Chapter 9. Risk Characterization

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Chapter 9

RISK CHARACTERIZATION OF DIOXIN AND RELATED COMPOUNDS

Introduction

Chlorinated dibenzo-p-dioxins and related compounds (commonly known simply as dioxins) are environmental contaminants present in a variety of environmental media. This class of compounds has caused great concern in the general public as well as intense interest in the scientific community. Much of the public concern revolves around the characterization of these compounds as among the most potent "man-made" toxicants ever studied. Indeed, these compounds are extremely potent in producing a variety of effects in experimental animals based on traditional toxicology studies at levels hundreds or thousands of times lower than most chemicals of environmental interest. In addition, human studies demonstrate that exposure to dioxin and related compounds is associated with subtle biochemical and biological changes and with chloracne, a serious skin condition associated with these and similar organic chemicals . Laboratory studies suggest the probability that exposure to dioxin-like compounds may be associated with other serious health effects including cancer. Human data are supportive of these concerns. Recent laboratory studies have provided new insights into the mechanisms involved in the impact of dioxins on various cells and tissues and, ultimately, on toxicity. Dioxins have been demonstrated to be potent modulators of cellular growth and differentiation, particularly in epithelial tissues. These data coupled with assumptions and inferences regarding extrapolation from experimental animals to humans and from high doses to low doses allow a characterization of dioxin hazards.

This chapter presents a risk characterization for dioxin and related compounds. In the risk characterization, key findings pertinent to understanding the hazards and risks of dioxin and related compounds are described and integrated. All of the available information is considered in proposing hypotheses or in reaching conclusions. The risk characterization is not meant to be an executive summary of the extensive data base which has been analyzed in detail in preceeding chapters and in

the exposure document. Risk characterization requires a discussion of likely routes, patterns and levels of exposure as well as aspects of hazard and dose response. Information contained in the document entitled, Estimating Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin and Related Compounds (EPA,1994), hereafter referred to as the Exposure Document, will be integrated with the health effects information on this class of compounds found in previous chapters of this assessment. The risk characterization contains an articulation of the strengths and weaknesses of the available evidence, as well as clearly presenting assumptions made and inferences used. Risk is characterized in both qualitative and quantitative terms, as appropriate. Finally, overall conclusions regarding the health risks of dioxin and related compounds are presented.

The process for development of this risk characterization of dioxin and related compounds has been an open and participatory one. The health assessment and exposure documents which provide the basis for this characterization have been developed in collaboration with scientists from within and from outside of the Federal government. Each of these has undergone extensive internal and external review including review at a meeting of experts after a first draft was completed. Additional input to this characterization comes from comments on those draft chapters as well as from the panel of experts who met in September, 1992. This panel was asked to provide their perspective on themes to be carried into the characterization and their contributions are reflected here. Finally the characterization, as presented here, represents review and comment by both those Federal scientists involved in development of the health assessment and exposure chapters as well as representatives of other Federal agencies. However, the views expressed in this characterization are those of the collective authors and, as a draft undergoing public comment and further external review, no Agency-level endorsement should be inferred at this time.

Once fully peer reviewed and revised accordingly, this risk characterization is meant to provide a balanced picture of the scientific findings of the health and exposure assessments for use by risk managers in selecting risk management options. As an integrated presentation of a complex data base, it is meant to answer key questions concerning the science behind concerns for dioxins and should be

useful in developing strategies for risk communication.

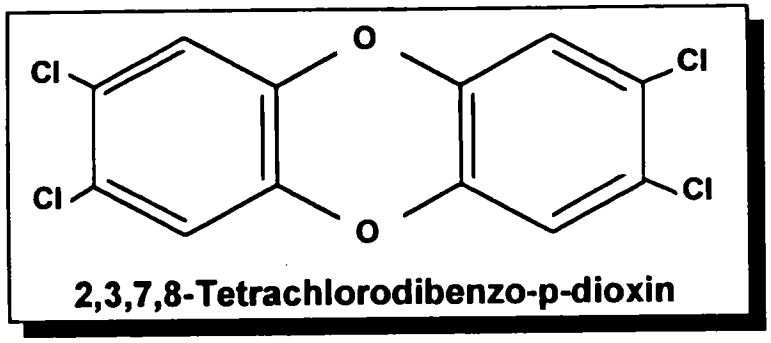
CHEMICAL STRUCTURE AND PROPERTIES

Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) are chemically classified as halogenated aromatic hydrocarbons (HAH). The chlorinated and brominated dibenzodioxins and dibenzofurans are tricyclic aromatic compounds with similar physical and chemical properties, and both classes are similar structurally. Certain of the PCBs (the so-called co-planar or mono-ortho co-planar congeners) are also structurally and conformationally similar. The most widely studied of these 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 9-I.

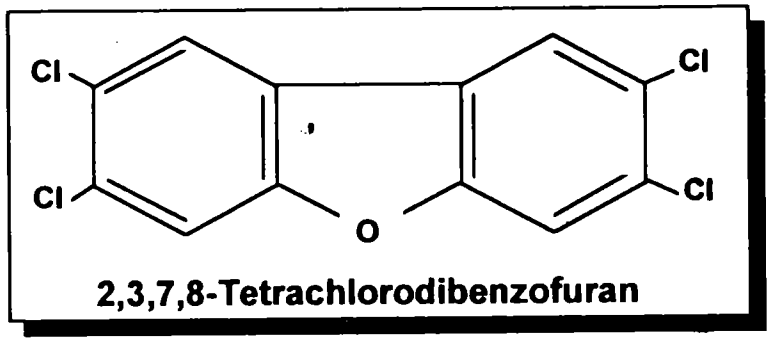
For purposes of this document, dioxin-like compounds are defined to include the subset of this class of compounds which are generally agreed to produce dioxin-like toxicity. These compounds are assigned individual Toxicity Equivalency Factor (TEF) values as defined by international convention (EPA, 1989) in proportion to their toxicity relative to TCDD. Results of *in vitro* and *in vivo* laboratory studies contribute to the assignment of a relative toxicity value. All chlorinated dibenzodioxins (CDDs) and chlorinated dibenzofurans (CDFs) with chlorines substituted in the 2,3,7, and 8 positions are assigned TEF values. Additionally, the analogous brominated dioxins and furans (BDDs and BDFs) and certain polychlorinated biphenyls (PCBs) have recently been identified as having dioxin-like toxicity and thus are also included in the definition of dioxin-like compounds. Generally accepted TEF values are shown in Table 9-1. A recent World Health Organization/International Program on Chemical Safety meeting (Dec.,1993) held in the Netherlands considered the need to derive internationally acceptable interim TEFs for the dioxin-like PCBs. Recommendations arising from that meeting of experts suggest that in general only a few of the dioxin-like PCBs are likely to be significant contributors to general population exposures to dioxin-like compounds. It is estimated that these dioxin-like PCBs may be responsible for approximately 1/4 to1/3 of the total toxicity equivalence associated with general population environmental exposures to this class of related compounds. Both the

Fig. 9-1

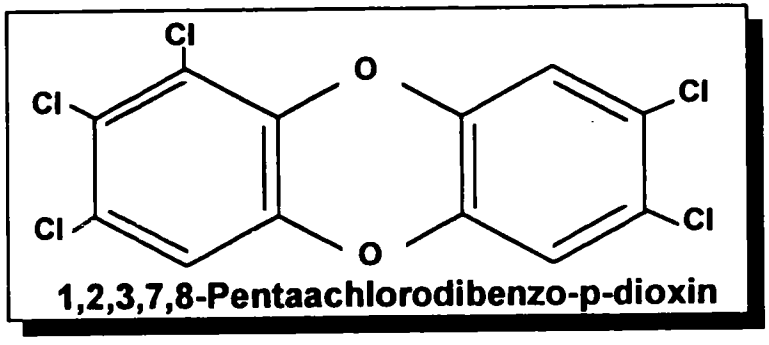
Dioxin and Similar Compounds - Chemical Structure



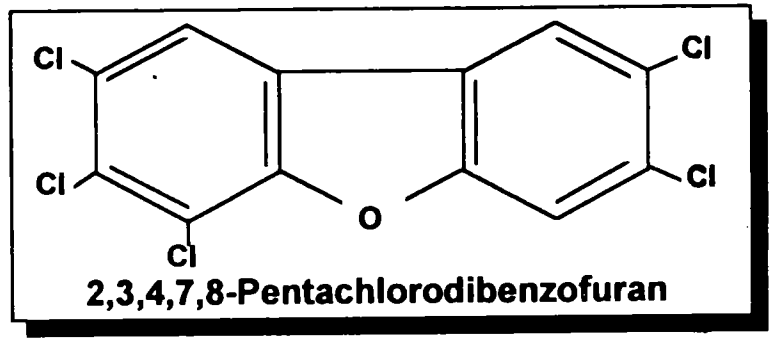
2,3,7,8-Tetrachlorodibenzo-p-dioxin



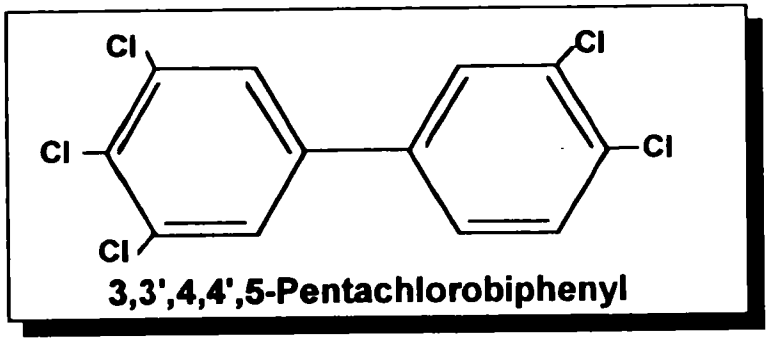
2,3,7,8-Tetrachlorodibenzofuran



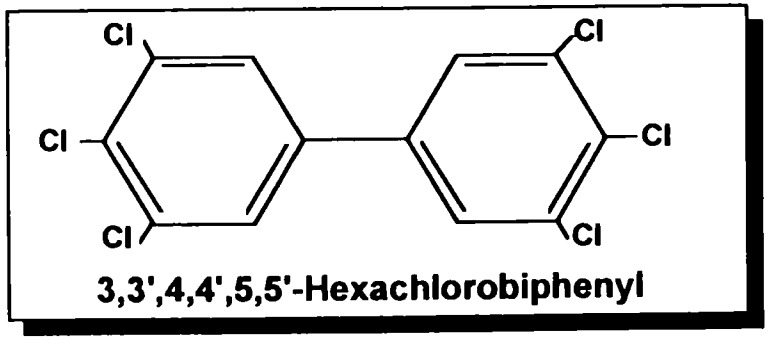
1,2,3,7,8-Pentaachlorodibenzo-p-dioxin



2,3,4,7,8-Pentachlorodibenzofuran



3,3',4,4',5-Pentachlorobiphenyl



3,3',4,4',5,5'-Hexachlorobiphenyl

Table 9-1. Toxicity Equivalency Factors (TEF) for CDDs and CDFs.

Compound	TEF
Mono-, Di-, and Tri-CDDs	0
2,3,7,8-TCDD	1
Other TCDDs	0
2,3,7,8-PeCDD	0.5
Other PeCDDs	0
2,3,7,8-HxCDD	0.1
Other HxCDDs	0
2,3,7,8-HpCDD	0.01
Other HpCDDs	0
OCDD	0.001
Mono-, Di-, and Tri-CDFs	0
2,3,7,8-TCDF	0.1
Other TCDFs	0
1,2,3,7,8-PeCDF	0.05
2,3,4,7,8-PeCDF	0.5
Other PeCDFs	0
2,3,7,8-HxCDF	0.1
Other HxCDFs	0
2,3,7,8-HpCDF	0.01
Other HpCDFs	0
OCDF	0.001

Source: EPA, 1989.

refinement of the toxicity equivalence factors for dioxin-like PCB congeners (DeVito et al., 1993) as well as a compilation and analysis of all of the available data on relative toxicities of dioxin-like PCBs with respect to a number of endpoints (Ahlborg et al., 1994) support these findings. Although these findings have been published recently, additional review and data collection will be needed . The panel specifically recommended that theseTEFs be used as intake values and urged caution in their use with regard to toxicity equivalence in body burden measurements. In addition, the panel urged investigation of companion TEFs for ecotoxicological use, based on data from ecotoxicological studies.

There are 75 possible individual compounds comprising the CDDs depending on the positioning of the chlorine(s) and 135 different CDFs. These are called individual congeners. Likewise, there are 75 possible different positional congeners of BDDs and 135 different congeners of BDFs (see Exposure Document, Table 2-1). Only 7 of the 75 possible congeners of CDDs or of BDDs are thought to have dioxin-like toxicity; these are ones with chlorine/bromine substitutions in, at least, the 2,3,7,8-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 2,3,7,8 -positions. While this suggests 34 individual CDDs, CDFs, BDDs or BDFs with dioxin-like toxicity, inclusion of the mixed chloro/bromo congeners substantially increases the number of possible congeners with dioxin-like activity. There are 209 possible PCB congeners. Only 13 of the 209 possible congeners are thought to have dioxin-like toxicity, these are ones with 4 or more chlorines with just 1 or no substitutions in the ortho position. These compounds are sometimes referred to as coplanar since they can assume a flat configuration with rings in the same plane. Similarly configured polybrominated biphenyls are likely to have similar properties although the data base on these compounds with regard to dioxin-like activity has been less extensively evaluated. Mixed chlorinated and brominated congeners will increase the number of compounds considered dioxin-like. The physical/chemical properties of each congener vary according to the degree and position of chlorine and/or bromine substitution.

In general these compounds have very low water solubility, high octanol-water partition coefficients, low vapor pressure and tend to bioaccumulate. Volume II of the Exposure Document presents congener specific values for water solubility, vapor

pressure, partition coefficients and photo quantum yields and discusses other physico-chemical characteristics of the chlorinated dioxins and dibenzofurans. These physico-chemical properties result in the environmental fate and transport discussed below. Expanded discussions will be required in future documents to account for dioxin-like PCB's and for brominated or mixed halogenated congeners.

ENVIRONMENTAL FATE

Despite a growing body of literature from laboratory, field, and monitoring studies examining the environmental fate and environmental distribution of CDDs and CDFs, the fate of these environmentally ubiquitous compounds is not yet fully understood and the following represents our best understanding, based on available data. In soil, sediment, the water column, and probably air, CDDs/CDFs are primarily associated with particulate and organic matter because of their high lipophilicity and low water solubility. They exhibit little potential for significant leaching or volatilization once sorbed to particulate matter. The available evidence indicates that CDDs and CDFs, particularly the tetra- and higher chlorinated congeners, are extremely stable compounds under most environmental conditions, with environmental persistence measured in decades. The only environmentally significant transformation process for these congeners is believed to be photodegradation of chemicals not bound to particles in the gaseous phase or at the soil- or water-air interface. Brominated congeners are significantly more readily transformed by photodegradation. CDDs/CDFs entering the atmosphere are removed either by photodegradation or by dry or wet deposition. Burial in-place or erosion of soil to water bodies appears to be the predominant fate of CDDs/CDFs sorbed to soil. CDDs/CDFs entering the water column primarily undergo sedimentation and burial. The ultimate environmental sink of CDDs/CDFs is believed to be aquatic sediments.

Little specific information exists on the environmental transport and fate of the dioxin-like PCBs. However, the available information on the physical/chemical properties of dioxin-like PCBs coupled with the body of information available on the widespread occurrence and persistence of PCBs in the environment indicates that these PCBs are likely to be associated primarily with soils and sediments, and to be thermally and chemically stable. Soil erosion and sediment transport in water bodies

and emissions to the air (via volatilization, dust resuspension, or point source emissions) followed by atmospheric transport and deposition are believed to be the dominant transport mechanisms responsible for the widespread environmental occurrence of PCBs. Photodegradation to less chlorinated congeners followed by slow anaerobic and/or aerobic biodegradation is believed to be the principal path for destruction of PCBs. Similar situations exist for the polybrominated biphenyls (PBBs). Little information is available on the occurrence and fate of biphenyl congeners containing both chlorine and bromine but their contribution to dioxin-like activity in the environment is thought to be small.

SOURCES

The chlorinated and brominated dioxins and furans have never been intentionally produced other than on a laboratory scale basis for use in chemical analyses. Rather, they are generated as byproducts from various combustion and chemical processes. PCBs were produced in relatively large quantities for use in such commercial products as dielectrics, hydraulic fluids, plastics and paints. They are no longer produced, but continue to be released to the environment through the use and disposal of these products. A similar situation exists for the commercially produced PBBs which were produced for a number of uses like flame retardants.

Dioxin-like compounds are released to the environment in a variety of ways and in varying quantities depending upon the source. Studies of sediment cores in lakes near industrial centers of the United States have shown that historical environmental deposition of dioxins and furans was quite low until about 1920, peaked around 1980 and has declined thereafter. This trend suggests that the presence of dioxin-like compounds in the environment has occured primarily as a result of industrial practices and is likely to reflect changes in release over time. Although these compounds are released from a variety of sources, the congener profiles of CDDs and CDFs found in sediments have been linked to combustion sources (Hites, 1991). Three theories have been suggested to explain formation of CDDs and CDFs during combustion: 1) The CDDs and CDFs are present in the fuels or feed materials and pass through the combustor intact; 2) precursor chemicals are present in the fuels or feed material and undergo reactions catalyzed by particulates and other chemicals to form CDDs and

CDFs; and 3) the CDDs and CDFs are formed *de novo* from organic and inorganic substrates bearing little resemblance in molecular structure.

The principal identified sources of environmental release of CDDs and CDFs may be grouped into four major types:

- Combustion and Incineration Sources: Dioxin-like compounds can be generated and released to the environment from various combustion processes when chlorine donor compounds are present. These sources can include incineration of wastes such as municipal solid waste, sewage sludge, hospital and hazardous wastes; metallurgical processes such as high temperature steel production, smelting operations, and scrap metal recovery furnaces; and the burning of coal, wood, petroleum products, and used tires for power/energy generation. Even cigarette smoke, crematories, volcanoes, and forest fires have been shown to be minor sources.

- Chemical Manufacturing/Processing Sources: Dioxin-like compounds can be formed as by-products from the manufacture of chlorine and such chlorinated compounds as chlorinated phenols, PCBs, phenoxy herbicides, chlorinated benzenes, chlorinated aliphatic compounds, chlorinated catalysts, and halogenated diphenyl ethers. Although the manufacture of many chlorinated phenolic intermediates and products, as well as PCBs, was terminated in the late 1970s in the United States, production continued elsewhere around the world until 1990 and continued, limited use and disposal of these compounds can result in releases of CDDs, CDFs, and PCBs to the environment.

- Industrial/Municipal Processes: Dioxin-like compounds can be formed through the chlorination of naturally occurring phenolic compounds such as those present in wood pulp. The formation of CDDs and CDFs resulting from the use of chlorine bleaching processes in the manufacture of bleached pulp and paper has resulted in the presence of CDDs and CDFs in paper products as well as in liquid and solid wastes from this industry. Municipal sewage sludge has been found to occasionally contain CDDs and CDFs.

- Reservoir Sources: The persistent and hydrophobic nature of these compounds cause them to accumulate in soils, sediments and organic matter and to persist in waste disposal sites. The dioxin-like compounds in these "reservoirs" can be

redistributed by various processes such as dust or sediment resuspension and transport. Such releases are not original sources in a global sense, but can be on a local scale. For example, releases may occur naturally from sediments via volatilization or via operations which disturb them such as dredging. Aerial deposition and accumulation on leaves can lead to releases during forest fires or leaf composting operations.

As awareness of these possible sources has grown in recent years, a number of changes have occurred which should reduce the release rates. For example, releases of dioxin-like compounds have been reduced due to the switch to unleaded automobile fuels (and associated use of catalytic converters and reduction in halogenated scavenger fuel additives), process changes at pulp and paper mills, new emission standards and upgraded emission controls for incinerators, and reductions in the manufacture of chlorinated phenolic intermediates and products.

Although dioxins in the environment may arise from a number of sources as discussed above, the Exposure Document presents recent analyses of air emissions of CDDs and CDFs for several European countries in terms of total toxic equivalents (TEQs) based on international TEFs. These studies assume that emissions to air make up the major portion of dioxins released to the environment. Estimates of total release in these countries range from approximately 100-1000 g TEQ/year in West Germany and 100-200g TEQ/year in Sweden to approximately 1000 and 4000 g TEQ/ year maximum emissions in the Netherlands and United Kingdom respectively. Similar nationwide estimates for the U.S. have not been compiled prior to this reassessment effort. The Exposure Document estimates an upper end on U.S. emissions to be in the range of 14,000 g TEQ/year. Qualitatively speaking, major contributors to this total include medical waste incinerators, municipal waste incinerators, cement kilns,and industrial wood burning. Because of the limited number of measurements and the large number of potential sources for each of these emissions, total estimated emissions from these sources are considered highly uncertain. Municipal waste incineration has a better data base of measurement data than other air sources but emissions are highly variable among facilities so that the overall estimate remains uncertain. Diesel-fueled vehicles, hazardous waste burning, forest fires and metal smelting are more moderate contributors of dioxin-like

compounds but the magnitude of the contribution is also highly uncertain. Sewage waste incineration and residential wood burning as well as a few minor processes round out the current analysis and provide lower range estimates of medium to low certainty. Although still other sources are recognized and releases to land and water in addition to air are briefly mentioned in the Exposure Document, it is clear from this exercise that additional measurement data will be needed to gain an adequate appreciation for the nature and magnitude of major U.S. sources of CDD and CDF emissions.

Several investigators have attempted to conduct "mass balance" checks on the estimates of national dioxin releases to the environment. Basically, this procedure involves comparing estimates of the emissions to estimates of aerial deposition. Such studies in Sweden (Rappe, 1991) and Great Britain (Harrad and Jones, 1992) have suggested that the deposition exceeds the emissions by about 10 fold. These studies are acknowledged to be quite speculative due to the strong potential for inaccuracies in emission and deposition estimates. In addition, the apparent discrepancies could be explained by long range transport from outside the country, resuspension and deposition of reservoir sources or unidentified sources. Bearing these limitations in mind, this procedure has been used in this reassessment to compare the estimated emissions and deposition in the U.S.

Deposition measurements have been made at a number of locations in Europe and two places in the US (See discussion of these studies in Volume II of the Exposure Document). These limited data suggest that a deposition rate of 1 ng TEQ/m^2-yr is typical of remote areas and that 2-6 ng TEQ/m^2-yr is more typical of populated areas. Applying the values of 1 ng TEQ/m^2-yr to Alaska and 2-6 ng TEQ/m^2-yr to the contiguous 48 states, the total U.S. deposition can be estimated as 20,000 to 50,000 g TEQ/yr. While this range is higher than the upper estimate of emissions for the US (<14,000 g TEQ/yr) as presented in the Exposure Document, the upper estimate may account for >30-70% of predicted deposition. As noted above, interpreting such comparisons is highly speculative and supports the need to conduct further emissions testing into all media and deposition measurement, if we are to understand emisssions and deposition in terms of a mass balance.

Levels in the Environment and in Food

CDDs, CDFs and PCBs have been found throughout the world in practically all media including air, soil, water, sediment, fish and shellfish, and other food products such as meat and dairy products. The highest levels of these compounds are found in soils, sediments, and biota; very low levels are found in water and air. The widespread occurrence observed, particularly in industrialized countries, is not unexpected considering the numerous sources that emit these compounds into the atmosphere, and the overall resistance of these compounds to biotic and abiotic transformation.

The average levels of these compounds found in the various media in North America have been compiled in the Exposure Document. The levels shown for environmental media and for food are based on few samples and must be considered quite uncertain. However, they seem reasonably consistent with levels measured in a number of studies in Western Europe and Canada. The consistency of these levels across industrialized countries adds some confidence to the limited data from the U.S. and provides some reassurance that these estimates are reasonable.

This assessment proposes the hypothesis that the primary mechanism by which dioxin-like compounds enter the terrestrial food chain is via atmospheric deposition. Dioxin and related compounds enter the atmosphere directly through air emissions or indirectly, for example through volatilization from land or water or from re-suspension of particles. Deposition can occur directly onto soil or onto plant surfaces. Soil deposits can enter the food chain via direct ingestion (e.g. grazing animals, earth worms, fur preening by burrowing animals). Dioxin-like compounds in soil can become available to plants by volatilization and vapor absorption or particle resuspension and adherence to plant surfaces. In addition, dioxin-like compounds in soil can adsorb directly to underground portions of plants. Uptake from soil via the roots into above ground portions of plants is thought to be insignificant.

Support for this air-to-food hypothesis is provided by Hites (1991) who concluded that "background environmental levels of dioxin-like compounds are caused by dioxin-like compounds entering the environment through the atmospheric pathway." His conclusion was based on demonstrations that the congener profiles in lake sediments could be linked to congener profiles of combustion sources. Further

arguments supporting this hypothesis include: 1) numerous measurements show that emissions occur from multiple sources and deposition occurs in most areas including remote locations, 2) atmospheric transport and deposition is the only mechanism that could explain the widespread distribution of these compounds in soil, and 3) other mechanisms of uptake into food, for instance from direct contamination or through packaging, are much less plausible. Direct uptake into food from soil or sediments is possible and could be important for "local" exposures. These routes are less likely to explain the general background level of dioxin and related compounds found in the diet of the general population.

At present, it is unclear whether atmospheric deposition represents primarily "new" contributions of dioxin and related compounds from all media reaching the atmosphere or whether it is "old" dioxin and related compounds which persist and recycle in the environment. Understanding the relationship between these two scenarios will be particularly important in understanding the relative contributions of individual point sources of these compounds to the food chain and assessing the effectiveness of control strategies attempting to reduce the levels in food.

Background Exposure Levels

The term "background" exposure has been used throughout this reassessment to describe exposure of the general population who are not exposed to identifiable point sources. For the purposes of calculating background exposures to dioxin-like compounds via dietary intake the upper-range background toxicity equivalent values (TEQs) (i.e., those calculated using one-half the detection limit for the non-detects) were used in the Exposure Document. The estimates are based on intake of dioxin-like CDDs and CDFs and do not include estimates for dioxin-like PCBs or other dioxin-like compounds. Inclusion of dioxin-like PCBs could raise these estimates by 35-50%. A background exposure level of 119 pg TEQ/day for the U.S. was estimated. These estimates are comparable to analogous estimates for European countries. These include estimates for Germany, which range from 79 pg TEQ/day based on Furst, et al. (1990) to 158 pg TEQ/day based on Furst, et al. (1991), 118-126 pg TEQ/day exposure via numerous routes in the Netherlands (Theelen, 1991), and 140-290 pg TEQ/day for the typical Canadian exposed mainly through food ingestion (Gilman and Newhook,

1991). It is generally concluded by these researchers that dietary intake is the primary pathway of human exposure to CDDs and CDFs. These investigators among others suggest that greater than 90 percent of human exposure occurs through the diet, with foods from animal origins being the predominant sources.

This hypothesis remains to be validated. Although data are derived from multiple studies from around the world, they represent limited numbers of samples. Use of one-half of the detection level for non-detects is a reasonable but conservative approach to estimating low levels in samples. For some data sets, use of zero values for non-detects could result in significantly lower estimates. However, it is widely held that such an approach would most likely underestimate true levels of exposure. Similar estimates derived from different data sets, developed by different investigators in several countries, strengthen the probability that this inference represents the true picture for exposure of the general population in industrialized countries to dioxin and related compounds.

Data on human tissue levels suggest that body burden levels among industrialized nations are reasonably similar (Schecter, 1991). These data can also be used to estimate background exposure through the use of pharmacokinetic models. Using this approach, exposure levels to 2,3,7,8-TCDD in industrialized nations are estimated to be about 20 to 40 pg/day (.3-.6 pg TCDD/kg/day). This is generally consistent with the estimates derived using diet based approaches to estimate total TCDD intake.

The U.S. study of CDD/F body burdens contained in the National Human Adipose Tissue Survey (NHATS) (EPA, 1991) analyzed for CDD/Fs in 48 human tissue samples which were composited from 865 samples. These samples were collected during 1987 from autopsied cadavers and surgical patients. While this was an important study of chemical residues occuring in human fat , numerous technical shortcomings of this study have been described. For instance, the sample compositing prevents use of these data to examine the distribution of CDD/F levels in tissue among individuals. However, it did allow conclusions in the following areas:

- **National Averages:** The national averages for all TEQ congeners (but excluding dioxin-like PCBs) were estimated and totaled to 28 pg TEQ/g lipid adjusted value (28 ppt).

•**Age Effects:** Tissue concentrations of CDD/Fs were found to increase with age.

•**Geographic Effects:** In general, the average CDD/F tissue concentrations appeared fairly uniform geographically.

•**Race Effects:** No significant difference in CDD/F tissue concentrations were found on the basis of race.

•**Sex Effects:** No significant difference in CDD/F tissue concentrations were found between males and females.

•**Temporal Trends:** The 1987 survey showed decreases in tissue concentrations relative to the 1982 survey for all congeners. However, it is not known whether these declines were due to improvements in the analytical methods or actual reductions in body burden levels. The percent reductions among individual congeners varied from 9 percent to 96 percent.

More recent data (Patterson et al., 1994) show similar trends with regard to decreasing levels of dioxin-like PCBs in blood and fat. In addition, they showed a wide variability of PCB congeners in human adipose tissue sample as compared to concentrations of CDDs and CDFs which were less variable.

Inclusion of dioxin-like PCBs in TEQ calculations raises the average body burden to 40-60 pg TEQ/g (40-60 ppt). Since available data from the two studies discussed above do not provide a representative population sample, these conclusions must be regarded as somewhat uncertain. Additional measurements will be necessary to confirm these findings. Use of a protocol for sampling which allows an evaluation of age adjusted population averages will be critical for understanding the current body burden situation and evaluating impacts of future efforts to further reduce exposures to this class of compounds.

Levels of dioxin-like compounds found in human tissue/blood appear similar in Europe and North America. Schecter (1991) compared levels of dioxin-like compounds found in blood among people from U.S. pooled samples (100 subjects) and Germany (85 subjects). Although mean levels of individual congeners differed by as much as a factor of two between the two populations, the total TEQ averaged 42 pg TEQ/g (42 ppt) in the German subjects and was 41 pg TEQ/g (41 ppt) in the pooled U.S. samples. These values do not include TEQs for PCBs.

New information on levels of dioxin-like compounds in human adipose tissue

and blood has recently been published (Patterson et al, 1994). This study reports measurements of dioxin-like PCB congeners as well as CDD and CDF levels in samples from 28 Atlanta residents. These measurements show that concentrations of dioxin-like PCBs can be more than an order of magnitude higher than concentrations of TCDD. Comparison with other published information suggests much higher levels of non-dioxin-like congeners of PCBs and the possibility that concentrations of both types of congeners will depend heavily upon previous human activities such as fish consumption. These data are consistent with the previous statement that dioxin-like PCBs may account for approximately 1/3 of the total TEQ in the general population. Values in Patterson's study calculated TEQs for PCBs using the data of Safe (1990) which were acknowledged by the author as being conservative and, based on more recent data, are likely to overestimate the contribution of dioxin-like PCBs.

Highly Exposed Populations

Certain groups of people may have higher exposures to dioxin-like compounds than the general population. This issue has been discussed previously in terms of increased exposure due to dietary habits (See Exposure Document) or due to occupational conditions or industrial accidents (See Chapter 7).

Consumption of breast milk by nursing infants may lead to higher levels of exposure during the early postnatal period as compared to intake in the diet later in life. Schecter et al. (1992) reports that a study of 42 U.S. women found an average of 16 pg TEQ /g (16 ppt), 3.3 ppt of which was 2,3,7,8-TCDD, in the lipid portion of breast milk. A much larger study in Germany (n= 526) found an average of 29 pg TEQ / g (29 ppt) in the lipid portion of breast milk. These estimates do not include a contribution to total TEQ from dioxin-like PCBs. The level in human breast milk can be predicted on the basis of the estimated dioxin intake by the mother. Such procedures are presented in Volume II of the Exposure Document.

Using these procedures and assuming that an infant breast feeds for one year, has an average weight during this period of 10 kg, ingests 0.8 kg/d of breast milk and that the dioxin concentration in milk fat is 20 pg /g (20 ppt) of TEQ, the average daily dose to the infant over this period is predicted to be about 60 pg TEQ/kg/d, not including dioxin-like PCBs. This value is 10 to 20 times higher than the estimated

range for background exposure to adults (i.e. 3-6 pg TEQ/kg/d). However, if a 70 yr averaging time is used to obtain an added increment of lifetime daily dose, then the increment of lifetime average daily dose is attributable to this nursing scenario is estimated to be 0.8 pg of TEQ/kg/d. On a mass basis, the cumulative dose to the infant under this scenario is about 210 ng compared to a lifetime background dose of about 1700 to 5100 ng (suggesting that 4 to 12 percent of the lifetime dose may occur as a result of breast feeding for the first year of life). Traditionally, EPA has used the lifetime average daily dose as the basis for evaluating incremental cancer risk and the average daily dose (i.e., the daily exposure per unit body weight occurring during an exposure event) as the more appropriate indicator of risk for certain noncancer endpoints. The use of a lifetime average daily dose for high level, early exposures may underestimate cancer risk if dose rate or perinatal sensitivity is important in the ultimate carcinogenic outcome. The average daily dose approach may be particularly important for the evaluation of non-cancer endpoints if exposure is occurring during windows of sensitivity during prenatal and postnatal development.

In addition, consumption of unusually high levels of fish or meat containing elevated levels of dioxin and related compounds can lead to elevated blood levels in comparison to the general population. Most people eat fish from multiple sources where levels of dioxin-like compounds are likely to be low. Even if large quantities of fish are consumed, they are not likely to have unusually high exposures. However, individuals who fish regularly for purposes of basic subsistence are likely to obtain their fish from a few sources and may have the potential for elevated exposures. Such individuals may also consume large quantities of fish. Although average consumers may eat a few fish meals a month (an average intake of 6.5 grams of fish a day), many recreational anglers near large water bodies may consume, on average, 4 to 5 times as much (approximately 30 grams per day); some individuals at the high end of the consumption range may eat, on average, as much as140 grams per day. Certain members of ethnic groups who are subsistence fishers may consume 2 to 3 times this amount as an upper estimate. Svensson et al (1991) found elevated blood levels of PCDDs and PCDFs in high fish consumers living near the Baltic Sea in Sweden. The highest consumers, fishermen or workers in the fish industry, had blood level TEQs that were approximately 3 times that of non-fish consumers (60 pg TEQ/g lipid versus

20 pg TEQ/g lipid). The difference in levels of dioxin-like compounds was particularly apparent for the PCDFs. Dioxin-like PCBs were not accounted for in this study. Studies are currently underway to examine fish consumption patterns in several Native American groups. Recent results (Columbia River Intertribal Fish Commission, 1994) suggest that Native Americans living along the Columbia River may consume an average of 30 grams of fish a day; some individuals consume much higher levels. Studies are currently underway to determine levels of dioxin-like compounds in fish from this region. No measurements of dioxin-like chemicals in the blood of these Native American populations are currently available.

Dewailly et al. (1994) observed elevated levels of coplanar PCBs in the blood of fishermen on the north shore of the Gulf of the St. Lawrence River who consume large amounts of seafood. Coplanar PCB levels were 20 times higher among the 10 highly exposed fishermen than among controls. This study also reported elevated levels of of coplanar PCBs in the breast milk of Inuit women of Arctic Quebec. The principal source of protein for the Inuit people is fish and sea mammal consumption.

The possibility of high exposures to dioxin-like chemicals as a result of consuming meat and dairy products is most likely to occur in situations where individuals consume large quantities of these foods from a locality where the level of these compounds is elevated. Most people eat meat and dairy products from multiple sources and, even if large quantities are consumed, are not likely to have unusually high exposures. However, individuals who raise their own livestock for basic subsistence have the potential for higher exposures if local levels of dioxin-like compounds are high. Volume III of the Exposure Document presents methods for evaluating this type of exposure scenario, but no studies were found in the literature to demonstrate this potential based on measurements of dioxin-like chemicals from source to livestock to humans.

Although the subpopulations discussed above have the potential for high exposure to dioxin-like compounds, a careful evaluation of dietary habits is needed to confirm this possibility. It would generally be inappropriate to compute the total intake of dioxin-like compounds in a subpopulation by simply adding the dioxin intake from highly consumed food to the general population intake level. The general population background estimate assumes a typical pattern of food ingestion, whereas a

subpopulation who has a high consumption rate of one particular food type is likely to eat less of other food types. Ideally, the evaluation should be based on the entire diet of the subpopulation and use case-specific values for food ingestion rates and concentrations of dioxin-like compounds.

High blood levels of dioxin and related compounds based on high levels of exposure have been documented for industrial exposures in segments of the chemical industry and for industrial accidents. Health effects studies in human populations have focused on these groups of highly exposed individuals. Results of these studies are described in detail in Chapter 7. Other populations in proximity to industrial sites have been evaluated for elevated blood levels of dioxin and related compounds. Higher levels have been measured in a few situations.

DISPOSITION AND PHARMACOKINETICS

The disposition and pharmacokinetics of 2,3,7,8-TCDD and related compounds have been investigated in several species and under various exposure conditions. These data and models derived from them are critical in understanding the sequelae of human exposure. Data related to disposition and pharmacokinetics of dioxin and related compounds and efforts to develop models to further understand tissue dosimetry are described in detail in Chapter 1 of the Health Assessment document.

The gastrointestinal, dermal and transpulmonary absorption of these compounds represent potential routes for human uptake. Findings of studies in experimental animals indicate that oral exposure to 2,3,7,8-TCDD in the diet or in an oil vehicle results in the absorption of >50%, and often closer to 90%, of the administered dose. Gastrointestinal absorption of related compounds is variable, incomplete and congener specific. More soluble congeners, such as 2,3,7,8-TCDF, are almost completely absorbed, while the extremely insoluble OCDD is very poorly absorbed. In some cases, absorption has been found to be dose dependent, with increased absorption occurring at lower doses (2,3,7,8-TBDD, OCDD). The limited data base also suggests that there are no major interspecies differences in the gastrointestinal absorption of these compounds among mammals. Limited data from a single human volunteer suggests a high level (> 87%) of absorption of 2,3,7,8-TCDD in corn oil from the gastrointestinal tract. Following absorption, a half life for

elimination was estimated to be 2120days.

Additional data also indicate the importance of the formulation or vehicle containing the toxicant(s) on the relative bioavailability of 2,3,7,8-TCDD and related compounds after exposure. For instance, rodent feeding studies indicate that the bioavailability of 2,3,7,8-TCDD from soil varies between sites and 2,3,7,8-TCDD content alone may not be indicative of potential human hazard from contaminated environmental materials. Although data indicate that substantial absorption may occur from contaminated soil, soil type and duration of contact may substantially affect the absorption of 2,3,7,8-TCDD from soils obtained from different contaminated sites. This uncertainty should be kept in mind as intake values are often used to estimate potential risk from environmental samples.

In experiments measuring dermal absorption for 2,3,7,8-TCDD and several CDFs, the percentage of administered dose absorbed decreased with increasing dose while the amount absorbed (μg/kg) increased with dose. Results also suggest that the majority of the compound remaining at the skin exposure site was associated with the the outer skin layer (the stratum corneum) and did not penetrate through to the dermis. Together, these results on dermal absorption indicate that at lower doses (≤0.1 μmol/kg), a greater percent of this administered dose of 2,3,7,8-TCDD and three CDFs was absorbed. Nonetheless, even following a low dose dermal application of 200 pmol (1 nmol/kg), the rate of absorption of 2,3,7,8-TCDD is still very slow (rate constant of 0.005 $hour^{-1}$). Dermal exposure of humans to 2,3,7,8-TCDD and related compounds usually occurs as a complex mixture of these contaminants in soil, oils or other mixtures which would be expected to alter absorption. Available data suggest that the dermal absorption of 2,3,7,8-TCDD depends on the formulation (vehicle or adsorbent) containing the toxicant. Although no data are available to directly evaluate human dermal absorption, the data available from *in vitro* and animal studies suggest slow dermal absorption of these compounds which is likely to be dependent on the vehicle or adsorbent containing the compounds and the duration of the contact.

The use of incineration as a means of solid and hazardous waste management results in the emission of contaminated particles that may contain TCDD and related compounds into the environment. Thus, exposure to TCDD and related compounds may result from inhalation of contaminated fly ash, dust and soil. Systemic effects

occur in animals after pulmonary exposure to TCDD, suggesting that transpulmonary absorption of TCDD does occur. Further results suggest that the transpulmonary absorption of 2,3,7,8-TCDD and 2,3,7,8-TBDD was similar to that observed following oral exposure. These limited data provide evidence of efficient transpulmonary absorption after intratracheal instillation in laboratory animals. No data from humans or primates are available to address this issue. However, these data provide support for the inference that efficient absorption will occur when particles containing dioxin and related compounds are inhaled by humans.

Once absorbed into blood, 2,3,7,8-TCDD and related compounds readily distribute to all organs. Tissue distribution within the first hour after exposure parallels blood levels and reflects physiological parameters such as blood flow to a given tissue and relative tissue size. There do not appear to be major species or strain differences in the tissue distribution of 2,3,7,8-TCDD and 2,3,7,8-TCDFin mammals, with the liver and adipose tissue being the primary disposition sites although human data to address this issue are quite limited. The tissue distribution of the coplanar PCBs and PBBs also appears to be similar to that of 2,3,7,8-TCDD and 2,3,7,8-TCDF based on evaluation in experimental animals.

Multiple studies suggest that distribution of this class of compounds to internal organs is likely to be dose dependent. At low doses in animal studies, adipose tissue serves as the major depot; at high doses, a major fraction is sequestered in the liver. The biochemical basis for this observation is under investigation. Induction of a binding protein has been hypothesized to play a major role.

As discussed above, levels of 2,3,7,8-TCDD averaging 5-10 pg/g lipid (ppt) have been reported for background populations. Sielken (1987) evaluated these data and concluded that the levels of 2,3,7,8-TCDD in human adipose are log-normally distributed and positively correlated with age. Among the observed U.S. background levels of 2,3,7,8-TCDD in human adipose tissue, more than 10% were >12 pg/g (ppt). Paired human serum and adipose tissue levels of 2,3,7,8-TCDD have been compared by Patterson et al. (1988) and Kahn et al. (1988). Both laboratories reported a high correlation between adipose tissue and serum 2,3,7,8-TCDD levels when the samples were adjusted for total lipid content. This correlation indicates that serum 2,3,7,8-TCDD provides a valid estimate of the 2,3,7,8-TCDD concentration in adipose tissue

under steady-state, low-dose conditions.

In a study of potentially heavily exposed Vietnam veterans, the Centers for Disease Control (MMWR, 1988) reported on an Air Force study of Ranch Hand veterans who were either herbicide loaders or herbicide specialists in Vietnam. The herbicide, 2,4,5,T (Agent Orange) that was used in Viet Nam was contaminated with a low percentage of 2,3,7,8-TCDD. The mean serum 2,3,7,8-TCDD levels of 147 Ranch Hand personnel was 49 pg/g (ppt) in 1987, based on total lipid-weight, while the mean serum level of the 49 controls was 5 pg/g (ppt). In addition, 79% of the Ranch Hand personnel and 2% of the controls had 2,3,7,8-TCDD levels ≥10 pg/g (ppt). The distribution of 2,3,7,8-TCDD levels in this phase of the Air Force health study indicates that , while Ranch Hand veterans have higher lifetime exposures than controls, only a small number of Ranch Hand personnel had unusually heavy 2,3,7,8-TCDD exposure. This report also estimated the half-life of 2,3,7,8-TCDD in humans to be ~7 years on the basis of 2,3,7,8-TCDD levels in serum samples taken in 1982 and 1987 from 36 of the Ranch Hand personnel who had 2,3,7,8-TCDD levels >10 pg/g (ppt) in 1987. Similar results were obtained by Kahn et al. (1988) who compared 2,3,7,8-TCDD levels in blood and adipose tissue of Agent Orange-exposed Vietnam veterans and matched controls. This study also examined moderately exposed Vietnam veterans who handled herbicides regularly while in Vietnam. Although this study can distinguish moderately exposed men from others, the data do not address the question of identifying persons whose exposures are relatively low and who constitute the bulk of the population, both military and civilian, who may have been exposed to greater than background levels of 2,3,7,8-TCDD.

Although early *in vivo* and *in vitro* investigations were unable to detect the metabolism of 2,3,7,8-TCDD, there is now evidence that a wide range of mammalian and aquatic species are capable of slowly biotransforming 2,3,7,8-TCDD to polar metabolites. Although metabolites of 2,3,7,8-TCDD have not been directly identified in humans, recent analytic data from feces samples from an individual in a self-dosing experiment suggests that humans can metabolize 2,3,7,8-TCDD (Wendling et al., 1990). The metabolism of 2,3,7,8-TCDD and related compounds is required for urinary and biliary elimination and therefore plays a major role in regulating the rate of excretion of these compounds. Direct intestinal excretion of parent compound is

another route for excretion of 2,3,7,8-TCDD and related compounds that is not regulated by metabolism.

Structure-activity studies of 2,3,7,8-TCDD and related compounds support the widely accepted principle that the parent compound is the active species, and the relative lack of biological activity of readily excreted monohydroxylated metabolites of 2,3,7,8-TCDD and 3,3'4,4'-TCB suggests that metabolism is a detoxification process necessary for the biliary and urinary excretion of these compounds. This concept has also been generally applied to 2,3,7,8-TCDD-related compounds, although data are lacking on the structure and toxicity of metabolites of other CDDs, BDDs, CDFs, BDFs, PCBs and PBBs. It is still possible,however quite unlikely, that low levels of unextractable and/or unidentified metabolites may contribute to one or more of the toxic responses of 2,3,7,8-TCDD and related compounds.

Due to the lipophilic nature of dioxins and related compounds, lactation can provide a mechanism for decreasing the body burden of these compounds in females. This elimination of 2,3,7,8-TCDD through mother's milk can result in high exposure levels in the infant, as discussed above. Since milk is highly absorbable, it would be likely that this source would provide 2,3,7,8-TCDD and related compounds in a form that is readily bioavailable to the nursing infant.

Physiologically-based pharmacokinetic (PB-PK) models have been developed for 2,3,7,8-TCDD in mice, rats and humans. PB-PK models incorporate known or estimated anatomical, physiological and physicochemical parameters to describe quantitatively the disposition of a chemical in a given species. PB-PK models can assist in the extrapolation of high-to-low dose kinetics within a species, estimating exposures by different routes of administration, calculating effective doses and extrapolating these values across species. These models are particularly important given the limited empirical data on individual dioxin-like congeners.

Kedderis (1994) has recently reviewed biologically-based models of dioxin pharmacokinetics. The early studies in rodents have recently been extended to describe protein induction and tissue distribution data in the mouse (Leung et al.,1990b) and rat (Leung et al., 1990a). Anderson and coworkers (Anderson et al.,1993) refined the model to relate protein induction to interactions between dioxin, the Ah receptor and DNA. This model also incorporated the concept of diffusion-

limited tissue distribution. The model described by Kedderis et al. (1993) for 2,3,7,8-tetrabromodibenzo-p-dioxin (TBDD) extended the use of PBPK models to the brominated congener of TCDD and designated the inducible cytochrome, CYP1A2, as the dioxin binding protein in the liver. Kohn et al. (1993) used similar approaches to describe tissue dosimetry of TCDD and additionally incorporated dioxin mediated effects on growth factors. Other models have been proposed recently to describe effects of TCDD on lipid metabolism (Roth et al.,1993). An empirical dose dependent model by Carrier (1991) related the varying fraction of the body burden of TCDD associated with the liver in humans to the total body burden of TCDD. Kedderis (1994) has suggested that, with our current understanding of the biologic determinants driving the hepatic sequestration of dioxin, this empirical description may now be interpreted in terms of a biologically-based model. The fact that the Carrier (1991) model deals with a relatively large data base of human exposures to dioxin and related compounds may facilitate predictions of human risk in terms of dosimetry as well as biologic response.

Our uncertainty in the validity of predictions from PB-PK models is primarily driven by the limited availability of congener and species-specific data that accurately describe the dose- and time-dependent disposition of 2,3,7,8-TCDD and related compounds. As additional data become available, particularly on the dose-dependent disposition of these compounds, more accurate models can be developed. In developing a suitable model in the human, it is also important to consider that the half-life estimate of 7.1 years for 2,3,7,8-TCDD was based on two serum values taken 5 years apart, with the assumption of a single compartment, and assuming a first-order elimination process (Pirkle et al., 1989). It is likely that the excretion of 2,3,7,8-TCDD in humans is more complex, involving several compartments, tissue-specific binding proteins and a continuous daily background exposure. Furthermore, changes in body weight and body composition should also be considered in developing PB-PK models for 2,3,7,8-TCDD and related compounds in humans.

It is known that some exposure occurs to the developing fetus through placental transfer of dioxin-like compounds in maternal blood via the placenta. In addition, exposure is likely to increase in the early post-natal period through intake of mother's milk containing dioxin-like compounds. Re-distribution of body burdens is likely to

occur with growth and development depending on relative intakes and changes in body fat content. Fasting, aging and disease are all thought to alter steady state levels of dioxin during life. These changes complicate standard pharmacokinetic models and present the possibility for transient but potentially important increases in blood or tissue levels of dioxin-like compounds during critical periods of development, growth and aging. Additional data on both pharmacokinetics and pharmacodynamics in relation to development and growth will be required to refine our perspectives on the importance of these issues in evaluating dioxin hazards and risks.

MECHANISMS OF DIOXIN ACTION

Knowledge of the mechanisms of dioxin action may facilitate the risk assessment process by imposing bounds upon the assumptions and models used to describe possible responses to exposure to dioxin. In this document, current knowledge of dioxin action has been reviewed, with emphasis on the contribution of the specific cellular receptor for dioxin and related compounds, the Ah receptor, to the mechanism. Other reviews referenced in Chapter 2 provide additional background on the subject.

The remarkable potency of TCDD in eliciting its toxic effects suggested the possible existence of a receptor for dioxin. Biochemical and genetic evidence implicate the TCDD-receptor in the biological responses to dioxin-like compounds. For example, studies of structure-activity relationships among congeners of TCDD reveal a correlation between a compound's specific binding affinity and its potency in eliciting biochemical responses, such as enzyme induction. Furthermore, inbred mouse strains in which TCDD binds with lower affinity to the receptor exhibit decreased sensitivity to dioxin's biological effects, such as thymic involution, cleft palate formation and hepatic porphyria.

Electrophoretic studies to evaluate the properties of specific proteins from inbred mouse strains reveal the existence of several forms of the TCDD-binding protein. These observations imply the existence of multiple alleles at the Ah locus in mice. The biochemical properties of the different forms of the Ah receptor remain to be described. In particular, the extent to which the different receptor forms affect the sensitivity to TCDD is not known.

Human cells contain an intracellular protein whose properties resemble those of the Ah receptor in animals. Binding studies and hydrodynamic analyses have identified an Ah receptor-like protein(s) in a variety of human tissues. Functional Ah receptors have been found in many human tissues including lymphocytes, liver, lung, and placenta. By analogy with the existence of multiple receptor forms in mice, it is reasonable to anticipate that the human population will also be polymorphic with respect to Ah receptor structure and function. Therefore, it is also reasonable to expect humans to differ from one another in their susceptibilities to TCDD. The binding and hydrodynamic properties of the Ah receptor differ relatively little across species and tissues yet responses vary widely; it is difficult to, therefore, account for the diversity of TCDD's biological effects by characteristics of the receptor alone.

The Ah receptor exists in cells as a complex of proteins. Upon binding of dioxin-like compounds (the "ligands" for this receptor), the Ah receptor dissociates from the complex and interacts with a protein designated "Aryl hydrocarbon Receptor Nuclear Transferase," or Arnt, forming a heterodimer (Hoffman, et al.,1991). Although originally thought to participate in transfer of the dioxin-bound Ah receptor to the nucleus, more recent studies suggest that Arnt is a nuclear protein that interacts with the liganded Ah receptor to form a heteromeric, DNA-binding complex that can activate gene transcription. Neither the ligand receptor nor Arnt exhibit substantial DNA-binding in the absence of the other; the presence of both proteins is required to generate a specific DNA-binding species and to activate the expression of specific genes. Both the Ah receptor and Arnt belong to a class of transcription factors which function as heterodimers and which contribute to the control of numerous genes (Kadesch, 1993). By analogy to the multiple alleles that exist for the ligand-binding component of the Ah receptor, it is reasonable to expect that the DNA-binding component of the receptor will also exhibit polymorphisms and exist in multiple forms. In principle, such a situation raises the possibility that different functional forms of the receptor complex can exist, created by the association of receptor subunits in different combinations. Such combinatorial diversity could contribute to the variety of biological responses produced by TCDD.

The evidence to date implies that the Ah receptor participates in every biological response to TCDD. A simplified diagram of this hypothesis is presented in

Figure 9-2. This hypothesis predicts that TCDD will be found to activate the transcription of other genes via a receptor- and enhancer-dependent mechanism analogous to that described for the cytochrome P4501A1 (CYP1A1) gene. CYP1A1 is one of a family of proteins involved in the activation and detoxification of both endogenous and exogenous chemicals. Preliminary data from a number of laboratories suggest that this is the case. For example, TCDD induces the expression of the cytochrome P4501A2 gene, the glutathione S-transferase Ya subunit gene, an aldehyde dehydrogenase gene, and a quinone reductase gene; in some cases, induction is known to occur at the transcriptional level, to be Ah receptor-dependent, and to involve a genomic regulatory element(s) analogous to that found upstream of the CYP1A1 gene. In addition, recent observations suggest that, in human keratinocytes, TCDD activates the transcription of plasminogen activator inhibitor-2 and interleukin-1ß, as well as other genes (Sutter et al., 1991). Recent data describe the complete cDNA sequence of the mRNA of one of these genes as a new gene subfamily of cytochrome P450 (Sutter et al., 1994). The mechanism by which dioxin activates the expression of these genes is currently unknown. For dioxin-responsive genes other than CYP1A1, and especially for those genes that respond in tissue-specific fashion, the presence of the receptor/enhancer system may not be sufficient for dioxin action, and other, tissue-specific regulatory components may play a dominant role in governing the response to TCDD. Thus, future research may reveal the existence of additional positive or negative gene regulatory components that can influence the response of the cell to TCDD.

Recent observations have suggested the presence of Ah-mediated changes in phosphotyrosyl proteins following TCDD treatment. These changes may be due to increased phosphorylation of preexisting proteins, increased synthesis of proteins that are phosphorylated, decreased phosphatase activity or a combination of all three mechanisms (DeVito et al., 1994). Protein tyrosine phosphorylation is known to play a critical role in signal transduction and regulation of cellular events, such as entry into the cell cycle. Changes in protein tyrosine phosphorylation following TCDD treatment may indicate additional changes in signal transduction pathways which alone or in combination with trancriptional alterations may result in altered cellular differentiation or proliferation. Further research will be required to test this hypothesis and further

elucidate the interactions among these regulatory processes.

Compensatory changes, which occur in response to TCDD's primary effects, can complicate the analysis of dioxin action in intact animals. For example, TCDD can produce changes in the levels of steroid hormones, peptide growth factors and/or their cognate cellular receptors. In turn, such alterations have the potential to produce a series of subsequent biological effects, which are not directly mediated by the Ah receptor. Furthermore, the hormonal status of an animal appears to influence its susceptibility to the hepatocarcinogenic effects of TCDD (Lucier et al., 1991). Likewise, exposure to other chemicals can alter the developmental toxicity of TCDD (Couture et al., 1990). Therefore, in some cases, TCDD may act in combination with other chemicals to produce its biological effects. Such phenomena increase the difficulty of analyzing dioxin action in intact animals and increase the complexity of risk assessment, given that humans are routinely exposed to a wide variety of chemicals.

The fact that TCDD may induce a cascade of biochemical changes in the intact animal raises the possibility that dioxin might produce a response such as cancer by mechanisms that differ among tissues. For example, in one case, TCDD might activate a gene(s) that is directly involved in tissue proliferation. In a second case, TCDD-induced changes in hormone metabolism may lead to tissue proliferation secondary to increased secretion of a trophic hormone. In a third case, TCDD-induced changes in hormone receptors for growth factors or hormones may alter the sensitivity of a tissue to proliferative stimuli. In a fourth case, TCDD-induced toxicity may lead to tissue death, followed by regenerative proliferation. Thus, while this reassessment has identified a number of hypothetical mechanisms for cancer induction by TCDD, there remains considerable uncertainty about which mechanisms occur, with what levels of sensitivity, and in which species.actually occurs, whether they would exhibit similar sensitivities to TCDD, or whether they would occur in all animal species exposed to the dioxin. Advances in knowledge regarding the role of such activities in dioxin toxicity will facilitate the development of more definitive biologically-based models of dioxin action.

Under some circumstances, exposure to TCDD elicits beneficial effects. For example, TCDD can protect against the carcinogenic effects of polycyclic aromatic hydrocarbons in mouse skin; this may reflect the induction of detoxifying enzymes by

dioxin (Cohen et al., 1979; DiGiovanni et al., 1980). In other situations, TCDD-induced changes in hormone metabolism may alter the growth of hormone-dependent tumor cells, producing a potential anti-carcinogenic effect (Spink et al., 1990). There is considerable uncertainty about the magnitude and importance of these effects in relation to both dose and response characteristics of dioxins in various species. Nonetheless, these (and perhaps other) potentially beneficial effects of TCDD complicate the risk assessment process for dioxin.

A substantial body of biochemical and genetic evidence indicates that the Ah receptor mediates the biological effects of TCDD. This evidence implies that a response to dioxin requires the formation of ligand-receptor complexes. TCDD-receptor binding appears to obey the law of mass action and, therefore, depends upon (1) the concentration of ligand in the target cell; (2) the concentration of receptor in the target cell; and (3) the binding affinity of the ligand for the receptor. In principle, some TCDD-receptor complexes will form even at very low levels of dioxin exposure. However, in practice, at some finite concentration of TCDD, the formation of TCDD-receptor complexes will be insufficient to elicit detectable effects. Furthermore, biological events subsequent to TCDD-receptor binding may or may not exhibit a linear response to dioxin. In some experimental systems with no direct relationship to dioxin-induced responses, the induction of gene transcription appears to require a threshold concentration of transcription factor(s) (Fiering et al., 1990). However, recent studies in several laboratories have indicated no evidence of a threshold for relatively simple responses to dioxin-like compounds such as CYP1A1 induction and others. Further information will be required to determine if other responses to dioxin-like compounds requiring gene transcription will also demonstrate low-dose linear behavior.

While much of our understanding of TCDD impacts on genetic activity is derived from studies on liver, studies of other tissues (e.g., skin, thymus) are likely to reveal additional TCDD-responsive genes, which exhibit tissue-specific expression (Sutter et al., 1991). Analyses of the mechanism of dioxin action in such systems appear likely to reveal additional factors that influence the susceptibility of a particular tissue to TCDD. In addition, studies of other TCDD-inducible genes, such as glutathione S-transferase, quinone reductase, and aldehyde dehydrogenase, may

reveal whether differences in enhancer structure, receptor-enhancer interactions, or promoter structure affect the responsiveness of the target gene to TCDD (Whitlock, 1990).

Further analyses of dioxin action may provide more insight into the mechanisms by which TCDD and related compounds produce immunological effects, reproductive and/or developmental effects or cancer, effects which are of particular public health concern. A major challenge for the future will be the establishment of experimental systems in which such complex biological phenomena are amenable to study at the molecular level.

TOXIC EFFECTS OF DIOXIN

A.) General Comments

It is clear from the evaluation of the toxicologic literature that dioxin and related compounds have the ability to produce a plethora of responses in animals and, presumably, in humans (Table 9-2). Relatively few have been demonstrated to occur in humans because of lack of knowledge regarding levels of dioxin exposure in the general population, few comprehensive studies of more highly exposed populations, the inherent insensitivity of epidemiologic studies, and the inability to rule out confounding exposures. Evaluation of hazard and risk for dioxin and related compounds must rely on a weight of the evidence approach in which all available data are brought to bear on these issues. This often necessitates cross-species extrapolation of effects.

The reliability of using animal data to estimate human hazard and risk has often been questioned for this class of compounds. Although human data are limited, evidence suggests that animal models are appropriate for estimating human risk if all available data are considered. Humans have a fully functional Ah receptor and both *in vivo* and *in vitro* studies demonstrate comparability of biochemical responses in humans and animals. When comparing species and strains for their responses to these compounds, a wide range of sensitivity to TCDD-induced toxicities has been noted. Qualitatively speaking, however, almost every response can be produced in every species if the appropriate dose is administered. Although outliers, i.e. species which are either very sensitive or refractory, can be identified for a particular response,

Table 9-2. Effects of TCDD and Related Compounds in Different Animal Species

Effect	Human	Monkey	Guinea Pig	Rat	Mouse	Hamster	Cow	Rabbit	Chicken	Fish
Presence of AhR	+	+	+	+	+	+	+	+	+	+
Binding of TCDD: AhR Complex to the DRE (enhancer)	+		+	+	+	+	+	+	+	+
Enzyme induction	+		+	+	+	+		+	+	+
Acute lethality		+	+	+	+	+		+	+	+
Wasting syndrome		+	+	+	+	+		+		
Terato-genesis/fetal toxicity, mortality	+/-	+	+	+	+	+		+	+	+
Endocrine effects	+/-	+		+	+					
Immuno-toxicity	+/-	+	+	+	+	+	+		+	
Carcino-genicity	+/-			+	+	+				+
Chlor-acnogenic effects	+	+			+			+		

+ = observed.
+/- = observed to limited extent, or +/- results.
0 = not observed.

Table 9-2. (continued)

Effect	Human	Monkey	Guinea Pig	Rat	Mouse	Hamster	Cow	Rabbit	Chicken	Fish
Porphyria		0	0	+	+	0			+	
Hepato-toxicity		+	+/-	+	+	+/-	+	+	+	
Edema		+	0	0	+	+			+	+
Testicular atrophy		+	+	+	+					
Bone marrow hypoplasia		+	+		+/-				+	

+ = observed.
+/- = observed to limited extent, or +/- results.
0 = not observed.

no species is consistently sensitive or refractory for all effects. In addition, the majority of species cluster in sensitivity for a given effect within approximately one order of magnitude (factor of 10). Therefore, despite a range of sensitivities across species, it is reasonable to assume that humans will not be refractory to all effects nor that they will be as sensitive as the most sensitive responder for each effect. Humans are likely, because of interindividual variability, which is greater than that found in individual species of laboratory animals, to show a wide range of sensitivities for various dioxin-induced toxicities. For purposes of the current assessment, therefore, unless there are data to identify a particular species as being representative of humans for a particular effect, average humans can be reasonably assumed to be of average sensitivity for various effects, recognizing that individuals in the population might vary widely in their sensitivity to individual effects. The uncertainty introduced by this assumption i.e. that, on average, humans will respond as do average animal models for individual effects of exposure to dioxin-like compounds and that an unknown range of variability exists in the human population for individual effects, should be carefully considered as results of this characterization are applied to individuals or specific subpopulations.

B.) Chloracne

Chloracne and associated dermatologic changes are widely recognized responses to TCDD and other dioxin-like compounds in humans. Chloracne is a severe acne-like condition which develops within months of first exposure to high levels of dioxin. For many individuals, the condition disappears after discontinuation of exposure, despite serum levels of dioxin in the thousands of parts per trillion; for others, it may remain for many years. The duration of persistent chloracne is on the order of 25 years although cases of chloracne persisting over 40 years have been noted. There are very little human data from which to determine definitively the doses at which chloracne is likely to occur. Data from occupational studies suggest that persistent chloracne is more often associated with exposures of high intensity, for long duration and commencing at an early age. Acute exposures, or chronic lower level exposures, if resulting in chloracne, have generally resulted in a condition which resolves itself in a matter of months to a few years. Details of cloracnegenic response in occupationally-exposed humans are described in detail in Chapter 7 of the Health Assessment document

Induction of chloracne in humans after exposure to dioxin and related compounds is supported by studies in laboratory animals. Rabbits, monkeys and hairless mice have all proved useful in investigating this response. In addition, cellular systems provide a research tool in elucidating the chloracne response at the cellular level. Keratinocytes, the principal cell type in the epidermis, have been used as an *in vitro* model for studies of TCDD-induced hyperkeratosis, a feature of chloracne, in human- and animal-derived cell cultures. The response in these systems is analogous to the hyperkeratinization observed *in vivo* as a part of chloracne.

There is little doubt that chloracne is a human condition often attributable to exposure to dioxin and related compounds. The specific risk factors associated with this response are still obscure. Recognition of chloracne has been associated with high level exposure to these compounds, and as such may represent a biomarker of exposure. Because of the wide variability of the chloracnegenic response in humans and its varied persistence, however, the absence of chloracne is not a reliable indicator of low exposure to dioxin and related compounds.

C.) Carcinogenicity

Since the last EPA review of the human data base relating to the carcinogenicity of TCDD and related compounds in 1988, several new followup mortality studies have been completed. Among the most important of these are a study of 5,172 workers by Fingerhut et al. (1991), a study with 1,583 workers by Manz et al. (1991), a smaller study of 247 workers by Zober et al. (1990), and a study of over 18,000 workers by Saracci et al. (1991). Although uncertainty remains in interpreting these studies because not all potential confounders have been ruled out and coincident exposures to other carcinogens is likely, all provide support for an association between exposure to dioxin and related compounds and increased cancer mortality. With the exception of the study by Saracci et al. (1991), these studies have some exposure information that permits an assessment of dose response. These data have in fact served as the basis for fitting the additive and multiplicative risk models in Chapter 8. In addition, more limited results have been presented recently on the Seveso cohort (Bertazzi et al., 1993) and on women exposed to chlorophenoxy herbicides, chlorophenols and dioxins (Kogevinas et al., 1993). While these two

studies have methodologic short-comings which are described in Chapter 7, they provide findings, particularly for exposure to women, which warrant additional follow-up.

While the data base from epidemiologic studies remains controversial, it is the view of this reassessment that this body of evidence support the laboratory data indicating that TCDD probably increases cancer mortality of several types. Although not all confounders were ruled out, positive associations between surrogates of dioxin exposure, either occupational or proximity to a known source combined with some information on body burden, and cancer have been reported. These data alone suggest a role for dioxin exposure to contribute to a carcinogenic response but do not confirm a causal relationship between exposure to dioxin and increased cancer incidence. Available human studies alone cannot demonstrate whether a cause and effect relationship between dioxin exposure and increased incidence of cancer exists. Therefore, evaluation of cancer hazard in humans must include an evaluation of all of the available animal and *in vitro* data as well as the data from exposed human populations. The 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 epidemiology studies, representing the range of opinion that still exists on the interpretation of the cancer epidemiology studies.

Many of the earlier epidemiological studies that suggested an association with soft tissue sarcoma (STS) were criticized for a variety of reasons. Nonetheless, the incidence of soft tissue sarcoma is elevated in several of the recent studies, supporting the findings from previous studies. The fact that similar results were obtained in independent studies of differing design and evaluating populations exposed to dioxin-like compounds under varying conditions, along with the rarity of this tumor type, weighs in favor of a consistent and real association. On the other hand, arguments regarding selection bias, differential exposure misclassification, confounding, and

chance in each individual study have been presented in the scientific literature which increase uncertainty around this association. In addition, excess respiratory cancer was noted by Fingerhut, Zober, and Manz. These results are also supported by observations subsequent to the Japanese rice oil poisoning accident where exposure to PCDFs and PCBs occurred. Again, while smoking as a confounder can not be totally eliminated as a potential explanation of these results, analyses conducted to date suggest that smoking is not likely to explain the entire increase in lung cancer. The question of multiple confounders, such as exposure to asbestos and other chemicals, in addition to smoking has not been entirely ruled out and must be considered as potentially adding to the observed increases. Although increases of cancer at other sites (e.g. non-Hodgkin's lymphoma, stomach cancer) have been reported, the data for an association with exposure to dioxin-like chemicals is less compelling. What emerges from an analysis of the epidemiology data is a view of dioxin-like compounds as potentially multi-site carcinogens in more highly exposed human populationsthat have been studied, consisting primarily of men. There are currently very few data for xposed women and children. Although uncertainty in this view remains, the cancer findings are generally consistent with results from studies of laboratory animals, and appears to be plausable given what is known about mechanisms of dioxin action.

While both past and more recent human studies have focused on males, there are some, limited data suggesting responses in females. Because both laboratory animal data and mechanistic inferences suggest that males and females may respond differently to dioxin-like activity, further data will be needed to address this question.

An extensive data base on the carcinogenicity of dioxin and related compounds in laboratory studies exists and is described in detail in 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. All studies have produced positive results, leading to the 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 (MTD). Since this issue was last reviewed by the Agency in 1988, TCDD has been shown to be a carcinogen in hamsters, which are relatively resistant to the lethal effects of TCDD. Recent 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 hexachlorodibenzodioxin (HCDDs) which produced liver tumors in both sexes of rats and mice (NTP, 1980), the more highly chlorinated CDDs and CDFs have not been studied in long-term animal cancer bioassays. However, it is generally recognized that these compounds bioaccumulate and exhibit toxicities similar to TCDD and are, therefore, also likely to be carcinogens (EPA Science Advisory Board, 1989).

In addition to the demonstration of TCDD as a complete carcinogen in long term cancer bioassays, a number of dioxin-like PCDDs and PCDFs, as well as several PCBs, have also been demonstrated to be tumor promoters in two stage (initiation-promotion) protocols in rodent liver and skin. In addition, recent data have demonstrated the ability of TCDD to neoplastically transform immortalized human cells in culture at very low concentrations of TCDD. While dioxin and related compounds are not generally considered to be "genotoxic" in traditional terms, both empirical data and the results of modeling efforts suggest that they may be functioning indirectly to produce irreversible genetic changes in exposed cells. All of these data add substantially to the weight of the evidence that dioxin and related compounds are likely to possess carcinogenic potential in humans, at least under some circumstances.

Despite the relatively large number of bioassays on TCDD, the study of Kociba et al. (1978) and those of the NTP (1982), because of their multiple dose groups and large dose range, continue to be the focus of additional review. Sauer (1990) re-evaluated 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). While this finding has little impact on the question of carcinogenic hazard, since TCDD induced tumors in multiple sites in this study, it does 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 chapter.

One of the more interesting findings in the Kociba bioassay was reduced tumor incidences of the pituitary, uterus, mammary gland, pancreas and adrenals. These findings, coupled with the sex specificity of the TCDD-induced liver tumors in rats

emphasize that the carcinogenic actions of TCDD involve a complex interaction of hormonal factors. Moreover, it is hypothesized that cell-specific factors modulate TCDD/hormone actions relevant to cancer. The findings of reduced tumor incidence in certain tissues suggest that dioxin exposure may be exerting an anti-carcinogenic effect under certain circumstances or in certain tissues. The complex interplay between dioxin and hormones in terms of both carcinogenic and anti-carcinogenic responses will continue to a be matter of hypothesis until such data to address these issues are obtained.

D.) Reproductive and Developmental Effects

The potential for dioxins and related compounds to cause reproductive and developmental toxicity in animals has been recognized for many years and the data base regarding these effects is analyzed in Chapter 5. Recent laboratory studies have suggested that altered development may be among the most sensitive TCDD endpoints in laboratory animal systems. Although the discussion of these effects is divided into developmental toxicity and male and female reproductive toxicity, it is important to recognize the interrelatedness of developmental and reproductive events at all levels of biological complexity. For example, effects of TCDD on circulating levels of sex hormones and/or on responsiveness to sex hormones in laboratory animals or humans may be translated into reproductive dysfunction if exposure occurs in adulthood as well as abnormal development if exposure occurs perinatally. Likewise, even though organ structure and growth are considered separate manifestations in developmental toxicity that are associated with perinatal exposure to TCDD in laboratory animals, the development of an organ in all biological systems is dependent on normal growth processes and inhibiting prenatal growth can significantly disrupt the structural integrity of an organ system.

In the current data base, developmental toxicity endpoints are observed at lower TCDD exposure levels than are endpoints of male and female reproductive toxicity in a number of animal systems. The lowest effective TCDD egg burden for causing developmental toxicity in fish and birds and the lowest effective maternal TCDD body burden for producing a wide range of developmental responses in mammals are summarized in Chapter 5. Of particular interest to the risk assessment

process is the fact that a wide variety of developmental events, crossing three vertebrate classes and several species within each class, can be perturbed, suggesting that dioxin has the potential to disrupt a large number of critical developmental events at specific developmental stages. Not only can these changes lead to increases in embryo/fetal mortality, but they can disrupt organ system structure and irreversibly impair organ function.

Since 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 humans in the population will respond with adverse impacts on development or reproductive function. Data analyzed in Chapter 5 and Chapter 7 suggest, however, that adverse effects may be occuring at levels lower than originally thought to represent "no observed adverse effect levels." Related effects in human infants exposed to a complex mixture of PCBs, CDFs and PCQs in the Yusho and Yu-Cheng poisoning episodes were probably caused by the combined exposure to those PCB and CDF congeners that are Ah receptor agonists. Similarity of the effects observed in human infants perinatally exposed to this complex mixture, with those reported in adult monkeys exposed only to TCDD, increases the probability of at least some of the effects in the Yusho and Yu-Cheng children being due to the TCDD-like congeners in the contaminated rice oil ingested by the mothers of these children. Most significant is a clustering of effects in organs derived from the ectodermal germ layer, a syndrome referred to as ectodermal dysplasia. Included in this syndrome are effects on the skin, nails, and meibomian glands that occur in both adult monkeys exposed to TCDD and in Yusho and Yu-Cheng infants exposed transplacentally to PCB, CDF and PCQ contaminated rice oils. In addition, accelerated tooth eruption has been reported both in human infants affected by the Yusho and Yu-Cheng exposures and in neonatal mice exposed to TCDD. Yu-Cheng children exposed to PCB, CDF and PCQ contaminated rice oil transplacentally have also exhibited developmental and psychomotor delay during developmental and cognitive tests. Monkeys perinatally exposed to TCDD are also affected by a deficit in cognitive function. The concept that the ectodermal dysplasia syndrome in Yusho and Yu-Cheng infants may be caused by the combination of PCB and CDF congeners in the rice oil that are Ah receptor agonists, but are less potent

than TCDD, is consistent with structure activity results for various developmental endpoints in different species of fish, birds, and mammals.

In mammals, postnatal functional alterations involving learning behavior and the developing reproductive system appear to be the developmental events most sensitive to perinatal dioxin exposure. The developing immune system may also be highly sensitive. Alterations in structural endpoints and diminished prenatal viability and growth begin to predominate at maternal TCDD body burdens and/or daily TCDD doses during gestation that are above 100 ng/kg in virtually every species tested. These doses of TCDD are not maternally toxic. Higher dose levels can be demonstrated to result in prenatal mortality. A general finding in fish, bird, and mammalian species is that the embryo or fetus is more sensitive to TCDD-induced mortality than the adult. Thus, the timing of TCDD exposure during the life history of an animal can greatly influence its susceptibility to overt dioxin toxicity.

With respect to male and female reproductive endpoints, there are clear effects following dioxin exposure of the adult animal. Such reproductive effects generally occur at TCDD body burdens that are higher than those required to cause the more sensitive developmental endpoints. For example, TCDD exposure of the adult male rodent causes reduced testis and accessory sex organ weights, abnormal testis structure, decreased spermatogenesis, reduced fertility, decreased testicular testosterone synthesis, reduced plasma androgen concentrations, and altered regulation of pituitary LH secretion. However, in laboratory animal studies, these effects are detectable only at TCDD exposure levels that are overtly toxic to the animal. In the more limited studies focusing on female reproduction, the primary effects include decreased fertility, inability to maintain pregnancy, and in the rat, decreased litter size. Signs of ovarian dysfunction and alterations in hormone levels have also been reported.

Exposure of female mice and rats to TCDD has an antiestrogenic effect. The dose of TCDD required to produce this response is generally higher than that needed to cause the most sensitive signs of developmental toxicity in these species. More specifically, hydronephrosis and cleft palate in mice and reductions in spermatogenesis in rats occur at maternal doses of TCDD which are far less than those needed to exert a demonstrable antiestrogenic effect when adult female mice

and rats are exposed to dioxin. The precise mechanism of TCDD's antiestrogenic effect is not fully understood. It may be caused by both a decrease in available estrogen receptor number and/or by an increase in cytochrome P-4501A-mediated estrogen metabolism within the target cell.

These studies indicate that while there is variability between species in the profile of developmental responses elicited by TCDD, essentially all dioxin-like PCB, CDD, and CDF congeners that have Ah receptor affinity and intrinsic activity produce the same pattern of developmental effects within a given vertebrate species if a sufficiently high dose of the congener is given. Data to support these conclusions regarding reproductive and developmental hazards of dioxin and related compounds continue to accumulate, but the weight of the evidence is still a subject of much scientific debate.

E.) Immunotoxicity

Concern over the potential toxic effects of chemicals on the immune system arises from the critical role that the immune system plays in maintaining health. It is well recognized that suppressed immunological function can result in increased incidence and severity of infectious diseases as well as some types of cancer. Conversely, the inappropriate enhancement of immune function or the generation of misdirected immune responses can precipitate or exacerbate the development of allergic and autoimmune diseases. Thus, suppression as well as enhancement of immune function are considered to represent potential immunotoxic effects of chemicals.

Extensive evidence has accumulated over the past 20 years to demonstrate that the immune system is a target for toxicity of TCDD and structurally related compounds, including PCDDs,PCDFs,PCBs, and PBBs. This evidence is described in detail in Chapter 4. The evidence has derived from numerous studies in various animal species, primarily rodents, but also guinea pigs, rabbits, monkeys, marmosets, and cattle. Epidemiological studies also provide some evidence for the immunotoxicity of HAH in humans. In animal studies, relatively high doses of HAH produce lymphoid tissue depletion, except in the thymus where cellular depletion occurs at lower doses. Alterations in specific immune effector functions and increased susceptibility to

infectious disease have been identified at doses of TCDD well below those which cause lymphoid tissue depletion. 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. In addition, in parallel with increased understanding of the cellular and molecular mechanisms involved in immunity, studies on TCDD are beginning to establish biochemical and molecular mechanisms of TCDD immunotoxicity.

The ability of an animal to resist and/or control viral, bacterial, parasitic, and neoplastic diseases is determined by both nonspecific and specific immunological functions. Decreased functional activity in any immunological compartment may result in increased susceptibility to infectious and neoplastic diseases. In terms of risk assessment, host resistance is often accorded the "bottom line" in terms of relevant immunotoxic endpoints. Animal host resistance models that mimic human disease are available and have been used to assess the effect of TCDD on altered host resistance. Results from host resistance studies provide evidence that exposure to TCDD results in increased susceptibility to bacterial, viral, parasitic, and neoplastic disease. These effects are observed at relatively low doses and likely result from TCDD-induced suppression of immunological function. The specific immunological functions targeted by TCDD in each of the host resistance models remain to be fully defined.

The difficulty in demonstrating consistent, direct effects of TCDD in vitro on lymphocytes, the dependence of those effects on serum components, and the requirement for high concentrations of TCDD are all consistent with the potential for an indirect mechanism of TCDD on the immune system. One potentially important indirect mechanism is via effects on the endocrine system. Several endocrine hormones have been shown to regulate immune responses, including glucocorticoids, sex steroids, thyroxine, growth hormone, and prolactin. Importantly, TCDD and other related compounds have been shown to alter the activity of all of these hormones.

It is important to consider, however, that if an acute exposure to TCDD even temporarily raises the TCDD body burden at the time when an immune response is initiated, there may be a risk of adverse impacts even though the total body burden

may indicate a relatively low average TCDD level. Furthermore, since TCDD alters the normal differentiation of immune system cells, the human embryo may be very susceptible to long-term impairment of immune function from in utero effects of TCDD on developing immune tissue. There are currently no data to directly support this hypothesis. Concern arises as a consequence of inferences derived from an understanding of dioxin action and observations in humans and laboratory animals.

F.) Other Effects

A number of other effects of dioxin and related compounds have been discussed in some detail throughout the chapters in this assessment. While they serve to illustrate the wide range of effects produced by this class of compounds, some may be specific to the species in which they are measured and may have limited relevence to the human situation. On the other hand, they may be indicative of the fundamental level at which dioxin produces its biological impact and may represent a continuum of response expected from these fundamental changes. While all may not be adverse effects (some may be adaptive and of neutral consequence, and some may be beneficial), several effects have been noted in human studies or in primates which deserve special mention:

Circulating Reproductive Hormones

Two cross-sectional epidemiologic studies have detected an association between levels of reproductive hormones and exposure to TCDD. Decreased testosterone levels were detected in two of the three studies where testosterone was evaluated and luteinizing hormone (LH) was increased in one of the two studies evaluating that endpoint. Animal data are available to support the plausability of these findings. The mechanism(s) responsible for this effect are largely unknown but changes in receptor level or function, and hormone metabolism and homeostasis need to be investigated. If these data continue to hold up in future observations, their clinical significance will need to be further evaluated. Follow-up studies are currently underway.

Diabetes and Fasting Serum Glucose Levels

Epidemiologic evidence has been presented to suggest an increased risk of diabetes and for an elevated prevalence of abnormal fasting serum glucose levels with dioxin exposure. Three studies found that individuals with elevated serum levels

of TCDD had a slight but statistically significant or borderline significant increased risk for developing diabetes or having elevated fasting serum glucose. There are virtually no animal data to corroborate these finding although some data have indicated effects of TCDD on glucose metabolism. While the findings of a greater prevalence of elevated fasting glucose may presage the development of diabetes, in the NIOSH study of chemical workers, the traditional risk factors for diabetes (age, body mass index or weight, and family history of diabetes) appear substantially more influential than TCDD exposure in the development of the disease.

Enzyme induction - One of the best characterized effects of exposure to dioxin-like compounds is the induction of cytochrome P-450 1A1 (CYP1A1). CYP1A1 is one of a family of proteins involved in the activation and detoxification of both endogenous and exogenous chemicals. Dioxin also increases the activity of a number of other enzymes involved in biotransformation reactions. Increased activity of these enzymes has been implicated mechanistically in the toxic responses seen in animals in response to dioxin-like compounds. For example, it has been hypothesized that increases in UDP-glucuronyltransferases, which metabolize thyroxine, may lead indirectly to increased Thyroid Stimulating Hormone (TSH) synthesis by the pituitary and subsequent hyperplastic and hypertrophic responses by the thyroid. There is speculation that such prolonged stimulation may lead to the thyroid tumors seen in both rats and mice exposed to TCDD. Therefore, while changes in enzyme activity in response to dioxin and related compounds may result in detoxification of certain chemicals, examples exist in experimental animals of changed metabolism leading directly or indirectly to adverse effects, some as severe as cancer. Data to confirm this effect of dioxin and related compounds in humans are not available.

Gamma glutymyl transferase (GGT) activity - GGT is one of the many hepatic enzymes that are measured in humans to evaluate liver toxicity. Of these, GGT is the only hepatic enzyme found in a number of human studies to be chronically elevated in adults exposed to high levels of TCDD. The consistency of the findings in a number of studies suggests that the finding may reflect a true effect of exposure but for which the clinical significance is unclear. Long term, pathologic 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. There are few

animal data to support these findings.

Endometriosis

Endometriosis is a serious disorder of the female reproductive system which is of unknown etiology and a major cause of infertility in women. The prevalence of endometriosis in the general population is unknown but is estimated to be 10% among reproductive-age women, indicating that endometriosis may be present in 6.6 million women in the U.S. (Wheeler, 1992). Recent studies have determined that chronic exposure to TCDD increases the risk of endometriosis in rhesus monkeys (Rier et al,1993). The severity of the disease was dependent on the dose given. Previous work has described an association between endometriosis in rhesus monkeys and exposure to polychlorobiphenyl (PCB) compounds (Campbell et al, 1985). Additional studies are underway which may confirm these observations in rhesus monkeys and studies are planned to evaluate women exposed at Seveso for any correlation between dioxin body burden and incidence or severity of endometriosis. Further evaluation of this important health endpoint awaits reports from these studies.

DOSE-RESPONSE CONSIDERATIONS

The current efforts to evaluate the risks of dioxin and related compounds have focussed on the understanding of the biological basis of response as well as evaluation of the weight of the empirical observations on inferences regarding hazard and risk. Previous sections have discussed the relationship of binding of this class of compounds to a specific receptor and subsequent events. It is generally accepted that all well-studied responses to dioxin appear to be mediated by receptor binding. This situation is not unlike the signal transduction pathways which have been described for hormone action, particularly exemplified by the well studied family of steroid hormones, although the dioxin receptor does not belong to the steroid receptor family.

As with the steroid hormones, the earliest events in the biochemical signal transduction process are likely to be linearly related to ligand concentration. The fact that much of the biological activity of this class of compounds follows the rank order of binding affinity of the congeners to the Ah-receptor supports the concept that these earliest steps play a determining role in the probability that later responses will occur. This does not suggest that a simple proportional relationship between receptor

binding and biological response can explain the diversity of biological responses described for dioxin and related compounds. It is likely that differences in response will be due to tissue and cell-specific factors that modulate the qualitative relationship between receptor binding, or more precisely, occupancy and response. It is expected that there may be markedly different dose response relationships for different effects of dioxin depending on the respective roles of modulating activities. Coordinated biological responses, such as TCDD-mediated increases in cell proliferation, likely involve other cellular factors and hormone systems. This means that the dose-response for relatively simple sequelae of the early binding events such as cytochrome (CYP1A1) induction may not accurately predict dose-response relationships for more complex responses such as cancer. Much additional knowledge will be required before we can more accurately predict these complex dose-response relationships.

Development of biologically-based dose response models for dioxin and related compounds as a part of this reassessment has led to considerable and valuable insights regarding both mechanisms of dioxin action and dose response relationships for dioxin effects. These are described in some detail in Chapter 8. These efforts have not resulted in an alternative model to replace the linearized multistage (LMS) procedure for estimating cancer potency or the uncertainty factor approach for estimating levels below which non-cancer effects are not likely to occur. These efforts have, however, provided additional perspectives on these traditional methods and have provided a biological-based rationale for what had been primarily statistical approaches. The development of models allows for an iterative process of data development and hypothesis testing. These efforts will result in incorporation of more of the available biological data into models to predict human risk at low increments of exposure.

Table 9-2 summarizes estimated body burdens and effect levels for a variety of species, including the low observed effect levels (LOELs) for some of the more sensitive indicators of biological response induced by dioxin and related compounds. Important assumptions used in deriving these values are included as part of this Table. It is particularly important to note that the estimated body burdens associated with several of these doses are quite low relative to background body burdens in the

general human population. The implications of this observation will be discussed later in this chapter. **[Note to reviewers: This Table will be modified in the external review draft to make it easier to understand. It is included in its entirety here for your comment.]**

Comparison of recent cancer modeling efforts using rodent data with the LMS procedure show no compelling arguments for use of alternative slope factors to estimate upper bounds on potential human cancer risk. All of these methods, when incorporating data from the most recent pathology re-evaluation of the Kociba rat study, result in upper bound estimates of a one in a million (10^{-6}) risk specific dose of approximately .01 pg TEQ/kg bw/day and an upper bound unit risk estimate of approximately 1×10^{-4} per pg/kg bw/day. Analysis of human data from several epidemiology studies yield similar, but slightly higher, estimates, although lack of sufficient knowledge regarding human hazard, exposure and potential confounders makes these estimates highly uncertain. Modeling efforts have indicated the sensitivity of certain model parameters to choice of data sets and/or assumptions. Particularly with regard to the slope of the response for surrogate markers of low dose response such as enzyme induction or indirect mutagenic activity, estimates of cancer risk are highly dependent on these assumptions and could predict very different, generally lower, risks if other parameters are shown to be more appropriate.

An additional consideration regarding the evaluation of dose-response for dioxin and related compounds involves the ubiquity of background exposure to these compounds. Body burdens of these compounds have been discussed previously in several parts of this assessment. In all studies, both in laboratory animals and in humans, incremental exposures are being added onto an existing body burden which is present at birth and appears to increase with age. This background is often insignificant from the standpoint of added dose in experimental studies or for highly exposed human cohorts. On the other hand, it has real implications relative to the detectability of response at low incremental exposures and may have implications for the use of models which assume additivity to ongoing processes which may have been stimulated by background levels. Modeling estimates suggest that, if dioxin and related compounds are adding to human cancer burden, current background exposures may result in upper bound population cancer risk estimates in the range of

Table 9-3

ESTIMATED BODY BURDENS OF EXPERIMENTAL ANIMALS AND HUMANS EXPOSED TO LOW EFFECT LEVELS OF 2,3,7,8-TCDD.

EFFECT	SPECIES	EXPERIMENTAL DOSE	BODY BURDEN	REF/note
CHLORACNE	HUMANS		36-3,000 ng/kg	1,2/a
CHLORACNE	MONKEY	1,000n g/kg	1,000 ug/kg	3/b
CHLORACNE	RABBITS	4 ng/kg 5d/wk/4wk	220 ng/kg	4/c
CHLORACNE	MICE	5,000 ug/kg 3d/wk/2wk	17,000 ng/kg	5/d
DECREASE TESTOSTERONE	HUMANS		13 ng/kg	6/e
DECREASE TESTOSTERONE	RATS	12,500 ng/kg sac day 7	10,200 ng/kg	7/f
ALTERED GLUCOSE TOLERANCE	HUMANS		110 ng/kg	8/g
ALTERED GLUCOSE TOLERANCE	HUMANS		14 ng/kg	9/h
DECREASE GLUCOSE UPTAKE ADIPOCYTES	GUINEA PIGS	30 ng/kg sac day 1	30 ng/kg	10/i
DECREASE SERUM GLUCOSE	RATS	100 ng/kg/d 30 days	1,900 ng/kg	11/j
DECREASE BIRTH WEIGHT	HUMANS	Mother body burden 1,400 ng/kg	1,400 ng/kg	12/k
DECREASE GROWTH	HUMANS		47 ng/kg	13/l
DECREASE GROWTH	RATS	125 ng/kg/d maternal dose gd day 6-15	1,250 ng/kg	14/m

EFFECT	SPECIES	EXPERIMENTAL DOSE	BODY BURDEN	REF/note
DECREASE GROWTH	RATS	400 ng/kg maternal dose gd 15	400 ng/kg	15/m
ALTERED LYMPHOCYTE SUBSETS	RHESUS MONKEYS	25 ppt in diet for 4 years	270 ng/kg	16/n
ALTERED LYMPHOCYTE SUBSETS	MARMOSETS	0.3 ng/kg/wk for 24 weeks 1.5 ng/kg/wk for 12 weeks	6-8 ng/kg	17/o
ENHANCED VIRAL SUSCEPTIBILITY	MICE	10 ng/kg sac day 7	7 ng/kg	18/p
ENDOMETRIOSIS	MONKEYS	5 ppt in diet 4 years	27 ng/kg	19/n
DECREASED SPERM COUNT	RATS	64 ng/kg maternal dose gd 15	64 ng/kg	20/m
CANCER	HUMANS		100-7,000 ng/kg	21,22,23/q
CANCER	HAMSTERS	100 ug/kg 6 doses (600 ug/kg total dose)	~~1 ng~~ 100 ng/kg	24/r
CANCER	RATS	100 ng/kg/d for 2 years	1,400 ng/kg	25/s
TUMOR PROMOTION	RATS	125 ng/kg/d 30 weeks	24,000 ng/kg	26/t

EFFECT	SPECIES	EXPERIMENTAL DOSE	BODY BURDEN	REF/note
CANCER SKIN TUMOR PROMOTION	MICE	7.5 ng/kg/wk for 20 wks dermal exposure	1,100 ng/kg	27/u
DOWN REGULATION OF EGFR IN PLACENTA (MAXIMAL EFFECT)	HUMANS		1,400 ng/kg	12/k
DOWN REGULATION OF EGFR IN LIVER 28/t (MAXIMAL EFFECT)	RATS	125 ng/kg/d 30 weeks	24,000 ng/kg	
INCREASE IN PLACENTAL CYP1A1 (MAXIMAL EFFECT)	HUMANS		1,400 ng/kg	12/k
INCREASE LIVER CYP1A1 (MAXIMAL EFFECT)	RATS	125 ng/kg/d 30 weeks	24,000 ng/kg	29/t
ENZYME INDUCTION CYP1A1 (LOEL)	RATS	1 ng/kg single dose sac 24 hr	1 ng/kg	30/v
ENZYME INDUCTION CYP1A1/1A2 (LOEL)	MICE	1.5 ng/kg/d 5 d/wk 13 wk	23 ng/kg	31/w
BACKGROUND	HUMAN	60 TEQ ppt in serum	9 ng/kg	x
BACKGROUND	MOUSE		4 ng/kg	y

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FOOTNOTES

a The two values presented from this data are from the persons with chloracne who had the lowest exposure (2) and the average level of persons with chloracne from Yu Cheng (1). Estimates of body burden for the average Yu Cheng pateint with chlroacne were determined by authors (1). In the patient with the lowest value, adipose tissue levels at the time of exposure are estimated by the authors (2) assuming a half-life for TCDD of 5.8 years and are expressed as pg/g of lipid. Body burdens are estimated from serum levels at time of exposure (2) assuming that all TCDD in the body is equally distributed in the lipid of the body. The average worker is assumed to be a male weighing 70 kg with 15% of the weight as lipid (31).

b Animal administered 1ug/kg TCDD and it is assumed that essentially no TCDD was eliminated when the animal developed a chloracnegenic response.

c Assumes the same rate of elimination as the rat and that the animals weights 2.5 kg throughout the experiment.

d Assumes a half-life of 11 days and an average weight of the animal at 25 grams.

e From reference (6) in which workers with levels of TCDD of 76 ppt in serum or higher had lower testosterone levels. Also assumed that the background TEQ was 60 ppt so that the total serum TEQ was 136 ppt (lipid adjusted). Average worker was male weighing 70 kg with 15% body fat.

f Animals received single exposure of 12.5 ug/kg (LOAEL) and sacrificed 7 days after dosing. Assumes a half-life of 23.4 days and body burden corrected for elimination.

g Same assumptions in e except average serum levels in affected workers is 640 ppt.

h From Ranch Hand study (8), assumes that high exposed group (>33 ppt) had a background of 60 TEQ ppt. Thus this group had at least 93 TEQ ppt. Assumes average ranch hand patient was male weighing 70 kg with 15% body fat.

i Guinea pigs received 0.03 ug TCDD/kg ip. and sacrificed 24 hours after dose. Assumes that no TCDD was eliminated at this time.

j Animals were treated with 0.1 ug/kg/day for 30 days and assumes half-life of TCDD in the rat is 23.4 days.

k According to the author (12), there is a decrease in birth weights of children born from these patients and that the epidermal

growth factor receptor (EGFR) and CYP1A1 are maximally affected in these patients. Body burdens determined based on levels of 2,3,4,7,8-pentachloro-dibenzofuran (TEF = 0.1) and 1,2,3,4,7,8-hexachlorodibenzofuran in placenta tissue. Assumes placenta is 1% lipid (34) and that women have a fat content of 21% of body weight (31).

l Body burdens estimated from serum levels presented by authors (6). The authors (6) published that the average body weight for the children was 30 kg with 25% of the weight as body fat. All the dioxins are assumed to be equally distributed in the body fat.

m Assumes pups exposed to an equal dose of TCDD as are the dams on a weight basis and that the pups do not eliminate any of the TCDD.

n Assumes a single first-order elimination rate constant and a a half-life for the whole body elimination of 400 days (3) and a gastrointestinal absorption of 86% (33).

o Assuming a single first-order elinination rate constatne and a half-life of 6-8 wks. Body Burdens calculated by authors (17).

p Body burden determined in these animals (32). Approximately 70% of the body burden remains at 7 days after dosing.

q Estimated highest body burden at time of last exposure. calculations based on measured TCDD levels in serum (lipid adjusted) and assuming a first-order elimination kinetics and a half-life for eliniationof 7.1 years. Also assumes a vody weight of 70 kg and 22% body fat. Calculations for estimated serum concentrations at last time of exposure performed by authors, not adjusted for background levels.

r Animals administered 100 ug/kg 6 times over a 4 week period. Assumes a half-life of 23.4 days and that animals are sacrificed at 10 months after the first dose.

s Assumes a single first-order elimination rate constant and a half-life for the whole body elimination of 23.7 days (33) and a gastrointestinal tract absorption of 86% (33).

t Liver levels measured in study at approximately 300 ppb (lipid adjusted). Also assumes animal is 10% body fat by weight.

u Assumes an elimination rate of 11 days and a body weight of 20 grams.

v Animals received a single dose and were sacrificed 24 hours later. Assumes no TCDD eliminated at this time.

w Animals received 1.5 ng/kg/d 5d/wk for 13 wk. Animals

sacrificed 3 days after last dose. Hepatic, dermal and pulmonary EROD activity induced at this dose. Tissue levels measured in liver, skin and fat. Assumes that this is the LOEL and that 100% of the dose is in liver, skin and fat.

x Assumes a background TEQ of 60 ppt for dioxins, dibenzofurans and PCBs. Also assumes a body weight of 70 kg with 15% body fat.

y Data from DeVito and Birnbaum. TEQ for TCDD, 1,2,3,7,8-PCDD; 2,3,7,8-TCDF; 1,2,3,7,8-PCDF; 2,3,4,7,8-PCDF; and OCDF in 150 day old female B6C3F1 mice. Chemicals were determined in liver, fat and skin of these animals. Assumes that 100% of the body burden is in liver, fat, and skin.

one in ten thousand (10^{-4}) to 1 in a thousand (10^{-3}) attributable to exposure to dioxin and related compounds. Actual risk for individuals in the population is likely to be less and, for some, may even be zero.

Background levels also complicate the evaluation of "No Observed or Low Observed Adverse Effect Levels" (NOAELs or LOAELs). Incremental exposures must be considered in light of existing body burdens in determining whether increased probability of effects having biological thresholds are likely. The concept that an incremental exposure is below an experimental threshold is moot unless the combined background and incremental exposure or dose are below the threshold level. This has important consequences for the assessment of compounds like dioxin where certain effects can be detected at or near equivalent human background body burden levels.

KEY ASSUMPTIONS AND INFERENCES

One of the primary functions of the risk characterization is to present key assumptions and inferences which are used to reach conclusions in the absence of definitive information. Not all scientists may agree with the use of these specific assumptions and inferences. The degree to which thereis disagreement will have profound effects on the acceptance of this analysis. While many of these assumptions and inferences are discussed in previous sections, it is important that they be recognized in order to put our overall conclusions in a proper perspective. Some of the key assumptions and inferences are:

- *The limited information on sources, fate and transport in the environment provide a reasonable basis for predicting human exposure.* While data are limited and, therefore, uncertain, information from a variety of studies in industrialized countries coupled with our detailed knowledge of physico-chemical properties for this class of compounds allows reasonable assumptions to be made regarding relative ranking of sources with regard to their contribution to environmental loading, the persistence of this class of compounds under specific environmental conditions and the likelihood that the chemical will be transferred from the environment to biological systems. Nonetheless, these are assumptions which are arguable and which will be refined as more data become available.

• *The air to food hypothesis is plausible and is supported by enough data to warrant its use in the absence of more complete information.* The air-to-food hypothesis is founded on data evaluating deposition, environmental transport, bioaccumulation and consumption patterns. It is supported by studies from Europe and Canada. While individual measurement data are still quite limited, the consistency of the evidence supporting the validity of the hypothesis is compelling. The hypothesis has been accepted by a large segment of the knowlegable scientific community. Because airborne dioxin may come from direct releases to air or from re-cycling of dioxin-like compounds released into various environmental media from a number of sources, this hypothesis provides a perspective on how dioxin-like compounds move through the environment to humans but does not allow attribution of exposure to particular sources.

• *Toxicity equivalence (TEQs) is a valid, interim method for assessing exposure to a complex mixture of dioxin and related compounds and predicting likely health outcomes.* The EPA and the international scientific community have agreed that the use of toxicity factors (TEFs) to predict relative toxicities of mixtures of this class of compounds has an adequate empirical basis, is thoeretically sound, and, in the absence of more complete data sets on the toxicity of individual members of this class, is a useful procedure. This is not to say that the use of TEFs is a certain procedure. Since 1986 when the first Agency-wide consensus on the use of TEFs was published, additional refinements to the data bases and to the use of TEFs have occurred. Published revisions in accord with international agreement appeared in 1989. In the course of this reassessment, critical data were collected and agreement was reached regarding the contribution of dioxin-like PCBs to overall TEQs. Additional validation of the TEQ concept in predicting effects of this class of compounds on wildlife species lends further support to the use of this approach. It must be recognized that this relatively simple, additive approach does not take into account interactions between dioxin-like compounds and other chemical exposures. These interactions may result in either an overestimate or an underestimate of likely effects of the complex mixture.

• *Use of one-half the non-detect level for estimating low levels of exposure is a reasonable but conservative approach to evaluating limited blood and tissue level data.* For some data sets, use of zero values for non-detects could result in

significantly lower estimates. However, it is widely held that such an approach would most likely underestimate true levels of exposure. Similar estimates derived from different data sets, developed by different investigators in several countries, strengthen the probability that this inference represents the true picture for exposure of the general population in industrialized countries to dioxin and related compounds.

• *The limited data available from studies of levels of dioxin and related compounds in humans provides an adequate basis to infer general population body burdens.* Although there are still limited measurements of general population body burdens, the data provide a consistent picture of background body burdens for industrialized countries. While additional data will help to refine the range of general population body burdens as a function of location, human activity, age and the like, there are adequate data to estimate current body burdens in the general population for the purposes of this assessment. If estimates were to change with new data, it is not likely that we would be far offand it is highly unlikely that these estimates would represent a sensitive parameter in estimating margins-of-exposure within an order of magnitude.

• *Laboratory animal studies provide useful information in evaluating potential human responses to dioxin and related compounds.* Based on our knowledge of the biochemical and biological similarities between laboratory animals and humans, our understanding of some of the fundamental impacts of this class of compounds on biological systems, and comparable responses from animal and human studies both *in vitro and in vivo,* our decision to use laboratory animal data to contribute to weight-of- the-evidence conclusions on human hazard and risk is reasonable. Humans do not appear to be an outlier for dioxin effects, that is, they do not, on average, appear to be either refractory to or exquisitly sensitive to the effects of dioxin-like compounds. While positive human data is preferable for ascribing hazard or risk, the lack of adequate human data to demonstrate causality for many suspected dioxin effects is assumed not to negate the findings from laboratory animal and *in vitro* studies. Although some scientists may disagree, in our estimation, the data base on dioxin and related compounds is one of the most comprehensive among all environmental chemicals. The fundamental understanding of mechanisms of dioxin action provides a unifying theory for the mechanisms for observed effects in laboratory animals and

humans, and for using a weight-of-the-evidence approach considering all relevent data to infer the human health impacts of dioxin and related compounds.

• *Observations of effects from exposure to dioxin and related compounds in humans and other animals suggest that fundamental changes in cellular biochemistry and biology may be related to frankly adverse effects which can be more readily observed at higher levels of exposure.* Observations described in this assessment suggest a continuum of response to exposure to dioxin-like chemicals. This continuum provides a basis for inferring a relationship between some early events which are not necessarily considered to be adverse effects with later events which are adverse effects. Considerable uncertainty remains in inferring how these events are related, although we know more about how dioxin-like compounds may elicit effects than we know about the mechanisms of action for most chemicals. This inference may be the most contentious of all and it is likely that a wide range of opinion will be provided by the scientific community regarding the relationship of these mechanistic observations and prediction of potential for adverse effects in exposed humans.

OVERALL CONCLUSIONS REGARDING THE IMPACT OF DIOXIN AND RELATED COMPOUNDS ON HUMAN HEALTH

Dioxin exposure from multiple sources may result in a number of biochemical and biological effects in both humans and other animals, many of which are considered adverse or toxic effects, and some of which occur at very low levels of exposure. A large variety of sources of dioxin have been identified and others may exist. Because dioxin-like chemicals are persistent and accumulate in biological tissues, particularly in animals, the major route of human exposure is through ingestion of foods containing minute quantities of dioxin-like compounds. This results in wide-spread exposure of the general population of industrialized countries to dioxin-like compounds. Certain sub-populations may be exposed to additional increments of exposure by being in proximity to point sources or because of dietary practices. Some of the effects of dioxin and related compounds have been observed in laboratory animals and humans at or near levels to which people in the general population are exposed. Other effects are detectable only in

highly exposed populations, and there may or may not be a likelihood of response in individuals experiencing lower levels of exposure. Evaluation of effects in this health assessment document are based on the concept that lipid adjusted serum levels approximate the body burden of dioxin and related compounds, and that there will be a dose-response relationship between effects and body burden. Adverse effects associated with temporary increases in dioxin blood levels based on short term high level exposures, such as those that might occur in an industrial accident scenario or infrequent contact with highly contaminated environmental media, may be dependent on exposure coinciding with a window of sensitivity of biological processes. It is reasonable to assume that developing organisms may be particularly sensitive to adverse impacts from fluctuations in exposure levels. Such exposures may also lead to higher tissue levels over the long term because of the long half-life for elimination of dioxin and related compounds.

The scientific community has identified and described a common initiating mechanism that may account for most if not all of the observed effects in vertebrates including humans. This mechanism involves binding of dioxin-like compounds to a cellular receptor called the "Ah receptor." Binding to the Ah receptor appears to be necessary for all well-studied effects of dioxin but is not sufficient to elicit these responses. Receptor binding represents the first step in a cascade of events attributable to exposure to dioxin-like compounds including biochemical, cellular and tissue-level changes in normal biological processes. The effects elicited by exposure to 2,3,7,8-TCDD are shared by other chemicals which have a similar structure and Ah receptor binding characteristics. Consequently, the biological system responds to the cumulative exposure of Ah receptor-mediated chemicals rather than to the exposure to any single dioxin-like compound. The concept of toxicity equivalence within this class of compounds and the use of toxicity equivalence factors (TEFs) is widely accepted by the scientific community. While some uncertainty remains with regard to the additivity of complex mixtures of these compounds and with the impacts of co-exposure to non-dioxin-like compounds, the use of this approach is consistent with the Agency's guidance on the evaluation of complex mixtures in the absence of data on the impact of the actual mixture. This approach to the evaluation of dioxin and related compounds represents one of the

best studied and most widely accepted applications of this guidance although additional validation studies to reduce uncertainty would be welcome.

There is adequate evidence from studies in human populations as well as in laboratory animals and from ancillary experimental data to support the inference that humans are likely to respond with a plethora of effects from exposure to dioxin and related compounds. These effects will likely range from adaptive changes at or near background levels of exposure which may be adverse or may be beneficial, to adverse effects with increasing severity as exposure increases above background levels. Induction of activating/metabolizing enzymes, for instance, can lead to increases in reactive intermediates and may potentiate toxic effects, or may lead to more rapid metabolism and elimination of potentially toxic compounds. Demonstration of examples of both of these situations is available in the published literature. The mechanistic relationships of biochemical and cellular changes seen at very low levels of exposure to production of adverse effects detectible at higher levels remains uncertain and controversial.

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 suggest that humans, in general, are neither extremely sensitive nor refractory 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 previous sections, although the influence of variability among humans remains difficult to assess. Discussions in previous chapters have highlighted certain prominent biologically significant effects of TCDD and related compounds to emphasize some of the more sensitive indicators of toxicity in animals and also, potentially, in humans. These endpoints have been shown to be affected by TCDD, but specific data on the endpoints of concern do not generally exist for other congeners. Concern for these effects based on the concept of toxicity equivalence remains,however, for all dioxin-like compounds.

In humans, subtle changes in enzyme activity indicating liver changes, in levels of circulating reproductive hormones in males, in reduced glucose tolerance, and in cellular changes related to immune

function suggest the potential for adverse impacts on human metabolism, reproductive biology and immune competence at or within one order of magnitude of average background body burden levels. Average human daily intakes of dioxin and related compounds, including the dioxin-like PCBs, are in the range of 3-6 pg TEQ/ kg BW/day. This results in average body burdens estimated to be in the range of 30-60 pg TEQ/g lipid(30-60 ppt) or 5-10 ng/kg body weight. The effects described above are seen at or just several fold above these average levels. Since exposures within the general population are thought to be log-normally distributed, individuals at the high end of the general population range may be experiencing some of these effects. Some more highly exposed members of the population may be at risk for a number of adverse effects including developmental toxicity, reduced reproductive capacity in males based on decreased sperm counts, higher probability of experiencing endometriosis in women, reduced ability to withstand an immunological challenge and others. This inference is supported by observations in animals, by some human information from highly exposed cohorts and by scientific inference. Fortunately, there have been few human cohorts identified with exposures in the high end of this range. While the lack of adequate human information and the insensitivity of epidemiologic studies makes validation of these inferences difficult, they are not unreasonable given the weight-of-the-evidence from available data. They represent testable hypotheses which may be strengthened by further data collection.

The background levels in humans described above would be well within a factor of 100 of levels representing low observed adverse effect levels (LOAELs) in laboratory animals. For several of the effects noted in animals, a "margin of exposure" (MOE) of less than an order of magnitude, based on intake levels or body burdens, is likely to exist. A MOE is calculated by dividing the human-equivalent animal LOAEL or no observed adverse effect level (NOAEL) with the human exposure level. The original basis for MOE calculations was the observation that exposure in the range of 1-10 ng TEQ/kg/day represented a no observed adverse effect level (NOAEL) for a sensitive non-cancer endpoint and, therefore, that an intake of up to 10 pg TEQ/kg/day might represent an adequate MOE for all other non-cancer effects. Recent data suggest that "high end" average exposures in the general population are likely to approach this

intake level and that several effects, both subtle and frank, can be demonstrated to occur in animals at intake values significantly lower than 1-10 ng TEQ/kg/day. It is, therefore, highly unlikely that a margin of exposure (MOE) of 100 or more currently exists for these effects at background intake levels, at least for some members of the human population. We need to continue to monitor trends in human body burden for dioxin and related compounds. If levels are declining, the relationship of background body burdens to observed effect levels in animal and human studies will need to be re-evaluated.

The USEPA has frequently defined a reference dose (RfD) for toxic chemicals to represent a scientific estimate of the dose below which no appreciable risk of non-cancer effects is likely to occur following chronic exposures. In the case of dioxin and related compounds, calculation of an RfD based on human and animal data and including standard uncertainty factors to account for species differences and sensitive subpopulations would result in reference intake levels on the order of 10-100 times below the current estimates of daily intake in the general population. For most compounds where RfDs are applied, background exposures are generally low, are not persistent and are not taken into account. Dioxin and related compounds presents an excellent example of a case where background levels in the general population are likely to have significance for evaluation of the relative impact of incremental exposures associated with a specific source. Since RfDs refer to the total chronic dose level, the use of the RfD in evaluating incremental exposures in the face of a background intake exceeding the RfD would be inappropriate.

In addition to the concern for various non-cancer health endpoints discussed above, the potential immunotoxicity of dioxin and related compounds represent a special situation. Impairment of the immune system can be considered an adverse outcome in its own right, being responsible for induced pathologies. At the same time, immunotoxicity can function as a modulator of the disease process. The immune system functions to protect against both pathogenic challenge and continued growth of malignant cells. Alterations in the ability of the immune system to perform these primary functions would result in either the promotion of the pathogenic process or the progression of cancer. While it is relatively simple to determine experimentally the effects of TCDD on the ability of the immune system to respond to a variety of specific

antigens or immunogens in laboratory animals, it is much more difficult to establish the effects on longer term immune surveillance and the effect of dioxin-like compounds on the immune system of humans.

Nonetheless, it has been clearly established that TCDD is immunotoxic and that it can impair normal immune function in laboratory animals and that it is likely to do so in humans as well. Although it is difficult to identify the cell type that is primarily affected in each of the testing protocols, it is clear that several animal species are sensitive to the immunotoxic effects of TCDD at single doses below 1 ug/kg. Although it is possible that humans may be less sensitive than animal models to dioxin immunotoxicity, there are currently limited data to evaluate the impact of immunotoxic responses to dioxin and related compounds in humans.

With regard to carcinogenicity, a weight-of-the-evidence evaluation suggests that dioxin and related compounds are likely to present a cancer hazard to humans. This hazard is likely by oral and inhalation routes of exposure and is less likely, although possible, by the dermal route of exposure based on bioavailability and uptake studies. As daily doses through these routes and subsequent body burdens approach those seen in occupational studies, the uncertainty of the hazard characterization is reduced. While the epidemiological data alone are not yet deemed sufficient to characterize the cancer hazard of this class of compounds as being "known," the unequivocal evidence in animal studies, inferences drawn from mechanistic data and the suggestive evidence of recent epidemiology studies support the characterization of dioxin and related compounds as likely cancer hazards. Extent of cancer risk will depend on such parameters as route and level of exposure, overall body burden, dose to target tissues, and hormonal status.

The current evidence suggests that both receptor binding and early biochemical events such as enzyme induction are likely to demonstrate low-dose linearity. The relationship of these early events to the complex process of carcinogenesis remains to be determined. If these findings imply low-dose linearity of biologically based cancer models under development, then probability of cancer risk will be linearly related to exposure to TCDD at low doses. However, until the 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 in the low-dose region can only be inferred with uncertainty. However, given that background exposures to dioxin are ubiquitous and associations between exposure to dioxin-like compounds and certain types of cancer have been noted at body burdens within 1-2 orders of magnitude (10-100 times) of average background body burdens, there is no need for large scale low dose extrapolations. However, since human data to support this conclusion remain limited and based on individuals who were highly exposed for some time in their life, the relationship of apparent increases in cancer mortality in these populations to calculations of general population risk remains uncertain.

The fact that dioxin-like compounds are ubiquitous in the environment may have further implications for low-dose risk assessment. Specifically, humans are currently exposed to background levels of dioxin-like compounds on the order of 3-6 pg TEQ/kg bw/day, including dioxin-like PCBs. This is more than 500-fold higher than the EPA's 1985 risk-specific dose associated with an upper bound one in a million ($1x10^{-6}$) risk of 0.006 pg TEQ/kg bw/day and 75-150-fold higher than revised risk specific dose estimates presented in Chapter 8 of this reassessment. For populations who are more highly exposed based on proximity to specific sources or specific human activity patterns such as consumption of higher amounts of foods containing average or higher levels of dioxin-like compounds, the additive background model of Crump et al. (1986) implies that the addition of an incremental dose to an existing background exposure would support the assumption of linearity within the exposure range, particularly if that background exposure is within 1-2 orders of magnitude (10-100 times) of the range of observation of purported dioxin-induced tumors in highly exposed humans.

TCDD has been clearly shown to increase malignant tumor incidence in laboratory animals. In addition, a number of studies have been conducted which elucidate other biological effects of dioxin. These studies have been used to develop biologically-based models of the pharmacokinetics of dioxin, of binding to the Ah receptor and of induction of various proteins. In addition, bioassay data on TCDD reported by Kociba have been analyzed using the two-stage clonal expansion model of carcinogenesis. There is evidence to suggest that hormonal factors may be involved in TCDD carcinogenesis. The role of such factors warrants additional study.

Ideally, a biologically-based model for cancer induction by TCDD should explicitly consider hormonal influences. Initial attempts to construct a biologically-based model for certain dioxin effects as a part of this re-assessment 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.

Based on all of the the data reviewed in this reassessment, a picture emerges of TCDD and related compounds as potent toxicants producing a wide range of effects at very low levels when compared with other environmental contaminants. The fundamental level at which these compounds act on biological systems is analogous to several well studied hormones. Dioxin and related compounds have the ability to alter the pattern of growth and differentiation of a number of cellular targets by initiating a cascade of biochemical and biological events resulting in a wide range of responses. While not all of these responses are adverse, and some may even be beneficial, the weight of the evidence suggests concern for the impact of these chemicals on humans at or near current background levels. Additional, incremental exposures occurring as a result of proximity to a point source of release or specific human activity patterns, such as consumption of high levels of more highly contaminated foods, should be evaluated relative to background levels and the impact of the incremental exposure on both transient and steady-state body burdens. This situation is somewhat akin to the scientific approach taken for evaluating and characterizing lead exposure in children. This approach has been useful in providing public health-based advise to decision-makers faced with difficult regulatory choices.

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