1. INTRODUCTION

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

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

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

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

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

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

This document (Part III) is organized as follows:

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

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

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

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

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

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

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

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

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

1.1. DEFINITION OF DIOXIN-LIKE COMPOUNDS

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

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

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

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

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

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

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

1.2. TOXIC EQUIVALENCY FACTORS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

1.3.1. Administered Dose

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

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

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

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

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

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

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

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

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

1.3.2. Area Under the Curve

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

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

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

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

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

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

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

1.3.3. Plasma or Tissue Concentrations

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

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

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

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

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

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

1.3.4. Steady-State Body Burdens

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

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

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

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

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

1.3.5. Mechanistic Dose Metrics

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

1.3.6. Summary

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

TEF = toxic equivalency factor

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

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

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

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

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

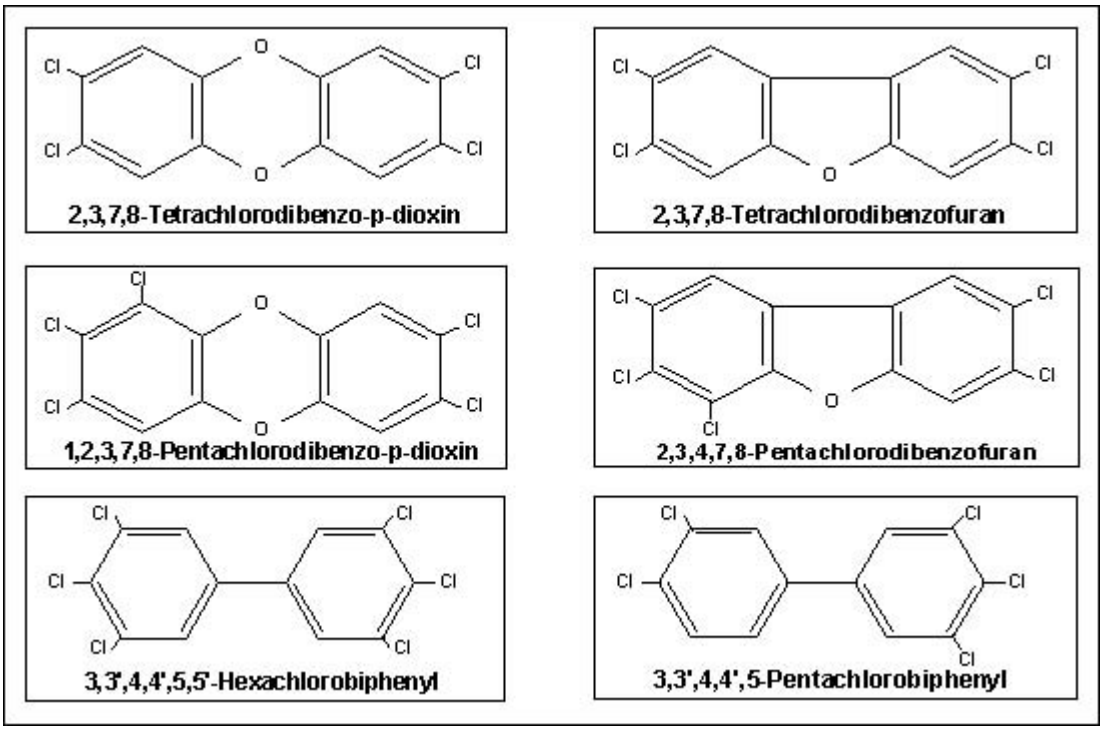

Figure 1-1. Chemical structure of 2,3,7,8-TCDD and related compounds.