of the mother. It is important to recognize that the greatest impact on the mother's body burden is from

of her lifetime exposure history rather than from the individual meals she eats during pregnancy. Good nutrition, including a diet with appropriate levels of fat, has consequences on dietary intake and consequent body burdens of dioxin and related compounds. Nursing infants represent special cases because for a limited portion of their lives they may have elevated exposures on a body-weight basis when compared with non-nursing infants and with adults (see discussion below).

In addition to breast milk exposures, intakes of CDD/CDFs and dioxin-like PCBs are more than three times higher for a young child than for an adult, on a body-weight basis. Table 4-7 in Section 4 of this document describes the variability in average intake values as a function of age using age-specific food consumption rates and average food concentrations, as was done for adult intake estimates. However, as with the nursing infants, the differences in body burden between children and adults are expected to be much less than the differences in daily intake. Assuming that body burden is the relevant dose metric for most if not all effects, there is some assurance that these short-term increased intake levels will have limited additional impact on risk as compared with overall lifetime exposure.

Background exposures to dioxin and related compounds need to be considered when evaluating both hazard and risk.

The term "background exposure" has been used throughout this reassessment to describe exposure of the general population to environmental media (food, air, soil, etc.) that have dioxin concentrations within the normal background range. Adult daily intakes of CDD/CDFs and dioxin-like PCBs are estimated to average 43 and 23 pg TEQ_{DFP}-WHO_{98}/day, respectively, for a total intake of 66 pg/day TEQ_{DFP}-WHO_{98}. On a body-weight basis, this corresponds to approximately 1 pg TEQ_{DFP}-WHO_{98}/kg-day. Daily intake is estimated by combining exposure media concentrations (food, soil, air) with contact rates (ingestion, inhalation). Table 4-6 summarizes the intake rates derived by this method. The intake estimate is supported by an extensive database on food consumption rates and food data. Pharmacokinetic modeling provides further support for the intake estimates. Current adult tissue levels reflect intakes from past exposure levels, which are thought to be higher than current levels.

CDD/CDF and dioxin-like PCB intakes for the general population may extend to levels at least three times higher than the mean. Variability in general population exposure is primarily a result of differences in the dietary choices that individuals make in terms of both quantity and types of food consumed. A diet that is disproportionately high in animal fats will result in an increased background exposure over the mean. Data on the variability of fat consumption

indicate that the 95th percentile is about twice the mean and the 99th percentile is approximately three times the mean. Additionally, a diet that substitutes meat sources that are low in dioxin (e.g., beef, pork, or poultry) with sources that are high in dioxin (e.g., freshwater fish) could result in elevated exposures.

Evidence of widespread background exposure can also be seen by examining data on human tissue. These data indicate that the average CDD/CDF tissue level for the general adult U.S. population appears to be declining. A pharmacokinetic modeling evaluation of this declining trend suggests that the CDD/CDF tissue level will drop below 10 ppt TEQ_{DF}-WHO_{98}, lipid basis, by 2030 (Lorber, 2002). The best estimate of current (mid to late 1990s) levels is 25 ppt (TEQ_{DFP}-WHO_{98}, lipid basis). The tissue samples collected in North America in the late 1980s and early 1990s showed an average TEQ_{DFP}-WHO_{98} level of about 55 pg/g lipid. This finding is supported by a number of studies, all conducted in North America, that measured dioxin levels in adipose tissue, blood, and human milk. However, the number of people in most of these studies is relatively small, and the participants were not statistically selected in ways that ensured their representativeness of the general U.S. adult population. One study, the 1987 National Human Adipose Tissue Survey (NHATS), involved more than 800 individuals and provided broad geographic coverage, but it did not address coplanar PCBs. Similar tissue levels of these compounds were measured in Europe and Japan during similar time periods.

Because dioxin levels in the environment have been declining since the 1970s, it is reasonable to expect that levels in food, human intake, and, ultimately, human tissue have also declined over this period. The changes in tissue levels are likely to lag the decline seen in environmental levels, and the changes in tissue levels cannot be assumed to occur proportionally with declines in environmental levels. CDC (2000) summarized levels of CDDs, CDFs, and PCBs in human blood collected between 1995 and 1997. The individuals sampled were all U.S. residents who had no known exposures to dioxin other than normal background. The blood was collected in six different locations from 316 individuals ranging in age from 20 to 70 years. All TEQ calculations were made assuming that nondetects were equal to half the detection limit. Although these samples were not collected in a manner that can be considered statistically representative of the national population and they lack wide geographic coverage, they are judged to provide a better indication of current tissue levels in the United States than the earlier data (see Table 4-5).

PCBs 105, 118, and 156 are missing from the blood data for the comparison populations reported by CDC (2000). These congeners account for 62% of the total PCB TEQ estimated in the early 1990s. Assuming that the missing congeners from the CDC study data contribute the

same proportion to the total PCB TEQ as in earlier data, they would increase the estimate of current body burdens by another 3.3 pg TEQ/g lipid, for a total PCB TEQ of 5.3 pg/g lipid and a total TEQ_{DFP}-WHO_{98} of 25.4 pg/g lipid.

As noted, characterizing national background levels of dioxins in tissues is uncertain because the current data cannot be considered statistically representative of the general population. The task is also complicated by the fact that tissue levels are a function of both age and birth year. Because intake levels have varied over time, the accumulation of dioxins in a person who turned 50 in 1990 is different from that in a person who turned 50 in 2000. Future surveys should help to characterize national levels of CDD/CDF/PCBs during the last years of the 20th century and into the 21st century. The National Health and Nutrition Examination Survey (NHANES) conducted in 1999-2000 included measurements of dioxin blood levels in 1921 individuals, aged 12 and higher, from numerous locations around the country (CDC, 2003). Unfortunately, not enough blood serum was available per individual to be able to quantify the dioxin concentrations at low background levels, so the majority of measurements were nondetects. An effort is currently underway to pool remaining NHANES 1999-2000 samples and reanalyze them. This will allow for an estimate of average background body burdens of dioxin-like compounds representative of the turn of the century, and in future years should provide a picture of dioxin levels in the general U.S. population.

As described above, current intake levels from food sources are estimated in this reassessment to be approximately 1 pg TEQ/kg body weight/day. Certain segments of the population may be exposed to additional increments of exposure by being in proximity to point sources or because of dietary practices. These types of exposure are described below.

Evaluating the exposure of "special" populations and developmental stages is critical to risk characterization.

As discussed above, background exposures to dioxin-like compounds may extend to levels at least three times higher than the mean. This upper range is assumed to result from the normal variability of diet and human behaviors. Exposures from local elevated sources or unique diets would be added to this background variability. Elevated exposures may occur in small segments of the population, such as individuals living near discrete local sources or subsistence or recreational fishers. Nursing infants represent a special case. For a limited portion of their lives, they may have elevated exposures on a body-weight basis when compared to non-nursing infants and to adults. This exposure will be discussed in a separate section.

Dioxin contamination incidents involving the commercial food supply have occurred in the United States and other countries. For example, in the United States, contaminated ball clay was used as an anticaking agent in soybean meal, resulting in elevated dioxin levels in some poultry and catfish. This incident involved only a small fraction of national poultry production and the practice has since been eliminated. Elevated dioxin levels have also been observed in a few beef and dairy animals, where the contamination was associated with contact with pentachlorophenol-treated wood. This type of elevated exposure was not detected in the national beef survey; consequently, its occurrence is likely to be low, although it has not been determined.

These incidents may have led to small increases in dioxin exposure to the general population; however, it is unlikely that they have led to disproportionate exposures to populations living near where they occurred because, in the United States, meat and dairy products are highly distributed on a national scale. If contamination events were to occur in foods that are predominantly distributed on a local or regional scale, then such events could lead to higher exposure among local populations.

Elevated exposures associated with the workplace or with industrial accidents have also been documented. U.S. workers in certain segments of the chemical industry had elevated levels of TCDD exposure, with some tissue measurements in the thousands of parts per trillion TCDD. There is no clear evidence that elevated exposures are currently occurring among U.S. workers. Documented examples of past exposures for other groups include certain Air Force personnel exposed to Agent Orange during the Vietnam War and individuals exposed as a result of industrial accidents in Europe and Asia.

The discussion in Section 4.5 identified the general population distribution of exposure as extending up to roughly three times the mean. Most people will have exposures within this range even if they have unusual diets in terms of meat and dairy products because most people eat food from multiple sources, which tends to average out the contamination levels, and meat and dairy products have similar dioxin levels, so substitution of one type of meat for another should not have a great impact on total exposure. Clearly elevated exposures are possible in unusual situations where an individual consumes high quantities of meat or dairy products that have significantly increased dioxin levels. Elevated exposures resulting from fish consumption can occur in different situations because concentrations in freshwater fish are significantly greater than in meat and dairy products. Therefore, people who consume large quantities of freshwater fish at background contamination levels may have intakes elevated above the general population distribution.

Consumption of fish, meat, or dairy products containing elevated levels of dioxins and dioxin-like PCBs can lead to elevated exposures in comparison to the general population. Most people eat some fish from multiple sources, both fresh and salt water. If individuals obtain their fish from areas where the concentration of dioxin-like chemicals is elevated, they may constitute a highly exposed subpopulation. Although this scenario seems reasonable, very little supporting data could be found for such a highly exposed subpopulation in the United States. One study that measured dioxin-like compounds in blood of sports fishers in the Great Lakes area showed elevations over mean background but within the range of normal variability.

Another study that measured 90 PCB congeners—of which 7 were dioxin-like mono-ortho PCBs (although PCB 126 was not measured)—in Lake Michigan "sport-fish eaters" showed a significant elevation in these PCBs versus a control group (little or no sport fish consumption). Significantly elevated concentrations of dioxins, furans, and coplanar PCBs were measured in Great Lakes fish by the Ontario Ministry of the Environment, although this study was conducted in known or suspected hot spots for the purpose of setting consumption advisories. It is not known to what extent individuals would be consuming fish at the high concentrations measured. Elevated CDD/CDF levels in human blood have been measured in Baltic fishermen. Similarly, elevated levels of coplanar PCBs have been measured in the blood of fishers on the north shore of the Gulf of the St. Lawrence River who consume large amounts of seafood.

High exposures to dioxin-like chemicals as a result of consuming meat and dairy products would most likely occur in situations where individuals consume large quantities of these foods and the level of these compounds is elevated. Most people eat meat and dairy products from multiple sources, and even if large quantities are consumed, unusually high exposures are not likely. Individuals who raise their own livestock for basic subsistence have the potential for higher exposures if local levels of dioxin-like compounds are high. One study in the United States showed elevated levels in chicken eggs near a contaminated soil site. European studies at several sites have shown elevated CDD/CDF levels in milk and other animal products near combustion sources.

In summary, in addition to general population exposure, some individuals or groups of individuals may also be exposed to dioxin-like compounds from local discrete sources or pathways within their environment. Examples of these "special" exposures include contamination incidents, occupational exposures, direct or indirect exposure to local populations from discrete sources, or exposures to subsistence or recreational fishers.

Breast-feeding infants have higher intakes of dioxin and related compounds for a short but developmentally important part of their lives; however, the benefits of breast feeding are widely recognized to outweigh the risks.

Three studies have compared dioxins in infants who were breast fed with those who were formula fed, and all have shown elevations in the concentrations of dioxins in infants being breast fed. Formula-fed infants had lipid-based concentrations < 5 ppt TEQ_{DF}-WHO_{98}, whereas breast-fed infants had average lipid-based concentrations > 20 ppt TEQ_{DF}-WHO_{98}. A similar disparity is seen in more limited data on dioxin-like PCBs.

The dose to the infant varies as a function of infant body weight, the concentration of dioxins in the mother's milk, and the trend of dioxins in the mother's milk to decline over time. Using typical values for these parameters, dioxin intakes at birth were estimated to equal 242 pg TEQ_{DFP}-WHO_{98}/kg/day, which would drop to 18 pg TEQ_{DFP}-WHO_{98}/kg/day after 12 months. The average infant dose over a year was calculated to be 87 pg TEQ_{DFP}-WHO_{98}/kg/day. Although this dose exceeds the currently estimated adult dose of 1 pg TEQ_{DFP}-WHO_{98}/kg/day, the effect on infant body burdens is expected to be less dramatic, that is, infant body burdens will not exceed adult body burdens by 87 times. This is due to the rapidly expanding infant body weight and lipid volume, the decrease in concentration of dioxins in the mother's milk over time, and more rapid elimination in infants.

A pharmacokinetic exercise comparing 6-month, 1-year, and 2-year nursing scenarios with formula feeding showed peak infant lipid concentrations of 44 ppt TEQ_{DFP}-WHO_{98} at 9 weeks of age, compared with peak lipid concentrations of less than 10 ppt for the formula-fed infants and average adult lipid concentrations of 25 ppt TEQ_{DFP}-WHO_{98}. The dioxin concentrations in breast-fed and formula-fed children were predicted to merge at about 10 years of age, at a lipid concentration of about 13 ppt TEQ_{DFP}-WHO_{98}. Breast feeding for 1 year was predicted to result in a lifetime accumulated exposure about 13% higher as compared to formula feeding only.

The American Academy of Pediatrics (1997) has made a compelling argument for the diverse advantages of breast feeding for infants, mother, families, and society. These include health, nutritional, immunologic, developmental, psychological, social, economic, and environmental benefits. Breast milk is the point of comparison for all infant food, and the breast-fed infant is the reference for evaluation of all alternative feeding methods. In addition, increasing the rates of breast-feeding initiation is a national health objective and one of the goals of the United States Government's Healthy People 2010. WHO (1988) maintained that the

evidence did not support an alteration of its recommendations that promote and support breast feeding. A more recent consultation in 1998 (WHO, 2000) reiterated these conclusions.

Although it is important that the recommendations of these groups continue to be reevaluated in light of emerging scientific information, the Agency does not believe that the findings contained in this reassessment provide a scientific basis for initiating such a reevaluation. This conclusion is based on the fact that stronger data have been presented that body burden, not intake, is the best dose metric; that many of the noncancer effects, particularly those seen in children, are more strongly associated with prenatal exposure and the mother's body burden than with postnatal exposures and breast milk levels; and that dioxin-like compounds are strong promoters of carcinogenicity, a mode of action that depends on late-stage impacts rather than on early-stage impacts on the carcinogenic process.

Many dioxin sources have been identified and emissions to the environment are being reduced.

Current emissions of CDDs/CDFs/PCBs to the United States environment result principally from anthropogenic activities. Evidence for this finding includes matches in time of the rise of environmental levels with the rise in general industrial activity (see discussion in Section 4.1), lack of any identified large natural sources, and observations of higher CDD/CDF/PCB body burdens in industrialized versus less industrialized countries (see discussion on human tissue levels in Section 4.4).

The principal identified sources of environmental releases are (1) combustion and incineration sources; (2) chemical manufacturing/processing sources; (3) industrial/municipal processes; (4) biological and photochemical processes; and (5) reservoir sources. Development of national estimates of annual environmental releases to air, water, and land is complicated by the fact that only a few facilities in most industrial sectors have been evaluated for CDD/CDF emissions. Thus, an extrapolation is needed to estimate national emissions. The extrapolation method involves deriving an estimate of emissions per unit of activity (i.e., an emission factor) at the tested facilities and multiplying this by the total activity level in the untested facilities.

In order to convey the level of uncertainty in both the measure of activity and the emission factor, EPA developed a qualitative confidence rating scheme. The confidence rating scheme, presented in Section 4, Table 4-1, uses qualitative criteria to assign a high, medium, or low confidence rating to the emission factor and activity level for those source categories for which emission estimates can be reliably quantified. The dioxin reassessment has produced an inventory of source releases for the United States (Table 4-2). The inventory is limited to

sources whose releases can be reliably quantified (i.e., those with confidence ratings of A, B, or C, as defined in Table 4-1). The inventory presents the environmental releases in terms of two reference years: 1987 and 1995. For both of these periods, emissions from combustion and incineration sources dominated total releases. EPA's best estimates of releases of CDD/CDFs to air, water, and land from reasonably quantifiable sources were approximately 3300 g (7 pounds) TEQ_{DF}-WHO_{98} in 1995 and 14,000 g (31 pounds) TEQ_{DF}-WHO_{98} in 1987. The decrease in estimated releases of CDD/CDFs between 1987 and 1995 (approximately 76%) was due primarily to reductions in air emissions from municipal and medical waste incinerators.

Although this inventory is one of the most comprehensive and well-documented in the world, it is likely to underestimate total releases because a number of known sources lacked sufficient data to be included in the inventory and the possibility remains that truly unknown sources exist.

Further reductions in environmental releases since the inventory for 1995 can be anticipated as a result of EPA regulations for waste combustion sources and pulp and paper facilities. EPA's regulatory programs estimate that, under full compliance with these regulations, an additional 1800 g I-TEQ reduction in CDD/CDF emissions should occur. With these anticipated emission reductions, uncontrolled burning of household waste would become the largest quantifiable source. Although the full magnitude of reservoir releases remains uncertain, their relative contribution to total annual releases be can reasonably anticipated to increase as contemporary formation sources continue to decrease.

No significant release of newly formed dioxin-like PCBs is occurring in the United States. Unlike CDD/CDFs, PCBs were intentionally manufactured in the United States in large quantities from 1929 until production was banned in 1977. Although it has been demonstrated that small quantities of coplanar PCBs can be produced during waste combustion, no strong evidence exists that the dioxin-like PCBs make a significant contribution to TEQ releases during combustion. The occurrences of dioxin-like PCBs in the U.S. environment most likely reflect past releases associated with PCB production, use, and disposal. Further support for this finding is based on observations of reductions since the 1980s in PCBs in Great Lakes sediment and in other areas.

As described in Section 4.1, combustion appears to be the most significant process of CDD/CDF formation today. Important factors that can affect the rate of dioxin formation include overall combustion efficiency, post-combustion flue gas temperatures and residence times, and the availability of surface catalytic sites to support dioxin synthesis. Although chlorine is an essential component for the formation of CDDs/CDFs in combustion systems, the empirical

evidence indicates that, for commercial-scale incinerators, chlorine levels in feed are not the dominant controlling factor for rates of CDD/CDF stack emissions. The conclusion that chlorine in feed is not a strong determinant of dioxin emissions applies to the overall population of commercial scale combustors. For any individual commercial-scale combustor, circumstances may exist in which changes in chlorine content of feed could affect dioxin emissions. For uncontrolled combustion, such as open burning of household waste, chlorine content of wastes may play a more significant role than commercial-scale combustors in levels of dioxin emissions.

Dioxins are widely distributed in the environment at low concentrations, primarily as a result of air transport and deposition.

The dioxin-like compounds are essentially insoluble in water, they are generally classified as semivolatile, and they tend to bioaccumulate in animals. Once introduced into the environment, they are widely distributed in the environment as a result of a number of physical and biological processes. There is some evidence that these compounds can degrade in the environment, but in general they are considered very persistent and relatively immobile in soils and sediments.

The dioxin-like compounds are transported through the atmosphere as vapors or attached to airborne particulates and they can be deposited on soils, plants, or other surfaces (by wet or dry deposition).

They enter water bodies primarily via direct deposition from the atmosphere or by surface runoff and erosion. From soils, these compounds can reenter the atmosphere as resuspended soil particles or as vapors. In water, they can be resuspended into the water column from sediments, volatilized out of the surface waters into the atmosphere, or buried in deeper sediments. Immobile sediments appear to serve as permanent sinks for the dioxin-like compounds. Anthropogenic materials (such as pentachlorophenol), although not always considered an environmental compartment, may also contain these compounds, and they have the potential to be released from these materials into the broader environment.

The two primary pathways by which dioxin-like compounds enter the ecological food chains and human diet are air to plant to animal and water/sediment to fish. Vegetation receives these compounds via atmospheric deposition in the vapor and particle phases. The compounds are retained on plant surfaces and bioaccumulated in the fatty tissues of animals that feed on these plants. In the aquatic food chain, dioxins enter water systems via direct discharge or deposition and runoff from watersheds. Fish accumulate these compounds through direct contact

with water, suspended particles, and bottom sediments and through the consumption of aquatic organisms.

Although these two pathways are thought to normally dominate contribution to the commercial food supply, others can also be important. Animal feed contamination episodes have led to elevations of dioxins in poultry in the United States, in milk in Germany, and in meat/dairy products in Belgium. Gaining a quantitative understanding of how dioxin moves in the environment will be particularly important in understanding the relative contributions of individual point sources to the food chain and assessing the effectiveness of control strategies to reduce human exposure. Although the emissions inventory shows the relative contribution of various sources to total emissions, it is unlikely that these sources make the same relative contributions to human exposure.

It is quite possible that the major contributors of dioxin to food may not be those sources that represent the largest fractions of total emissions in the United States (see discussion in Section 4.4 indicating that the diet is the dominant exposure pathway for humans). The geographic locations of sources relative to the areas from which much of the beef, pork, milk, and fish are produced should be considered. Most of the agricultural areas that produce dietary animal fats are not located near or directly downwind of the major sources of dioxin and related compounds.

The contribution of reservoir sources to human exposure is likely to be significant. Several factors support this finding. First, human exposure to the dioxin-like PCBs is thought to be derived almost completely from reservoir sources. Because approximately one-third of general population TEQ intake is due to PCBs, then at least one-third of the calculated overall risk from dioxin-like compounds comes from reservoir sources. Second, CDD/CDF releases from soil via soil erosion and runoff to waterways appear to be greater than releases to water from the primary sources included in the inventory. CDD/CDFs in waterways can bioaccumulate in fish, leading to human exposure via consumption of fish. This suggests that a significant portion of the CDD/CDF TEQ exposure could be due to releases from the soil reservoir. Finally, soil reservoirs could have vapor and particulate releases that deposit on plants and enter the terrestrial food chain. However, the magnitude of this contribution is unknown. Collectively, these three factors suggest that reservoirs are a significant source of current background TEQ exposure, perhaps contributing half or more of the total.

Environmental levels, emissions, and human exposures have declined during recent decades.

The most compelling supportive evidence of a general decline in environmental levels for CDD/CDF/PCBs comes from dated sediment core studies. CDD/CDF/PCB concentrations in sediments began to increase around the 1930s and continued to increase until about 1970. Decreases began in 1970 and have continued to the time of the most recent sediment samples (about 1990). Sediment studies in lakes located in several European countries have shown similar trends.

It is reasonable to assume that sediment core trends are driven by a similar trend in emissions to the environment. The period of increase generally matches the time when a variety of industrial activities began rising, and the period of decline appears to correspond with growth in pollution abatement. Decreases in dioxin emissions will presumably have resulted from many of these abatement efforts, which included elimination of most open burning, particulate controls on combustors, phase-out of leaded gas, and bans on PCBs, 2,4,5-T, hexachlorophene and restrictions on the use of pentachlorophenol. Also, the national source inventory of this assessment documented a significant decline in emissions from the late 1980s to the mid-1990s.

Evidence of declines in human exposure can be inferred from the overall declines in environmental levels and emissions, and it is directly supported by limited data on concentrations in food and human tissues (see Sections 4.3 and 4.4). Because of the lag between environmental levels and body burdens, it is anticipated that further declines in tissue concentrations should occur as individuals with higher body burdens from past exposure age out of the population. A pharmacokinetic modeling exercise suggested that levels of TEQ_{DF}-WHO_{98} in the U.S. population should decline from levels of about 20 ppt lipid-basis measured in the mid-1990s CDC study to below 10 ppt lipid-basis by 2030. This analysis includes CDD/CDFs only, not PCBs. Dioxin-like PCBs currently make up approximately 20% of the current total TEQ body burden but may increase in percentage as CDD/CDFs decline. This modeling result is based on the assumption that current CDD/CDF intakes remain the same into the 21st century.

Risk Characterization Summary Statement

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD; "dioxin") is highly toxic to many animal species, producing a variety of noncancer and cancer effects. Other 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins and dibenzofurans and coplanar polychlorinated biphenyls (PCBs) exhibit similar effects, albeit at different doses and with different degrees of confidence in the database.

The similarities in toxicity between species and across different dioxin congeners stem from a common mode of action via initial binding to the aryl hydrocarbon (AhR) receptor. This common mode of action is supported by the consistency in effects evident from multiple congener databases, although uncertainty remains due to data gaps for some congeners. The databases supportive of dioxin-like toxicity, both cancer and noncancer, are strongest for those congeners that are the major contributors to the risk to human populations. This has led to an international scientific consensus that it is prudent science policy to use the concept of toxic equivalency factors (TEFs) to sum the contributions of individual PCDD, PCDF, and coplanar PCB congeners with dioxin-like activity.

In addressing receptor-mediated responses resulting from complex mixtures of dioxin-like congeners, this assessment has provided a basis for the use of integrated measures of dose such as lifetime average body burden as more appropriate default metrics than average lifetime daily intake. Although average body burden over a lifetime appears to be the most useful dose metric for chronic effects, average body burden during the window of sensitivity may be the most appropriate metric for developmental effects. The Agency recognizes, therefore, that the final choice of the appropriate metric may depend on the endpoint under evaluation.

Dioxin and related compounds have been shown to be developmental, reproductive, immunological, endocrinological, and cancer hazards, among others in multiple animal species. There is no reason to expect, in general, that humans would not be similarly affected at some dose, and indeed, a growing body of data supports this assumption. On the basis of the animal data, current margins of exposure are lower than generally considered acceptable, especially for more highly exposed human populations. The human database supporting this concern for potential effects near background body burdens is less certain. Occupational and industrial accident cohorts exposed at higher levels show correlations with exposure for cancer and a number of noncancer effects consistent with those seen in the animal studies.

For cancer outcomes, the epidemiological evidence provides consistent findings of statistically significant elevations, with dose-response trends for all cancers combined and lung cancer risk in occupational cohorts along with evidence of possible additional tissue-specific cancer rate elevations. Given this substantial yet still not definitive epidemiological data, the positive cancer bioassays at multiple sites and in all animal species tested, in vitro studies, and the mechanistic considerations common to animals and humans for dioxin carcinogenicity, EPA characterizes 2,3,7,8-tetrachlorodibenzo-*p*-dioxin as "carcinogenic to humans." On the basis of similarities of response in multiple positive animal bioassays for non-TCDD congeners and mixtures, mode of action studies, and consistent with the concept of toxic equivalency, complex mixtures of dioxin and related compounds are considered highly potent "likely" carcinogens.

The calculated body burdens of dioxin and dioxin-like substances leading to an estimated 1% increase (ED_{01}) in the lifetime risk of cancer in the three occupational studies with the best exposure information fall within a 10-fold range, and those calculated from the animal bioassay data fall in the middle of this range. The ED_{01} for all cancers combined from the three occupational cohorts range from 6 to 62 ngTCDD/kg body weight (excluding the NIOSH power model calculation), depending on the study and the model used. By comparison, current background body burdens in the United States are approximately 5 ngTEQ/kg body weight, suggesting little margin of exposure (MOE) at today's body burden levels.

From these same occupational and animal cancer studies, EPA estimates an upper bound on the lifetime risk of all cancers combined of 1×10^{-3} per pgTEQ/kg/day. This cancer slope factor is based on a statistical estimate of risks from occupational exposures—principally to healthy, adult, male workers—and it must be coupled with a recognition that a small number of people may be both more susceptible and consume up to three times the average level of fat per day (the principal exposure pathway for dioxins in the general population). Conversely, this risk estimate is based on assumptions that the extra cancer risk seen in the occupational cohorts is attributable to dioxin and not other chemical agents present; that the appropriate metric for cancer risk is lifetime average body burden and not a measure of peak exposure, which would tend to mitigate risks at low exposures; and that the dose-response model curve continues below the range of statistically significant data and does not then exhibit some nonlinearity. Using the best available estimates of cancer risks, the upper bound on general population lifetime risk for all cancers might be on the order of 1 in 1000 or more. Upper-bound risk estimates allow the calculation of the high end of the probability of cancer risk in the population. This means that there is greater than a 95% chance that cancer risks will be less than the upper bound, and it could be as low as zero in some individuals.

For noncancer effects, EPA generally calculates an RfD/RfC value that represents an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. RfD/RfCs are generally calculated by estimating a point of departure dose just below the lower end of the range of observed adverse effects, and dividing this by uncertainty factors to account for extrapolation issues and database deficits. Applying these standard procedures to the data reviewed in this assessment would result in an RfD/RfC below the current estimated average dose to the U.S. population (~1 pgTEQ/kg/day), and would, therefore, be uninformative for a safety assessment.

EPA has chosen instead to characterize the MOEs for noncancer endpoints in order to better inform risk management decisions. The MOE is the ratio of the effect level in the

comparison species (ED_{01} or low effect level; animal or human) to the human body burden. For the most sensitive endpoints identified, MOEs range from, for example, less than 1 for enzyme induction in mice and rats, < 4 for developmental effects, and 4 for endometriosis in non-human primates. In evaluating MOEs, consideration should be given to uncertainties in distinguishing between adaptive biochemical changes and adverse effects, both on an individual level and as these changes impact whole populations. The risks from dioxin and related compounds may be greater for children than for adults, but more data are needed to fully address this issue.

Releases of dioxins to the environment from characterized sources have decreased significantly over the last decade and are expected to continue to decrease. Other sources are still poorly characterized, and an environmental reservoir of dioxins from both man-made and natural sources has been recognized. Human body burdens have also declined and are anticipated to be further reduced as additional, recently implemented, dioxin emission controls impact environmental and food levels and, ultimately, human exposure, although the relationship with reservoir sources remains uncertain.

APPENDIX A

Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake

Animal	Endpoint	Study	Estimated body burden (ng/kg)			Human equiv.[a] intakes (pg/kg/day)
			LOAEL	NOAEL	ED01	
Rats	Cancer	Kociba et al. (1978)[1]	180	18	32	60; 6; 11
Rhesus monkeys	Fetal Mortality	Bowman et al. (1989)[2]	90	21	NC	30; 7
	Developmental Neurotoxicity	Schantz et al. (1992)[3]	21	–	NC	7
	Endometriosis	Rier et al. (1993)[4]	21	–	NC	7
Rats	Reproductive Tox. (multigenerational)	Murray et al. (1979)[5]	180	18	NC	60; 6
Rats	Developmental/ Reproductive Toxicity	Mably et al. (1992a, b, c)[6]	38	–	0.34	13; 0.1
		Gray et al. (1997)[7]	30	–	0.08	10; 0.03
		Faqi et al. (1998)[8]	25	–	0.6	8; 0.2
		Ohsako et al. (2001)[9]	30	8	NC	10; 3
Rats	Developmental Immunotoxicity	Gehrs and Smialowicz (1999)[10]	60	–	NC	20
Rats	Developmental Neurotoxicity	Markowski et al. (2001)[11]	108	36[b]	0.7	36; 12; 0.2
Mice	Immunological Effects (adult)	Burleson et al. (1996)[12]	6	3	NC	2; 1
		Smialowicz et al. (1994)[13]	300	–	2.9	100; 1
		Narasimhan et al. (1994)[14]	100	50[b]	1.5	33; 17; 0.5
		Vecchi et al. (1983)[15]	1200	–	7	401; 2
Rats	Thyroid Effects	Sewall et al. (1995)[16]	76	22	26	25; 7; 8
Mice	CYP1A1/1A2 Enzyme Induction	DeVito et al. (1994)[17]	24	–	22	8; 7
		Diliberto et al. (2001)[18]	2.8	–	67	0.9; 22
		Vogel et al. (1997)[19]	5.1	0.51	0.003	1.6; 0.16; 0.001
		Narasimhan et al (1994)[14]	25	10	3	8; 3; 2; 1
Rats	CYP1A1/1A2 Enzyme Induction	van Birgelen et al. (1995)[20]	243	–	19	81; 6
		Schrenk et al. (1994)[21]	72	–	26	24; 9
		Sewall et al. (1995)[16]	8	2	3.5	3; 0.7; 1
		Walker et al. (1999)[22]	76	–	59	25; 20

Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

[a] Human equivalent intakes were estimated according to the following equation: daily intake (pg/kg/day) = (body burden (ng/kg)*Ln2*1000)/(t½*absorption) where t½ = 2593 days and absorption fraction = 0.8 (Poiger and Schlatter 1986; see Section II). Corresponding human equivalent intake values are arranged in sequence from the previous three columns.

[b] NOAEL values are based on the highest individual dose group in which there are no statistically significant changes. Statistically significant dose response trends plus apparent declines are also evident at all dose levels—20 and 60 ng/kg orally—in all fixed-ratio test groups in Markowski et al. (2001) and in the 50 ng/kg dose group in Narasimhan et al. (1994).

- - = No NOAEL value, as effects seen in the lowest dose group in the study.

NC = Not calculated due to insufficient dose response information (less than three doses and a control) or due to presentation of the data in graphical form without tabulation of mean and variance estimates.

1. **Kociba et al. (1978)**. Increased cancer in female Sprague-Dawley rats exposed for 2 years to TCDD in the food matrix. Statistical LOAEL and NOAEL body burden estimates modeled assuming 50% absorption from the food matrix and a 25-day half-life. Compare to measured lipid levels in the Kociba et al. (1978) rats of 540 and 1700 ppt at 1 and 10 ng/kg/day dose rates and to measured body burdens in the Hurst et al. (2000) subchronic 5/7 day gavage study in female Long-Evans rats of 19 and 120 ng/kg at 1 and 10 ng/kg/day dose rates. ED_{01} calculated for female rat tumors using a multistage formula and EPA Benchmark Dose Software result in an ED_{01} (LED_{01}) of 31.9 (22) ng/kg body burden using Kociba et al. (1978) data and Goodman and Sauer (1992) pathology.

Kociba et al. 1978: Tumors

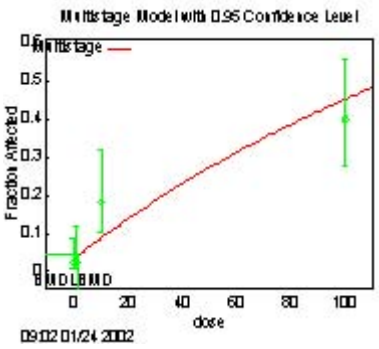
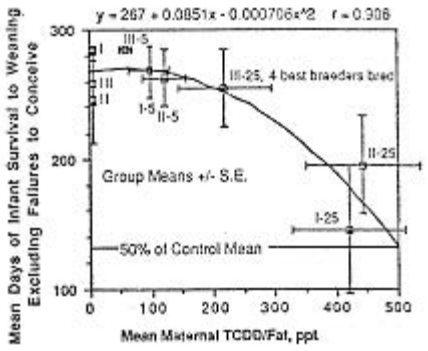


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

2. **Bowman et al. (1989)**. Offspring per cohort significantly reduced at the 25 ppt dose group in cohorts I and II (LOAEL) but not in the 5 ppt dose group (NOAEL; publication Fig. 5 attached). Estimated maternal body burdens are calculated at parturition of the 25 ppt cohort II group for the LOAEL (lowest value of 25 ppt cohorts I and II) and the 5 ppt cohort I for the NOAEL (highest value of 5 ppt cohorts I and II). Maternal TCDD fat levels are estimated according to the empirical formula and data supplied in Bowman et al. (1989; see publication figures 3 and 5): y = 14.9 + 4.29 x (r=0.924), where y=PCDD/fat ppt infant at weaning and x=TCDD/fat ppt mother at parturition. The measured TCDD fat levels in offspring ("y" value) of the 5 ppt cohorts I and II at parturition were 377±141 ppt and 323±70 ppt, respectively, resulting in estimated maternal fat levels at parturition of cohorts I and II of 84 and 72 ppt, respectively. Following the authors' recommendation, the fat level in the 25 ppt dose group is calculated following a 5:1 ratio to the 5 ppt groups, i.e., 420 and 360 ppt for cohorts I and II respectively. Measured maternal data in the 25 ppt dose group at the time of birth of cohort III (488 days post cessation of TCDD dose) were 335±119 ppt (3 non-bred females) and 219±75 ppt (all 7 monkeys) in fat. A 25% body lipid was assumed in converting to human equivalent body burden.

Bowman et al. 1989: Infant Survival



3. **Schantz et al. (1992)**. Increased rough-tumble play (publication Fig. 2 attached), fewer retreats during play bouts, and fewer displacements from preferred positions in the 5 ppt cohort I offspring. Maternal TCDD fat levels are estimated according to the empirical formula and data supplied in Bowman et al. (1989; see Figs. 3 and 5): y = 14.9 + 4.29 x (r=0.924), where y=PCDD/fat ppt infant at weaning and x=TCDD/fat ppt mother at parturition. The measured TCDD fat level in offspring ("y" value) of the 5 ppt cohort I group at parturition was 377±141 ppt, resulting in an estimated maternal fat level at parturition of 5 ppt cohort I of 84 ppt. Fat level converted to body burden by dividing by 4, approximating 25% body fat in a human equivalent comparison.

Schantz et al. 1992: Rough-tumble Play

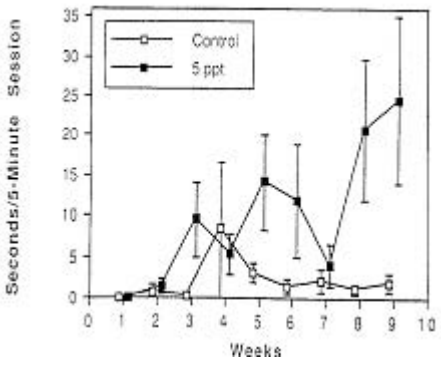
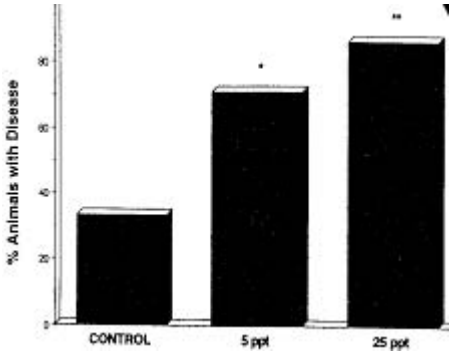


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

4. **Rier et al. (1993)**. Increased incidence, severity and dose-response for rhesus monkeys with endometriosis in the 5 and 25 ppt dose groups (rAFS classification; publication figure 2 attached, * $p<0.17$, ** $p<0.05$). LOAEL (no NOAEL) body burden adopted from the highest maternal fat level calculated according to the formula supplied by Bowman et al. (1989; see footnote 2) of 84 ppt for the 5 ppt dose group occurring at the parturition of cohort I. For comparison, the average of eight measured maternal fat levels at the birth of the 5 ppt cohort III (488 days post cessation of TCDD) was 54±11 ppt fat. A 25% body lipid was assumed in converting to human equivalent body burden.

Rier et al. 1993: Endometriosis



5. **Murray et al. (1979)**. Significant reductions in fertility (graph of publication table 1 data attached), litter size, gestation survival, and neonatal survival and growth in the 10 ng/kg/day food matrix maternal dose group in a three-generation reproduction study in Sprague-Dawley rats. Mathematically estimated body burden of 180 ng/kg at 10 ng/kg/day (half-life = 25 days, 50% absorption from food matrix). Comparison empirical measurements from a similar dose regimen in the related cancer study by Kociba et al. (1978) were 1700 ppt TCDD in lipid in the 10 ng/kg/day dose group, and the measured body burden in Hurst et al. (2000) subchronic 5/7 day gavage study in female Long-Evans rats was 120 ng/kg at the 10 ng/kg/day dose rate. The fertility index in the f_0 generation was so low that further studies with this dose group were discontinued. Thus, the study is essentially two dose levels and a control and was not modeled because of the limited dose response relationship data.

Murray et al. 1979: Rat Fertility Index

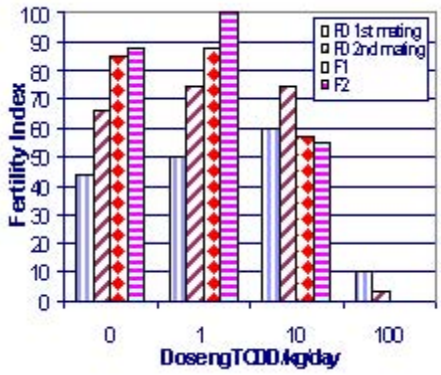
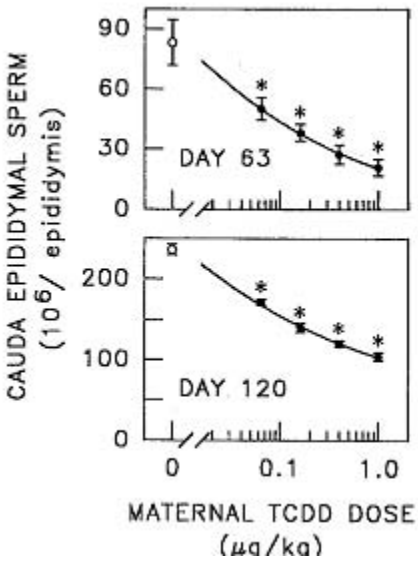


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

6. **Mably et al. (1992a,b,c)**. Decreased daily sperm production (publication Fig. 5 attached), cauda epididymal sperm, epididymis weights and altered sexual behavior in offspring at 64 ng/kg orally to Holtzman rat dams on gestation day 15. LOAEL (no NOAEL) body burden based on Hurst et al. (2000) GD16 body burden fraction of 60% following single GD15 50 ng/kg gavage dose to female Long-Evans rats. ED_{01} value modeled for caudal sperm count of 0.34 ng/kg body burden at day 63 using EPA Benchmark Dose Software Version 1.3, 60% absorption. ED_{01} modeling of Mably et al. (1992) using EPA Benchmark Dose Software Version 1.3 results in a broad range of ED_{01}s, from 0.34 ng/kg for daily sperm production on PND 63 to 461 ng/kg for pinna detachment, with a median value of 3.1 ng/kg for 15 different endpoints.

Mably et al. 1992: Epididymal Sperm



7. **Gray et al. (1997)**. Decrease in ejaculated sperm numbers in male offspring, pooled results from two studies (see publication Fig. 1; results pooled with Gray et al. 1995; attached graph of data from publication text p.15) at 50 ng/kg single dose, day 15 of gestation to female Long-Evans rats. LOAEL (no NOAEL) body burden based on Hurst et al. (2000) GD16 body burden fraction of 60% following single GD15 50 ng/kg gavage dose to female Long-Evans rats. ED_{01} modeling of Gray et al. (1997) using EPA Benchmark Dose Software Version 1.3, 60% absorption, results in a broad range of ED_{01}s from 0.08 ng/kg for epididymal sperm count on D49 to 327 ng/kg for daily sperm production on D49, with a median value of 80 ng/kg for 32 different endpoints.

Gray et al. 1997: Ejaculated Sperm

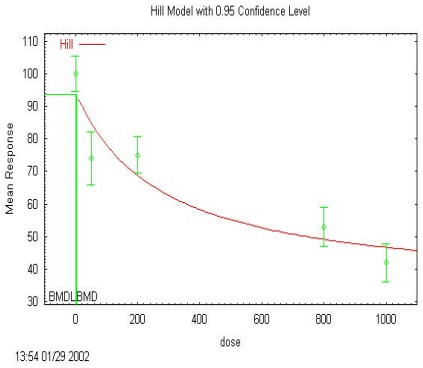
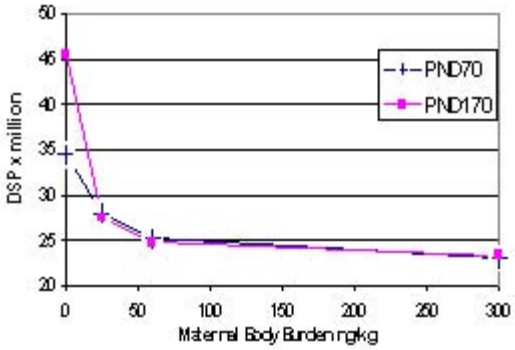


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

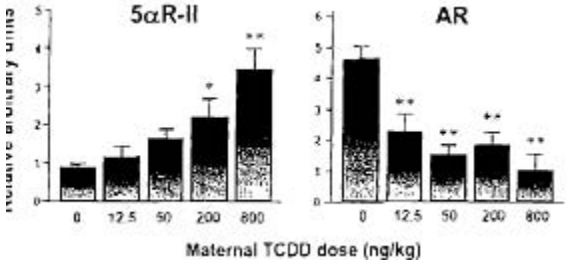
8. **Faqi et al. (1998)**. Decreased daily sperm production (graph attached of data from publication Table 3), cauda epididymus sperm, sperm transit rate, and percent abnormal sperm in offspring of 25/5 ng/kg (loading/weekly maintenance) maternal Wistar rat group. Maintenance dose of 5 ng/kg/week subcutaneous administered to maintain body burden of 25 ng/kg. Additional data on TCDD levels measured in maternal fat at gestation day 21 estimated from publication Figure 1 at 150 ng/kg in 25/5 group. Decreases in cauda epididymal sperm numbers (PND170) and daily sperm production (PND 70 and 170) were observed at all doses. In addition, increases in sperm transit rate and percent abnormal sperm were observed at all dose levels at PND 170. ED_{01} value of 0.6 ng/kg for decreases in daily sperm production, on PND70 and modeled using EPA Benchmark Dose Software Version 1.3.

Faqi et al. 1998; Daily Sperm Production



9. **Ohsako et al. (2001)**. Decreased ano-genital distance in male offspring of Holtzman rat dams receiving 50 ng/kg single dose or greater on gestation day 15 (publication Fig. 7 attached). NOAEL at 12.5 ng/kg single dose. Dose-dependent decreases in androgen receptor mRNA levels in ventral prostate in all dose groups (publication Fig. 8 attached). No changes in daily sperm production or sperm reserve. LOAEL/NOAEL body burdens based on Hurst et al. (2000) gestation day (GD) 16 body burden fraction of 60% following single GD15 50 ng/kg gavage dose to female Long-Evans rats. ED_{01} values for this study were not calculated because the significant data were not presented in tabular format.

Ohsako et al. 2001: Androgen Receptor



Ohsako et al. 2001: Ano-genital Distance

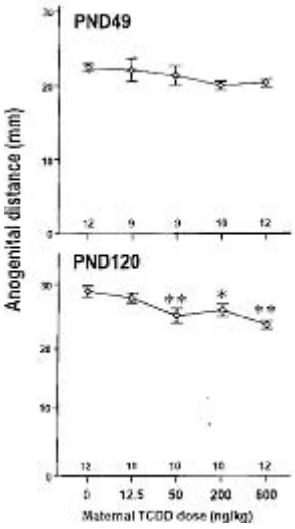
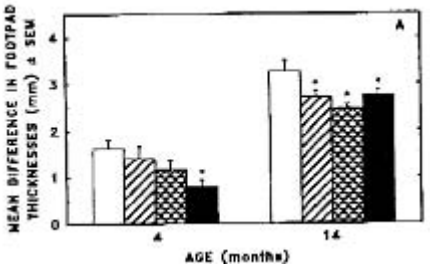


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

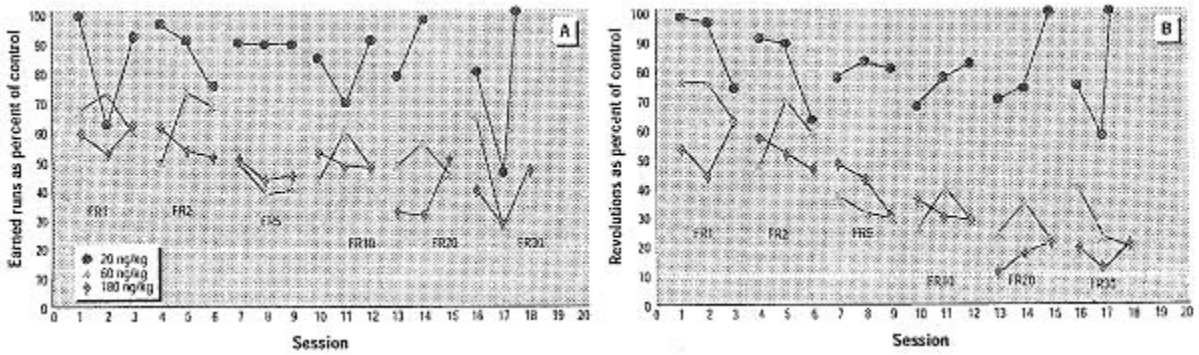
10. **Gehrs and Smialowicz (1999)**. Decreased delayed-type hypersensitivity (DTH; publication Fig. 2a attached; dose units for columns are 0, 100, 300, and 1000 ng/kg) in male offspring following single maternal oral dose of 100 ng/kg on gestation day 14 to F344 rats. LOAEL (no NOAEL) body burden based on Hurst et al. (2000) GD16 body burden fraction of 60% following single GD15 50 ng/kg gavage dose to female Long-Evans rats. Benchmark dose analysis was not performed on this study because the data were presented in graphical format.

Gehrs and Smialowicz 1999: Delayed-Type Hypersensitivity



11 **Markowski et al. (2001)**. Perinatal TCDD exposure produced a significant dose-related reduction in the number of earned opportunities to run, lever response rate, and total number of revolutions in the wheel in offspring of Holtzman rats exposed to single oral TCDD doses on GD18. Statistically significant dose group effects at 180 ng/kg dose (LOAEL). NOAEL at 60 ng/kg dose group, where apparent declines are not statistically significant (see publication Fig. 2 attached; publication Table 3). ED_{01} results modeled by the authors. Table includes result for total wheel revolutions. Body burdens based on 180 and 60 ng/kg single oral doses and Hurst et al. (2000) GD16 body burden fraction of 60% following single GD15 50 ng/kg gavage dose to female Long-Evans rats.

Markowski et al. 2001: Operant Conditioning



12. **Burleson et al. (1996)**. Increased susceptibility to influenza infection challenge in B6C3F1 mice following 10 ngTCDD/kg (LOAEL) and higher single oral gavage dose to 8-week-old mice (publication Fig. 1 attached). No significant effects seen at 1 and 5 ng/kg doses (NOAEL). Assume 60% absorption. Benchmark dose analysis was not performed on study because the data were not presented in tabular format.

Burleson et al. 1996: Influenza Susceptibility

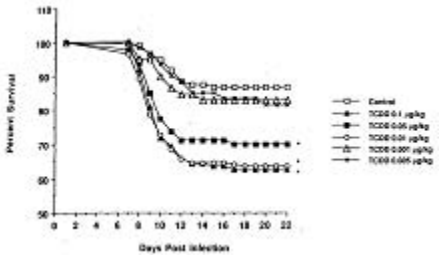
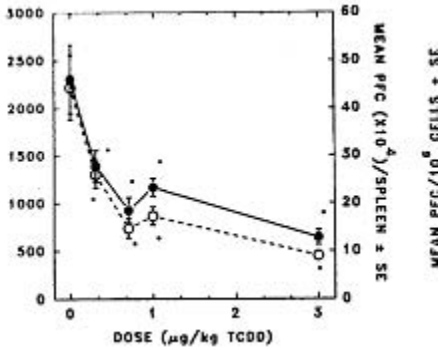


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

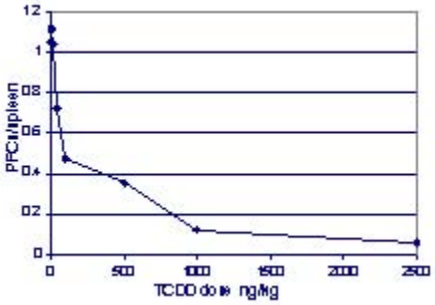
13. **Smialowicz et al. (1994)**. Dose-related suppression of antibody plaque forming cell (PFC; publication Fig. 1 attached) response in adult female B6C3F1 mice at 300 ng/kg single intraperitoneal injection and higher. PFC increases reported in high-dose-group male F344 and female Long-Evans rat species tested, accompanied by alterations to splenic $CD4^{-}CD8^{+}$ lymphocytes. ED_{01} values for mice calculated for plaque forming cells per million cells of 2.9 ng/kg body burden using EPA Benchmark Dose Software Version 1.3.

Smialowicz et al. 1994: PFC Immune Response



14. **Narasimhan et al. (1994)**. Decreased splenic antibody plaque-forming cell (PFC; graph of publication Table 5 data attached) response following single intraperitoneal dose administered to female B6C3F1 mice (7–9 weeks old). LOAEL for decreased SRBC and splenic PFC responses at 100 ng/kg, nonstatistically significant decrease evident at 50 ng/kg, NOAEL at 25 ng/kg. CYP1A1 LOAEL (NOAEL) at 25 (10) ng/kg dose (graph of publication table 1 data attached). ED_{01} values calculated for spleen PFC/million cells of 1.5 ng/kg body burden and for CYP1A1 mRNA induction of 3 ng/kg using EPA Benchmark Dose Software Version 1.3.

Narasimhan et al. 1994: PFC Immune Response



CYP1A1 mRNA Induction

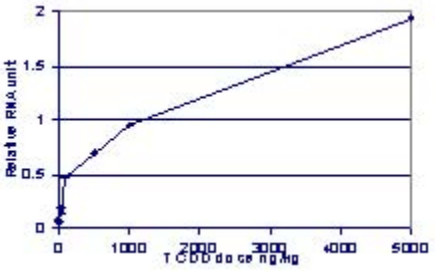
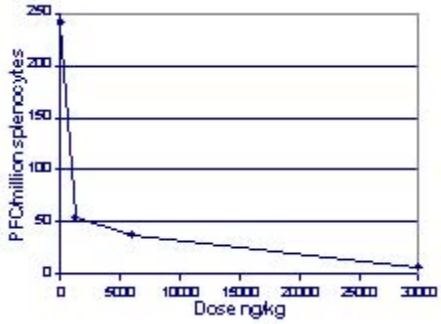


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

15. **Vecchi et al. (1983)**. Decreased plaque-forming cells (PFC) per million and PFC/spleen (graph of publication table 2 data attached) at all doses tested in aryl hydrocarbon hydroxylase sensitive mouse strains (B6, C3) following single intraperitoneal doses. Less sensitivity in other strains (e.g. DBA/2 and AKR). LOAEL (no NOAEL) of 1200 ng/kg. ED_{01} calculated for PFC/million splenocytes of 7 ng/kg for B6 mice using EPA Benchmark Dose Software Version 1.3.

Vecchi et al. 1983: PFC Immune Response



16. **Sewall et al. (1995)**. Statistically significant decreased ratio of thyroid parenchymal area to thyroid follicle area (publication Fig. 6 attached) was reported in female Sprague-Dawley rats following oral gavage biweekly dosing for 30 weeks at daily equivalent doses of 0.1–125 ng/kg/day. LOAEL (NOAEL) of 3.5 (1) ng/kg/day for thyroid parenchyma/follicle ratio, calculating to approximate body burdens of 76 and 22 ng/kg for the LOAEL and NOAEL, respectively. These calculations assume a half-life of 25 days and 60% body burden fraction following gavage dose, based on Hurst et al. (2000). Significant increases were also reported for thyroid stimulating hormone, with a LOAEL of 3.5 ng/kg/d and a NOAEL of 1 ng/kg/d. Serum thyroxine was significantly decreased at 10.5 ng/kg/day and at higher doses. ED_{01} values were not calculated for thyroid parenchymal/follicle ratio. ED_{01} for decreases in serum thyroxin of 43 ng/kg body burden using EPA Benchmark Dose Software Version 1.3. The ED_{01} for increased serum thyroid stimulating hormone is 26 ng/kg. LOAEL(NOAEL) for CYP1A1 mRNA induction of 0.35 (0.1) ng/kg/day, approximating to 8 (2) ng/kg body burden (assuming 60% absorption, 25 day halflife). ED_{01} for increases in CYP1A1 mRNA was 3.5 ng/kg using EPA Benchmark Dose Software Version 1.3.

Sewall et al. 1995: Thyroid Histology

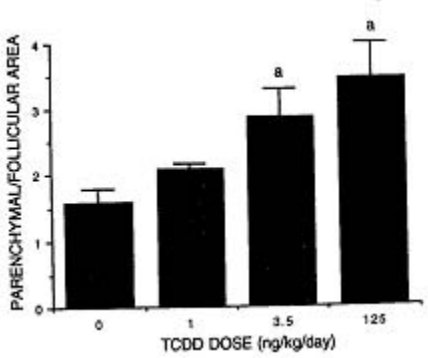
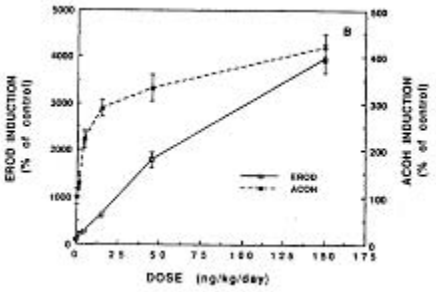


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

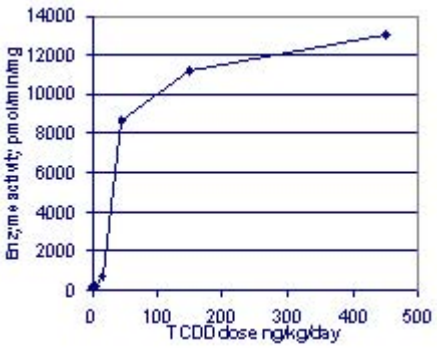
17. **DeVito et al. (1994)**. LOAEL (no NOAEL) of 1.5 ng/kg/day for induction of CYP1A1 and CYP1A2 (publication Fig. 2 attached) and increased phosphorylation of phosphotyrosyl proteins in female B6C3F1 mice gavage fed 1.5–150 ng/kg/day, 5 days per week, for 13 weeks. Approximate body burden after 13 weeks at 1.5 ng/kg/day of 24 ng/kg, based on Diliberto et al. (2001). ED_{01} value calculated at 22 ng/kg for CYP1A1 induction in the liver using EPA Benchmark Dose Software Version 1.3.

DeVito et al. 1994: Enzyme Induction



18. **Diliberto et al. (2001)**. Dose response relationship for CYP1A1 induction in female B6C3F1 mice (60 days old) at all oral gavage doses from 0.15 ng/kg/day (5/7 days, 13 weeks) and higher, corresponding to a radiolabel measured body burden of 2.75 ng/kg. Hepatic CYP1A1 activity (publication Table 5, graph of liver EROD data attached) modeled using EPA BMDS Software Version 1.3 results in an ED_{01} of 9.7 ng/kg/day. Body burden interpolated using linear regression of data from Diliberto et al. (2001; Table 4) with formula: body burden = 6.8782 * daily dose (M-F) (R^2 = 0.9994; Microsoft Excel), resulting in estimated ED_{01} body burden of 67 ng/kg.

Diliberto et al. 2001: EROD Induction



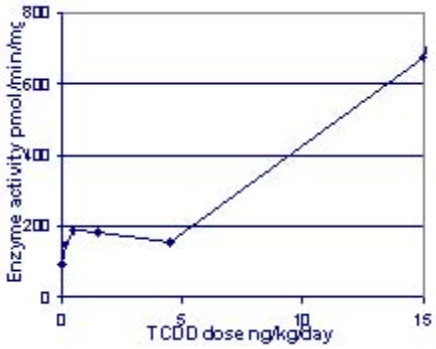
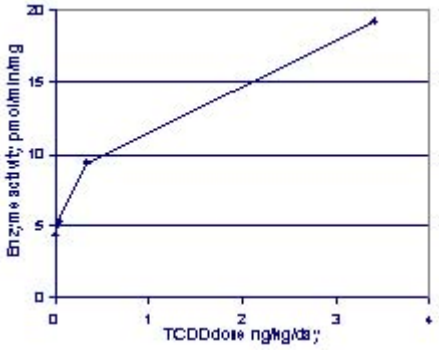


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

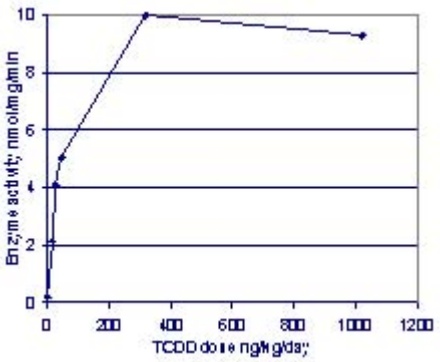
19. **Vogel et al. (1997)**. LOAEL(NOAEL) for CYP1A1 EROD induction (graph of publication Table 3 data attached) at 0.34 (0.034) ng/kg/day to C57 female mice administered 1, 10, 100 ng/kg loading doses followed by weekly injections of 0.2, 2, and 20 ng/kg for 135 days, calculating to 4.9 (0.49) ng/kg body burden (assuming 100% absorption, 10 day halflife). ED_{01} value calculated for CYP1A1 EROD induction of 0.003 ng/kg body burden using EPA Benchmark Dose Software Version 1.3.

Vogel et al. 1997: EROD Induction



20. **van Birgelen et al. (1995)**. Significant increases in CYP1A1 (graph of liver EROD data from publication table 3 attached) and CYP1A2, plus decreased relative thymus weights and loss of hepatic retinoids, at all doses tested in 8 week old female Sprague-Dawley rats exposed to dietary matrix intakes of 0, 0.2, 0.4, 0.7, 5, 20 µgTCDD/kg diet, corresponding to 0, 13.5, 26.4, 46.9, 320 and 1024 ng/kg/day oral intake. LOAEL (no NOAEL) calculated from 13.5 ng/kg/day dose to be 243 ng/kg body burden (50% absorption from dietary matrix, 25 day halflife). Calculated no effect levels (CNEL) by the authors of 0.7 to 4 ng TCDD/kg/day (Hill and Weibull models, based on the measured control value plus twice the standard deviation: mathematical calculated corresponding body burdens are 13 and 72 ng/kg at 50% absorption, halflife 25 days). Measured levels by authors in liver and fat of 1400 and 620 ppt, respectively. ED_{01} value calculated for CYP1A1 of 19 ng/kg body burden using EPA Benchmark Dose Software Version 1.3.

van Birgelen et al. 1995: EROD Induction



21. **Schrenk et al. (1994)**. CYP1A1 induction in female Wistar rats exposed via biweekly subcutaneous injection to average daily doses of 2, 20, and 200 ng/kg/day for 13 weeks. LOAEL (no NOAEL) for CYP1A1 EROD induction of 2 ng/kg/day (graph of publication table 1 data attached), approximating to 72 ng/kg body burden (25 day halflife, 100% absorption). ED_{01} for CYP1A1 induction of 26 ng/kg body burden using EPA Benchmark Dose Software Version 1.3.

Schrenk et al. 1994: EROD Induction

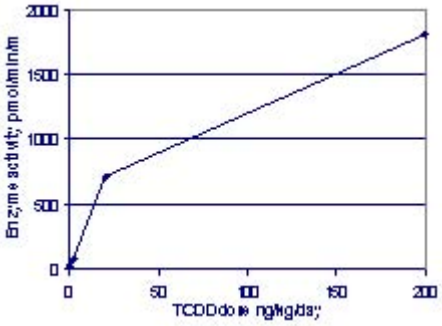
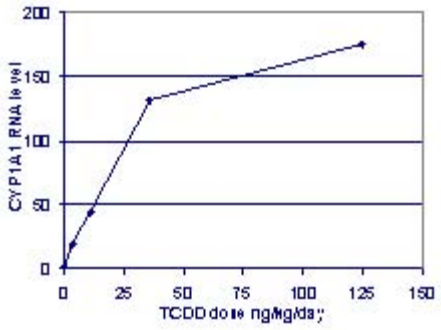


Table A-1. Body burdens for critical endpoints in animals with human equivalent daily intake (continued)

22. **Walker et al. (1999)**. Dose-dependent expression of CYP1A1 (graph of publication table 3 data attached) and CYP1A2 RNA (CYP1B1 less sensitive) in female Sprague-Dawley rats gavage fed biweekly for 30 weeks to average daily doses of 3.5 - 125 ng/kg/day. LOAEL value of 3.5 ng/kg/day (no NOAEL), calculates to 76 ng/kg body burden (assuming halflife of 25 days, 60% absorption following gavage). Measured liver level of 447 ng/kg. ED_{01} values calculated for CYP1A1 mRNA and CYP1A2 mRNA at 59 and 270 ng/kg body burdens, respectively, using EPA Benchmark Dose Software Version 1.3. High maximal induction potential of enzymes contributes to high 1% effective dose (ED_{01}).

Walker et al. 1999: CYP1A1 mRNA



GLOSSARY

Adverse effect: A biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism or reduces an organism's ability to respond to an additional environmental challenge.

Area Under the Curve (AUC): Area under the concentration versus time curve. The AUC is a summary measure that integrates serial assessments of a dose over the duration of the study.

Aryl hydrocarbon receptor (AhR): An intracellular protein that is a ligand-dependent transcription factor that functions in partnership with a second protein, the aryl hydrocarbon receptor nuclear translocator (Arnt).

Aryl hydrocarbon receptor nuclear translocator (Arnt): An intracellular protein that functions as a transcription factor in the cell in partnership with a second protein, the aryl hydrocarbon receptor (the AhR).

Background exposure: The exposure that regularly occurs to members of the general population from exposure media (food, air, soil, etc.) that have dioxin concentrations within the normal background range. Most (> 95%) of background exposure results from the presence of minute amounts of dioxin-like compounds in dietary fat, primarily from the commercial food supply. The origin of this background exposure is from three categories of sources: naturally formed dioxins, anthropogenic dioxins from contemporary sources, and dioxins from reservoir sources. The term "background exposure" as used in this document should not be interpreted as indicating the significance or acceptability of risk associated with such exposures.

Benchmark dose (BMD): A statistical lower confidence limit on the dose that produces a predetermined change in response rate of an adverse effect, typically 1–10%, compared to background.

Body burden: Body burden is defined as the concentration of TCDD and related chemicals in the body and is typically expressed as ng/kg body weight. In animals, these values are calculated from studies at or approaching steady-state and are associated with either biochemical or toxicological responses. In addition, these values are calculated on the basis of knowledge of the species-specific half-life and the exposure, or they are estimated on the basis of the TCDD tissue concentration, the size of the tissues, and the weight of the animal. In humans the values are typically presented as steady-state body burdens and are estimated on the basis of an intake rate and the half-life of TCDD in humans. Alternatively, body burdens in humans are estimated on the basis of lipid adjusted serum or adipose tissue TCDD or TEQ concentrations.

Cancer: A family of diseases affecting cell growth and differentiation, characterized by an abnormal, uncontrolled growth of cells.

Carcinogen: An agent capable of inducing cancer.

Carcinogenesis: The origin or production of a benign or malignant tumor. The carcinogenic event modifies the genome and/or other molecular control mechanisms of the target cells, giving rise to a population of altered cells.

Chronic effect: An effect that occurs as a result of repeated exposures over a long period of time in relation to the lifetime of the organism.

Chronic exposure: Multiple exposures occurring over an extended period of time or a significant fraction of the animal's or the individual's lifetime.

Chronic study: A toxicity study designed to measure the (toxic) effects of chronic exposure to a chemical.

Chronic toxicity: The capacity of a substance to cause adverse human health effects as a result of chronic exposure.

Cohort: A group of animals of the same species, including humans, that is identified by a common characteristic and that is studied over a period of time as part of a scientific or medical investigation.

Confidence interval (CI): A range of values for a variable of interest, for example, a rate, constructed so that this range has a specified probability of including the true value of the variable.

Confounder: A condition or variable that is both a risk factor for disease and is associated with an exposure of interest. This association between the exposure of interest and the confounder (a true risk factor for disease) may make it falsely appear that the exposure of interest is associated with disease.

Congeners: Compounds that have similar chemical structures or belong to closely related chemical families

Coplanar: Descriptive term referring to the fact that multi-ringed chemical structures can assume a flat configuration, with rings in the same spatial plane.

Dioxin-like: An adjective that describes compounds that have similar chemical structure and physical-chemical properties and invoke a common battery of toxic responses as does 2,3,7,8-TCDD. Because of their hydrophobic nature and resistance towards metabolism, these chemicals persist and bioaccumulate in fatty tissues of animals and humans. Certain members of the dioxin, furan, and PCB family are termed “dioxin-like” in this reassessment.

Effective dose (ED): The dose that corresponds to an increase, expressed as a percent response, in relation to expected levels of an adverse effect that can be defined as a percent increase over background rates or a percent increase between background and maximal rates.

Effective dose$_{01}$ (ED$_{01}$): The dose corresponding to a 1% increase in an adverse effect. Effective dose evaluation at the 10% response level (ED$_{10}$ or lower bound on ED$_{10}$ [LED$_{10}$]) is somewhat the norm, given the power of most chronic toxicology studies to detect an effect. In cases where the data allow evaluation at a lower effective dose level, the Agency suggests using the lower value. Such is the case for 2,3,7,8-TCDD.

Epidermal growth factor (EGF): A mitogenic polypeptide active on a variety of cell types, especially, but not exclusively, epithelial.

Follicle stimulating hormone (FSH): FSH is an acidic glycoprotein secreted by the anterior pituitary gland. In women, follicle stimulating hormone stimulates the development of ovarian follicles (eggs) and stimulates the release of estrogens. In men, follicle stimulating hormone stimulates the production of sperm.

Half-life: A measure of the time required to reduce to one-half the original concentration of a specified chemical in the body.

Hormone: Control chemicals produced by tissues or organs specialized for that function and that exert their highly specific effects on other tissues of the body.

Latency Period: The time between first exposure to an agent and manifestation or detection of a health effect of interest.

Ligand: Any molecule that binds to another. In normal usage, a soluble molecule such as a hormone or neurotransmitter that binds to a receptor, usually with high affinity.

Lower limit on effective dose$_{01}$ (LED$_{01}$): The 95% lower confidence limit of the dose of a chemical needed to produce a 1% increase of an adverse effect in those exposed to the chemical or to 1% of the maximal response relative to control.

Lowest-observed adverse effect level (LOAEL): The lowest exposure level at which there are statistically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group.

Luteinizing hormone (LH): A hormone that acts with the follicle stimulating hormone (FSH) to stimulate sex hormone release.

Margin of exposure (MOE): The LED$_{10}$, LED$_{01}$, or other point of departure divided by the actual or projected environmental exposure/dose of interest, expressed as a ratio.

Minimal risk level (MRL): An estimate of daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

No-observed-adverse effect level (NOAEL): The highest exposure level at which there are no statistically significant increases in the frequency or severity of adverse effect between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered adverse or to be precursors to adverse effects.

No-observed-effect level (NOEL): An exposure level at which there are no statistically significant increases in the frequency or severity of any effect between the exposed population and its appropriate control.

Pharmacokinetics: The quantitative description of the process of chemical disposition: absorption, distribution, metabolism, and excretion (metabolism and excretion equal elimination).

Physiologically based pharmacokinetic (PBPK) model: Physiologically based model used to characterize pharmacokinetic behavior of a chemical. Available data on blood flow rates and metabolic and other processes that the chemical undergoes within each compartment are used to construct a mass-balance framework for the PBPK model.

Point of departure (POD): The dose-response point that marks the lower end of the range of observation and the beginning of a low-dose extrapolation. This point is most often the upper bound on an observed incidence or on an estimated incidence from a dose-response model or the lower bound on the dose associated with such an incidence.

Promoter: An agent that is not carcinogenic itself but that when administered after an initiator of carcinogenesis stimulates the clonal expansion of the initiated cell to produce a neoplasm.

Receptor: A molecular structure within a cell or on the cell's surface that is characterized by selective binding of a specific substance and a specific physiologic effect that accompanies the binding (for example, see aryl hydrocarbon receptor).

Receptor site: The portion of the receptor molecule or structure with which the compound (ligand) interacts.

Reference dose (RfD): An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, a LOAEL, or a benchmark dose, with uncertainty factors generally applied to reflect limitations of the data used. Generally used in EPA's noncancer health assessments.

Relative potency (REP): The ratio of the potency of the congener to the standard toxicant in that specific study; a concept similar to toxic equivalency but based on a single study, species, or matrix, etc., and not averaged to obtain a general toxic equivalency value.

Relative risk (RR): The relative measure of the difference in risk between the exposed and unexposed populations in a cohort study. The relative risk is defined as the rate of disease among the exposed divided by the rate of the disease among the unexposed. A relative risk of 2 means that the exposed group has twice the disease risk as the unexposed group.

Reservoir sources: Reservoirs are materials or places that contain previously formed CDD/CDFs or dioxin-like PCBs and have the potential for redistribution and circulation of these compounds into the environment. Potential reservoirs include soils, sediments, biota, water, and some anthropogenic materials. Reservoirs become sources when they have releases to the circulating environment.

Risk (in the context of human health): The probability of injury, disease, or death from exposure to a chemical agent or a mixture of chemicals. In quantitative terms, risk is expressed in values ranging from zero (representing the certainty that harm will not occur) to one (representing the certainty that harm will occur).

Slope factor: An upper bound, generally approximating or exceeding a 95% confidence limit, on the increased cancer risk from a lifetime exposure to an agent. This estimate, usually expressed in units of proportion (of a population) affected per mg/kg/day, is generally reserved for use in the low-dose region of the dose-response relationship, that is, for exposures corresponding to risks less than 1 in 100.

Standardized mortality ratio (SMR): This is the relative measure of the difference in risk between the exposed and unexposed populations in a cohort study. The SMR is similar to the relative risk in both definition and interpretation. This measure is usually standardized to control for any differences in age, sex, and/or race between the exposed and the reference populations. It is frequently converted to a percent by multiplying the ratio by 100.

Statistical significance: The probability that a result may be due to chance alone. By convention, a difference between two groups is usually considered statistically significant if chance could explain it only 5% of the time or less. Study design considerations may influence the a priori choice of a different statistical significance level.

Thyroid stimulating hormone (TSH): A hormone secreted by the anterior pituitary gland that activates certain actions in thyroid cells leading to production and release of the thyroid hormones (T3 and T4). T3 and T4 blood levels feed back on the hypothalmus/pituitary gland and decrease TSH production when T3 and T4 levels are high.

Tolerable daily intake (TDI): A TDI is an estimate of the amount of a contaminant in food or drinking water that can be ingested daily over a lifetime without a significant health risk. The term is used frequently in World Health Organization (WHO) health assessments. The

term "tolerable" is used, as contaminants do not serve an intended function and as intake is unavoidably associated with the basic consumption of food and water. Tolerable does not generally connote "acceptable" or "risk free."

Toxic equivalence (TEQ): The toxic equivalency factor (TEF) of each dioxin-like compound present in a mixture multiplied by the respective mass concentration. The products are summed to represent the 2,3,7,8-TCDD toxic equivalence of the mixture.

Toxic equivalency factor (TEF): TEFs compare the potential toxicity of each dioxin-like compound present in a mixture to the well-studied and well-understood toxicity of 2,3,7,8-TCDD, the most toxic member of the group, with the TEF of 2,3,7,8-TCDD being 1. TEFs are the result of expert scientific judgment using all of the available data and taking into account uncertainties in the available data.

Transcription: The process of constructing a messenger RNA molecule using a DNA molecule as a template, with resulting transfer of genetic information to the messenger RNA.

Transcription factor: A substance, usually a protein, that is developed within the organism and that is effective in the initiation, stimulation, or termination of the genetic transcription process.

Upper bound: A plausible upper limit to the true value of a quantity or response. This is usually not a true statistical confidence limit.

Weight-of-evidence: An approach used for characterizing the extent to which the available data, including human, animal, and mechanism of action, support the hypothesis that an agent causes an adverse effect, such as cancer, in humans. The approach considers all scientific information, both positive and negative, in determining whether and under what conditions an agent may cause disease in humans.

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