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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

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National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

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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
kd	kilodalton
kg	kilogram
L	liter
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)
NAS	National Academy of Sciences
NHANES	National Health and Nutrition Examination Survey
NHATS	National Human Adipose Tissue Survey
ng	nanogram
NIOSH	National Institute for Occupational Safety and Health
NRC	National Research Council
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
POTW	publicly-owned treatment works
ppt	part per trillion
PVC	polyvinyl chloride
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-trichorophenoxyacetic acid
TBD	thyroid binding globulin
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TCP	trichlorophenol
TDI	tolerable daily intake
TEF	toxic equivalency factor
TEQ	toxic equivalent
$TEQ\text{-}WHO_{94}$	1994 WHO extension of the I-TEF scheme to include 13 dioxin-like PCBs
$TEQ\text{-}WHO_{98}$	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 it summarizes key findings developed in the exposure and health assessment portions (Parts I and II, respectively) of the Agency's 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 for further information on the topics covered here and to see complete literature citations. These documents are:

Estimating Exposure to Dioxin-like Compounds: This document, hereafter referred to as Part I, the Exposure Document, is divided into four volumes: (1) Executive Summary; (2) Sources of Dioxin-Like Compounds in the United States; (3) Properties, Environmental Levels, and Background Exposures; and (4) Site-Specific Assessment Procedures.

Health Assessment for 2,3,7,8-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

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[1]The term "background" exposure has been used throughout this reassessment to describe exposure which 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.

articulates the strengths and weaknesses of the available evidence for possible sources, exposures and health effects, and 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 EPA and by the general public.

This document (Part III) is organized as follows:

1. Introduction - This section 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 section 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 section 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's (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 Summary - This section summarizes the key findings of the Exposure Document and links them to the effects, mechanisms, and dose-response characterization.

5. Dose Response Summary - This section 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 section presents conclusions 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 1994, drafts of each document, including an earlier version of this risk characterization, were made available for public review and comment. This 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 is the final step in this open and participatory process of reassessment. When complete, and following final SAB review, the comprehensive set of background documents and this integrative summary and risk characterization will be published as final reports and replace the previous dioxin assessments as the scientific basis for EPA decision-making.

1.1. DEFINITION OF DIOXIN-LIKE COMPOUNDS

As defined in Part I, 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), and describes this subset of chemicals as "dioxin-like." Dioxin-like refers to the fact that these compounds have similar chemical structure, similar physical-chemical properties, and 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; these are ones 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; these also are ones 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 have not been explicitly considered in this assessment.

There are 209 PCB congeners. Only 12 of the 209 congeners are thought to have dioxin-like toxicity; these are PCBs with 4 or more lateral chlorines with 1 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 have not been 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 have not been 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. While recognizing 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., halogenated naphthalenes or benzenes, azo- or azoxybenzenes), the evaluation of less than two dozen chlorinated congeners is generally considered sufficient to characterize environmental "dioxin."

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 called simply "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 comprising the mixture to the well-studied and understood toxicity of TCDD, the most toxic member of the group. 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, 1989, 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 along with considerations of 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 using all of the available data and 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. To apply this TEF concept, the TEF of each congener present in a mixture is multiplied by the respective mass concentration and the products are summed to represent the 2,3,7,8-TCDD Toxic Equivalence (TEQ) of the mixture, as determined by Equation 1-1.

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

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). 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). 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. As an 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 its 20-year history, the approach has evolved, and decision criteria supporting the scientific judgment and expert opinion used in assigning TEFs has become more transparent. Numerous states, countries, and several international organizations have evaluated and 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 all chemicals are equally potent to 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 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 U.S. EPA Science Advisory Board (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 assure 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 based on all the available data comparing the chemical to either TCDD or PCB 126. In addition, there are weighting criteria that place more emphasis on chronic and subchronic studies examining 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 in vivo endpoints examining responses as diverse as enzyme induction, developmental toxicity, immunotoxicity, hepatic toxicity, alterations in hormones and tumor promotion, while the TEF for 3,4,4',5-tetrachlorobiphenyl (PCB 81) is based on in vitro CYP1A induction and QSAR calculations. Fortunately, PCB 81 does not significantly contribute to human TEQ exposures. There are 5 congeners that 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 3 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 from multiple studies performed in different laboratories (**Table 1-4**). The TEF for 1,2,3,6,7,8-HxCDD is based 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 12378-PCDD is based largely on its REP for tumor promotion in rats. From these data, it is clear that the chemicals that contribute approximately 80% to the total human TEQ are well studied and the assigned TEFs provide reasonable estimates of the relative potency of these chemicals. In contrast, while there are some chemicals in the TEF methodology which have minimal data sets to reliably assess their relative potency, these chemicals do not contribute substantially to the 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). In addition, there are examples of non-additive interactions between dioxins and non-dioxins. Both greater than additive and less than additive interactions have been observed in these studies. In general the non-additive interactions between the dioxins and non-dioxins 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 AhR and induce some dioxin-like effects. It has been proposed by some scientists that these chemicals contribute significantly to the total TEQ exposures and that these exposures far out weigh those from PCDDs, PCDFs and PCBs (Safe, 1995a). While this hypothesis is intriguing, there are several

limitations to these analyses (Part II, Chapter 9,Section 9.3.). 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. The natural ligands also have significant 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. Clearly this issue requires further research in order to better understand the relative potential health effect 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 non-dioxin-like chemicals is not evaluated in concert with that of dioxin-like chemicals. Another limition of the TEF methodology is their application to non-biological samples. The fate and distribution of PCDDs, PCDFs and PCBs are not necessarily related to their TEF. Thus, the use of the TEF for non-biological media must be done cautiously. 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.

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 of 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 Ah receptor 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 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 dose and time related to effects in humans are known.

The choice of dose metric not only considers mechanistic data but must also consider pragmatic approaches as well. The use of the dose metric plays a role in its choice. Because of

differences in life-span and uncertainties in the windows of sensitivity for various endpoints, AUC may not be a useful dose metric for cross species extrapolation in the risk assessment of dioxin and related compounds. However, 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 since cross species scaling is not required. The choice of dose metric is also dependent upon the data available. A number of dose metrics, such as Ah receptor occupancy, induction of CYP1A2, and decreases in EGF receptor have been proposed based on PBPK models (Jusko et al., 1995; Andersen et al.,1997; Kohn et al., 1993; Portier and Kohn, 1996). While 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 will be 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, 1992; 1996) to estimate the human equivalent dose when scaling across species is to use allometric scaling based on the following equation:

$$Dose_{human} = Dose_{rat} (BW_{rat}/BW_{human})^{0.25}$$

where BW is the body weight in kg and Dose is the daily administered dose in rats or the scaled human daily dose expressed as ng/kg/d. This method 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), taking both pharmacokinetics and pharmacodynamics into account. Using this equation, a dose of 1 ng TCDD/kg/d in a 0.35 kg rat would result in a scaled human dose of 0.27 ng TCDD/kg/d for a 70 kg human. If this scaling method applies to TCDD and related chemicals, then 1 ng TCDD/kg/d in the rat should produce similar effective doses in a human exposed to 0.27 ng TCDD/kg/d, some 3.8 times lower. 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.

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

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 F is 50% and the species specific half-life of TCDD is 25 days for rats and 2593 days for humans. Starting with an administered dose of 1 ng/kg/d in rats and the scaled human dose of 0.27 ng/kg/d, 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 the equation above 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/d, 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). One reason for the discrepancy of the scaling method is that the half-life of TCDD in rodents and humans is much longer than is typically observed for other xenobiotics (Bachmann et al., 1996). 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 a the EPA default methodology. However, administered dose can be used to compare 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.

1.3.2. Area Under the Curve

Area under the curve or 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. Mechanistic considerations also suggest that AUC can be a useful dose metric for carcinogenesis. TCDD and related chemicals are thought to induce tumors through promotional mechanisms as opposed to acting as 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. It is

likely that AUC would be an appropriate dose metric for cancer in humans, and 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. While 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, the window of sensitivity is clearly defined in rodents and humans, such as induction of cleft palate. 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 carcinogenesis, 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 the 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 life-span 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. However, because the plasma concentrations were 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 life-span must be applied. Typically, this involves the derivation of a lifetime average serum lipid concentration (Cavg), 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.

While AUC may not be an appropriate dose metric for animal to human extrapolations, it is 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) successfully used this approach to examine dose response relationships for cancer in an occupationally exposed cohort. 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 since 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 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 is based predominately 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 well as a number of assumptions used in the PBPK models.

The use of tissue concentrations as a dose metric has been examined by van Birgelen et al. (1996) and Hurst et al. (1999). van Birgelen and coworkers (1996) 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. (1999) 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.

While 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 concentrations are also a useful tool to compare 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 comparing steady-state in humans and animals. Comparing plasma or blood concentrations following acute exposures in experimental animals to steady-state human blood or plasma concentrations would not be appropriate. One limitation of the use of either plasma, blood or target tissue concentrations as dose metrics is the lack of human PBPK models to predict these values based on changes in intake patterns.

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 and are associated with either biochemical or toxicological responses. In addition, these values are calculated based on either knowledge of the species-specific half-life and the exposure or they are estimated based on 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 based on an intake rate and the half-life of TCDD in humans. Alternatively, body burdens in humans are estimated based on lipid adjusted serum or adipose tissue TCDD or TEQ concentrations (See Part I, Volume 3, 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 is accounted for because these body burdens are estimated at steady-state conditions. Third, DeVito et al. (1995) have demonstrated that for some biochemical responses, 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 based on either intake rates or on measured tissue concentrations.

Body burdens also have some limitations. In order to estimate 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. It should be noted that there are human populations with body fat compositions less than 10% and greater than 35%. Also, when estimating the body burden based on intake rates and half-lives, the uncertainty of these parameters should be considered. In the reassessment, the estimated 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. While measured concentrations should eliminate some of the uncertainties around 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. 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 experimental 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 epidermal growth factor receptor (EGFR) (Portier and Kohn, 1996). While 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 these dose metrics have limitations that prohibit their use, such as tissue concentrations and the mechanistic dose metrics. Other dose metrics, such as AUC have limited utility for species extrapolations because of our limited understanding of the concept of physiological time. Some dose metrics can be used to compare different human exposures, such as AUC and administered dose, but are not necessarily suitable for species extrapolations. Other dose metrics, such as steady-state body burdens or blood concentrations are useful dose metrics for species extrapolations because of they are directly related to tissue concentrations, and can be estimated in both animals and humans. The use of any of these dose metrics requires a number of assumptions discussed above. 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 on the issue of dose metrics could provide better guidance in choosing the dose metrics for dioxins and related chemicals. However, in the mean time, the use of steady-state body burdens can provide a reasonable description of dose for use in species extrapolations and risk assessments.

2. EFFECTS SUMMARY

Since the identification of 2,3,7,8-TCDD as a chloracnegen in 1957, more than 5,000 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. They 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.

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; Steenland et al., 1999; Manz et al., 1991; Eriksson et al., 1990). 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; Centers for Disease Control (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. In more recent analyses of cohorts (Fingerhut et al., 1991; 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" effects (i.e., some may be responses within the normal range, 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 dose associated with this plethora of effects is 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. 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, relatively small margins of exposure (MOE) 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. This concept is illustrated in **Table 2-2.** This point will be discussed further in Sections 5.2 and 6.0 in this document.

The effects discussed in the following sections are focused on development of 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 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 (the aryl hydrocarbon receptor [AhR]), which is a ligand-dependent transcription factor that functions in partnership with a second protein (known as the Ah receptor nuclear translocator, Arnt) to alter gene expression. In addition, receptor binding may result in release of cytoplasmic proteins which, 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, 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 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 but within normal homeostatic limits and, therefore, are often not considered adverse in and of themselves. However, many of these biochemical changes are potentially on a continuum of dose-response relationships which leads to adverse responses and, considering the potential to shift population distributions in response, may be of concern. At this time, caution must be used when describing these events as adaptive.

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. 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 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 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).

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 over 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 the 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 Ah receptor 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. **Figure 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). 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 presumed, 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 the evidence for the involvement of the AhR in all of these processes has not always been ascertained, structure-activity relationships, genetic data, and reports from the use of biological models like "knockout" mice that are lacking the AhR ($AhR^{-/-}$) are consistent with the involvement of the AhR as the initial step leading to many of 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, occurring at body burdens as low as 1-10 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 upon 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 contrast 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, 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 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 coordinate 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 U.S. EPA review of the human database relating to the carcinogenicity of TCDD and related compounds in 1988, 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 has recently been published as part of an International Agency for Research on Cancer (IARC) Monograph (1997) and the Agency for Toxic Substances and Disease Registry (ATSDR) ToxProfile (ATSDR, 1999a). Among the most important of these are the studies of 5,172 U.S. chemical manufacturing workers by Fingerhut et al. (1991a) and Steenland et al. (1999) from NIOSH and an independent study by Aylward et al. (1996); a study of 2,479 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 2,000 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 of 247 workers involved in a chemical accident cleanup by Zober et al. (1990) and subsequent follow-up (Ott and Zober, 1996b); and an international study of more than 18,000 workers exposed to phenoxy herbicides and chlorophenols by Saracci et al. (1991), with subsequent follow-up and expansion by Kogevinas et al. (1997). Although uncertainty remains in interpreting these studies because not all potential confounders have been ruled out and coincident exposures to other carcinogens are likely, 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. They focused their 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 subcohorts with 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 (SMRs) 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. They 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 in all cancers combined of this magnitude 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).

While smoking as a confounder cannot be totally eliminated as a potential explanation of the occupational studies results, analyses (Fingerhut, 1991b; Ott and Zober, 1996b) conducted to date 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 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.

Some studies that have been discussed in Part II, Chapter 7a, report little 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 relevance to providing information that could assist in determining the carcinogenic hazard of dioxins. These deficiencies fall into the following categories: little statistical power to detect an effect of exposure since the measured exposures are lower than those seen in the studies cited above and more similar to that of the comparison population; no measurements of in vivo exposure to 2,3,7,8-TCDD and potential for misclassification of exposure; and inadequate latency or follow-up. In short, these mostly non-positive studies lack one or more strengths of the cohort studies discussed above.

For example, substantial exposures to dioxin were also experienced by U.S. Air Force Ranch Hand personnel spraying the defoliant Agent Orange during the Vietnam war. In this study, there is no statistically significant increase in all cancers in the exposed population. 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. 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. A relative risk for all cancers combined can be estimated for the Ranch Handers 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), 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 ngTCDD/kg lipid * years, and for the high dioxin group the median is 2,280 ngTCDD/kg lipid * years (note from Joel Michalek, U.S. Airforce, to Bruce Rodan, U.S. EPA, dated September 8, 2000). Using the Becher et al. (1998) linear formula (RR

= 1 + 0.000016 x AUC ng-TCDD/kg lipid * Years; ~ 3×10^{-3} risk/pg/kg/day) described in Section 5.3 and **Table 5-4** of this document, the estimated all cancers relative risk for the overall Ranch Hand cohort is approximately 1.01, and for the high exposure group1.04 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 percent increases in relative risk. Data on the overall prevalence of cancer in the comparison group (18.9%) and sample sizes (all Ranch Hand 845 v. 1224 controls; high category 241 v. 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). The lack of a statistically significant positive response in this study is consistent with the lack of power of this study to detect an increase in all cancer risks, based on observations on cancer risk emerging from the analysis of the more highly exposed occupational cohorts.

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 other recent evaluations of the epidemiologic database, many of the earlier epidemiological studies that suggested an association between dioxin exposure and soft tissue sarcoma are criticized for a variety of reasons. Arguments regarding selection bias, lack of exposure or differential exposure misclassification, confounding, and chance in each individual study have been presented in the scientific literature, which increases uncertainty around this association. Nonetheless, the incidence of soft tissue sarcoma is elevated, but not statistically, 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 are difficult to define from exposure to TCDD.

The accidental exposure of the population at Seveso serves as an example of a more highly exposed group where, to date, latency is considered to be inadequate. Although Bertazzi and coworkers have 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 accepted for publication.

As mentioned above, 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 reported female cohort with good TCDD exposure surrogate information was that of Manz et al. (1991), which had a borderline statistically significant increase in breast cancer. Although Saracci et al. (1991) did report reduced female breast and genital organ cancer mortality, this 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 produced 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 contaminated by dioxin. 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 his small cohort. In sum, 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, recent studies of Brown et al. (1998) demonstrate that prenatal exposure of rats to 2,3,7,8-TCDD enhances their sensitivity as adults to chemical carcinogenesis. 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. Presently, the epidemiological data 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 that have been studied, consisting primarily of adult males. Although the epidemiologic data are not sufficient by themselves to infer a causal association between exposure to TCDD and other dioxin-like chemicals and increased cancer in humans (IARC, 1997; ATSDR, 1999a), this "limited" epidemiologic data base has been strengthened by emerging data reflecting 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. In addition, 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. While several studies exhibit 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), 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. This issue will be 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 rats and mice (U.S. EPA, 1985; Huff et al., 1991; Zeise et al., 1990; IARC, 1997). All studies have produced positive results, leading to conclusions that TCDD is a multistage carcinogen increasing 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). Few attempts have been made to demonstrate the carcinogenicity of other dioxin-like compounds. Other than a mixture of two isomers of hexachlorodibenzo-*p*-dioxin (HCDDs), which produced liver tumors in both sexes of rats and mice (NTP, 1980) when given by the gavage route, but not by the dermal route in Swiss mice (NTP, 1982a,b) and recent reports from Rozman (Rozman, 1999; Rozman, 2000; Rozman et al., 2000) attributing lung cancer in female rats to gavage exposures of 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin(HpCDD), neither the more highly chlorinated PCDDs/PCDFs nor the coplanar PCBs have been studied in long-term animal cancer bioassays. The National Toxicology Program (NTP) is currently testing the relative carcinogenic potency of four dioxin-like congeners (PeCDF, PeCDD, and PCB 118 and PCB 126), both alone and in combination. These data, when they are available, should add to our understanding regarding the carcinogenicity of these dioxin-like congeners.

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 and for skin. 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** 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; Teegarden et al., 1999), exposure-duration dependent (Dragan et al., 1992; Teegarden 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 show 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 also induce 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 demonstrate the influence that the intact hormonal system has on AHF development. AHF are 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 (TPA) (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 of 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 rat lung. Perhaps use of transgenic animal models will allow further understanding of the complex interaction of factors associated with carcinogenesis in rodents as well, presumably 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 have 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 are also tumor promoters, like TCDD (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 dioxins, furans, and coplanar PCBs that comprise approximately 80% of the current, total dioxin/furan TEQ in human blood, are all positive in either rodent bioassays or rodent liver tumor promotion studies, or mouse skin tumor promotion studies. These data suggest that while the majority of dioxin-like congeners have not been tested for carcinogenicity in chronic rodent bioassays, 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; their Table 1) present a summary of the data relied on by the European Centre for Environment and Health of the World Health Organization (WHO-ECEH) and the International Programme on Chemical Safety (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 II, Chapter 4). 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 of Waern et al. (1991) and for PCB 126 similar dose adjustments are included from Hemming et al. (1995; their figure 4). For the comparison of TCDD to the HxCDDs, in addition to the NTP studies, (U.S. EPA, 1980) 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 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 2-4** 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 90% 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 based on a tumor promotion or carcinogenic endpoint. 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 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 are biologically plausible as contributors to the carcinogenic process 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 will be 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. 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 therefore 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 progression of clonally expanded cell populations into tumors. Within this framework, it is hypothesised that TCDD may be acting as a tumor promoter through multiple mechanisms. Primarily, the activation of the AHR leads to alteration in genes involved in normal cell growth response pathways.

TCDD may contribute to the formation of 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 altered expression and activity of a number of genes regulating the cell-cycle. Activation of the AhR by TCDD results in altered expression or activity in for 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 and colleagues 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 (Portier et al., 1996). In contrast, Conolly and Andersen, 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 (Conolly and Andersen, 1997). Descriptions of these models are provided in Part II, Chapter 8. Interestingly, the use of the model by Portier and colleagues, leads to a model that is consistent with low-dose linearity, whereas the Andersen and Conolly model predicts highly non-linear 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, PM, 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 thyroid stimulating hormone 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. modeled the effect of 2,3,7,8-TCDD on UGTS, and thyroid hormones in female rats within the framework of a pharmacologically based pharmacokinetic (PBPK) model (Kohn et al., 1996). 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 and colleagues, which demonstrated the extrathyroidal effects of 2,3,7,8-TCDD on thyroid hormone turnover (Schuur et al., 1997).

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). In each case, critical "key events"can be identified and measured which correlate with carcinogenicity. While

these relationships are still uncertain, 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 epidermal growth factor receptor 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, the study of Kociba et al. (1978) and those of the 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 will be 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 (Kociba et al., 1978). While these findings, coupled with evaluation of epidemiologic data, have led some authors to conclude that dioxin possesses "anticarcinogenic" activity (Kayajanian, 1997; Kayajanian, 1999), it should be noted that, in experimental studies, with the exception of mammary gland tumors, the decreased incidence of 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). As discussed later 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). Because the mechanism of the decreases in the tumors 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, positively or negatively, it is unlikely that such data will be available to argue that dioxin exposure provides a net benefit to human health.

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). Under EPA's current approach, 2,3,7,8-TCDD is best characterized as a "human carcinogen." 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 2,3,7,8-TCDD exposure and cancer hazard. The guidance suggests that "human carcinogen" 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 "human carcinogen" descriptor is suggested for 2,3,7,8-TCDD because *all* of the following conditions are met:

- Occupational epidemiologic studies show an association between 2,3,7,8-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 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 epidermal growth factor receptor and estrogen receptor.
- The human AhR and 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" human carcinogens 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 data base demonstrating AhR-mediated dioxin-like activities. Each has physico-chemical properties which 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 toxicity equivalency for 2,3,7,8-TCDD and related compounds. For the congeners most frequently encountered in human blood, milk and adipose tissue, the data base 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. Based on 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 OCDD 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. 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 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*

The literature base with regard to potential human effects is detailed in Part II, Chapter 7b, Section 7.13. In general, there is little epidemiological evidence that makes 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). Particularly intriguing in this latest evaluation is the observation that exposure before and during puberty is linked to this sex ratio effect. Other sites have been examined for the effect of TCDD exposure on sex ratio with mixed results, but with smaller numbers of offspring. Continued evaluation of the Seveso population may provide other indications of impacts on reproduction and development but, for now, such data are very 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) have been 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) suggest impacts of background levels of dioxin and related compounds on neurobehavioral outcomes, thyroid 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 Ah-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 Ah-receptor 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 birthweight 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 all of these effects in the Yusho and Yu-Cheng cohorts do not correlate with TEQ, some of the effects are exclusively due to nondioxin-like PCBs or a combination of all the congeners. It is still not clear to what extent there is an association between overt maternal toxicity and embryo/fetal toxicity in humans.

Of particular interest is the common developmental origin (ectodermal layer) of many of the organs and tissues that are affected in the human. An ectodermal dysplasia syndrome has been clearly associated with the Yusho and Yu-Cheng episodes, involving hyperpigmentation, deformation of the fingernails and toenails, conjunctivitis, gingival hyperplasia, and abnormalities of the teeth. An investigation of 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 (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 (Alaluusua et al., 1996, 1999). 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. The developmental effects that can be associated with the nervous system are also consistent with this pattern of impacts on tissues of ectodermal origin, as the nervous system is of ectodermal origin. These data are limited but are discussed in Part II, Chapter 7b, Section 7.13.

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 (Egeland et al., 1994), in Army Vietnam veterans (CDC Vietnam Experience Study, 1988), and in Air Force personnel, known as "Ranch Hands," who handled and/or sprayed Agent Orange during the Vietnam War (Roegner et al., 1991; Grubbs et al., 1995). The risk of abnormally low testosterone was two to four times higher in exposed workers with 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 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).

In summary, the results from both the NIOSH and 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*

The extensive experimental animal database with respect to reproductive and developmental toxicity of dioxin and dioxin-related agents has been discussed in Part II, Chapter 5. Dioxin exposure has been observed to result in both male and female reproductive effects, as well as effects on development. 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 comes from animals exposed only to TCDD, more recent studies of animals exposed to mixtures of PCDD/PCDF isomers 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, including 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 and sexual behavior (rat and monkey), and changes in the development of the reproductive system (rat, hamster) occur at the lowest exposure levels tested (see also Part II, Chapter 8, Section 8.3).

Dioxin exposure results 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 et al. (1989) in the monkey were suggestive 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. While 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 low as 0.064 μg/kg could alter normal sexual development in the male offspring. A dose of 0.064 μg/kg in these studies results 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), as well as extended to females and another strain and species (hamster) (Gray et al., 1995b). 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 normal. Most of these effects occur in a dose-related fashion, some occurring at 0.05 μg/kg and 0.064 μg/kg, the lowest TCDD doses tested (Mably et al., 1992c; Gray et al., 1997a).

In the developing female rat, Gray and Ostby (1995) have 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 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) which demonstrated the same incidence of adverse effects in rat pups born to dams with a single exposure of 0.2 μg TCDD/kgBW on gestation day 15 (GD 15) versus 1.0 μg TCDD/kgBW on gestation day 8 (GD 8). Both of these experimental paradigms result in the same fetal tissue concentrations and body burdens during the critical window of sensitivity. For example, exposure to 0.2 μg TCDD/kgBW on GD 15 results in 13.2 pg TCDD/g fetal tissue on GD16; exposure to 1.0 μg TCDD/kgBW on gestation GD 8 resulted in 15.3 pg TCDD/g fetus on GD 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 earlier 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 doses of TCDD. The findings, while 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 with a greater affinity for the AhR are more developmentally toxic than those with 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.

2.2.2.2.2. ***Adult female reproductive toxicity.*** The primary effects of TCDD on female reproduction 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). While 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.

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 endometriosis was noted (Rier et al., 1993). A recent study in Cynomolgus monkeys has shown 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 which failed to detect dioxin promotion of surgically induced endometriosis only 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. 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, while non-AhR ligands, including a non-dioxin-like PCB, had no effect on surgically induced endometriosis. Dioxin has also been shown to result in endometriosis in human endometrial tissue implanted in nude mice (Bruner-Tran et al., 1999).

Data on the relationship of dioxins to endometriosis in people is intriguing, but preliminary. Studies in the early 1990s suggested that women with 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 reported from other populations (Koninckx et al., 1994). A study from Israel then demonstrated that there was a correlation between detectable TCDD in women with surgically confirmed endometriosis, in comparison to those with no endometriosis (Mayani et al., 1997). Recent studies from Belgium have indicated 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, which 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 animal results lend biological plausibility to the epidemiology findings. Endometriosis is not only an endocrine disorder, but is also associated with immune system alterations (Rier et al., 1995). Dioxins are known to be potent modulators of the animal immune system, as well as affecting 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 (DHT) 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 μgTCDD/kgBW) 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 exposure 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 still does not address the issues surrounding the potentially different responses humans (or animals) might have to the more complex and variable environmental mixtures of dioxin-like compounds.

TCDD and related compounds have reproductive and developmental toxicity potential in a broad range of wildlife, 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 these studies, effects were often observed at the lowest exposure level tested, thus a no-observed adverse effect level (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.

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.

Although there is evidence in experimental animals that exposure to dioxin-like chemicals during development produces neurobehavioral effects, 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 non-dioxin-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 non-dioxin-like PCBs. At present there are limited data that define the roles of the dioxins versus the non-dioxin-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 cleft palate in the mouse. However, a direct relationship with Ah binding is less clear for other effects, including 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, one exposed to 2,3,7,8-TCDD and the other to 2,3,7,8-tetrabrominated dioxin and furan, observed dose-related increases of complements C3 or C4 (Zober et al., 1992; Ott et al., 1994), while the Ranch Hands continue 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 immunosuppression have

been observed in a small group of exposed workers as a result of a single test (Tonn et al., 1996), providing support for a testable hypothesis to be evaluated in other exposed populations.

Comprehensive evaluation of immunologic status and function of the NIOSH, Ranch Hand, and Hamburg chemical worker cohorts found no consistent differences between exposed and unexposed groups for lymphocyte subpopulations, response to mitogen stimulation, or rates of infection (Halperin et al., 1998; Michalek et al., 1999b; Jung et al., 1998; Ernst et al., 1998).

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 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 (TNP-LPS) 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 evidence of 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. 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.

Although the immunosuppressive potency of individual dioxin-like compounds in mice is related to their structural similarity to TCDD, this pattern of suppression is observed only following exposure to an individual congener. The immunotoxicity of TCDD and related congeners can be modified by co-exposure to other congeners 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.

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 no-observed effect levels (NOEL). 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 is inconsistent across studies. However, 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. 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 acnelike 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 (ppt); 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 over 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 have 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 have 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, only in two studies were risk

estimates calculated for chloracne. Both were studies of different cohorts of TCP production workers (Suskind and Hertzberg, 1984; Bond et al., 1989); one group was employed in a West Virginia plant, the other in a plant in Michigan. 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 ($p<0.001$) (Suskind and Hertzberg, 1984). 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 (≥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) (Bond et al., 1989).

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, 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., 1999; Longnecker and Michalek, 2000). An increase in diabetes in other occupational cohorts (Steenland et al., 1999; Vena et al., 1998), as well as 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, it is well understood that mortality studies are limited in their ability to assess risk from diabetes mellitus. The recent 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. The most recent update of the Ranch Hand study shows a 47% excess of diabetes in the most heavily exposed group of veterans (Michalek et al., 1999).

Most of the data suggest that the diabetes 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 with less weight. Dioxin alters lipid metabolism in multiple species, including humans (Sweeney et al., 1997; Pohjanvirta and Tuomisto, 1994). Dioxin 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, such as the hypoxia-inducible factor (HIF) pathway, for Arnt (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 the toxicologic data are suggestive of a plausible mechanism. Many questions are yet to be answered. 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. As adult-onset diabetes is also associated with overweight, and body composition has been shown to modify the apparent half-life of dioxin, could the rate of elimination of dioxins be lowered in people with diabetes, causing them to have higher body burdens? This may be relevant to the background population, but is hardly likely to be an explanation in highly exposed populations. Key research needs are twofold. The first is to develop an animal model in which to study the association between dioxins and diabetes and glucose perturbation. Several rodent models for Type II diabetes exist and may be utilized. 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 have been 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 to Army Vietnam veterans, GGT was increased, at borderline significance, among Vietnam veterans compared to non-Vietnam veterans (CDC Vietnam Experience Study, 1988). In addition, despite the increases observed in some 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, e.g., AST and ALT, and metabolites, e.g., 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 2,3,7,8-TCDD 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 (ALK), have been observed after exposure to 2,3,7,8-TCDD 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 in a cross-sectional study, for example, liver enzymes, does not obviate the possibility that the enzyme levels may have increased concurrent to 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 examined the relationship between 2,3,7,8-TCDD exposure and hormone concentrations in adults, the results are mostly equivocal (CDC Vietnam Experience Study, 1988; Roegner et al., 1991; Grubbs et al., 1995; Suskind and Hertzberg, 1984). However, concentrations of thyroid binding globulin (TBG) appear 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. In fact, thyroid hormones are so important in development that in the United States all infants 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 accounting 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. Reduced T4 levels stimulate the pituitary to secrete more thyroid stimulating hormone (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, and TSH levels remain elevated and T4 levels decrease. A plausible mode of action for thyroid effects is described in Section 2.2.1.3 above.

2.2.6.3. *Cardiovascular Disease*

Elevated cardiovascular disease has been noted in several of the occupational cohorts (Steenland et al., 1999; Sweeney et al., 1997; Flesch-Janys et al., 1995) and in Seveso (Pesatori et al., 1998), as well as in the rice oil poisonings. This appears to be associated with ischemic heart disease and in some cases with hypertension. Recent data from the Ranch Hand study indicates 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 people 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). 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 the 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 shown 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 is caused in peritoneal macrophages by exposure to TCDD (Alsharif et al., 1994). A recent report that low-dose (0.15 ng TCDD/kg/day) chronic 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.

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 recent 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).

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 mode(s) of action. This discussion will focus 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 will identify important and relevant knowledge gaps and uncertainties in the understanding of the mechanism(s) of dioxin action, and will indicate how these may affect the approach to risk characterization.

3.1. MODE VERSUS MECHANISM OF ACTION

In the context of revising its Cancer Risk Assessment Guidelines, the 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). 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 (NAS) National Research Council (NRC) 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, identify key events that can be measured, outline the information that is available to support the hypothesis, and discuss those data that are

inconsistent with the hypothesis or support an alternative hypothesis. Following this, the information is weighed to determine if 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 if 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, i.e., 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 were 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, i.e., 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 classical "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. Many of these issues may apply to dioxin-receptor interactions. To the extent that they do occur, they may present complications to use of the toxic equivalency approach, particularly for extrapolation purposes. 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 the face of incomplete mechanistic understanding to address uncertainty.

3.2.2. A Framework to Evaluate Mode of Action

EPA in its revised proposed guidelines for carcinogen risk assessment (U.S. EPA, 1999) 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 would now stop short of evaluating the association between the chemical or complex mixture and clearly adverse effects. Instead, the approach would apply to early events, e.g., 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. Several of these 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, 1994; 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). 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 of 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 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, involving 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., 2000a). AIP/XAP2/ara9 is a 37 kilodalton (kd) protein that is

related to known immunophilins and involved in 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 known 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., 2000b). 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., 2000a).

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) contributing 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 only apply 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 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 with 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 with 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. 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 periods of development, and might be modulated by different genetic and environmental factors. As such, this complexity increases the difficulty associated with assessing the human health risks from dioxin exposure.

Under certain circumstances, exposure to TCDD may elicit beneficial effects. 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 data base on mode(s) of dioxin action related to biochemical effects and to clearly adverse effects supports an understanding of dioxins' 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 should not detract from the recognition that, among data available to aid hazard characterization and risk assessment, these are remarkably consistent and useful findings.

4. EXPOSURE CHARACTERIZATION

This section summarizes key findings developed in the exposure portion of the Agency's dioxin reassessment. The findings are developed in the companion document entitled "Part I: Estimating Exposure to Dioxin-Like Compounds." This document is divided into four volumes: (1) Executive Summary; (2) Sources of dioxin in the United States; (3) Properties, Environmental Levels, and Background Exposures; and (4) 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 2: Sources of Dioxin-Like Compounds in the United States)

The CDD/CDFs have never been intentionally produced other than on a laboratory scale basis for use in scientific analysis. Rather, they are 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 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. These 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 bichloride).
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.

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 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 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 source 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. 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 (i.e., 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 3,300 gram (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 environmental releases of CDD/CDFs in the United States occur from a wide variety of sources, but are dominated by releases to the air from combustion sources.* The current (1995) inventory indicates 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 (representing 19% of total releases) and medical waste incinerators (representing 14% of total releases).
- *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

grams I-TEQ. These reductions will occur in the following source types: municipal waste combustors, medical waste incinerators, and various facilities which burn hazardous waste (see Part I, Volume 2 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 only available for chlorine bleached pulp and paper mills (356 g I-TEQ_{DF} or TEQ_{DF}-WHO_{98} for 1987 and 28 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 is achieved with limitations on effluent discharges of CDD/CDF from chlorine bleached pulp and paper mills, 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.* The only releases to land quantified in the national inventory are land application of sewage sludge or commercial sludge products (106.5 g I-TEQ_{DF} or 79 g TEQ_{DF}-WHO_{98} in 1995), land application of pulp and paper mill wastewater sludges (2.0 g I-TEQ_{DF} or TEQ_{DF}-WHO_{98} in 1995), use of 2,4-D pesticides (18.4 g I-TEQ_{DF} or 28.9 g TEQ_{DF}-WHO_{98}), and manufacturing wastes from EDC/VCM (<1 g I-TEQ_{DF} or TEQ_{DF}-WHO_{98}). 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 which are disposed of in approved landfills and are therefore outside the definition of annual environmental releases, and products which 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 suggesting that deposition exceeds emissions (Rappe,

1991; Harrad et al., 1992; Bruzy and Hites, 1995). The uncertainty, however, 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, however, indicate that the inventory could underestimate emissions of dioxin-like compounds:

- A number of sources lacked sufficient data to include in the inventory, but did have 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 only discovered 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 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 total magnitude of release for dioxin-like compounds into the U.S. environment from all sources. For example, in terms of 1995 releases from reasonably quantifiable sources, this document estimates releases of 3,300 g TEQ_{DF}-WHO_{98} (3,000 g I-TEQ_{DF}) for contemporary formation sources, and 2,900 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 estimate of 1995 poorly characterized contemporary formation sources is 1,500 g I-TEQ_{DF} or TEQ_{DF}-WHO_{98}. The preliminary release estimates for contemporary formation sources and reservoir sources are presented in **Table 4-3. Table 4-4** 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 contributions from point sources.* Current data are only sufficient to support preliminary estimates of nonpoint source contributions of dioxin-like compounds to water (i.e., 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 3, Chapter 6), lack of any identified large natural sources, and observations of higher CDD/CDF body burdens in industrialized vs. less industrialized countries (see discussion on human tissue levels in Section 4.4).
- *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 which 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 2, Chapter 2, Section 2.4.

- *Dioxins are present in some ball clays, but insufficient data are available to estimate whether environmental releases occur during the 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 2, Chapter 13, it is likely that 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 which 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 does, 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 (8,400 g I-TEQ_{DF} or 4,800 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 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 reflects past releases associated with PCB production, use, and disposal. Further support of this finding is based on observations of reductions since 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 Emissions 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 discussion in Part I, Volume 2, Chapter 2, Section 2.6 indicating that the 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. The geographic locations of sources relative to the areas from which much of the beef, pork, milk, and fish come is important to consider. That is,

much 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 Volume 3, Chapter 4, Section 4.4.2, 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 consumption of fish. As discussed in Volume 3, Chapter 4, Section 4.4.2, 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 which deposit on plants and enter the terrestrial food chain. The magnitude of this contribution, however, is unknown.

4.2. ENVIRONMENTAL FATE (Cross reference: Part I, Volume 3, 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. This is 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 which is available as a companion to this document (Database of Sources of

Environmental Releases of Dioxin-Like Compounds in the United States (EPA/600/P-98/002Ab). In Part I, Volume 4, 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, generally classified as semivolatile, and tend to bioaccumulate in animals. Some evidence has shown that these compounds can degrade in the environment, but in general they are considered 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 either 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 become buried in deeper sediments. Immobile sediments appear to serve as permanent sinks for the dioxin-like compounds. Though not always considered an environmental compartment, these compounds are also found in anthropogenic materials (such as PCP) and have the potential to be released from these materials into the broader environment.

Atmospheric transport and deposition of the dioxin-like compounds are a primary means of dispersal of these compounds throughout the environment. The dioxin-like compounds can be 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 with similar properties, and this 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, 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.

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

Background levels of dioxin-like compounds in various environmental media including food are presented in **Table 4-5** 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 these data were derived from studies that were not designed to estimate national background means. The environmental media concentrations were similar to studies in Western Europe. These data are the best available for comparing with site-specific values. Because of the limited number of locations examined, it is not known if 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 this document (Part I, Volume 3, 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 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 8 states (CA, OH, GA, NY, PA, OR, MN, WS; 2 samples/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 are 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 Food and Drug Administration during the mid to latter1990s (Jensen and Bolger, 2000; Jensen et al., 2000). An important aspect of the U.S. Food and Drug Administration (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 over 300 individual samples and good representation of the most highly consumed species. However, the levels of dioxins in fish remain more uncertain than 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 which measured the effects of cooking on the levels of CDDs, CDFs and PCBs in foods (see Part I, Volume 3, Chapter 3, Section 3.7.5). 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 that 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 has occurred in concentrations of dioxins and furans in food products, particularly dairy products (see Part I, Volume 3, Chapter 6, Section 6.5). For example, the United Kingdom's Ministry of Agriculture, Fisheries, and Food (MAFF) 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, while 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 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 twentieth century (Winters et al., 1998). It was found that meat samples of the 1950s through the 1970s had concentrations that were 2-3 times higher for the CDD/CDF TEQs and about 10 times higher for the PCB TEQs, as compared to current meat concentrations.

4.4. BACKGROUND EXPOSURES (Cross reference: Part I, Volume 3, 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 which measured dioxin levels in adipose, blood, and human milk, all conducted in North America. The number of people in most of these studies, however, is relatively small and the participants were not statistically selected in ways that assure their representativeness of the general U.S. adult population. One study, the 1987 National Human Adipose Tissue Survey (NHATS), involved over 800 individuals and provided broad geographic coverage, but 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 trends discussion in Part I, Volume 3, 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) summarized levels of CDDs, CDFs, and PCBs in human blood collected during the time period 1995 to 1997. The individuals sampled were all U.S. residents with no known exposures to dioxin other than normal background. The blood was collected from 316 individuals in six different locations with an age range of 20 to 70 years. While the samples in this data set were not collected in a manner that can be considered statistically representative of the national population and 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 the same proportion to the total PCB TEQ as in earlier data, they would increase our 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. A summary of the CDC (2000) data is shown in **Table 4-6.**

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 and also that the variability in blood levels increase with age.

This finding regarding 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 our best estimate of current adult intake, i.e., 65 pg WHO_{98}-TEQ_{DFP}/d. Using this intake in a one-compartment, steady-state pharmacokinetic model yields a tissue level estimate of about 11.1 pg TEQ/g lipid (assumes TEQ_{DFP} has an effective half-life of 7.1 yr, 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 that was observed, than 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 in a person who turned 50 in 2000. Future studies should help address these uncertainties. The National Health and Nutrition Examination Survey (NHANES)

began a new national survey in 1999 that will measure blood levels of CDDs, CDFs, and PCBs 126, 77, 169, and 81 in about 1,700 people per year (see http:www.cdc.gov/nchs/nhanes.htm). The survey is conducted at 15 different locations per year and is designed to select individuals statistically representative of the civilian U.S. population in terms of age, race, and ethnicity. These new data should provide a much better basis for estimating national background tissue levels and evaluating trends than the currently available data.

4.4.2. Intake Estimates

Adult daily intakes of CDD/CDFs and dioxin-like PCBs are estimated to average 41 and 24 pg TEQ_{DFP}-WHO_{98}/day, respectively, for a total intake of 65 pg/day TEQ_{DFP}-WHO_{98}. Daily intake is estimated by combining exposure media concentrations (food, soil, air) with contact rates (ingestion, inhalation). **Table 4-7** 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). Pharmacokinetic (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 yr, 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 65 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 that are thought to be higher than current levels (see Part I, Volume 3, 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 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 comes from several analyses of the Bogalusa Heart Study (Cresanta et al., 1988; Nicklas et al., 1993; Nicklas et al., 1995; Nicklas et al., 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 which 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. This is based on the observation that the 95th percentile blood level in the CDC (2000) study was almost twice the mean level.

Intakes of CDD/CDFs and dioxin-like PCBs are over three times higher for a young child as compared to that of an adult, on a body weight basis. This is based on combining age-specific food consumption rate and average food concentrations, as was done above for adult intake estimates (see **Table 4-8**).

Only four of the 17 toxic CDD/CDF congeners and one 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, 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 indicating that these congeners are also the primary contributors to dietary dose. These five compounds make up about 80% of the total WHO_{98}-TEQ tissue level.

4.5. POTENTIALLY HIGHLY EXPOSED POPULATIONS OR DEVELOPMENTAL STAGES (Cross reference: Part I, Volume 3, 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 non-nursing infants and adults.

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 anti-caking agent in soybean meal and resulted in elevated dioxin levels in some poultry and catfish. This incident, which occurred in 1998, involved less than 5% of the national poultry production and has since 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 such incidents have led to disproportionate exposures to populations living near where these incidents have 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 highly exposed local populations.

Elevated exposures associated with the workplace or 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 ppt TCDD. There is no clear evidence that elevated exposures are currently occurring among United States 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 non-nursing infants (Part I, Volume 3, Chapter 5, Section 5.2).

Three German studies have compared dioxin levels in infants who have been breast-fed with those who have been formula-fed. All have shown elevations in the concentrations of dioxins in infants being breast-fed. Collectively these studies included 99 infants and found that blood levels (in units of pg TEQ_{DF}-WHO_{98}/g lipid - i.e., dioxin-like PCBs not included) in infants aged 4-12 months were generally more than 20 in nursing infants and less than 5 in formula fed infants.

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 dropped to 22 pg TEQ_{DFP}-WHO_{98}/kg/day after 12 months of nursing. The average intake over one year of nursing was calculated to be 92 pg TEQ_{DFP}-WHO_{98}/kg/day. The cumulative intake for a one year nursing scenario represented about 12% of the total lifetime cumulative intake (see Part I, Volume 3, Chapter 5, Section 5.2 for details on these calculations).

The CDC (1997) reported that in 1995, 55% of all babies experience 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 is ideal nutrition and sufficient to support optimal growth and

development for 6 months after birth. They 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. Changes in TEQ tissue concentration over time were modeled for a variety of nursing scenarios: formula only, 6 weeks nursing, 6 months nursing, and one year. These scenarios reasonably capture the range of current nursing practice. This modeling effort required using the intake assumptions described earlier and a variety of additional assumptions including: the fraction of the oral dose which is absorbed into the body, changes in body weight over time, and changes in body fat fraction over time. Assumptions were also made about changes in the biological half-life of dioxins as a function of body fat fraction. For the infant, the half-life was less than one year, and during adulthood the half-life increased as the fraction of body fat increased. The short half-life at birth was based on a study by Kreuzer et al. (1997) and the longer half-life during the later years of life, when body fat fraction increased, was based on a model presented in Michalek et al. (1996). The complete set of input values are listed in Part I, Volume 3, Chapter 5, Section 5.2.

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

- For the 6 and 12 month nursing scenarios, lipid concentrations peaked at around 4 months at about 46 ppt TEQ_{DFP}-WHO_{98}. The formula-fed infants 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.

A sensitivity analysis was performed to test the assumptions about changes in breast milk concentrations during lactation and changes in half-life over time. In this analysis, breast milk concentrations were held steady at 25 pg TEQ_{DFP}-WHO_{98}/g lipid for a 6-month nursing scenario, and the half-life of dioxins in the body remained steady at 7.1 years from birth until 70 years of age. With these two changes, the maximum infant lipid concentration increased from 46 to 70 pg TEQ_{DFP}-WHO_{98}/g lipid. The major impact of a steady half-life assumption, instead of one which increased with increasing body lipid fractions in the aging adult, was that the lipid concentrations

stabilized at about 8 pg TEQ_{DFP}-WHO_{98}/g lipid in the adult, instead of rising to 13 pg TEQ_{DFP}-WHO_{98}/g lipid at age 70.

The above analysis indicates that the average annual infant intake resulting from one year of nursing, 92 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, i.e. infant body burdens will not exceed adult body burdens by 92 times. Rather, the modeling suggests that peak infant body burdens are only about 2 times current adult body burdens (46 vs 25 pg TEQ_{DFP}-WHO_{98}/g lipid). The reduced body burden impacts in nursing infants (relative to the intake) is thought to be due to the rapidly expanding infant body weight and lipid volume and the possibly faster elimination rate in infants. Impacts to 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. Most people eat some fish from multiple sources, both fresh and salt water. The estimated dioxin concentrations in these fish and the typical rates of consumption are included in the mean background calculation of exposure. People who consume large quantities of fish at estimated contamination levels may have elevated exposures. These kinds of exposures are addressed within the estimates of variability of background and are not considered to result in highly exposed populations. If individuals obtain their fish from areas where the concentration of dioxin-like chemicals in the fish 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 measuring 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. Another study measuring 90 PCB congeners (seven of which were dioxin-like PCBs, although PCB 126 was not measured) in the blood of sport fishers consuming high amounts of fish caught from Lake Michigan (>26 pounds of sport fish/yr) did, however, show significant elevations of PCBs in their blood as compared to a control population (individuals consuming < 6 pounds of sport fish/yr). The average total concentration of PCBs in the blood of these sport fishers was over 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 3, 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 have also documented elevations in the levels of dioxin-like compounds in blood from the families consuming their home products.

5. DOSE-RESPONSE CHARACTERIZATION

Previous sections of this integrated summary have 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? (2) What is a reasonable limit (critical dose or point of departure) 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)

In Part II, Chapter 8, the body of literature concerning dose-response relationships of TCDD is presented. 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 are amenable to dose-response modeling, more than 200 data sets were evaluated for shape, leading to an effective dose (ED) value expressed as a percent response being presented for the endpoint being evaluated (e.g., ED_{01} equals an effective dose for a 1% response). The analysis of dose-response relationships for TCDD, considered within the context of toxic equivalency, mechanism of action, and background human exposures, helps to 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 point of departure 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 point of departure, which can help inform choices for extrapolation models for both TCDD and total TEQ.

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). 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.

Physiologically based pharmacokinetic (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, its molecular actions with the AhR and other proteins, as well as numerous physiological parameters (Part II, Chapter 1). These have provided insights into key determinants of TCDD disposition in treated animals. The most complete PBPK models give similar predictions about TCDD tissue dose metrics. The PBPK models have been extended to generate predictions for early biochemical consequences of tissue dosimetry of TCDD, such as induction of CYP1A1. Nevertheless, extension of these models to more complex responses is more uncertain at this time. Differences in interpretation of the mechanism of action 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 the determination of the dose metric to use for animal-to-human extrapolations. To provide significant insight into differences in sensitivity among species, an appropriate animal-to-human extrapolation of tissue dose is required. As described in Section 1.3, the most appropriate dose metric should reflect both the magnitude and frequency of exposure, and should be clearly related to the toxic endpoint of concern by a well-defined mechanism. This is, however, 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).

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 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 evaluation of acute exposures, such as those occurring in the 1976 accidental exposure of some people living in Seveso, Italy (Bertazzi and di Domenico, 1994). In cases such as this, 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. However, this issue of acute exposure is not a major factor in the current analyses. 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 5-2**. 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 six months by a mother with a background dioxin body burden level, and 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 255 ng/kg*Y, equivalent to a lifetime average body burden of 3.6 ng/kg (~255/70 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 - one hundred times background - then ceasing. 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 peak body burden is ~40 times background, but the AUC and LABB are only 15 times background.

Table 5-1 and **Figure 5-1** 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, tabulating either current levels for the background population or back calculated peak levels for the exposed cohorts. **Figure 5-1** 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, and for this cohort the TCDD graph includes non-TCDD TEQ. 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.

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 to 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. As these are peak body burdens, measured at the time of the Seveso accident or back-calculated to the time of last known elevated exposure, being compared to background averages, average lifetime body burdens in these cohorts will be even closer to lifetime average background levels. This will be important if, as demonstrated for some chronic effects in animals and as assumed when relying on average body burden as a dose metric, cancer and other noncancer effects are a consequence of average tissue levels over a lifetime. Body burdens begin to decline slowly soon after elevated exposure ceases. Some data in humans and animals suggest that elimination half-lives for dioxin and related compounds may be dose-dependent, with high doses being eliminated more rapidly than lower doses. Nonetheless, the use of an approximately 7-year half-life of elimination presents a reasonable approach for evaluating both back-calculated and average lifetime levels, because for most cohorts the exposure is primarily to TCDD.

The ability to detect effects in epidemiologic studies is dependent on a sufficient difference between control and exposed populations. The relatively small difference (<10-100 fold) 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.

Characterization of the risk of exposure of humans today remains focused on the levels of exposure that occur in the general population, with particular attention given to special populations (see Part I, Volume 3, Chapters 4 and 5). For evaluation of multiple endpoints and considering the large differences in half-lives for TCDD across multiple species, it is generally best to use body burden rather than daily intake as the dose metric for comparison unless data to the contrary are presented. Further discussion of this point, which provides the rationale for this

science-based policy choice, is presented in Part II, Chapters 1 and 8, and is summarized in Section 1.3 of this document .

5.1.1. Calculations of Effective Dose (ED)

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, comparison of responses is made 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 or ED, which is the exposure dose resulting in an excess response over background in the studied population. EPA has suggested this approach in calculating benchmark doses (BMD) (Allen et al., 1994) and in its proposed approaches to quantifying cancer risk (U.S. EPA, 1996; U.S. EPA, 1999). 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 (2 times the background lifetime risk) represents approximately a 4% increased lifetime risk. Based upon 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}. The use of ED values below 10% is consistent with the Agency's guidance on the use of mode of action in assessing risk, as described the proposed Cancer Risk Assessment Guidelines (U.S. EPA, 1996; U.S. EPA, 1999) 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 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 on the mechanism of action of this chemical, 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). As such, 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 non-dioxin-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 recently summarized (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 a 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 therefore 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 described in Section 2.2.1.4, TCDD has been characterized as a human carcinogen, 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, in respiratory tumors and, perhaps, in soft-tissue sarcoma. 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, there exist three studies of human occupational exposure with enough information to perform a quantitative dose-response analysis.

Table 5-2 summarizes the epidemiology and bioassay studies used in the calculations of the all cancer mortality ED_{01}s/LED_{01}s. Results for three different occupational cohorts are

tabulated: Hamburg, NIOSH, and BASF, along with the bioassay results on liver cancer in female Sprague-Dawley rats (Kociba et al., 1978). In addition to the three dose-response results analyzed in Part II, Chapter 8 (Flesch-Janys et al., 1998; Aylward et al., 1996; Ott and Zober 1996a, b), two additional primary publications on these occupational cohorts are tabulated and graphed. Although these additional studies demonstrate dose-response relationships when using improved exposure metrics, neither can be used for the calculations in this assessment because of lack of an upper confidence interval on the risk provided in the original publication (Becher et al., 1998) or absence of a quantitative exposure metric (Steenland et al., 1999).

Modeling cancer in humans uses slightly different approaches from those used in modeling animal studies. The modeling approach used in the analysis of the human epidemiology data for all cancers combined and lung cancer involves applying estimated human body burden to cancer response and estimating parameters in a linear risk model for each data set. A linear risk model was used because the numbers of exposure groups available for analysis were too small to support more complicated models. Because of this, no evaluation of the shape of the dose-response data for the human studies was performed. Access to the raw data may make it possible to use more complicated mathematical forms that allow for the evaluation of shape. In the one case in which this has been done, the dose-response shape suggested a response that was supralinear (dose raised to a power <1) (Becher et al., 1998). For these studies, there are several assumptions and uncertainties involved in modeling the data, including extrapolation of dosage, both in back-calculation and in elimination kinetics, and the type of extrapolation model employed.

As described in Part II, Chapter 8, Section 8.3, the data used in the analyses are from Flesch-Janys et al. (1998) for the Hamburg cohort, Aylward et al. (1996) for the NIOSH study, and Ott and Zober (1996a,b) for the BASF cohort. The limited information available from these studies is in the form of standardized mortality ratios (SMRs) and/or risk ratios by exposure subgroups with some estimate of cumulative subgroup exposures. Exposure subgroups were defined either by number of years of exposure to dioxin-yielding processes or by extrapolated TCDD levels. No study sampled TCDD blood serum levels for more than a fraction of its cohort, and these samples were generally taken decades after 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. Aylward et al. (1996) used the average TCDD levels of those sampled in an exposure subgroup to represent the entire subgroup. Flesch-Janys et al. (1998) and Ott and Zober (1996a) performed additional calculations, using regression procedures with data on time spent at various occupational tasks, to estimate TCDD levels for all members of their respective cohorts. They then divided the cohorts into exposure groups based on the estimated TCDD levels. The information presented in

the literature cited above was used to calculate estimated average TCDD dose levels in Chapter 8, Section 8.3.

To provide ED_{01} estimates for comparison in Chapter 8, Section 8.3, Poisson regression (Breslow and Day, 1987) was used to fit a linear model to the data described above. A linear model was chosen for several reasons. Analysis of animal cancer data suggests a mixture of linear and nonlinear responses, with linear shape parameters predominating (Portier et al., 1984). Toxic responses to TCDD, both cancer and noncancer, are presumably more likely to result from multiple cellular and tissue-level perturbations and are less likely to follow linear relationships. This hypothesis was examined by empirical dose-response modeling of cancer and noncancer effects of TCDD in experimental animals (Part II, Chapter 8, Section 8.3). This empirical modeling exercise demonstrated that in general, the linear models provided the best fit to the biochemical response data and that more complex responses were generally fit best with non-linear models. Many examples of adverse effects experienced at these low levels have too much data variability to clearly distinguish on a statistical basis between dose-response curve options, and whether dose-response follows linear, supra/sub-linear, power curve, or threshold kinetics.

Besides the issue of use of a linear model, additional important uncertainties in the human epidemiological data discussed in Part II, Chapter 8, Section 8.3, 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. Nevertheless, with these qualifications, it is possible to apply simple empirical models to studies in which exposure data for TCDD are available in human populations.

The analysis of these three epidemiological studies of occupationally exposed individuals suggest an effect of TCDD on all cancers, and on lung cancers in the adult human male. The ED_{01}s based upon average excess body burden of TCDD ranged from 5.7 ng TCDD/kg to 250 ng TCDD/kg in humans. The lower bounds on these doses (based on a modeled 95% C.I.) range from 3.5 ng TCDD/kg to 120 ng TCDD/kg. For the effect of TCDD on all cancers combined, the human ED_{01}s ranged from 5.7 ng/kg to 80.2 ng/kg. The lower bounds on these doses (based on a modeled 95% C.I.) range from 3.5 ng TCDD/kg to 37.5 ng TCDD/kg. For the effect of TCDD on lung cancers, the only tumor site increased in both rodents and humans, the human ED_{01}s ranged from 36.6 ng/kg to 250 ng/kg. The lower bounds on these doses (based on a modeled 95% C.I.) range from 16.2 ng TCDD/kg to 120 ng TCDD/kg. These estimates of ED_{01}s are compared to animal estimates later in this discussion.

Both empirical and mechanistic models were used to examine cancer dose-response in animals. 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 of 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 the NTP (Osborne-Mendel rats and B6C3F$_1$ mice, 1982a). The findings from this analysis, which examined cancer dose-response within the range of observation, are presented in Part II, Chapter 8, Table 8.3.2., which is reproduced with slight modifications as **Table 5-3.** 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. (1978) study (feed experiment) and 100% for the NTP study (gavage experiment). Also presented in **Table 5-3** are the shapes of the dose-response curves as determined by Portier et al. (1984).

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 for these cases. These studies had only three experimental dose groups, hence these shape calculations are not based upon sufficient doses to guarantee a consistent estimate; they should be viewed with caution. The ED_{01} steady-state body burdens range from a low value of 14 ng/kg based upon the linear model associated with liver tumors in female rats to as high as 1,190 ng/kg based upon 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 0.8 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 0.6 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 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 2-year cancer bioassay of 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, different dose metrics can lead to widely diverse conclusions. For example, as described in Chapter 8, Section 8.2, 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 1,400 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 ngTCDD/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 ngTEQ/kg body weight (assuming about 25% of body weight is lipid), humans are exposed to about 5 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 between 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, since this accounts for the large differences in animal to 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 approximately equal potential for the carcinogenic effects of TCDD. In humans, restricting the analysis to log-linear models in Part II, Chapter 8, Section 8.3, resulted in cancer ED_{01}s ranging from approximately 6 ng/kg to 250 ng/kg. This was similar to the empirical modeling estimates from the animal studies, which ranged from 14 ng/kg to 1,190 ng/kg (most estimates were in the range from 14 to 500 ng/kg). The lower bounds on the human body burdens at the ED_{01}s (based on a modeled 95% C.I.) range from 3.5 ng TCDD/kg to 120 ng TCDD/kg. Lower bounds on the steady-state body burdens in the animals range from 10 ng TCDD/kg to 224 ng/kg. The estimate for the single mechanism-based model presented earlier (2.7 ng/kg) was approximately 2 times lower than the lower end of the range of human ED_{01} estimates and less than the lower bound on the LED_{01}. The same value was approximately 5 times lower than the lower end of the range of animal ED_{01} estimates and less than 4 times less than the LED_{01}.

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

[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.

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

$$\text{Intake (pgTEQ/kgBW/day)} = \frac{[\text{body burden at } ED_{01} \text{ (ng TEQ/kg)} * \text{Ln(2)}]}{\text{half-life (days)}} * 1/f \qquad (5\text{-}1)$$

half-life = 2,593 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 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 ED01, where: (5-2)

Risk at ED_{01} = 0.01

Use of these approaches reflects methodologies being developed within the context of the revised draft Cancer Risk Assessment Guidelines. Slopes are estimated by a simple proportional method at the "point of departure" (LED_{01}) at the low end of the range of experimental observation. As discussed below, these methods can be compared to previous approaches using the linearized multistage (LMS) procedure to determine if 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 $\times 10^{-4}$ per pgTCDD/kgBW/day which is equivalent to 1.6 $\times 10^{5}$ per mgTCDD/kgBW/day (U.S. EPA, 1985).

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

Estimates of upper bound slope factors (per pg TCDD/kgBW/day) calculated from the human ED_{01}s presented in Part II, Chapter 8, Table 8.3.1, range from 8.6 $\times 10^{-3}$, if the LED_{01} for all cancer deaths in the Hamburg cohort is used, to 2.5 $\times 10^{-4}$ if the ED_{01} for lung cancer deaths in

the smaller BASF cohort is used. All of the other slope factors for all cancer deaths or lung cancer deaths in the three cohorts would fall within this range. LED_{01}s for all cancer deaths span approximately an order of magnitude and would generate slope factors in the range of 8.6×10^{-3} to 8×10^{-4}. Slightly smaller slope factors are generated when LED_{01}s for lung cancer are used. The largest slope factors based on LED_{01}s come from the Hamburg cohort (8.6×10^{-3} and 1.9×10^{-3} respectively for all cancer deaths and lung cancer deaths.) 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). Thus, a meta-analysis was performed by combining all data sets into a single large data set and using Poisson regression procedures detailed in Part II, Chapter 8, Section 8.3, yielding a slope factor estimate of approximately 1×10^{-3} per pg TCDD/kgBW/day. This represents EPA's most current upper bound slope factor for estimating human cancer risk based on human data.

These estimates compare well with the estimates of cancer slope and risk associated with TCDD exposure in the Hamburg cohort published by Becher et al. (1998). The risk estimates of Becher et al.(1998) were derived from data on TCDD exposure to male workers with a 0 or 10-year latency and taking into account other factors affecting risk including choice of model, latency, job category, dose metric, and concurrent exposures. These estimates range from 1.3×10^{-3} to 5.6×10^{-3} per pg TCDD/kgBW/day. In this analysis all excess cancers are attributed to TCDD exposure, despite significant levels of other dioxin-like compounds in blood measurements of this cohort (see **Table 5-1**) with similar slope coefficients calculated for total TEQ. Although risk estimates using TCDD alone in this cohort might suggest an overestimate of risk because dose is underestimated, no evidence for this emerged from the analysis because TCDD dominates the total TEQ in this population. In the preparation of this document, an independent estimate of slope using the Becher models was performed consistent with the approaches suggested in Part II, Chapter 8, Section 8.2 (See Table 8.4 for more details). A slope of 3×10^{-3} per pg TCDD/kgBW/day was derived. This slope represents a central estimate since no upper bound could be calculated with the available data.

Taking into account different sources of variation, Becher et al. (1998) suggest a range of 10^{-3} to 10^{-2} for additional lifetime cancer risk for a daily intake of 1 pg TCDD/kg BW/day. By inference, that range could also apply to total TEQ intake. As described in Section 4.4.2, current intakes in the United States are estimated to be approximately 1 pg TEQ_{DFP}-WHO_{98}/kg BW/day. Using Equation 5-2 and based on all cancer deaths in the three cohorts, the upper bound range of risks estimated from current human body burdens of 5 ng TEQ_{DFP}-WHO_{98}/kgBW (which equates to a serum level of approximately 20 pg/g lipid [see **Table 4-7**]) ranged from 1.4×10^{-2} to 1.3×10^{-3}. Based on lung cancer deaths, the lower end of the upper bound on the estimates of excess risk extended to 4×10^{-4}. Using the LED_{01} estimate of 30.1 ng/kg from the meta-analysis yields

an upper bound risk estimate of 1.7×10^{-3} for an average lifetime body burden estimate of 5 ng/kg. Estimates using high end current or historical body burdens would be proportionately higher. The range of these estimates provides further support for the perspective on risk provided by Becher et al. (1998). Uncertainties associated with these estimates from human studies are discussed in Part II, Chapter 8, Section 8.3, and in Becher et al. (1998).

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/kgBW/day) for human cancer risk calculated from lower bounds in ED_{01}s (LED_{01}s) for the animal cancers presented in **Table 5-3** range from 3×10^{-3} to 1×10^{-4}. This spans a range from being 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 largest 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 largest slope factor.

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 (U.S. EPA, 1985). Despite the use of the same study results, a slope factor of 1.8×10^{-5} per pg TCDD/kgBW/day results using the linearized multistage (LMS) approach in Portier et al. (1984). This is a factor of approximately 10 lower than the EPA (U.S. EPA, 1985) estimate of the slope. The differences are attributable to the aims of the respective calculations at the time. Portier et al. (1984) calculated "virtually safe doses" assuming that rodent and human doses scaled on a mg/kg basis, and he used the original tumor counts from the study. EPA (U.S. EPA, 1985), on the other hand, used $(BW)^{2/3}$ to arrive at a human equivalent dose and used the pathology results from a reread of the original Kociba study (U.S. EPA, 1980). In addition, EPA (U.S. EPA, 1985) adjusted tumor counts for early mortality in the study. The factor to adjust for $(BW)^{2/3}$-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/kgBW/day) is multiplied by these two factors, a slope of 1.7×10^{-4} per pg TCDD/kgBW/day is calculated. This is essentially equivalent to the EPA (U.S. EPA, 1985) estimate of 1.6×10^{-4} per pg TCDD/kgBW/day. Reconciling these issues is important to ensure appropriate comparisons of slope factor estimates.

Calculating a Revised Estimate of Cancer Slope from Kociba et al. (1978)

More important 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 (2,593 days versus 25 days [see Part II, Chapter 8, Table 8.1]). 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 tumor counts. This compares favorably with the Portier estimate of 10 ng TEQ/kg found in **Table 5-3.** The difference is entirely accounted for by the early deaths adjustment by EPA (U.S. EPA, 1985). Use of these body burdens at the LED_{01} results in slope factor estimates of 3.3×10^{-3} per pg TCDD/kgBW/day and 4.9×10^{-3} per pg TCDD/kgBW/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 does the unadjusted estimate. EPA's revised slope factor (4.9×10^{-3} per pg TCDD/kgBW/day) would be 31 times greater than the slope factor from 1985.

However, a second issue with the modeling of the Kociba data relates to the appropriate tumor counts to use. As mentioned in Section 2.2, Goodman and Sauer (1992) reported a second re-evaluation of the female rat liver tumors in the Kociba 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 of 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 (U.S. EPA, 1985) slope factor estimate 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/kgBW/day. However, because the original estimates used a $(BW)^{2/3}$ scaling, this must be adjusted to use body burden and obtain an appropriate result. 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/kgBW/day are derived. These results can also be obtained using EPA's Bench Mark Dose (BMD) 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. <u>This represents EPA's most current upper bound slope factor for estimating human cancer risk based on animal data.</u> 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 use of the body burden dose metric (31 times greater) and the use of the Goodman and Sauer (1992) pathology (3.6 times less).

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 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 can be calculated. It is 7.1×10^{-3} per pg TCDD/kgBW/day. This estimate represents an example of the type of modeling, based on key events in a mode of action for carcinogenesis, which is consistent with 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 upon two types of initiated cells 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 while 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, while the other group is increasing in size. Their model is different from those of Portier and Kohn (1996) 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 (1997) did not apply their model to cancer risk estimation. Presently, there is insufficient experimental data to support or refute the use of either the Portier and Kohn (1996) model or that of Conolly and Andersen (1997).

5.2.2. Noncancer Endpoints

At this point, sufficient data are not available to model noncancer endpoints in humans. Many studies are available to estimate ED_{01} values for noncancer endpoints in animals. However, there are a number of difficulties and uncertainties that should be considered when comparing the same or different endpoints across species. Some of these include differences in sensitivity of endpoints, times of exposure, exposure routes, species and strains, 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 in comparing values from different designs. Estimates of ED_{01}s in Part II, Chapter 8 represent estimates of 1% of the maximal response in the studies being evaluated. In addition, caution should 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. Finally, background exposures are not often considered in these calculations simply because they were not known.

Nevertheless, given these considerations, several general trends were observed and discussed in Part II, Chapter 8. The lowest ED_{01}s tended to be for biochemical effects, followed by hepatic responses, immune responses, and responses in tissue weight. An analysis of shape parameters 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.

Overall shape parameter data suggest that biochemical responses to TCDD are more likely to be linear within the experimental dose range, while the more complex responses are more likely to assume a nonlinear shape. However, a large number (> 40%) of the more complex responses have shape parameters that are more consistent with linearity than nonlinearity.

The tissue weight changes seen for animals (using only data sets with good or moderate empirical fits to the model) yielded a median ED_{01} at average body burdens of 510 ng/kg in the multidose studies (range; 11 to 28000 ng/kg) and a median ED_{01} of 160 ng/kg (range 0.0001 to 9,700 ng/kg) in the single dose studies. Toxicity endpoints from the single dose studies resulted in a median value at average body burdens of 4,300 ng/kg (range 1.3 to 1,000,000 ng/kg). For tissue weight changes, 43% of the dose-response curves exhibited linear response. In contrast, the toxicity endpoints from the single-dose studies exhibited predominantly nonlinear responses (80%). All multidose studies demonstrated a greater degree of linear response than did single-dose studies, especially for tissue weight changes and toxicity endpoints (50% linear for multidose versus 34% for single dose). In general, it is not possible to dissociate the differences between cancer and noncancer dose-response as being due to differences in endpoint response or simply to differences in the length of dosing and exposure. Also, a greater percentage of the noncancer ED_{01}s were extrapolations below the lower range of the data (42%) than was the case for the cancer endpoints (8% in animals and no extrapolations in humans).

Results from the analysis of ED_{01}s and from examining 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 which may be on the critical path for both noncancer and cancer effects, such as enzyme induction or impacts on growth factors or their receptors. While 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 both many biochemical and some clearly adverse effects in animals (See **Table 2-2** for examples). The use of ED_{01}s and 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 reference dose calculations. The range of effects (biochemical, tissue or toxic responses) is presented and individual responses at the low end of the range in each of these categories are discussed in the development of the hazard characterization to demonstrate the potential significance of these responses in similarly exposed humans.

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

As described in 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 have 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.

As a part of this reassessment, the development of biologically based dose-response (pharmacodynamic) models for dioxin and related compounds has lead 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, hypotheses testing and model development.

5.4. SUMMARY DOSE-RESPONSE CHARACTERIZATION

All humans tested contain detectable body burdens of TCDD and other dioxin-like compounds that are likely to act through the same mode of action. Receptor modeling theory outlined in Chapter 8 indicates that xenobiotics which 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 may also follow linear dose-response kinetics, such as effects on DNA transcription and enzyme induction. 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 effect 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/sub-linear, 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 2-4 and 5-2**) and are insufficient to move from EPA's default linear extrapolation policy in the proposed Carcinogen Assessment Guidelines (U.S. EPA, 1996; 1999). This policy is that for cancer dose-response the data are to be modeled within the observed range, and a point-of-departure calculated from which a linear extrapolation to the origin is generated. For noncancer endpoints, EPA proposes using a margin of exposure 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 increments of dose to this background level. Margins of exposure between population levels and the empirically observed (not modeled) one percent 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 margins of exposure. 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 margin of exposure 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 uncertainties described above, a simple margin-of-exposure approach may be useful to decision-makers when discussing risk management goals. However, this decision would have to be based upon 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 point of departure 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 5.7 ng/kg to 250 ng/kg. This was similar to the estimates, from empirical modeling, from the animal studies which ranged from 14 ng/kg to 1,190 ng/kg (most estimates were in the range from 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. In addition to tabulating the results provided in Part II, Chapter 8, this table includes: 1) a further calculation of the central estimate from the Hamburg occupational cohort using formulae derived from Becher et al. (1998); 2) a Poisson regression analysis of all three occupational cohorts combined; and 3) benchmark dose (BMD) analyses of the Kociba rat bioassay using both daily dose and adipose tissue concentration as the metrics. The slope factor calculations are performed by linearly extrapolating the 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). A slope factor estimate of approximately 1×10^{-3} per pg TCDD/kgBW/day, based on the meta-analysis, 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/kgBW/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/kgBW/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 greater than a 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 2-3 times of 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. Based on these slope factor estimates (per pg TEQ/kgBW/day), upper bound cancer risk at average current background body burdens (5 ng TEQ/kgBW) exceed 10^{-3} (1 in a thousand). Current background body burdens reflect higher average intakes from the past (approximately 3 pgTEQ/kgBW/day). A very small percentage of the population (< 1%) may experience risks that are 2-3 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. This range of upper bound risk for the general population has increased from the risk described at background exposure levels based on EPA's draft of this reassessment (10^{-4}-10^{-3}) (U.S. EPA, 1994).

Estimates for noncancer endpoints showed much greater variability. In general, the noncancer endpoints displayed lower ED_{01}s for short-term exposures versus longer term exposures, and for simple biochemical endpoints versus more complex endpoints such as tissue weight changes or toxicity. In addition, the noncancer endpoints generally displayed higher estimated ED_{01}s than the cancer endpoints, with most estimates ranging from100 ng/kg to 100,000 ng/kg. The mechanism-based models for noncancer endpoints gave a lower range of ED_{01}s (0.17 to 105 ng/kg). Although most of these estimates were based upon 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.

These estimates, although highly variable, suggest that any choice of body burden, as a point of departure, above a body burden of 100 ng/kg would likely yield >1% excess risk for some endpoint in humans, including those with clear clinical significance. Also, choosing a point of departure below 1 ng/kg would likely be an extrapolation below the range of these data and would likely represent a risk of <1%. Any choice 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 in the range of 10 ng/kg 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.

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 is, for the most part, 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 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 or inferences have been used in the absence of data. Although characterization of risk is inherently a scientific exercise, by its nature it must 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 our confidence in a well structured and documented characterization of risk, but should serve to confirm the importance of considering risk assessment as an iterative process that benefits from evolving methods and data collection.

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

There is adequate evidence based on all available information discussed in Parts I and II of this reassessment, as well as that discussed in this Integrated Summary, to support the inference that humans are likely to respond with a broad spectrum of effects from exposure to dioxin and related compounds. These effects will likely range from biochemical changes at or near background levels of exposure to adverse effects with increasing severity as body burdens increase above background levels. 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 may be considered adverse. Induction may lead to more rapid metabolism and elimination of potentially toxic compounds, or may lead to increases in reactive intermediates and may potentiate toxic effects. Demonstrations of 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 Section 5. Subtle effects, such as the impacts on neurobehavioral outcomes, thyroid function, and immune system alterations seen in the Dutch children exposed to background levels of dioxin and related compounds, or changes in circulating reproductive hormones in men exposed to TCDD, illustrate the types of responses that support the finding of 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 background by one or two orders of magnitude (10 or 100 times). The

mechanistic relationships of biochemical and cellular changes seen at or near background body burden levels to production of adverse effects detectable at higher levels remain uncertain. Information on these mechanistic relationships is useful in hazard characterization and data are accumulating to suggest mode of action hypotheses for further testing.

It is well known that individual species vary in their sensitivity to any particular dioxin effect. However, the evidence available to date indicates that humans most likely fall in the middle of the range of sensitivity for individual effects among animals rather than at either extreme. 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 discussed in the reassessment, 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 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 carcinogenic, developmental, reproductive, immunological, and endocrinological hazards. The deduction that humans are likely to respond with noncancer effects from exposure to dioxin-like compounds is based on the fundamental level at which these compounds impact cellular regulation and the broad range of species that have been demonstrated to respond with adverse effects. For example, because developmental toxicity following exposure to TCDD-like congeners occurs in fish, 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 analysis of the Dutch cohort data and laboratory animal studies suggests 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 5-1** in this document) and when these cohorts have been 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 makes it difficult to evaluate the inference that noncancer effects associated with exposure to dioxin-like compounds may be occurring in humans. 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. Some have argued that to deduce that a spectrum of noncancer effects will occur in humans in the absence of better human data overstates the science; most scientists involved in the reassessment as authors and reviewers have indicated that such inference is reasonable given the weight-of-the-evidence from available data. As presented, this logical conclusion represents a testable hypothesis which 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 that will reduce our uncertainty regarding both hazard and risk characterization for dioxin and related compounds.

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 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 that does not allow us to rule out receptor-independent alternative pathways. However, these reported non-AhR mediated responses occur at doses that are orders of magnitude higher than human exposures and require much higher doses than other AhR mediated effects in animals. Thus, these non-AhR mediated mechanisms are unlikely to impact any of the assumptions made in this reassessment. The well-documented effects elicited by exposure of animals and, in some cases, humans, to 2,3,7,8-TCDD are shared by other chemicals with similar structure and AhR binding characteristics. In the past 5 years, significant data has 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 has been 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, 1989).

Analyses in this document demonstrate that, although variability in the data underpinning the scientific judgments regarding toxic equivalency exist, 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.

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 as a "useful, interim" way to deal with the complex mixture problem and 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 generally rejected as inadequate for risk assessment purposes.

Significant additional literature is now available on the subject of toxic equivalency of dioxin and related compounds, and Part II, Chapter 9 provides the reader with a summary that is up to date through 1999. A recent international evaluation of all of the available data (van den Berg et al., 1998) has reaffirmed the TEQ approach and has 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. Future research will be needed to address remaining uncertainties inherent in the current approach. The 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.

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

A weight-of-the-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 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" is appropriate when the available tumor effects and other key data are adequate to demonstrate carcinogenic potential to humans (U.S. EPA, 1999). Adequate data are recognized to span a wide range. The data for complex mixtures of dioxin and related compounds represents a case that, according to 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 human carcinogens.

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 indicating that TCDD probably 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 data 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.

As discussed earlier in Section 2.2.1.4, under EPA's current approach individual congeners can also be characterized as to their carcinogenic hazard. 2,3,7,8-tetrachlorodibenzo-

p-dioxin (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), 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 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 demonstrate a causal association.
- 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 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 epidermal growth factor receptor and estrogen receptor.
- The human AhR and 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 individual dioxin-like compounds are characterized as "likely" human carcinogens 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 lack of 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. On the basis of this logic, 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 OCDD as compared with one dominated by pentaCDF.

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 margin of exposure (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 indicates that many of these biochemical effects can be hypothesized as key events in a generalized dioxin mode-of-action model. These analyses do not argue for significant departures from linearity below a calculated ED_{01} for endpoints potentially related to cancer response, 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 in Section 5 for both animals and humans, fall in a range of approximately 1×10^{-3} to 9×10^{-3} per pg/TEQ/kgBW/day. The ranges of estimates of upper bound cancer potency calculated from the human and animal data analyzed in Part II, Chapter 8, Section 8.3, 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 estimate from the results of the meta-analysis of the three human studies and from reanalyzed Kociba study. 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/kgBW/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 meta-analytic estimate from the human studies and the comparability of the revised estimate from the animal data. 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 greater than a 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 2-3 times of 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. Based on these slope factor estimates (per pg/TEQ/kgBW/day), risks at average current background body burdens (5 ng TEQ/kgBW) that result from average intakes of approximately 3 pgTEQ/kgBW/day in the past exceed 10^{-3} (1 in a thousand). A very small percentage of the population (< 1%) may experience risks that are 2-3 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. This range of upper bound risk for the general population has increased from the risk described at background exposure levels based on EPA's draft of this reassessment (10^{-4}-10^{-3}) (U.S. EPA, 1994).

Despite the use of the epidemiology data to describe an upper bound on cancer risk, the Peer Panel that met in September 1993 to review an earlier draft of the cancer epidemiology chapter suggested that the epidemiology data alone were still not adequate to implicate 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 not be dismissed or ignored. Other scientists, including those who attended the Peer Panel meeting, felt either more or less strongly about the weight of the evidence from cancer epidemiology studies, representing the range of opinion that still exists on the interpretation of these studies. Similar opinions were expressed in the comments documented in the SAB's report in 1995 (U.S. EPA,1995). More recently, IARC (1997), in its reevaluation of the cancer hazard of dioxin and related compounds, found that whereas the epidemiologic database for 2,3,7,8-TCDD was still "limited," the overall weight of the evidence 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 has been proposed within the context of the Department of Health and Human Services' Report on Carcinogens (NTP, 2000). They too base their characterization on the broad base of human, animal, and mode-of-action information in humans and animals that supports this conclusion. 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 are likely to demonstrate low-dose linearity. The mechanistic relationship of these early events to the complex process of carcinogenesis remains to be established. If these findings imply low-dose linearity in biologically based cancer models under development, then the probability of cancer risk will be linearly related to exposure to TCDD at low doses. 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 only be inferred with uncertainty. Associations between exposure to dioxin and certain types of cancer have been noted in occupational cohorts with average body burdens of TCDD approximately 1- 3 orders of

magnitude (10-1,000 times) higher than average TCDD body burdens in the general population. The average body burden in these occupational cohorts level is within 1-2 orders of magnitude (10-100 times) of average background body burdens in the general population in terms of TEQ (see **Table 5-1** and **Figure 5-1**). Thus, there is no need for large-scale low-dose extrapolations in order to evaluate background intakes and body burdens, and little 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.

TCDD has been clearly shown to increase malignant tumor incidence in laboratory animals. In addition, a number of studies analyzed in this reassessment demonstrate other biological effects of dioxins related to the process of carcinogenesis. 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.

Use 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 is often evaluated using a MOE approach. The Agency has used this approach for a number of years in its assessment of the safety of pesticides. This concept has also been incorporated into the revised proposed Guidelines for Carcinogen Risk Assessment. A MOE is calculated by dividing a "point of departure" for extrapolation purposes at the low end of the range of observation in human or animal studies (the human-equivalent animal lowest observed adverse effect level (LOAEL), NOAEL, BMD, or effective dose [EDxx]) by the human exposure or body burden level of interest. Generally speaking, when considering either background exposures or incremental exposures plus background, MOEs in the range of 100-1,000 are considered adequate to rule out the likelihood of significant effects occurring in humans based on sensitive animal responses or results from epidemiologic studies. The adequacy of the MOE to be protective of health must take into account the nature of the effect at the "point of departure," the slope of the dose-response curve, the adequacy of the overall database, interindividual variability in the human population, and other factors. Considering MOEs based on incremental exposures 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 dioxins is that background exposures may not be insignificant when based on total TEQ. The average levels of background intake and associated body burdens of dioxin-like compounds in terms of TEQs in the general

population are well within a factor of 100 of human-equivalent exposure levels associated with NOELS, LOAELs, BMDs, or ED_{01} values in laboratory animals exposed to TCDD or TCDD equivalents. In many cases, the MOE compared to background using these endpoints is a factor of 10 or less (see **Tables 2-2 and 2-3**). These estimates, although variable, suggest that any choice of body burden, as a point of departure, above 100 ng/kg would likely yield >1% excess risk for some endpoint in humans (see Part II, Chapter 8). Also, choosing a point of departure below 1 ng/kg would likely be an extrapolation below the range of these data and would likely represent a risk of < 1%. Any choice for a 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 a point of departure in the range of 10 ng/kg 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.

Because of the relatively high background 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 is to evaluate increments of exposure from specific sources when background exposures are low and insignificant. Any RfD that the Agency would recommend under the traditional approach for setting an RfD is likely to be 2-3 orders of magnitude (100-1,000) below current background intakes and body burdens. Because exceeding the RfD is not a statement of risk, discussion of an RfD for an incremental exposure when the RfD has already been exceeded by average background exposures is meaningless.

When evaluating incremental exposures associated with specific sources, knowing the increment relative to background may help to understand the impact of the incremental exposure. For instance, it would be misleading to suggest that an incremental exposure of 0.001 pg TEQ/kg/day was below the RfD if "background" exposures were already at or above that level. On the other hand, as part of the total, the increment represents less than a 0.1% increase over average "background," and we estimate that individuals within the 50%-95% range of exposure within the population may be 2-3 times (200%-300%) higher. This has led us to suggest that perhaps the best information for a decision-maker to have is: (1) a characterization of average "background" exposures; (2) a characterization of the percent increase over background of individuals or subpopulations of interest; and (3) a policy statement about when increases over average "background" become significant for the decision. This is not easy because one could argue that, given high "background," any addition, if it is widespread, is too much. On the other hand, someone else could argue that a 10% increase in incremental exposure for a small population around a specific point source would be well within the general population exposures

and would not constitute a disproportionate exposure or risk. In this case, the strategy might be to bring average "background" exposures down and to focus on large incremental exposures or highly susceptible populations. This would be a strategy that would parallel the Agency's lead strategy. Other parallel issues between dioxin-like compounds and lead are under discussion within the Agency.

ATSDR (1999a) set a minimal risk level (MRL), which is defined similarly to the EPA's RfD, for dioxin and related compounds of 1.0 pg TEQ/kgBW/day. Some of the data regarding lower bounds on the ED_{01}s from various noncancer effects call that MRL into question. WHO (2000) has set a tolerable daily intake of 1-4 pg TEQ/kgBW/day and has indicated that, although current exposures in that range are "tolerable" (a risk management decision rather than a risk assessment), efforts should be made to ultimately reduce intake levels. Findings in this reassessment are supportive of that recommendation.

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 prenatally and postnatally. However, data are limited. Because evaluation of the impacts of early exposures on both children's health and health later in life is important to a complete characterization of risk, collection of additional data in this area should be a high priority to reduce uncertainties in future risk assessments.

Data from the Dutch cohort of children exposed to PCBs and dioxin-like compounds suggest impacts from exposure to background levels of dioxin and related compounds prenatally and, perhaps, postnatally on neurobehavioral outcomes, thyroid function, and immune system alterations. Although these effects cannot be attributed solely to dioxin and related compounds, several associations suggest that these are, in fact, likely to be Ah-mediated effects. 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 6-7 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 where significantly elevated exposure occurred. The incidents at Yusho and Yu-Cheng resulted in increased perinatal mortality and low birthweight 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 with those reported in adult monkeys exposed only to 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 these effects in the Yusho and Yu-Cheng cohorts do not correlate with TEQ, some of the effects are exclusively due to nondioxin-like PCBs or a combination of all the congeners. In addition, on the basis of these data, it is still not clear to what extent there is an association between overt maternal toxicity and embryo/fetal toxicity in humans. Further studies in the offspring as well as follow-up of the Seveso incident may shed further light on this issue. In addition to 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 may have increased concurrent to 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. 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 with 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 fact, thyroid hormones are considered so important in development that 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. The exact processes accounting 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. 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 in

response to the increased TSH. In animals, given higher doses of dioxin, the body is unable to maintain homeostasis, and TSH levels remain elevated and T4 levels decrease.

A large number of studies in animals have addressed the question of effects of dioxin-like chemicals after in utero or lactational exposure. These have included both single-congener studies and exposures to complex mixtures. However, the vast majority of the data are derived from studies of 2,3,7,8-TCDD, or 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 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. Given the relative impact of nursing on body burdens (see the discussion of breast milk exposures and body burdens below), direct impacts of increased early postnatal exposure on the carcinogenic process are expected to be small. This conclusion is based on the reasonable assumptions that cancer risk is a function of average lifetime body burden or 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.

In addition to potential vulnerability during development, 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 it is not the individual meals a pregnant woman eats during pregnancy that might affect development, but the consequence of her exposure history over her life, which has the greatest impact on her body burden. Again, 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 who, for a limited portion of their lives, may have elevated exposures on a body-weight basis when compared with non-nursing infants and adults (see discussion). 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 those of an adult, on a body-

weight basis. **Table 4-9** 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 for 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 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, who are exposed to levels in 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 41 and 24 pg TEQ_{DFP}-WHO_{98}/day, respectively, for a total intake of 65 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 4-7** summarizes the intake rates derived by this method. The intake estimate is supported by an extensive database on food consumption rates and food data. PK 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 dietary choices that individuals make. These are differences in 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 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 (i.e., beef, pork, or poultry) with sources that are high in dioxin (i.e., 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 on the average CDD/CDF tissue level for the general adult United States population appears to be declining; 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. The number of people in

most of these studies, however, is relatively small and the participants were not statistically selected in ways that assured their representativeness of the general United States adult population. One study, the 1987 National Human Adipose Tissue Survey (NHATS), involved more than 800 individuals and provided broad geographic coverage, but 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, 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 during the time period 1995 to 1997. The individuals sampled were all U.S. residents with no known exposures to dioxin other than normal background. The blood was collected in seven different locations from 316 individuals with an age range of 20 to 70 years. All TEQ calculations were made assuming 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 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 4-6**). 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 DFP TEQ 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 studies should help address these uncertainties. The National Health and Nutrition Examination Survey (NHANES) began a new national survey in 1999 that will measure dioxin blood levels in about 1,700 people per year (see http:www.cdc.gov/nchs/nhanes.htm). The survey is conducted at 15 different locations per year and is designed to select individuals statistically representative of the civilian U.S. population in terms of age, race, and ethnicity. These new data should provide a much better basis than the currently available data for estimating national background tissue levels and evaluating trends.

As described above, current intake levels from food sources are estimated in this reassessment to be approximately 1 pg TEQ/kgBW/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 will be described below.

Evaluation of 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 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, or subsistence or recreational fishers. Nursing infants represent a special case where, for a limited portion of their lives, these individuals may have elevated exposures on a body-weight basis when compared to non-nursing infants and 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 and resulted in elevated dioxin levels in some poultry and catfish. This incident involved less than 5% of national poultry production and 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 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 such incidents have led to disproportionate exposures to populations living near where these incidents have 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 highly exposed local populations.

Elevated exposures associated with the workplace or 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 ppt 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 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. The typical dioxin concentrations in these fish and the typical rates of consumption are included in the mean background calculation of exposure. People who consume large quantities of fish at typical contamination levels may have elevated exposures. These kinds of exposures are addressed within the estimates of variability of background and are not considered to result in highly exposed populations. 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 measuring 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 measuring 90 PCB congeners, of which 7 were dioxin-like mono-ortho PCBs (although PCB 126 was not measured), in Lake Michigan "sport-fish eaters" versus a control group (little or no sport fish consumption) showed a significant elevation in these PCBs. Significantly elevated concentrations of dioxins, furans, and coplanar PCBs were measured in Great Lakes fish by the Ontario Ministry of the Environment, although this was a study of known or suspected hot spots, with the purpose being to set 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, 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.

In summary, in addition to general population exposure, some individuals or groups of individuals may also be exposed to dioxin-like compounds from discrete sources or pathways locally 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 have been breast-fed versus those who have been 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 above 20 ppt TEQ_{DF}-WHO_{98}. 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 about 20 pg TEQ_{DFP}-WHO_{98}/kg/day after 12 months. The average dose over a year was calculated to be 92 pg TEQ_{DFP}-WHO_{98}/kg/day. Although this average annual infant dose of 92 pg TEQ_{DFP}-WHO_{98}/kg/day 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, i.e., infant body burdens will not exceed adult body burdens by 92 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 possibly more rapid elimination in infants. A pharmacokinetic exercise comparing 6- and 12-month nursing scenarios with formula feeding showed peak infant lipid concentrations to exceed 40 ppt TEQ_{DFP}-WHO_{98}, compared with peak lipid concentrations 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 these two hypothetical children merged at about 10 years of age, at a lipid concentration of about 13 ppt TEQ_{DFP}-WHO_{98}.

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 WHO 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 finding contained in this report provides 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 rather than 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 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 that supports this finding includes matches in time of the rise of environmental levels with 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 release may be grouped into five major types: (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 above). 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 dominate total releases. EPA's best estimates of releases of CDD/CDFs to air, water, and land from reasonably quantifiable sources were approximately 3,300 gram (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.

While this inventory is one of the most comprehensive and well-documented in the world, it is likely to underestimate total releases. This underestimate is likely because: 1) a

number of known sources lacked sufficient data to include in the inventory and 2) 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 grams 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 remain 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 other areas.

As described in Section 4.1, combustion appears to be the most significant process of formation of CDDs/CDDFs today. Important factors that can affect the rate of dioxin formation include the 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 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. 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 in affecting levels of dioxin emissions than observed in commercial-scale combustors.

Dioxins are widely distributed in the environment at low concentrations, primarily as a result of air transport and deposition.

Once introduced into the environment, 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, generally classified as semivolatile, and tend to bioaccumulate in animals. Some evidence has shown that these compounds can degrade in the environment, but in general they are considered 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 either 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 become buried in deeper sediments. Immobile sediments appear to serve as permanent sinks for the dioxin-like compounds. Though not always considered an environmental compartment, these compounds are also found in anthropogenic materials (such as pentachlorophenol) and have the potential to be released from these materials into the broader environment.

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. 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, milk in Germany, and 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 (see discussion in Section 4.4 indicating that the diet is the dominant exposure pathway for humans) may not be those sources that represent the largest fractions of total emissions in the United States. The geographic locations of sources relative to the areas from which much of the beef, pork, milk, and fish are produced are important to consider. 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 one-third of general population TEQ exposure is due to PCBs, at least one-third of the 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. 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.

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/CDFs and PCBs comes from dated sediment core studies. CDD/CDF and 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). Additionally, sediment studies in lakes located in several European countries have shown similar trends.

It is reasonable to assume that sediment core trends should be 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. Many of these abatement efforts should have resulted in decreases in dioxin emissions, i.e., 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 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 overall declines in environmental levels and emissions. Also, 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 in environmental levels and body burdens, it is anticipated that further declines in tissue concentrations should occur.

Risk Characterization Summary Statement

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (dioxin) is highly toxic to many animal species producing a variety of cancer and noncancer 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 (Ah) receptor. This common mode of action is supported by consistency in effects evident from multiple congener databases. 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. 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. 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 average body burden, as more appropriate default metrics than daily intake. The Agency recognizes, however, that the final choice of the appropriate metric may depend on the endpoint under evaluation.

Dioxin and related compounds have been shown in multiple animal species to be carcinogenic, developmental, reproductive, immunological and endocrinological hazards, among others. There is no reason to expect, in general, that humans would not be similarly affected at some dose, and indeed there is a growing body of data supporting this assumption. Based upon the animal data, current margins of exposure are too low, especially for more highly exposed human populations. The human database supporting this concern is less certain. Occupational and accidentally exposed cohorts exposed at higher levels show correlations with exposure for a number of cancer and noncancer effects, consistent with those seen in the animal studies.

For cancer outcomes, the epidemiological evidence provides consistent findings of statistically significant elevations and 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; and mechanistic considerations common to animals and humans for dioxin carcinogenicity, EPA characterizes 2,3,7,8-tetrachlorodibenzo-*p*-dioxin as "carcinogenic to humans." 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 one percent increase (ED_{01}) in the lifetime risk of cancer all fall within a 10-fold range when comparing the occupational studies, and are the same as those calculated based on the animal bioassay data. The ED_{01} for all-cancers combined from a metaanalysis of the three major occupational cohorts is 47

ngTCDD/kgBW, with a lower confidence limit of 30 ngTCDD/kgBW. By comparison, current background body burdens in the United States are approximately 5 ngTEQ/kgBW. Using 30 ngTEQ/kgBW as the point of departure for the slope calculation, EPA calculates an upper bound on the lifetime risk of all cancers combined of 1×10^{-3} risk/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 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). Using 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 1,000 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 could be as low as zero in some individuals.

For noncancer effects, EPA generally calculates an RfD/RfC value which 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. The current estimated average dose to the U.S. population (~1 pgTEQ/kg/day) is greater than RfD/RfC dose values that would be calculated given the data reviewed in this assessment, and, therefore, RfD/RfC values would be uninformative for safety assessment. EPA has chosen rather to characterize the margins-of-exposure (MOE) for noncancer endpoints in order to inform risk management decisions. MOE is the ratio of the human body burden to the effect level in the comparison species (ED_{01} or low effect level), animal or human. For the most sensitive endpoints identified, MOE's range from, for example, less than one for enzyme induction in mice, through 2.6 - 15 for enzyme induction in rats, <3 for developmental effects, and 5 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. Children's risks from dioxin and related compounds may be greater than for adults, but more data are needed to fully address this issue.

Releases of dioxins to the environment from sources that have been characterized 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, but their relationship to contemporary sources or reservoirs is uncertain.

Table 1-1. The TEF scheme for I-TEQ$_{DF}$[a]

Dioxin (D) congener	TEF	Furan (F) 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 (Ds) and furans (Fs) are included in the TEF scheme.

Table 1-2. The TEF scheme for TEQ_{DFP}-WHO_{94}[a]

Dioxin (D) congener	TEF	Furan (F) congener	TEF	Dioxin-like PCB (P)	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 (Ds), furans (Fs), 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 TEF scheme for TEQ_{DFP}-WHO_{98}[a]

Dioxin (D) congener	TEF	Furan (F) congener	TEF	Dioxin-like PCB (P)	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 (Ds), furans (Fs), 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 REP values for the major TEQ contributors

CHEMICAL	Number of in vivo endpoints	Range of REPs (mean±std)	Number of end points 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

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

	(A) Rat Daily Administered Dose/Body Burden	(B) Human Scaled Administered Dose/Body Burden[1]	(C) Human Equivalent Administered Dose/Body Burden[2]	(A/B) Ratio of Rat to Human Scaled Dose	(A/C) Ratio of Rat to Human Equivalent Dose
Dose (ng/kg/d)	1	0.27	0.0096	3.7	104
Body Burden (ng/kg)	18	505	18	0.036	1

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

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

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

Effect	Human	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		+	+		+/-				+				

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

Table 2-2. Examples of margins of exposure (M-O-E)

Effect	ED_{01} or Low Effect Level	M-O-E [1]
CYP1A1/1A2/1B1 *(Rats)*	13 - 74 ng/kg *(ED_{01})*	2.6 - 15
Tumors;Multiple sites *(Rats)*	14 -1190 ng/kg *(ED_{01})*	3 - 238
Endometriosis *(Rhesus Monkey)*	38ng/kg *(LOEL)*	5
Developmental Effects *(Humans)*	Dutch *background*; early '90s	<3
Tumors; All/lung *(Humans)*	6-250 ng/kg *(ED_{01})*	1.2 - 50

[1] MOE = $\frac{ED_{01} \text{ or Low Effect Level}}{\text{Current Human Body Burden (}\sim 5\text{ng } TEQ_{DFP}\text{-}WHO_{98}\text{ng/kg)}}$

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		
	Obs.	SMR	95% C.I.	Obs.	SMR	95% C.I.
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 & Zober (1996b)[f] (BASF accident)	18	1.9	1.1 - 3.0	7	2.4	1.0 - 5.0
TOTAL[g]	[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 lymphoma, soft-tissue sarcoma, and gastrointestinal results not shown.

[b] Kogevinas et al. (1997): 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] Fingerhut et al. (1991a): Men ≥20 years latency and ≥1 year exposure.

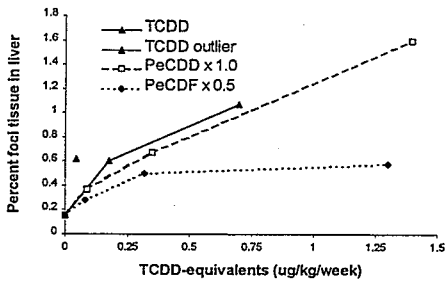
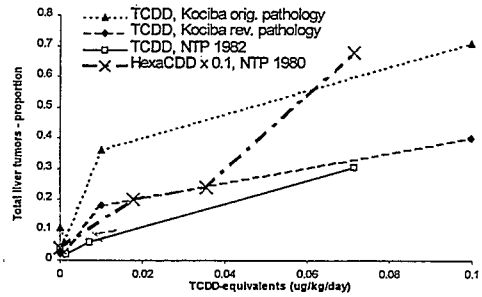
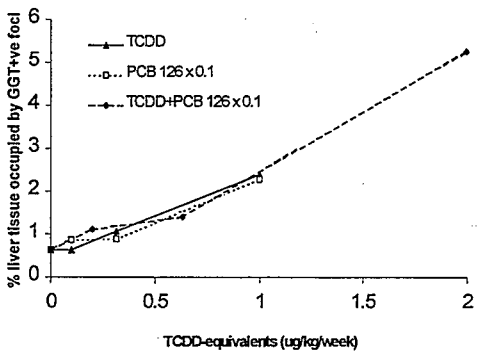
[d] Becher et al. (1996): Men, Cohort I and II, summed (Boehringer-Ingelheim, Bayer-Uerdingen cohorts).

[e] Hooiveld et al. (1996): Men and women, Factory A.

[f] Ott & Zober (1996b): Men, chloracne subgroup, ≥ 20 years latency. Data presented for lung cancer are all respiratory tract cancers combined.

[g] TOTALs in square brackets are those calculated by the IARC Working Group.

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[1]	TEF-WHO 98	% of Adipose TEQ_{DFP}-WHO_{98} Tissue Conc.[2]	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	

1. van den Berg, et al. 2000. Human risk assessment and TEFs. Food Additives and Contaminants, Vol. 17(4):347 - 358. Hexa-CDD referenced to previous TEF reviews.

2. See Part II, Chapter 4, Tables 4-46, 4-47

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

Diffusion into the cell
Binding to the AhR protein
Dissociation from hsp90
Active translocation from cytoplasm to nucleus
Association with Arnt protein
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

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

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. Quantitative inventory of environmental releases 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	A	B	C
Releases (g TEQ_{DF}-WHO_{98} /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			5.5
Sewage sludge incineration		14.8			6.1	
Tire combustion			0.11			0.11
Pulp and paper mill sludge incinerators[f]						
Power/Energy Generation						
Vehicle fuel combustion - leaded[b]			2			37.5
- unleaded			5.9			3.6
- diesel			35.5			27.8
Wood combustion - residential			62.8			89.6
- industrial		27.6			26.4	
Coal combustion - utility		60.1			50.8	
Oil combustion - industrial/utility			10.7			17.8
Other High Temperature Sources						
Cement kilns (hazardous waste burning)			156.1			117.8
Lightweight aggregate kilns burning hazardous waste			3.3			2.4
Cement kilns (nonhazardous waste burning)			17.8			13.7
Petroleum refining catalyst regeneration			2.21			2.24
Cigarette combustion			0.8			1
Carbon reactivation furnaces			0.08			0.06
Kraft recovery boilers		2.3			2	
Minimally Controlled or Uncontrolled Combustion						
Forest, brush, and straw fires[d]			208			170
Metallurgical Processes						
Ferrous metal smelting/refining						
- Sintering plants		28				32.7
Nonferrous metal smelting/refining						
- Primary copper		<0.5[e]			<0.5[e]	
- Secondary aluminum			29.1			16.3
- Secondary copper			271			983
- Secondary lead		1.72			1.29	
Drum and barrel reclamation			0.08			0.08
Chemical Manufac./Processing Sources						
Ethylene dichloride/vinyl chloride		11.2				
Total quantified releases to air[c]		2705			13081	

Table 4-2. Quantitative inventory of environmental releases 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	A	B	C
Releases (g TEQ/yr) to water						
Chemical Manuf./Processing Sources						
Bleached chemical wood pulp and paper mills	19.5			356		
Ethylene dichloride/vinyl chloride		0.43				
Total quantified releases to water[c]	19.93			356		
Releases (g TEQ/yr) to land						
Chemical Manuf./Processing Sources						
Bleached chemical wood pulp and paper mill sludge	1.4			14.1		
Ethlyene dichloride/vinyl chloride		0.73				
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 quantified releases to land[c]	110.23			126.7		
Overall quantified releases to the open and circulating environment	2835			13564		

Confidence Rating 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.**
Confidence Rating 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.**
Confidence Rating 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.

[a]A confidence rating reflects EPA's judgment as to the adequacy of information pertaining to the emission factor and activity level.
[b]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.)
[c]TOTAL reflects only the total of the estimates made in this report.
[d]It is not known what fraction, if any, of the estimated emissions from forest fires represents a "reservoir" source. The estimated emissions may be solely the result of combustion.
[e]Congener-specific emissions data were not available; the I-TEQ_{DF} emission estimate was used as a surrogate for the TEQ_{DF}-WHO_{98} emission estimate.
[f]Included within estimate for Wood Combustion - Industrial.

Table 4-3. Preliminary indication of the potential magnitude of TEQ_{DF}-WHO_{98} releases from "unquantified" (i.e., Category D) sources in reference year 1995

Emission source category	Release medium	Preliminary release estimate (g WHO_{98}-TEQ_{DF}/yr)
I. Contemporary Formation Sources Biogas Combustion	Air	0.22*
Oil Combustion-Residential	Air	6.0*
Coal Combustion - Commercial/Industrial	Air	39.6*
Coal Combustion - Residential	Air	32.0*
Asphalt Mixing Plants	Air	7*
Combustion of Landfill Gas	Air	6.6
Landfill Fires	Air	1,050*
Accidental Fires (Structural)	Air	>20*
Accidental Fires (Vehicles)	Air	28.3*
Forest and Brush Fires	Air	208
Primary Magnesium Production	Air	11.4*
Coke Production	Air	6.9*
Electric Arc Ferrous Furnaces	Air	44.3[a]
Ferrous Foundries	Air	17.5*
Municipal Wastewater	Water	12
II. Reservoir Sources Urban Runoff	Water	190*
Rural Soil Erosion	Water	2,700*

* Congener-specific emissions data were not available; the I-TEQ_{DF} emission factor was used as a surrogate for the TEQ_{DF}-WHO_{98} emission estimate.

Table 4-4. Sources that are currently unquantifiable [1] (i.e., Category E)

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

[1]/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 4-5. Summary of North American CDD/CDF and PCB TEQ-WHO$_{98}$ Levels in Environmental Media and Food (whole weight basis; concentrations provided in parenthesis for food products are calculated at ND = 0).

Media	CDD/CDFs[a]	PCBs[a]
Urban Soil, ppt	n=171 9.4 ± 11.2 Range = 2 - 21	NA[b]
Rural Soil, ppt	n = 292 2.5 Range = 0.1 - 6	NA[b]
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	0.0009
Rural Air, pg/m^3	n=7 0.017 Range = 0.01 - 0.02	NA[b]
Freshwater Fish and Shellfish, ppt	n=289 1.0 (NA[b])	n = 1 composite of 10 samples plus 6 composites 1.2[c] (NA[b])
Marine Fish and Shellfish, ppt	n=158 0.26 (NA[b])	n = 1 composite of 13 samples plus 5 composites 0.25[c] (NA[b])
Water, ppq	n=236 0.00056 ± 0.00079 (NA[b])	NA[b]
Milk, ppt (Note: each composite for CDD/F/PCB comprised of 40+ U.S. regional samples)	n=8 composites 0.031 ± 0.0022 (0.031)	n = 8 composites 0.016 (0.016)
Dairy, ppt[e]	n = 8 composites 0.12 ± 0.22 (0.12)	n = 8 composites 0.058 (0.058)
Eggs, ppt (Note: each composite for CDD/F data comprised of 24 eggs)	n=15 composites 0.081[c] (0.013)	n = 18 plus 6 composites 0.10[c] (NA[b])
Beef ppt	n=63 0.20 ± 0.12 (0.07) Range = 0.2 - 1.1	n = 63 0.094 (0.094)
Pork, ppt	n=78 0.22 ± 0.22 (0.06) Range = 0.12 - 1.4	n = 78 0.0093 (0.006)
Poultry, ppt	n=78 0.12 ± 0.12 (0.072) Range = 0.05 - 0.72	n = 78 0.044 (0.044)
Vegetable Fats, ppt	n=30 0.056 ± 0.24[d] (NA[b])	n = 5 composites 0.037[c]

[a] Values are the arithmetic mean TEQs, in ppt, 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.

[b] NA = not available; Congener-specific PCB data, and data to calculate TEQ concentrations at ND = 0, are limited.

[c] Standard deviations could not be calculated due to limitations associated with the data (i.e., composite analyses).

[d] TEQ calculated by setting nondetects to zero.

[e] Dairy concentration calculated from milk lipid concentrations and then assuming a fat fraction for dairy.

Table 4-6. Background serum levels in the United States 1995 - 1997

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

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

Source: CDC, 2000.

Table 4-7. Adult contact rates and background intakes of dioxin-like compounds

Exposure route	Contact rate	Dioxins and furans		Dioxin-like PCBS		Total intake (pg TEQ_{DFP}-WHO_{98}/kg-d)
		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)	
Soil ingestion	50 mg/d	9.4 pg/g	0.0067	NA	NA	0.0067
Soil dermal	12 g/d	9.4 pg/g	0.0016	NA	NA	0.0016
Freshwater fish and shellfish	5.9 g/d	1.0 pg/g	0.084	1.2 pg/g	0.1	0.18
Marine fish and shellfish	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	0.031 pg/g	0.078	0.016 pg/g	0.040	0.12
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	0.20 pg/g	0.13	0.094 pg/g	0.063	0.19
Pork	0.22 g/kg-d	0.22 pg/g	0.048	0.009 pg/g	0.0021	0.05
Poultry	0.49 g/kg-d	0.11 pg/g	0.054	0.044 pg/g	0.022	0.076
Vegetable fat	17 g/d	0.056 pg/g	0.014	0.037 pg/g	0.0090	0.023
Water	1.4 L/d	0.0005 pg/L	0.000011	NA	NA	0.000011
Total			**0.59 (41 pg/d)**		**0.34 (24 pg/d)**	**0.93 (65 pg/d)**

Table 4-8. Variability in average daily 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 yr	54	3.6
6-11 yr	59	2
12-19 yr	64	1.1
Adult	65	0.9

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

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 Tend.	Upper	Central Tendency	Mean TEQ	Central Tendency	
CDC comparison population, USA 1995 - 97; 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[5] median	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
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

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

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
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. Summary of Cancer Epidemiology and Bioassay Data in Dose-Response Calculations

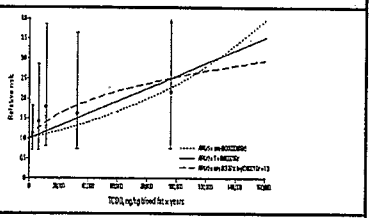
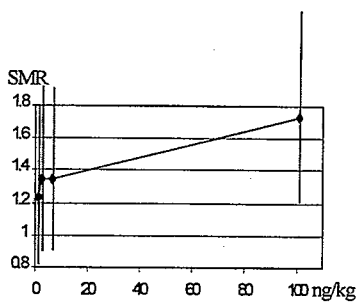
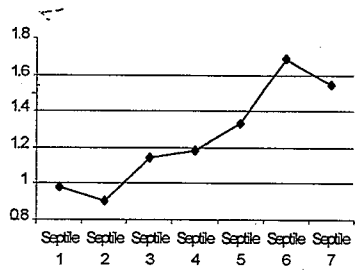
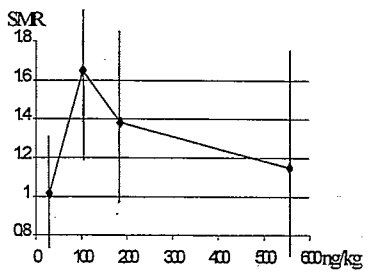
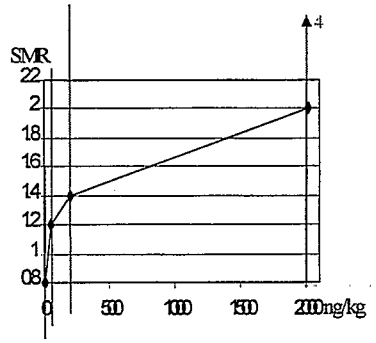
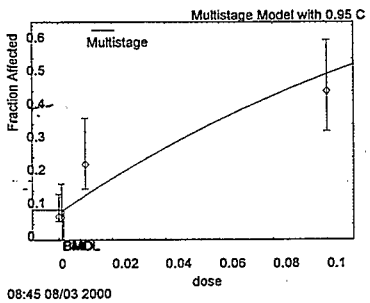
Study	Exposure Groups	Exposure Lifetime Ave. Body Burden ngTCDD/kg	All Cancer Deaths Observed (latency)	
Hamburg cohort, Becher et al., 1998	0 - 1 µg/kg fat*Years 1 - 4 µg/kg fat*Years 4 - 8 µg/kg fat*Years 8 - 16 µg/kg fat*Years 16 - 64 µg/kg fat*Years 64+ µg/kg fat*Years	0 - 3.6 3.6 - 14 14 - 28.6 28.6 - 57 57 - 229 229+	1.00 RR 1.12 (0 yr) 1.42 1.77 1.63 2.19	
Hamburg cohort, from Flesch-Janys et al., 1998	1st quartile 0 -125.2 2nd quartile 125.2- 627.1 3rd quartile 627.1 - 2503 4th quartile 2503+ ng/kgf*Y	1.4[1] 2.5 6.5 101.2	1.24 SMR 1.34 (0 yr) 1.34 1.73	
NIOSH cohort, Steenland et al., 1999	Septile 1 Septile 2 Septile 3 Septile 4 Septile 5 Septile 6 Septile 7		0.98 SMR 0.90 (15yr) 1.14 1.18 1.33 1.69 1.54	
NIOSH cohort, from Aylward et al., 1996	<1 year 1 -5 years 5 - 15 years > 15 years	27.8[2] 103.3 184.5 554.5	1.02 SMR 1.65 (20yr) 1.38 1.15	

Table 5-2. Summary of Cancer Epidemiology and Bioassay Data in Dose-Response Calculations (continued)

Study	Exposure Groups	Exposure Lifetime Ave. Body Burden ngTCDD/kg	All Cancer Deaths Observed (latency)	
BASF cohort, from Ott and Zober 1996	<0.1 µg/kg bw. peak 0.1 - 0.99 µg/kg bw. peak 1.0 - 1.99 µg/kg bw. peak 2.0+ µg/kg bw. peak	4.6[3] 51.9 200.1 2012	0.80 SMR 1.2 (0 yr) 1.4 2.0	
S-D Rats, Kociba et al., 1978; Goodman & Sauer, 1992 pathology	0 µg/kg/day 0.001 µg/kg/day 0.01 µg/kg/day 0.1 µg/kg/day	4 135 425 2025	2/86 Tumors 1/50 9/50 18/45	

1. For Flesch-Janys et al. (1998), the mean of the AUC in each exposure quartile was calculated as the mean of the lognormal distribution when restricted to that range. Time mean concentrations C_s were derived by dividing the mean AUCs by 63 years (derived by subtracting the mean year of birth of the study subjects, 1929, from the date of followup, 1992). Body burden was computed by multiplying this lipid concentration by 0.25 (assuming 25% lipid in the body) and adding 1.25 ng/kg (mean background lipid concentration of 5 ng/kg, times 0.25). Parameters for the fitted lognormal distribution are μ=6.3617, σ=2.2212.

2. Aylward et al., 1996, Table 5, Cavg/4, assuming 25% lipid

3. For Ott and Zober (1996), the lognormal fitting procedure described above was used to find mean values for each group. AUCs were then calculated for each group by integrating the solution to the first-order kinetics equation over time 39 years (the time from the 1953 accident to the 1992 followup). Using C_0 as the initial concentration (i.e., that given in the article), this gives AUC = $C_0/k_e[1-e^{-39ke}]$. The constant k_e is ln(2)/(half-life). The time-mean concentration is taken to be AUC

Table 5-2. Summary of Cancer Epidemiology and Bioassay Data in Dose-Response Calculations (continued)

divided by the age 71 years (mean age in 1954, 33 years, + 38 years from 1954 to the date of followup 1992). Parameters for the fitted lognormal distribution are μ=-1.8676, σ=2.2927. The half-life used for the calculation of k_e is 7.1 years.

4. Human equivalent assumption of 25% lipid in rats; empirical data in 22 month old female SD rats varies from 4 - 33 % (Birnbaum 1983).

Table 5-3. 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)	1,190 (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 Chapter 8, Table 8.3.2.

Table 5-4. Summary of All Site Cancer ED_{01}s and Slope Factor Calculations

Study	ED_{01}/LED_{01}[1] (95% lower bound) ng/kg	Upper bound[2] slope factor risk/pg/kg/day
Hamburg cohort, Becher et al., 1998	9.83	[3.0 E-3][4]
Hamburg cohort, from Flesch-Janys et al., 1998	5.7 (3.5)	8.6 E-3
NIOSH cohort, from Aylward et al., 1996	39.9 (23.0)	1.3 E-3
BASF cohort, from Ott and Zober ,1996	80.2 (37.5)	0.80 E-3
Poisson regression on combined Hamburg (Flesch-Janys et al., 1998), NIOSH (Aylward et al., 1996), and BASF (Ott and Zober, 1996) cohorts[5]	47.2 (30.1)	0.99 E-3
Sprague-Dawley rats, Kociba et al., 1978; Goodman & Sauer ,1992 pathology	31.9 (22)[6] BMD dose 38 (27.5) BMD adipose	1.4 E-3 1.1 E-3

See next page for footnotes.

Table 5-4. Summary of All Site Cancer ED_{01}s and Slope Factor Calculations (cont.)

1. Algorithms used for Poisson dose-response calculation for dioxin exposure and cancer EDs.
Data: exposures for each exposure group – X_j; number of deaths expected in each exposure group under conditions of background exposure – E_j; number of deaths observed in each exposure group – O_j.
The model assumes that the risk of death in an exposed group divided by the background risk of death (E_j) is a linear function of exposure, i.e., $R_j = E_j(1 + bX_j)$. The parameter b is the slope of the dose-response model. The observed number deaths in group j, O_j is assumed to be distributed as a Poisson random variable with expected value R_j. Under these assumptions, the solution by maximum likelihood proceeds as follows: The likelihood L is:

$$L = \prod_{j=1}^{N} \{\exp[-E_j(1+\beta X_j)]\}[-E_j(1+\beta X_j)]^{O_j}(O_j!)^{-1}$$

where N = the number of separate exposure groups. The maximum likelihood estimate (MLE), b, of the parameter β is obtained by taking the first derivative of the log-likelihood equation, setting it equal to 0 and solving for b.

$$\frac{d\ln L}{d\beta} = \sum_{j=1}^{N} -E_{0j}X_j + (O_jX_j/(1+bX_j)) = 0$$

The asymptotic variance of the estimate is given by $[-d^2\ln L/d\beta^2]^{-1}$, with the observed value O_j replaced by its expected value $E_j(1+bX_j)$:

$$\text{var}(b) = \left[\sum_{j=1}^{N}(E_jX_j^2)/(1+bX_j)\right]^{-1}$$

where b is the MLE. This variance can then be used to obtain approximate 95% upper and lower bounds for b. Lifetime incremental risk estimates per unit body burden are obtained by multiplying b by the background lifetime cause-specific risk of death, P_0. The ED_{01}s are also calculated from b. Calculations incorporate a lifetime risk of dying from cancer of 18.5%.

2. Formula for column entries: LED_{01} * ln2 * 1000/$T_{1/2}$/fraction absorbed = $Dose_{01}$ pg/kg/day. Cancer slope = $0.01/Dose_{01}$. Assumes $T_{1/2}$ = 7.1 years, or 2593 days. Assumes 80% absorption from human food supply.

3. Calculated from Becher et al., 1998:

i) Xs Risk (d) = $\frac{\text{Risk(d)} - \text{R(0)}}{\text{R}(\infty) - \text{R(0)}}$ see Chapter 8, Section 8.2.2.

ii) $RR_{(TCDD)} = 1 + 0.000016 * [\text{AUC TCDD ng/kg fat*Y}]$
$RR_{(TCDD)} = 1 + 0.000016 * 4 * 70$ [TCDD ng/kgBB] (25% lipid, 70 year lifespan)
$RR_{(TCDD)} = 1 + 0.00448 *$ [TCDD ng/kg lifetime ave. BB]
Note: Source Becher et al., 1998, table 8, 0 years latency, additive model. Similar results obtained from 10 year latency model, with AUC adjusted by 60/70 years.

iii) US Lifetime Risk of Dying from All-Sites Cancer ~18.5% during period of study

Calculation of Relative Risk Leading to a 1% Increase in Lifetime Risk of Cancer Mortality: Using the above formula and lifetime rates, 0.01 = (Risk(ED_{01}) - 0.185)/1-0.185, Risk(ED_{01}) = 0.19315 (i.e., a 19.315 % lifetime risk constitutes a 1% increase under the formula). Therefore, the Relative Risk (ED_{01}) = 0.19315/0.185 ~ 1.044.
Calculation of ED_{01} from Combining Relative Risk and Slope Formula: RR(ED_{01}) = 1.044 = 1 + 0.00448 * [TCDD ng/kgBB]; ED_{01} = 9.8 ng/kg BB. Data are not available to estimate the LED_{01}. $Dose_{01}$ = ED_{01}BB * ln2/$T_{1/2}$/abs. = 9.8 * 0.693/2593/0.8 = 0.0033 ng/kg/day. Cancer slope factor = $0.01/dose_{01}$ = 3.0 E-3 risk/pg/kg/day

4. Based on central estimate; upper confidence limit unavailable.

5. Metaanalysis performed by combining all data sets (i.e., collections of (Xj, Oj, Ej) into a single large data set and using procedures detailed above.

6. Modeled using EPA benchmark dose software, version 1.2, with either dose or adipose concentration as the metric. 50% absorption assumed from food pellets. 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

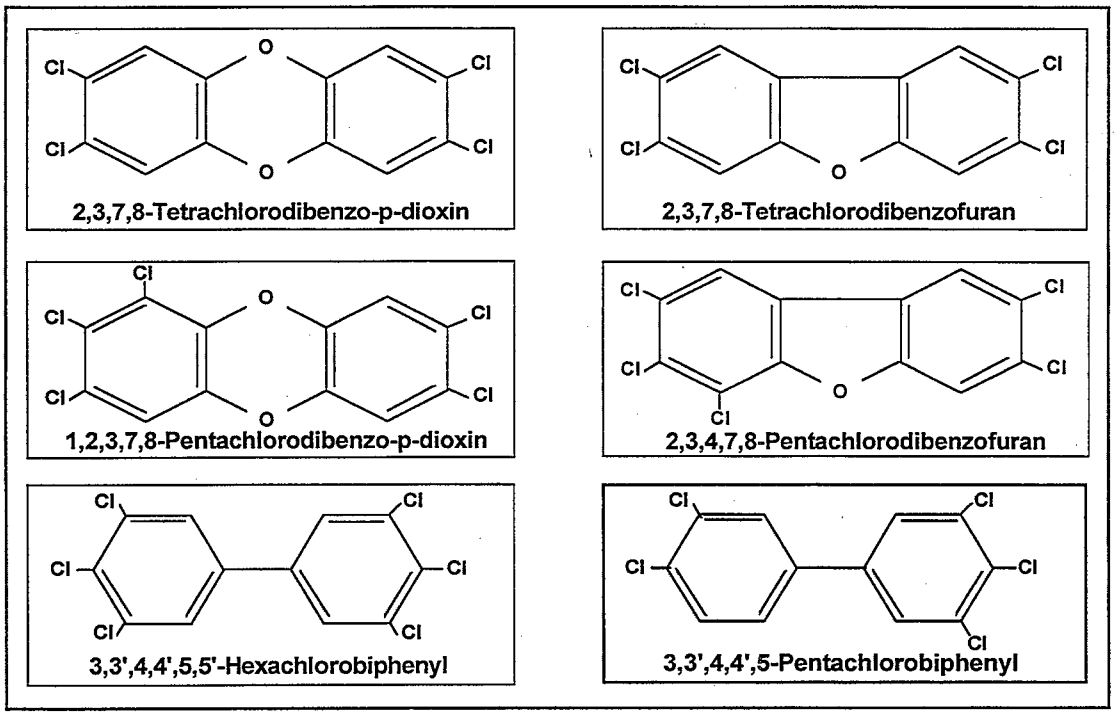


Figure 1-1. Chemical structure of 2,3,7,8-TCDD and related compounds.

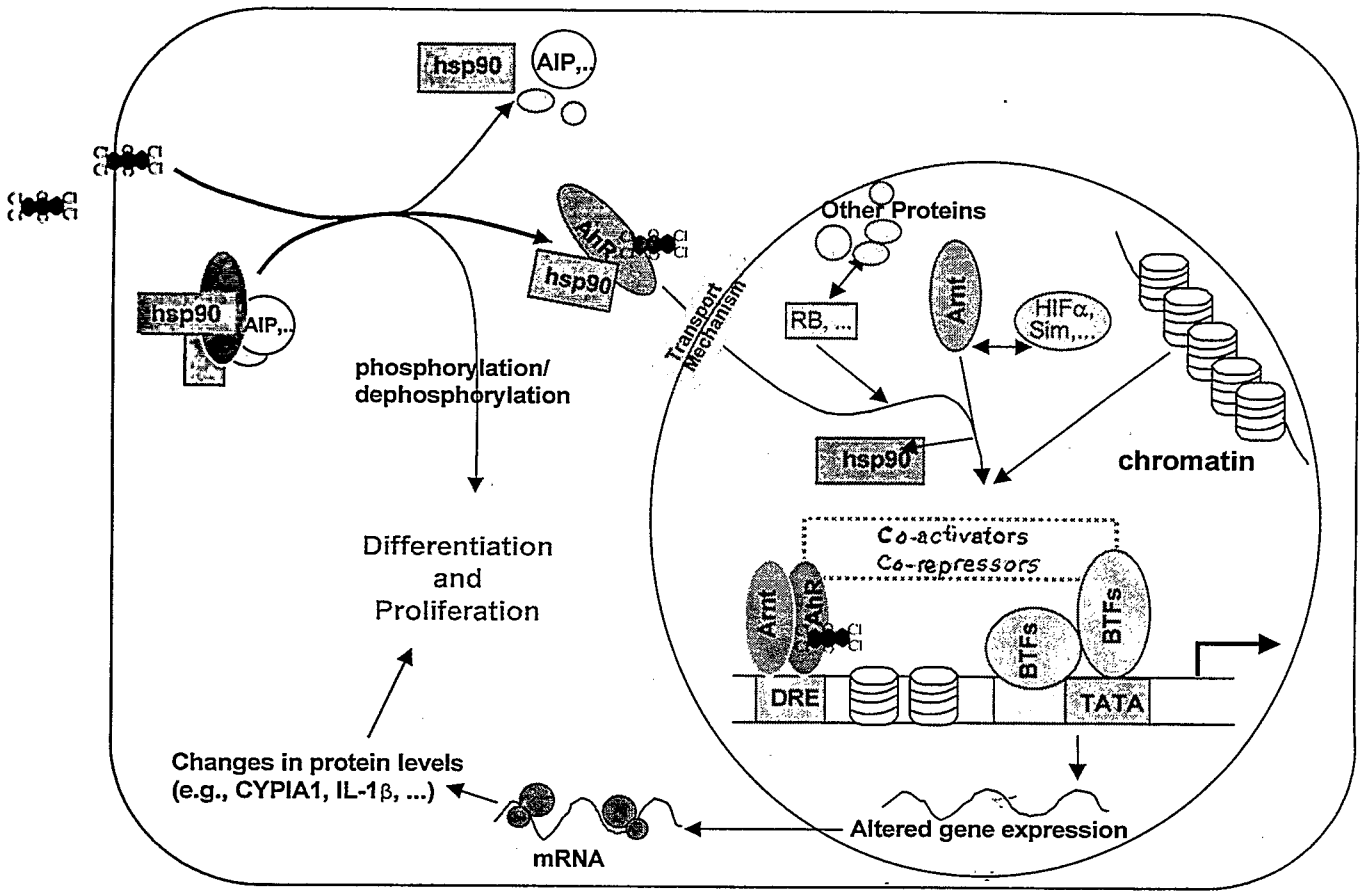
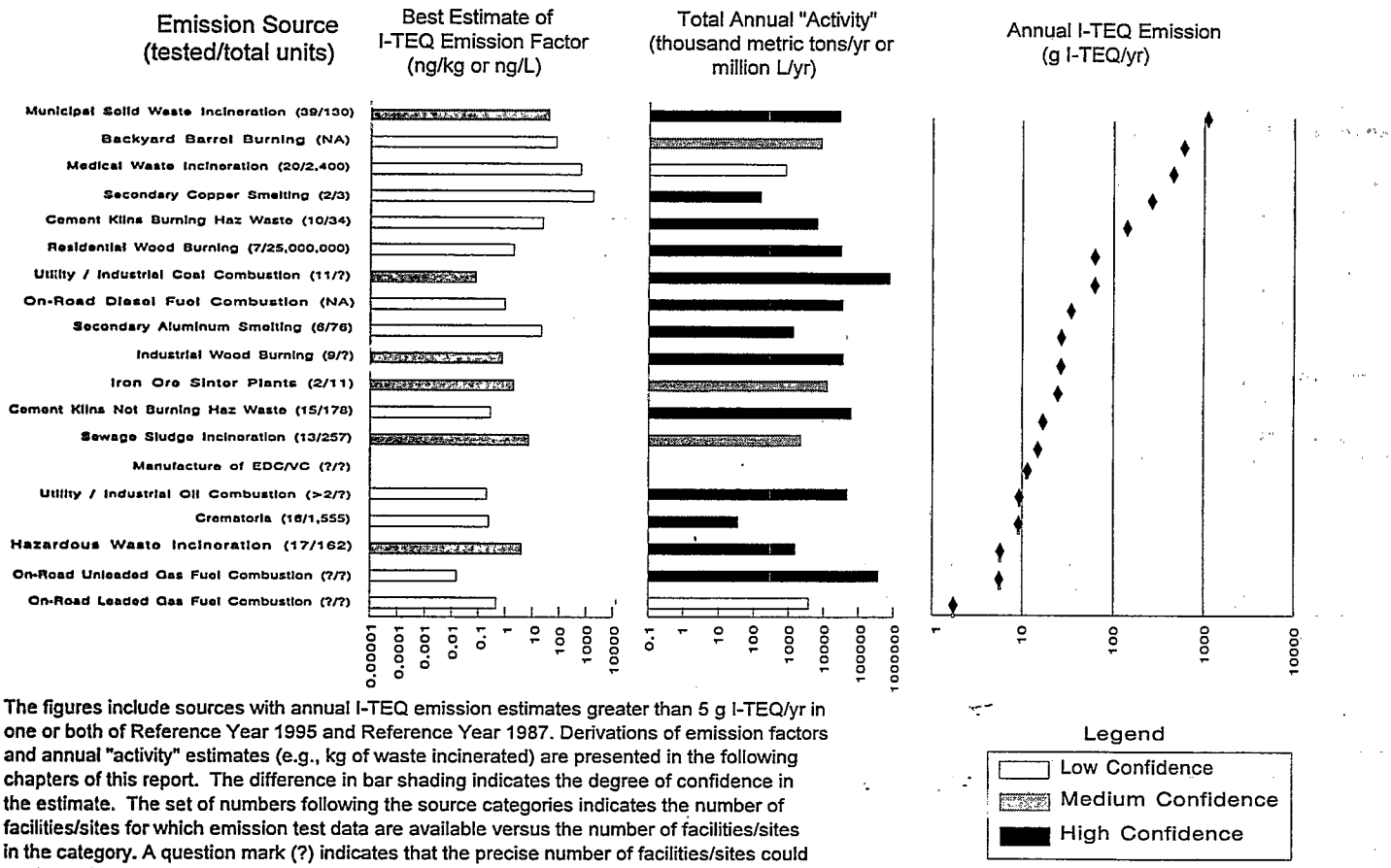


Figure 2-1. Cellular mechanism for AhR action.
TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; AhR, aryl hydrocarbon receptor; AIP, associated immunophilin-like protein; hsp90, 90 kilodalton heat shock protein; p, sites of phosphorylization; Arnt, AhR nuclear translocator protein; RB, retinoblastoma protein;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

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

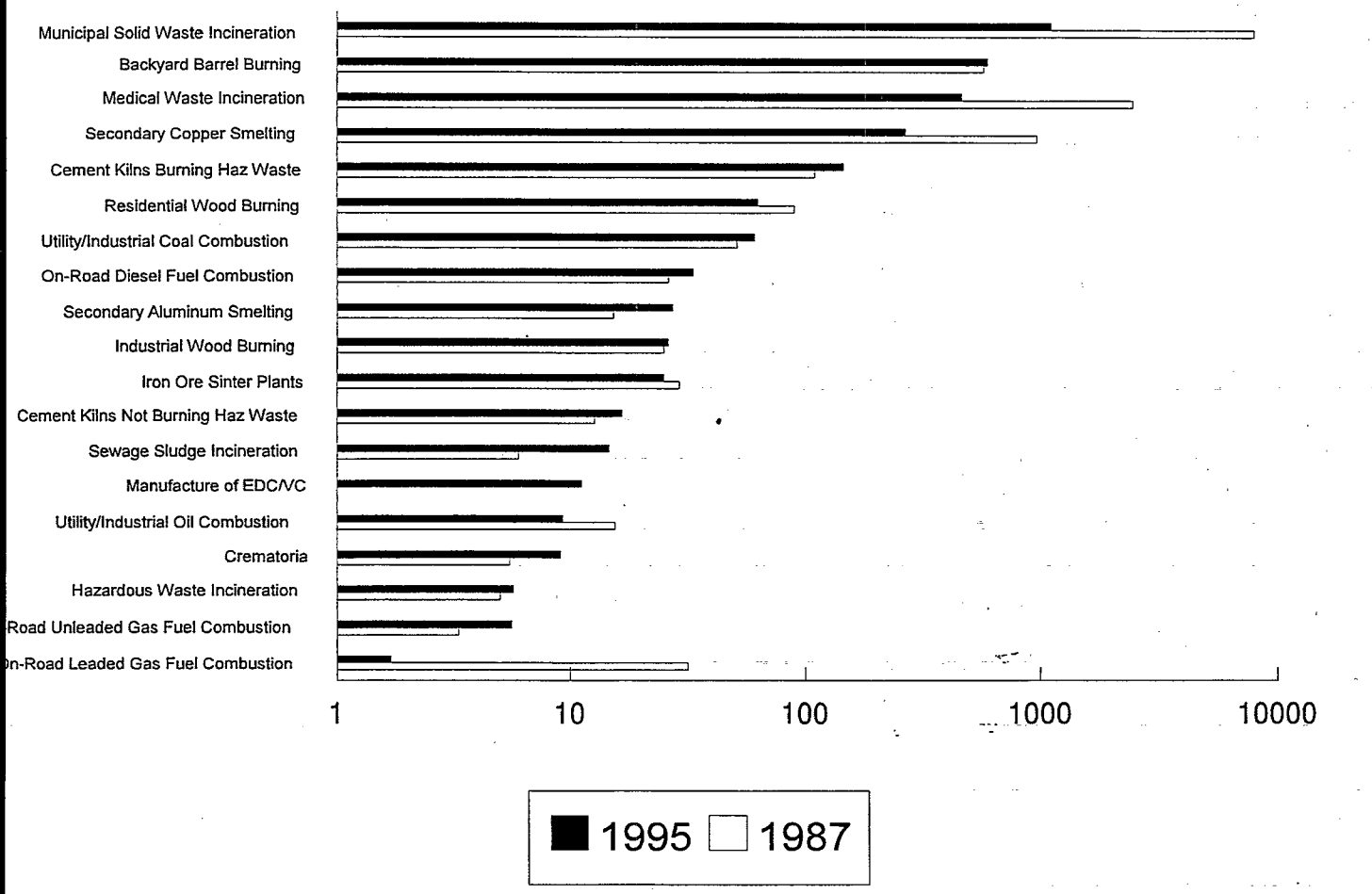
Note: This list is not a comprehensive list of all responses known to be affected by TCDD.
Source: Sutter et al., 1992; Lai et al., 1996.

Figure 2-2. Some biochemical responses to TCDD



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.



igure 4-2. Comparison of estimates of annual I-TEQ emissions to air (grams I-TEQ/yr) for reference years 987 and 1995.

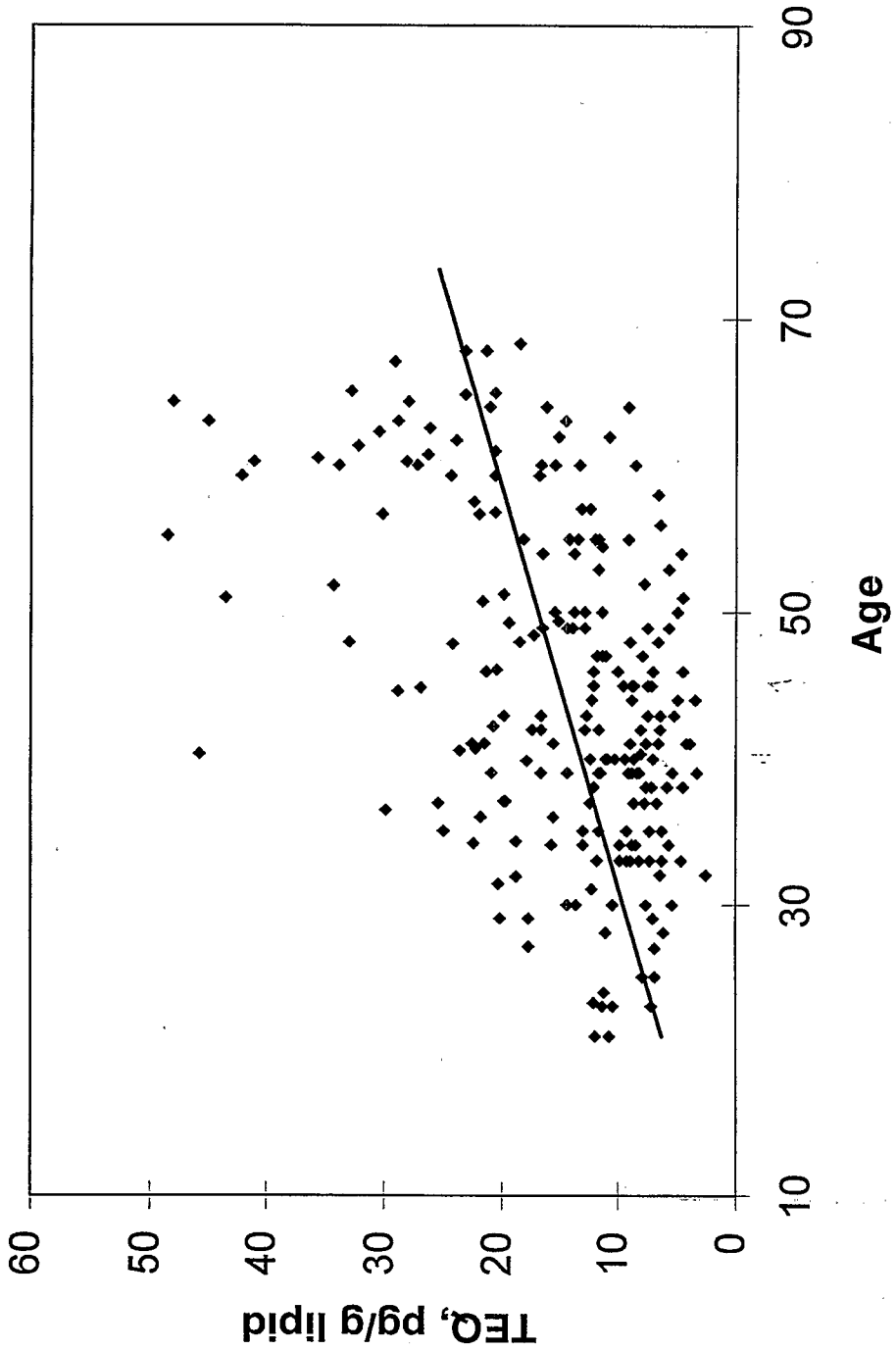
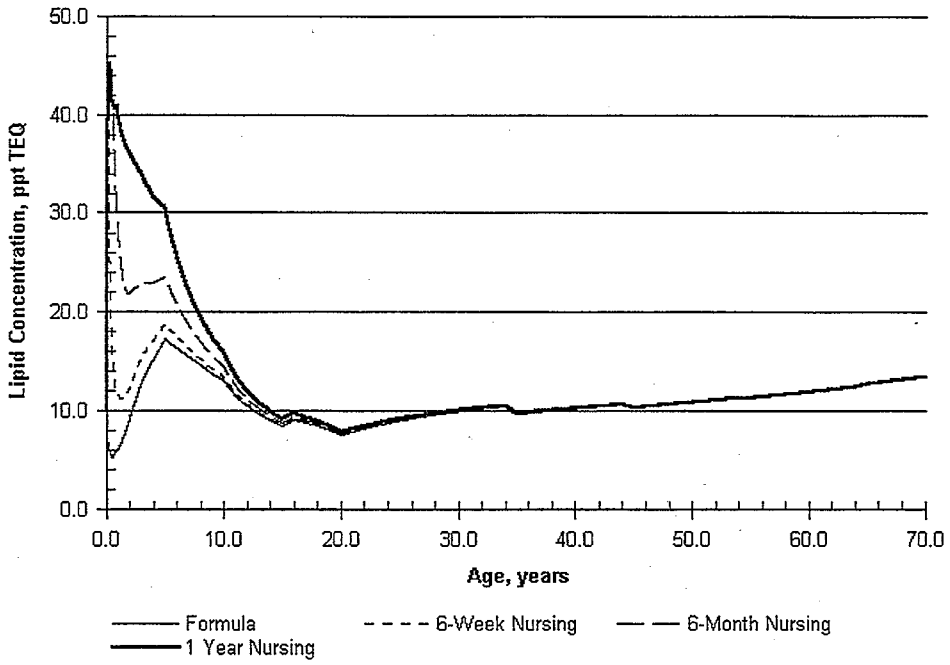


Figure 4-3. Blood levels (I-TEQ for CDD/CDF + WHO_{94}) versus age of a subset of participants in the CDC (2000).
Source: ATSDR (1999b)

(a)



(b)

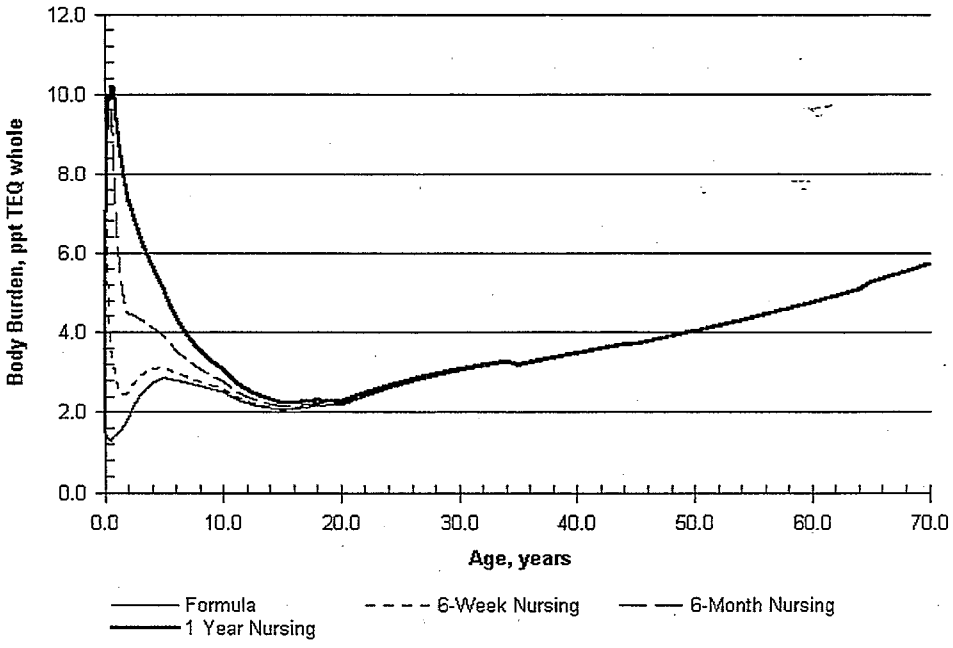


Figure 4-4. Lipid (a) and body burden (b) concentrations in a hypothethical female until age 70 under four nursing scenarios: formula only, and 6-week, 6-month, and 1 year nursing.

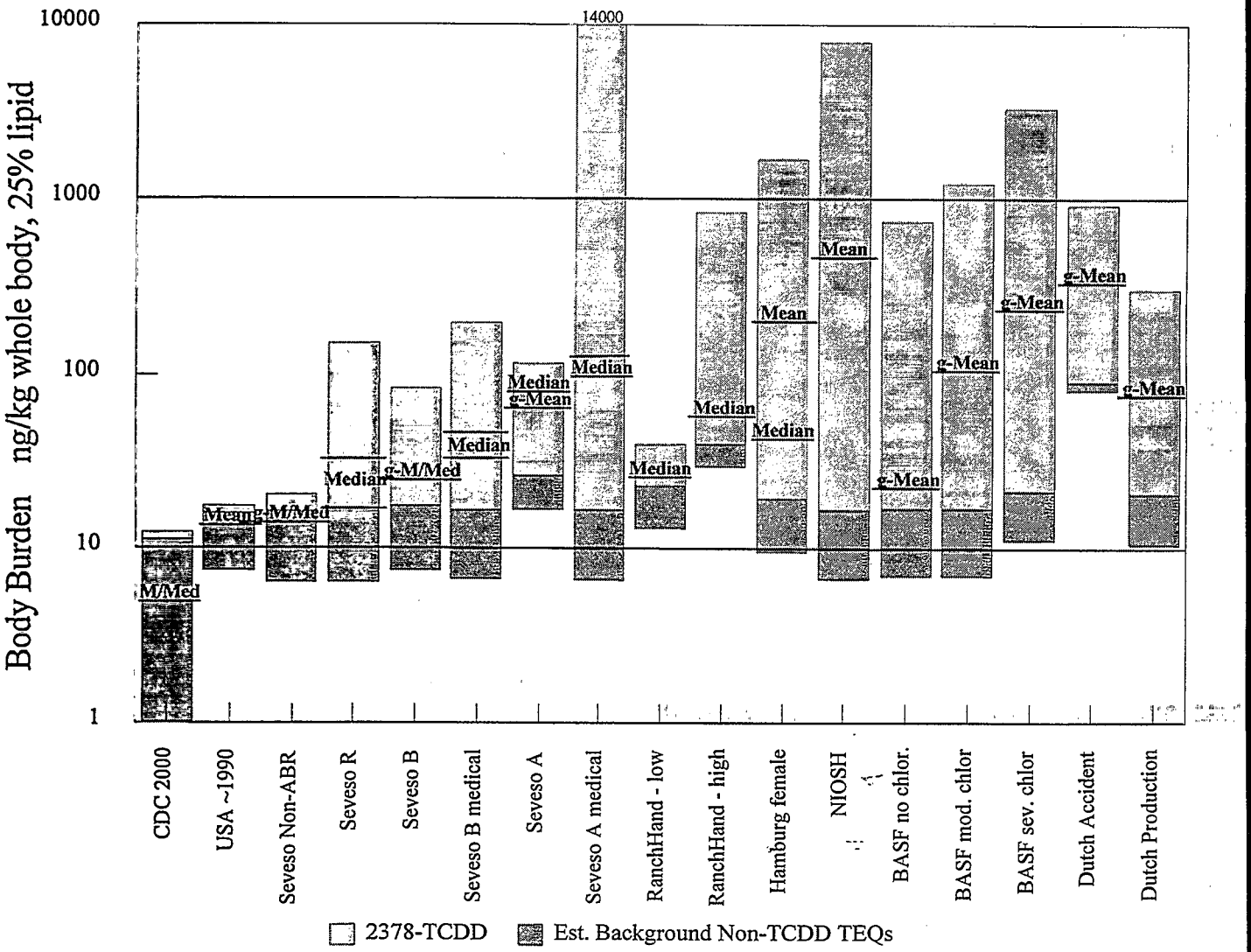


Figure 5-1. Peak dioxin body burden levels in background populations and epidemiological cohorts(back-calculated) (See Table 5-1).

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 based on 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.

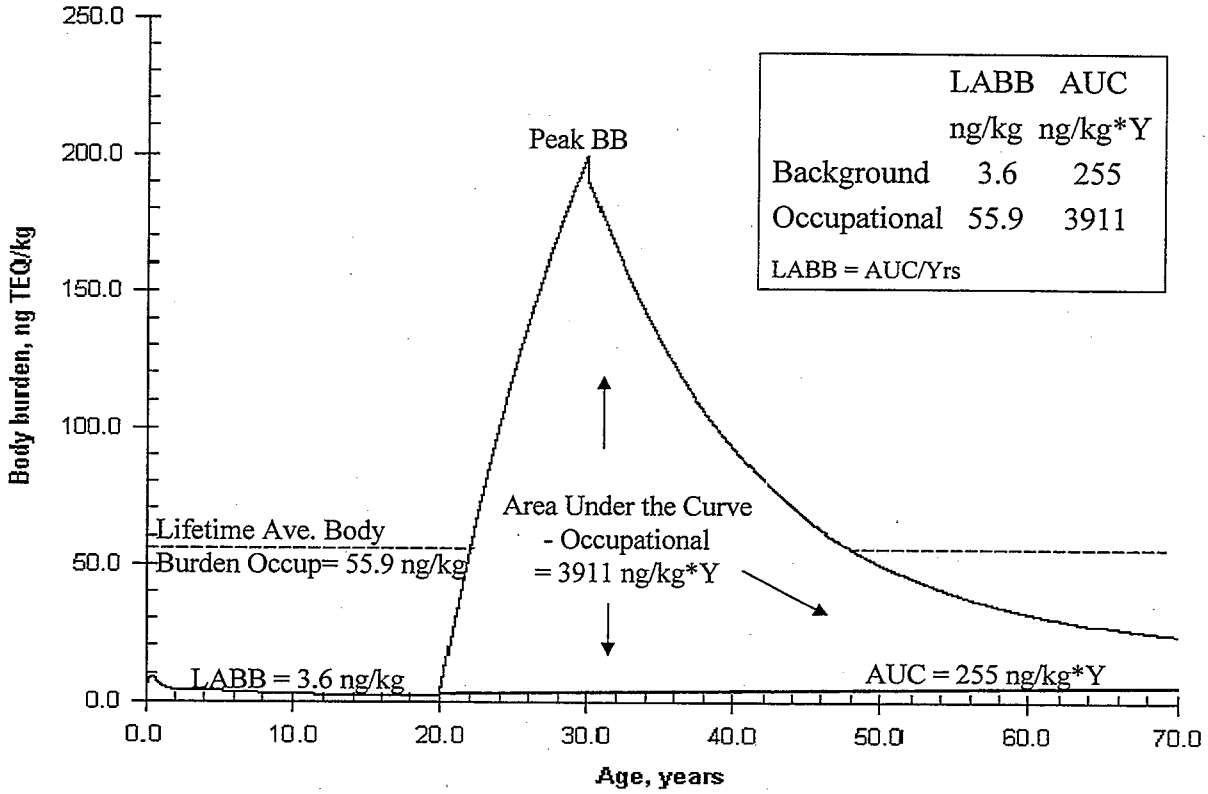


Figure 5-2. Comparison of lifetime average body burden and area under the curve in hypothetical background and occupational scenarios.

GLOSSARY AND DEFINITIONS

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 *vs.* 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, which 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: This is exposure which 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 associate with either biochemical or toxicological responses. In addition, these values are calculated based on either knowledge of the species-specific half-life and the exposure or they are estimated based on 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 based on an intake rate and the half-life of TCDD in humans. Alternatively, body burdens in humans are estimated based on 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 which 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 cohort is a group of animals of the same species, including humans, identified by a common characteristic, which is studied over a period of time as part of a scientific or medical investigation.

Confidence Intervals (CI): A range of values for a variable of interest, e.g., 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 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.

Congener: 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: Dioxin-like is an adjective that refers to the fact that these compounds have similar chemical structure, similar 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 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, nor 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 which the chemical undergoes within each compartment are used to construct a mass-balance framework for the PBPK model.

Point of Departure: 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 dose associated with such an incidence.

Promoter: An agent that is not carcinogenic itself, but 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, 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, LOAEL, or benchmark dose, with uncertainty factors generally applied to reflect limitations of the data used. Generally used in EPA's noncancer health assessments.

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 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 feedback 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 comprising the mixture to the well-studied and 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, 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, 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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