Environmental Toxicology of Polychlorinated Dibenzo-*p*-Dioxins and Polychlorinated Dibenzofurans

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Few environmental compounds have generated as much interest and controversy within the scientific community and in the lay public as polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Their ubiquitous presence in the environment and the risk of accidental exposure has raised concern over a possible threat of PCDDs or PCDFs to human health. The most extensively studied and potent isomer is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin). Dioxin is a multisite toxicant in laboratory rodents resulting in a number of tissue-, species-, and sex-dependent responses. Much has been learned about the mechanism of dioxin's effects, especially for the induction of cytochrome P-450 enzymes. Binding of PCDDs and PCDFs to a receptor protein, termed the dioxin or Ah receptor, is necessary for most biological and toxic responses. The most common toxic response used for evaluating the human health risk posed by PCDDs and PCDFs is the hepatocarcinogenic response observed primarily in rodents. Despite extensive research efforts, the effects of PCDDs and PCDFs on humans are not well characterized. However, available data indicate there is good agreement between known effects of dioxin in laboratory animals and those described in epidemiological studies for effects in humans. The sequence in events initiated by the Ah receptor interacting with dioxin-responsive genes and ending with altered patterns of differentiation and growth must be sought in order to understand tissue, species, sex, and interindividual variation in biological responses and the health risk posed by PCDDs and PCDFs.

Introduction

Over the past 20–30 years the public has become increasingly aware of the presence of toxic substances in the environment and the risk that these substances pose to human health. The polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are two series of polychlorinated aromatic compounds (Fig. 1) that are ubiquitous environmental contaminants. Of the 75 possible PCDDs and 135 PCDFs, the most extensively studied isomer is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), commonly referred to as dioxin. Since 1970, it has been estimated that more than $1 billion has been spent on researching the toxicity of PCDDs and PCDFs. These studies have produced considerable information on the properties and mechanism of action of PCDDs and PCDFs, yet there is no agreement by the health authorities on the risks posed by these substances.

In laboratory animals, dioxin is one of the most toxic chemicals ever described. The spectrum of toxic responses observed in rodents includes effects on immune function, reproduction, organogenesis, lipid and glucose metabolism, and behavior (*1*). In addition, TCDD is a multisite carcinogen in rodents and is classified as a tumor promoter in liver and skin. The hepatocarcinogenic effects in rodents have been the primary end point used to estimate human health risk associated with exposure to these compounds. However, due to the variety of quantitative models used to describe the same experimental data sets, an unusually large range of health risk estimates has been proposed by regulatory agencies throughout the world (Table 1). In addition, the estimated human consumption of dioxins and furans (approximately 1 pg/kg/day) is close to the tolerable daily intake set by the health authorities in several countries.

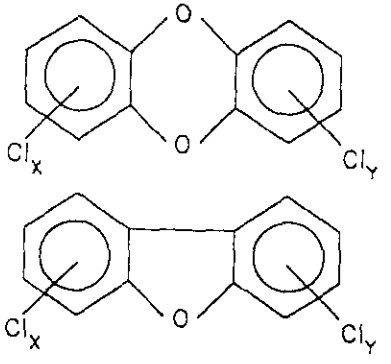

FIGURE 1. Structures of polychlorinated dibenzo-*p*-dioxins (top) and polychlorinated dibenzo-*p*-furans (bottom).

Conceding that humans are exposed to significant amounts of PCDDs and PCDFs in their day-to-day existence, the next issue faced by scientists is to determine the risk posed by this exposure

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Table 1. Extrapolation to humans from chronic animal studies with TCDD by various health agencies.[a]

Agency	Mechanistic basis of extrapolation	Results
U.S. EPA	Nonthreshold, linearized multistage model (dose/surface area)	VSD = 0.0064 pg/kg/day
U.S. CDC	Nonthreshold, linearized multistage model (liver concentration)	VSD = 0.0276 pg/kg/day
U.S. FDA	Nonthreshold, linearized multistage model (dose/body weight)	VSD = 0.0572 pg/kg/day
Germany	Threshold, safety factor applied to NOAEL	Maximal daily intake 1–10 pg/kg/day
U.K. Department of the Environment	Threshold, safety factor applied to NOAEL	Allowable daily intake 1–10 pg/kg/day
Dutch Institute of Natural Health	Threshold, safety factor applied to NOAEL	Allowable daily intake 4 pg/kg/day
Swiss Institute of Toxicology	Threshold, safety factor applied to NOAEL	Allowable daily intake 10 pg/kg/day
Ontario Ministry of the Environment	Threshold, safety factor applied to NOAEL	Allowable daily intake 10 pg/kg/day

Abbreviations: EPA, Environmental Protection Agency; CDC, Centers for Disease Control; FDA, Food and Drug Administration; NOAEL, no observable adverse effect level; VSD, virtually safe dose for upper-limit cancer risk of 10^{-6}.
[a]Source of the dose–response data is a 2-year study by Kociba et al. (67).

as well as incremental exposures over background levels. The purpose of the present article is to review the knowledge gained on the toxicity and mechanism of action of these important environmental contaminants. The appropriateness of extrapolating data obtained from laboratory animals to humans and the use of dose-response relationships is also discussed.

Sources and Environmental Fate of PCDDs and PCDFs

Sources

There are no specific commercial uses for PCDDs or PCDFs, and they arise primarily as unwanted contaminants. An important aspect in the evaluation of health risks associated with dioxin and related compounds is that they often exist as complex mixtures of polychlorinated species including polychlorinated biphenyls (PCBs), polychlorinated phenols, and polychlorinated tetraphenyls. Therefore, it is often difficult to dissociate the risk posed by trace contaminants such as dioxin from those species present in much higher proportions.

The primary sources of these contaminants includes chemical, enzymatic, thermal, and photochemical reactions (Table 2). It has been suggested that PCDDs and PCDFs are almost exclusively of anthropogenic origin (*2*), although forest fires may result in their formation as well (*3*). A series of combustion reactions have been found to result in the formation of PCDDs and PCDFs. The formation of PCDDs and PCDFs from waste incineration has been recognized for several years based on their determination in fly ash (*3*). PCDDs and PCDFs may form as a result of burning precursor products, i.e., chlorophenol compounds, or as a result of pyrolysis of unrelated chlorine compounds (*1*). Several factors determine the relative amount of PCDD and PCDF formed by combustion of organic materials including chlorine content of the fuel, mixing efficiency with air, and reaction temperature. In addition, the amount of lead present in petrol products is directly related to the formation of these chemical species (*4*). Very low levels of PCDDs and PCDFs have been observed in cigarette smoke and charcoal-grilled meats (*5*).

One source of aquatic dioxin contamination is the result of chlorine bleaching of paper pulp (*6*). Sediment analysis performed in the vicinity of paper mills using this process show high levels of TCDD and TCDF, as well as lesser chlorinated dioxins and furans (*6*). In addition, contamination of water and fish downstream from paper mills has been observed. Recent concern over the presence of these contaminants in bleached paper products has surfaced due to low levels of PCDDs and PCDFs found in coffee filters, facial tissue, and milk cartons.

Table 2. Sources of PCDDs and PCDFs.[a]

Thermal processes
Incineration of municipal solid waste
Production of steel and copper
Combustion of leaded gasoline
Accidental burning of PCB-containing electrical equipment
Incineration of coal, peat and wood
Cigarette smoke
Chemical processes
Chlorine bleaching of paper products
Intermediates in production of chlorophenol-based products

Abbreviations: PCDD, polychlorinated dibenzo-*p*-dioxin; PCDF, polychlorinated dibenzofuran; PCB, polychlorinated biphenyl.
[a]Adapted from Rappe (*2*).

The presence of PCDDs and PCDFs as impurities in the manufacture of chlorophenol and chlorinated aromatic hydrocarbons has added to the public concern about these substances. Chlorophenols are widely used as fungicides and herbicides and are key intermediates in the production of phenoxyacetic acid herbicides such as 2,4,-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). The herbicide known as "Agent Orange" used as a defoliant in Vietnam is a 1:1 mixture of *n*-butyl esters of 2,4-D and 2,4,5-T and is contaminated with up to 30 mg/kg with 2,3,7,8-TCDD. PCBs used in electrical transformers and capacitors have also been shown to be contaminated with high levels of PCDFs.

Fate in Environment

PCDDs and PCDFs are strongly bound to organic matter in the environment. These compounds have a high degree of hydrophobicity that generally increases with the degree of chlorination. Due to the strength of this binding, 2,3,7,8-TCDD is usually found in uppermost layers of the soil and undergoes very little vertical migration (*1*). Transport of these chemicals is generally governed by the mobilization of soil particles to which they are attached. Levels of PCDDs and PCDFs in aqueous environments is generally low due to preferential absorption to particulate matter. However, suspension of this particulate matter may occur. Animals living in this environment may become exposed to these chemicals either directly, i.e., by ingestion of contaminated sediment, or indirectly, i.e., through the food chain. Bioaccumulation of PCDDs and PCDFs in aquatic biota is highly dependent on the chemical species involved as well as the animal exposed (*6*).

Photolysis appears to be the primary route of environmental degradation of PCDDs and PCDFs. Exposure of dioxins to ultraviolet light in the presence of an electron donor [e.g., leaf

waxes, organic films, pesticides, and other co-pollutants (*1,7,8*)] results in the degradation of TCDD. Very few bacterial or fungal species are known to degrade dioxins. The half-life of TCDD in soil in the absence of UV light is approximately 10 years, indicating the general lack of alternative degradation pathways.

Human Exposure

Due to the numerous sources and the environmental persistence of PCDDs and PCDFs, these classes of compounds have ubiquitous distribution. Perhaps the earliest clinical description of dioxin toxicity in humans was in 1901 (*9*) when the skin condition chloracne, the most apparent toxic response of humans to PCDDS and PCDFs, was reported. The primary exposure of humans to these lipophilic compounds is via the food chain (*10*). Approximately 90% of the total daily intake of PCDDs and PCDFs is derived from food, particularly those of animal origin.

Another important source of contamination may be from packaging materials (*10*). The average daily intake via food in industrial countries has been estimated to be in 1–3 pg PCDDs and PCDFs/kg body weight/day. This level of consumption is higher than that recommended by the U.S. EPA as a "safe" level (1 in 1,000,000 cancer risk) of exposure (0.006 pg/kg/day).

Monitoring human tissues for the presence of PCDDs and PCDFs indicates that there are background levels of these compounds. The average levels of dioxin in human tissues are generally higher in industrial countries than those from developing nations (Table 3). Within a given country, the human tissue levels are generally consistent, although Vietnam may be the exception due to the high level exposure to PCDDs and PCDFs as a result of Agent Orange contamination. The tissue levels of PCDDs and PCDFs can be substantially elevated in poisoning cases. Several high-level exposures of dioxins have been reported in humans such as the toxic rice oil outbreak in Taiwan and Japan, the BASF plant explosion in Germany, the Sevaso incident in Italy, Agent Orange exposure in Vietnam,and the Missouri waste oil contamination in the United States [for review see Skene et al. (*1*)]. Due to the slow metabolism of PCDDs and PCDFs in humans (half-life 5–10 years) and sequestration in adipose tissue and liver, the body burden of these compounds can remain elevated for years after exposure.

Mechanism of Action of PCDDs and PCDFs

Discovery of Ah Receptor

The striking similarity in the biological effects of several polychlorinated compounds such as PCDDS, PCDFs, PCBs and polybrominated biphenyls (PBBs) lead Glover and Poland (*12*) to hypothesize that these xenobiotics elicit their effects through a common mechanism. The unusual potency of TCDD and the relationship between congener structure and potency were important clues that halogenated hydrocarbons may act through a specific receptor(s). This receptor was subsequently characterized, and its properties are discussed below.

The administration of 3-methylcholanthrene (3-MC) and structurally related polycyclic aromatic hydrocarbons to some inbred strains of mice leads to the induction of cytochrome P-450 and associated monooxygenase activities [i.e, aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-*O*-deethylase (EROD) activity]. In the following discussion, these enzymes will be called by their recommended nomenclature, i.e., Cyp representing cytochrome P-450, followed by an arabic number denoting the family and a letter to designate the subfamily (*13*). The induction of AHH activity by PCDDs and PCDFs is a result of increased CyplA1. Subsequently, AHH as well as EROD activites will be described in terms of the form of cytochrome P-450 associated with their activity, CyplA1.

Early studies of genetic polymorphisms in mice showed that 3-MC induced CyplA1 in C57BL/6 ("responsive") but not DBA/2 ("nonresponsive") inbred mouse strains (*14*). Crossbreeding studies showed that the responsive phenotype, i.e., 3-MC induction of CyplA1, segregated as a dominant trait and was governed by a single autosomal gene. The gene locus controlling this trait is designated the *Ah* (for *aryl*hydrocarbon) locus. Mouse strains that are nonresponsive to 3-MC are less sensitive to TCDD as well. Poland et al. (*15*) subsequently identified and characterized a receptor protein for TCDD, 3-MC, and other inducers of CyplA1 in C57BL/6J mice. This protein (designated the Ah or dioxin receptor) is apparently a product of the *Ah* locus (*16*). Discovery of the Ah receptor was a significant event in the maturation of toxicology as a scientific discipline and also helped to find a common focus for the disciplines of toxicology and molecular biology.

Table 3. Background PCDD and PCDF levels in human tissues.[a]

	Country						
Tissue	USA	Germany	China	Japan	Canada	S. Vietnam	N. Vietnam
Blood							
Total PCDDs	1499	788				1983	126
Total PCDFs	92	98				133	41
PCDDs + PCDFs	1591	886				2071	167
Adipose tissue							
Total PCDDs	558	942	247	1535	1217	814	133
Total PCDFs	32	140	53	92	65	57	21
PCDDs + PCDFs	590	1082	300	1627	1282	871	154
Milk							
Total PCDDs	367	289		1085	493	406	104
Total PCDFs	31	58		44	41	133	23
PCDDs + PCDFs	398	348		1128	534	559	127

Abbreviations: PCDD, polychlorinated dibenzo-*p*-dioxin; PCDF, polychlorinated dibenzofuran.
[a]Data are expressed as average parts per trillion relative to lipid content. Adapted from Schecter (*11*).

Role of Ah Receptor in Biological Responses to PCDDs and PCDFs

Presence of the Ah receptor confers sensitivity to several of the effects of PCDDs and PCDFs including enzyme induction, carcinogenesis, and immunotoxicity. Mason and Okey (*17*) demonstrated that in several tissues, nuclear Ah receptor levels were higher in responsive C57BL/6J mice than in nonresponsive DBA/2J mice. This is consistent with the fact that PCDDs and PCDFs elicit biological responses in both strains of mice, albeit at different dose levels. In addition, in several mammalian cell culture system there is an excellent correlation between CyplA1 induction (AHH activity) and number of Ah receptor molecules per cell (*18*).

Although these data support receptor-mediated specificity of biological responses, the presence of the Ah receptor by itself cannot explain observed species and tissue differences in the effects of PCDDs and PCDFs. Hepatic Ah receptor levels and K_d values for [^{3}H]2,3,7,8-TCDD binding in several species, (i.e., pigs, rats, hamsters, and nonhuman primates), are comparable, although there are marked differences in maximal CyplA1 induction and toxicity (*18*). For example, despite similar Ah receptor characteristics, the acute lethality and maximal CyplA1 induction by dioxin varies over a 5000-fold range between the guinea pig and the hamster (*19*). Also, there is little difference in Ah receptor concentration in various rodent tissues (*20*). Therefore, although the presence of the Ah receptor is necessary for biochemical and biological responses to PCDDs and PCDFs, it is not sufficient to explain the qualitative and quantitative differences in biological response. Recently, Denison et al. (*21*) have shown that although Ah receptor levels do not correlate with tissue and species responses to dioxin, there is good agreement between the binding of the TCDD–Ah receptor complex to specific segments of DNA and responsiveness of that cell to enzyme induction by dioxin. Hence, there are other potential sites of regulation of the cell-specific responses to dioxins and furans not solely governed by the concentration of the Ah receptor.

Properties of Ah Receptor

The Ah receptor is a high-molecular weight (110–150 KD) protein with reversible, high-affinity binding for TCDD [K_d 0.1–0.4 nM (*22*)]. This binding can be competed for by other inducers of CyplA1 but not by inducers of other forms of cytochrome P-450 such as phenobarbital (*23*) or the steroids dexamethasone, progesterone, estradiol, or testosterone (*15*). In addition, TCDD is not a ligand for any identified steroid hormone receptors. The Ah receptor is similar in its structure and mechanism to known steroid receptors, although no steroid or endogenous ligand has been found. The Ah receptor is markedly similar among species examined with a 5*S* sedimentation coefficient on sucrose density gradients and a stokes radius of 6.6 nm. The receptor is heat labile and inactivated by trypsin (*24*).

The ontogeny of the Ah receptor has been examined in several rodent species (*25,26*). Receptor levels and peak CyplA1 induction in lung and liver increased postpartum, reaching a maximum by 15–21 days and declining through adulthood. In contrast, Ah receptor levels in the thymus remained constant throughout the study. The expression of this receptor protein does not appear to be regulated by endogenous hormones, as orchiectomy, ovariectomy, adrenalectomy or hypophysectomy had no effect on Ah receptor concentrations. However, it has been noted that 2,3,7,8-TCDD may induce hepatic Ah receptor levels (*27*).

Structure–Activity Relationships

Since the initial studies by Poland et al. (*15*) several other investigators have examined the effect of chlorine substitution on Ah receptor binding and CyplA1 induction [reviewed in Safe (*18*)]. The results of these studies are summarized in Figure 2. With PCDDs, lateral chlorination (positions 2,3,7,8) is quite important for high-affinity binding to the receptor, whereas chlorination at nonlateral positions (1,4,6,9) decreases binding interaction with the receptor protein. Addition of chlorines at nonlateral positions in highly chlorinated PCDDs may decrease receptor binding by altering molecular size, lipophilicity, coplanarity, or aromatic ring electron density. Similar effects of chlorine substitution are noted with PCDFs, where the most active congeners are fully substituted in the 2,3,7, and 8 positions. It has been suggested that a planar ring structure and an ideal ligand binding area of 3 × 10 Å are critical structural features contributing to the high binding affinities of 2,3,7,8-TCDD and -TCDF (*24,28*).

Ah Receptor-Mediated Gene Regulation

The mechanism of action of dioxin and related compounds has been an area of intense scientific study [for review see Whitlock (*29*)]. The induction of CyplA1 is commonly used as the model system for TCDD's mechanism of gene regulation, although other proteins have been shown to be under direct control of the Ah receptor. As summarized in Figure 3, the induction of CypA1 requires multiple events, many of which may be under tissue- and species-specific regulation. As discussed above, TCDD and related compounds enter the cell through passive diffusion and bind to the Ah receptor, presumably in the cytosol. Similar to hormone receptors, upon ligand binding the receptor undergoes a transformation or activation step. The Ah receptor exists in its inactivated form as a large protein complex containing both 95 kD ligand binding and heat shock protein(s) [HSP 90 (*30,31*)]. Activation of the Ah receptor may involve dissociation of these heat shock proteins following the binding of TCDD.

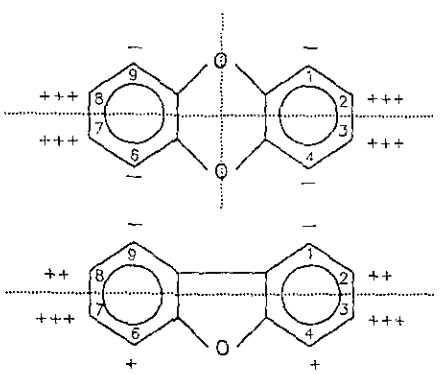

FIGURE 2. Summary of the effects of chlorine substituents on different position in the dibenzo-*p*-dioxin (top) and dibenzo-*p*-furan rings on the relative binding to the Ah receptor. The (+) indicates that addition of a chlorine increases affinity for the receptor; (−) denotes a decrease in affinity. Adapted from Safe (*18*).

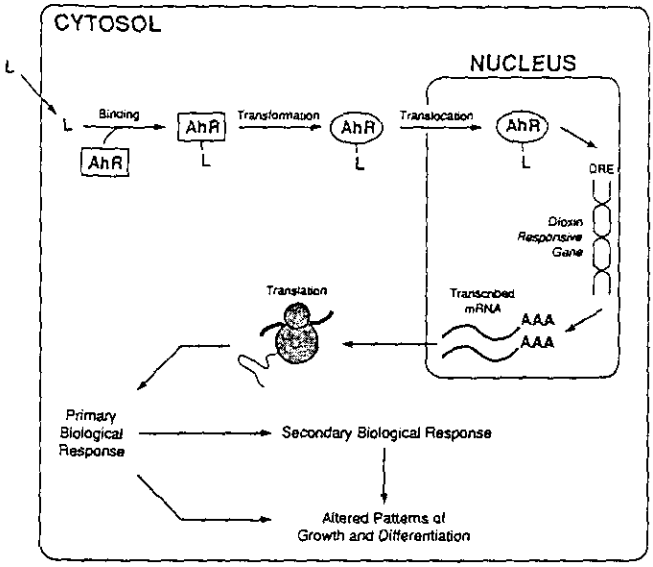

FIGURE 3. Proposed mechanism of action of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans. Ligand (L) passively enters the cell and encounters and binds to the Ah receptor (AhR). This ligand-AhR complex undergoes a structural transformation (or activation) followed by translocation to the nucleus. The activated receptor complex then recognizes and binds to specific regions (dioxin-responsive elements or DREs) 5′ to a dioxin-responsive gene. Binding to DREs results in an increase in gene transcription of several genes. The transcribed mRNA is translated in the cytosol resulting in the synthesis of cytochrome P-450s (primary biological responses) as well as a multitude of other biological responses such as altered patterns of growth and differentiation.

Subsequent to receptor transformation, nuclear translocation of the TCDD-AhR complex occurs before binding to DNA. Recently the cDNA and part of the gene for a 87 kD human protein required for nuclear translocation of the ligand-binding subunit has been cloned and named the arnt (Ah receptor nuclear translocation) protein (*29*). In the ligand-bound form, the nuclear Ah receptor functions as a *trans*-activator of several genes including *Cyp1A1* (*1*). The activated ligand-bound receptor binds to a core DNA recognition motif found within several enhancers, designated as dioxin responsive elements (DREs). In addition to induction of cytochromes Cyp1A1 and Cyp1A2, other primary biological responses include induction of glutathione-*S*-transferase, menadione oxidoreductase, and aldehyde dehydrogenase. Using a subtractive hybridization technique, a recent study has shown that plasminogen activator inhibitor-2 (PAI-2) and a yet unidentified clone are induced at the level of gene transcription by TCDD (*33*).

Primary biological responses such as those described above may result in secondary and tertiary effects. Many secondary biological responses exist due to dioxin's perturbation of endocrine systems, i.e., hormones and hormone receptors. Dioxin and structural analogs affect endocrine components such as the estrogen receptor (*34*) tumor necrosis factor α (*35*), c-*erb*-a (*36*), gastrin (*37*), and interlelukin 1β (*33*). The effects on many diverse endocrine systems shows a similarity between dioxin and endogenous hormones such as glucocorticoids. A good example of a secondary effect of dioxin on an endocrine system is the downregulation of the epidermal growth factor receptor (EGFR). A decrease in membrane-bound EGFR is a sensitive response of the liver to PCDDs and PCDFs, although it is not a result of decreased EGFR mRNA (*38*). Alternatively, the decreased membrane-associated EGFR may be due to internalization of the receptor as a result of dioxin-induced production of TGF-α, an alternative ligand for EGFR. The downregulation of EGFR has been implicated in the altered patterns of growth and differentiation characteristic of dioxin-related toxic effects. In fact, many of the endocrine effects of dioxin and related compounds, albeit secondary responses, may have profound effects on cell differentiation and proliferation.

Toxicity of PCDDs and PCDFs in Laboratory Animals

Carcinogenesis

The most common end point used in assessing the possible human health risk to PCDDs and PCDFs is carcinogenesis in laboratory animals following chronic exposure. The available data are summarized in Table 4. There is little information on the carcinogenicity of PCDFs. 2,3,7,8-TCDD and the hexachlorodibenzo-*p*-dioxin (HCDD) mixture act as complete carcinogens, producing both common and uncommon tumors at multiple sites; dibenzo-*p*-dioxin and 2,7-dichlorodioxin are weak or noncarcinogens. TCDD is an extremely potent carcinogen in animal studies, producing carcinogenic effects at doses as low as 0.001 μg/kg/day. Hepatocellular carcinomas have also been reported in mice for the related halogenated hydrocarbons polybrominated and polychlorinated biphenyls (*39–41*).

There is considerable evidence that PCDDs and PCDFs do not act as genotoxic carcinogens. That is, TCDD and its structural analogs do not form covalent DNA adducts in *in vitro* or *in vivo* systems and are negative for genetic toxicity in short-term tests (*42,43*). Several studies have shown that TCDD acts as a tumor promoter in multistage models for experimental carcinogenesis in liver (*44–47*) and in skin (*48*). In fact, TCDD is two to three orders of magnitude more potent than the prototypical promoting agent 12-*O*-tetradecanoylphorbol-13-acetate in skin (*48*).

Chronic bioassays as well as two-stage models for liver altered-enzyme foci demonstrate that female rats are more susceptible to TCDD-induced liver tumors than are male rats. Recent studies have shown that ovarian hormones are essential to the tumor-promoting actions of TCDD in rat liver (*46*). Although the presence of the ovaries is necessary for the hepatocarcinogenic effects, ovarian dependence was not observed in all tissues. In fact, ovariectomy increases the risk for lung tumors as a result of TCDD treatment (*46*). Taken together, these data suggest that tissue-specific carcinogenic effects of TCDD reflect a complex interaction with hormones and their receptors.

Noncarcinogenic End Points

Although most regulatory agencies use rodent carcinogenesis as the principal toxic end point by which to extrapolate human health risk, many noncarcinogenic effects exist and these are now receiving increased attention. In laboratory animals, TCDD and related halogenated hydrocarbons produce a multitude of toxic responses which vary both quantitatively and qualitatively with the species, strain, and sex of the animal examined. The results of enumerable studies are briefly summarized on next page.

Table 4. Carcinogenicity of PCDDs.[a]

Isomer	Dose	Species	Tumor type
2,3,7,8-TCDD	0.001 μg, 3 times per week, skin painting	Male mice	None
2,3,7,8-TCDD	0.005 μg 3 times per week, skin painting	Female mice	Fibrosarcoma of integumetry system
2,3,7,8-TCDD	0.01, 0.05, 0.5 μg/kg/week by gavage	Male rats	Thyroid follicular cell adenoma (0.5 μg)
2,3,7,8-TCDD	0.01, 0.05, 0.5 μg/kg/week by gavage	Female rats	Liver neoplastic nodule/carcinoma (0.5 μg), pituitary adenoma (0.01 μg)
2,3,7,8-TCDD	0.01, 0.05, 0.5 μg/kg/week by gavage	Male mice	Hepatocellular adenoma/carcinoma (0.5 μg), alveolar/bronchiolar adenoma (0.5 μg)
2,3,7,8-TCDD	0.04, 0.2, 2 μg/kg/week by gavage	Female mice	Hepatocellular adenoma/carcinoma (2 μg), Thyroid follicular cell adenoma (2 μg), histocytic lymphoma (2 μg)
2,3,7,8-TCDD	0.001, 0.01, 0.1 μg/kg,/day in diet	Male rats	Squamous cell carcinoma of hard palate/nasal turbinates and tongue (0.1 μg), adenoma of adrenal cortex (0.1 μg)
2,3,7,8-TCDD	0.001, 0.01, 0.1 μg/kg/day in diet	Female rats	Hepatocellular hyperplastic nodules (0.01 and 0.1 μg), squamous cell carcinoma of hard palate/nasal turbinates and tongue (0.1 μg), hepatocellular carcinoma (0.1 μg), keratinizing squamous cell carcinoma of lung (0.1 μg)
Dibenzo-*p*-dioxin	5000 or 10,000 ppm in diet	Rats and mice	None
2,7-DCDD	5000 or 10,000 ppm in diet	Rats	None
2,7-DCDD	5000 or 10,000 ppm in diet	Female mice	None
2,7-DCDD	5000 or 10,000 ppm in diet	Male mice	Hepatocellular adenoma (5000 and 10,000 ppm), leukemia/lymphoma (5000 ppm)
HCDD mixture[b]	1.5, 2.5, 5 μg/kg/week by gavage	Rats	Hepatocellular adenoma and carcinoma/neoplastic nodules (5 μg in males, all doses in females), follicular cell adenoma of thyroid (1.25 μg, males)
HCDD mixture	1.5, 2.5, 5 μg/kg/week by gavage	Male mice	Hepatocellular adenoma and carcinoma/neoplastic nodules (5 μg)
HCDD mixture	2.5, 5, 10 μg/kg/week by gavage	Female mice	Hepatocellular adenoma and carcinoma/neoplastic nodules (5 μ)
HCDD mixture	Skin painting	Mice	None

Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; DCDD, dichlorodibenzo-*p*-dioxin; HCDD, hexachlorodibenzo-*p*-dioxin.
[a]Adapted from Skene et al. (*1*).
[b]Mixture of 1,2,3,6,7,8 and 1,2,3,7,8,9 isomers.

Table 5. Toxic potency (LD_{50}) of various PCDDs.[a]

	LD_{50}, μg/kg, for					
Isomer	Guinea pig	Monkey	Rat	Rabbit	Mouse	Hamster
2,3,7,8	0.6–1	70	25–60	100	200–600	5500
1,2,3,4	—	—	800	—	—	—
2,4,8	—	—	5000	—	—	—
2,3,7	29,400	—	—	—	—	—
2,8	300,000	—	—	—	—	—
1,2,4,7,8	1,125	—	—	—	—	—
1,2,3,4,6,7,8,9	—	—	—	1000	—	—

[a]Adapted from Vickers et al. (*19*).

Acute Toxicity. Certain toxic responses are consistently observed regardless of the test animal studied, including progressive body weight loss and hypophagia, thymic atrophy. (especially of the cortex), gastrointestinal hemorrhage, and delayed lethality. The acute LD_{50} (dose associated with 50% lethality) of TCDD varies over a 5000-fold range, with the guinea pig being the most sensitive species and the hamster being the least sensitive (Table 5). A similar range (10^3–10^4 difference) in toxicity is observed for other PCDDs with the same general order of species' sensitivity and pattern of toxic responses. A common characteristic of TCDD-induced toxicity is a pathologic "wasting syndrome," or cachexia, with reduced feed intake and depletion of adipose fat stores. The decrease in feed intake is not wholly responsible for the decreased body mass and implicates altered energy metabolism (*49*). The cause for the cachexia and delayed lethality is not known; however, effects on vitamin A, thyroid hormones, and tumor necrosis factor (TNF) have been suggested as possible mediators. Recently it has been shown that antibodies to TNF can decrease TCDD lethality (*35*).

Many pathological changes are observed following administration of TCDD, including testicular degeneration, muscular necrosis, hepatomegaly, bile duct proliferation, fatty infiltration of tissues, and fluorescence of bones (indicative of porphyrin deposition). The production of chloracnelike lesions has been reported in hairless mice following dermal application of TCDD. The response of cells to PCDDs and PCDFs, i.e., hyperplasia versus hypoplasia, is highly species- and tissue-sensitive. For example, following exposure to TCDD, the rodent liver displays primarily a hyperplastic response, whereas the thymus shows an atropic response (*19*). Factors such as Ah receptor concentration, translocation, or DNA bindings as well as various endocrine effects of PCDDs and PCDFs, may influence this differential cellular response.

Immunotoxicity. Immunotoxicity testing with 2,3,7,8-TCDD was initially undertaken to further characterize the lymphoid involution and thymic atrophy seen in general toxicity testing [for review see Holsapple et al. (*50*)]. PCDDs and PCDFs have both direct and indirect effects on immunocompetence, and the extent of the effects vary with the developmental stage of the animal. Both T-cell-mediated and humoral (B cell) immunity are affected. TCDD prevents thymocyte maturation and induces terminal differentiation of thymic epithelial cells. The primary effect of TCDD on cell-mediated responses may be due to activation of T-suppressor cells (*50*). Humoral immune responses, i.e., production of antibodies by B-cells, are affected by TCDD in adult mice, but not in those exposed perinatally. The effect on humoral immune responses were shown to be a direct effect of the xenobiotic on B-lymphocytes.

The developing immune system appears to be particularly sensitive to the suppressive effects of TCDD. Perinatal exposure to TCDD in rats leads to a prolonged reduction in delayed hypersensitivity and lymphoproliferative responses (*51*). In addition, following perinatal exposure to TCDD, a significant increase in mortality due to endotoxin administration and reduction in plaque-forming cells was observed (*52*).

Structure–activity relationships of PCDDs demonstrate that effects on the immune system parallel those of CYP1A1 induction (*1*). Also, 2,3,7,8-TCDF has been shown to be an immunosuppressor in guinea pigs, although it is much weaker than TCDD (*52*).

Developmental and Reproductive Toxicity. As stated above, the developing organism is quite sensitive to the effects of PCDDs and PCDFs. TCDD is a potent fetotoxin and teratogen, although there are extreme species differences in responses. Exposure of mice to TCDD and related compounds results in a highly reproducible and characteristic teratogenic response including hydronephrosis and cleft palate (*53*). These structural malformations in mice are seen at doses much lower than needed to cause maternal or fetal toxicity and are some of the most sensitive effects known for dioxin exposure in laboratory animals (*54*). In other laboratory animals tested, TCDD causes maternal/fetal and developmental toxicity but does not lead to a significant increase in structural abnormalities, even at toxic doses.

Work done primarily with polychlorinated biphenyls suggests that PCDDs and PCDFs may also result in developmental neurotoxicity (*55*). Exposure of birds, rodents, and monkeys to complex mixtures of PCBs causes persistent changes in cognitive behaviors such as learning and memory and alterations in the rate of maturation of sensomotor reflexes in offspring. However, it is not known which PCBs are producing these effects and whether TCDD-like activity is necessary.

TCDD exposure has been shown to decrease female fertility and general reproductive performance (*1*). Although the male reproductive system in sexually mature rats is relatively insensitive to TCDD exposure, *in utero* and lactational exposure to TCDD inhibits sexual differentiation of the central nervous system (*56*). Exposure of dams to low doses of TCDD (0.064 μg/kg) had consequences in the male pups that extended into adulthood, including decreased sex organ weights, impaired spermatogenesis, and demasculinization and feminization of sexual behavior. Thus, the reproductive system of the male rat is highly sensitive to perinatal TCDD exposure.

Toxic Equivalency Factors

Although there are extensive data on the toxicity of 2,3,7,8-TCDD, toxicological information on the other 209 compounds in the PCDD and PCDF family is much more sparse. Consequently, the risk assessment of complex mixtures of environmental contaminants must be estimated based on limited experimental information.

In 1977, Donald Grant proposed a simple approach to this problem taking into account the mechanism of action of PCDDs and PCDFs (*57*). As stated above, 2,3,7,8-TCDD and other polycyclic aromatic hydrocarbons elicit their effects through interaction with a specific receptor. Although 2,3,7,8-TCDD is the most potent member of this family, other compounds that interact with the Ah receptor result in similar effects, albeit at higher doses. Grant hypothesized that the potency of PCDDs and PCDFs correlates with affinity for Ah receptor and early sequelae (i.e., CYP1A1 induction). These relative potencies are expressed as toxicity equivalency factors (TEFs), as calculated below:

$$\begin{aligned}&\text{Estimated 2,3,7,8-TCDD-like toxicity of a mixture of PCDDs and PCDFs}\\ &= \text{concentration of toxic equivalents} = [\text{TEFs}]\\ &= \Sigma_i(\text{TEF})_i \times [\text{PCDD or PCDF}]_i\end{aligned}$$

where $(\text{TEF})_i$ is the relative potency of the PCDD or PCDF compared with 2,3,7,8-TCDD, i.e.,

$$(\text{TEF})_i = \frac{(\text{potency of } i\text{th PCDD or PCDF})}{(\text{potency of 2,3,7,8-TCDD})}.$$

After examining the relative potency of different PCDDs and PCDFs for a variety of end points both *in vivo* and *in vitro*, such as cancer, reproductive effects, body weight loss, cell transformation, immunotoxicity and Ah receptor binding, a set of TEFs has been adopted by several regulatory agencies ("1988 International Toxic Equivalency Factors" or I-TEFs). As shown in Table 6, these relative potencies can be used to convert concentrations of PCDDs and PCDFs found in environmental samples to the equivalent concentration of 2,3,7,8-TCDD. For example, 2,3,7,8-TCDD is 10 times more potent than 2,3,7,8-TCDF assessed by lethality in rats. Therefore, the TEF for this furan congener is 0.1 "dioxin equivalents." By multiplying the TEF of a congener by its concentration in an environmental sample, an estimate of the toxicity can be obtained. In this instance it is estimated that 0.12 ppt TCDF will have effects equivalent to 0.012 ppt of TCDD. The sum of the toxic equivalents for all congeners present represents the estimated 2,3,7,8-TCDD-like toxicity of a mixture of PCDDs and PCDFs. A major drawback of the TEF approach is that an assumption is made that all isomers found in a mixture have additive effects. However, several researchers have shown additive, synergistic, and antagonistic effects within mixtures of PCDDs or PCDFs. Also, TEFs are often based on *in vitro* data, where possible effects on the rate of clearance of isomers is negated. For example, the rate of clearance of TCDF is much more rapid than that of TCDD. However, both congeners have equivalent affinity for the Ah receptor. Therefore, if Ah receptor binding *in vitro* is the sole determinant of TEF calculations, the risk posed by TCDF would be overestimated. Despite negative aspects to the use of TEFs, they have been shown to give

Table 6. Converting congener-specific source data to toxic equivalents.[a]

Congener	Concentration, ppt[b]	Toxic equivalency factor (TEF)	Toxic equivalents (TEQ)
2,3,7,8-TCDD	0.11	1	0.11
1,2,3,7,8-PeCDD	0.18	0.5	0.09
1,2,3,4,7,8-HxCDD	0.08	0.1	0.008
1,2,3,6,7,8-HxCdd	0.73	0.1	0.073
1,2,3,7,8,9-HxCDD	0.15	0.1	0.015
1,2,3,4,6,7,8-HpCDD	1.3	0.01	0.013
OCDD	5.7	0.001	0.0057
Total PCDDs			0.31
2,3,7,8-TCDF	0.12	0.1	0.012
1,2,3,7,8-PeCDF	0.022	0.05	0.0011
2,3,4,7,8-PeCDF	0.51	0.5	0.26
1,2,3,4,7,8-HxCDF	0.097	0.1	0.0097
1,2,3,6,7,8-HxCDF	0.078	0.1	0.0078
2,3,4,6,7,8-HxCDF	0.04	0.1	0.004
1,2,3,4,6,7,8-HpCDF	0.19	0.01	0.0019
OCDF	0.062	0.0008	0.000052
Total PCDFs			0.30
Total TEQ			0.61

Abbreviations: T, tetra; Pe, penta; Hx, hexa; Hp, hepta; O, octa; CDD, chlorodibenzo-*p*-dioxin; CDF, chlorodibenzofuran.
[a]Adapted from Barnes (*51*).
[b]Concentration of PCDDs and PCDFs found in an environmental sample.

a fair estimate of enzyme induction by complex mixtures of PCDDs (*58*). Thus, until more detailed data on the biological effects of other PCDD and PCDF congeners become available, especially within the framework of interactions between congeners, the TEF approach is based on a solid scientific foundation and provides our only means to estimate the health risk posed by complex mixtures of polychlorinated aromatic hydrocarbons.

Laboratory Animal–Human Concordance

Although much is known of the biological and toxic effects of dioxin and related compounds in experimental animals, little information is available on their effects in humans. The data available on humans are based on *in vitro* (i.e., in culture) as well as epidemiological studies. A comparison of the effects of PCDDs and PCDFs on laboratory animals versus humans is given in Table 7. *In vitro* systems such as keratinocytes or thymocytes in culture have clearly shown that not only do human cells possess Ah receptors, but they respond similarly to cells derived from rodents. Epidemiological studies suggest that humans exposed *in vivo* to PCDDs and PCDFs respond similarly to experimental animals, although the data available are not always clear.

A comparative study on the effects of TCDD on rat liver versus those in placentas of women exposed to PCDF-contaminated rice oil has recently been reported (*59*). Induction of CYP1A1 and effects on the epidermal growth factor receptor and glucocorticoid receptor were observed in both species. In fact, humans may be more sensitive to effects of toxic halogenated hydrocarbons than rats, although the correlation between these events (i.e., CYP1A1 induction) and toxic end points such as cancer are not known.

Several reports in the literature suggest that exposure of humans to dioxin and related compounds may be associated with cancer at many different sites including malignant lymphomas, soft tissue sarcomas, thyroid tumors, and lung tumors (*60–62*). Recently two large cohort studies performed by the International

Table 7. Similarities between laboratory animals and humans in biological effects of TCDD.[a]

Effect	Laboratory animals	Humans
In vitro		
Presence of Ah receptor	+	+
Enzyme induction	+	+
Altered patterns of growth and differentiation	+	+
Immunosuppression	+	+
Chloracnogenic response	+	+
In vivo		
Presence of Ah receptor	+	+
Enzyme induction	+	+
Altered lipid metabolism	+	+
Immune effects	+	+/–
Cancer	+	+/–
Reproductive effects	+	+/–
Teratogenic effects	+	+/–
Altered epithelial cell differentiation	+	?
Tumor promotion	+	?

[a]Adapted from Silbergeld and Gasiewicz (*77*). The (+) indicates a clear association; (+/–) indicates conflicting or unclear associations; (?) indicates that nothing is known about the effects of TCDD on the system.

Agency for Research on Cancer (IARC) and the National Institute of Occupational Safety and Health (NIOSH) have been completed. Both studies included individuals who were suspected to have been exposed to dioxin as a result of occupation. An increase in thyroid tumors was noted in the IARC registry. Increased risk of all cancers was observed in the NIOSH registry as well as increased risk of respiratory tract cancer (*63*). Mortality from several cancers in the Sevaso, Italy, area including biliary cancer has been reported (*64*). Although several earlier studies showed a lack of liver tumors in humans, the majority of cohorts were male. Based on data obtained in rats (*46*), tumor formation by TCDD is partially dependent on ovarian hormones, and male rats show relatively few altered hepatic foci. However, TCDD-induced lung tumors are much more prevalent in male rats. This increase in lung tumors in males has been reported in humans (*64*). Therefore, the human carcinogenicity data are consistent with the rodent data presented earlier (Table 4). In

fact, the carcinogenicity of dioxin in rodents may help point to cancers in humans for future epidemiological examination, such as those of the lung and thyroid gland.

Several noncarcinogenic effects of PCDDS and PCDFS show good concordance between laboratory species and humans as well. For example, in laboratory animals TCDD causes altered intermediary metabolism manifested by changes in lipid and glucose levels. In alliance with these results, workers exposed to TCDD 7–8 years previously during the manufacture of trichlorophenol showed elevated total serum triacylglycerides and cholesterol with decreased high-density lipoprotein concentration [HDL (*65*)]. Recently, the results of a statistical analysis of serum dioxin analysis and health effects in Air Force personnel following exposure to Agent Orange was reported (*66*). Significant associations between serum dioxin levels and several lipid-related variables were found, e.g., percent body fat, cholesterol, triacylglycerols, and HDL. Another interesting result of these studies was a positive relationship between dioxin exposure and diabetes, to our knowledge the first report of such an association.

The human-to-experimental animal comparison is confounded by at least two factors: *a*) For every toxic effect produced by dioxin, there is marked species variation. An outlier or highly susceptible species for one effect, e.g., guinea pigs for lethality or mice for teratogenicity, may not be an outlier for other responses. *b*) Human toxicity testing is based on epidemiological data comparing "exposed" to "unexposed" individuals. However, as shown in Table 2, the "unexposed" cohorts contain measurable amounts of background exposure to PCDDS and PCDFs. Also, the results of many epidemiological studies are hampered by small sample size, and in many cases the actual amounts of dioxin and related compounds in the human tissues were not examined. However, based on the available information, it appears that humans are sensitive to several of the toxic efffects of PCDDs and PCDFs and that there is good agreement with the effects observed in laboratory species.

Dose–Response Relationships

Receptor-Mediated Events

There is considerable controversy regarding the validity of various mathematical models used to estimate human health risk to dioxins and related chemicals. For example, the U.S. EPA (using a linear multistage model) and Canadian Health and Welfare Department (using a threshold model) set acceptable daily intakes at 6 fg/kg/day and 10,000 fg/kg/day, respectively. Amazingly, this enormous difference in acceptable daily intake between the agencies is derived from the same data, that of Kociba et al. (*67*).

Obviously there is great need to generate new models for risk assessment of PCDDs and PCDFs based on the increasing knowledge of the mechanism of action of these xenobiotics. The central hypothesis regarding the biological effects of TCDD and related chemicals is that the presence of the Ah receptor is necessary but not sufficient to result in a response. Therefore, the effects of TCDD and related chemcials can be summarized using classical pharmacology relationships:

$$R + A \rightleftharpoons RA \rightarrow\rightarrow\rightarrow \text{Effect}$$

$$\frac{E_A}{E_{max}} = \frac{[RA]}{R_T} = \frac{[A]}{K_d + [A]}$$

where R is the Ah receptor and A is a PCDD or PCDF. The simplist assumption based on these relationships is that the response is linear, i.e., response E_A is directly proportional to the fractional receptor occupancy and one-half of the maximal response occurs at the drug concentration equivalent to K_d. The log dose versus response curves for most drugs is sigmoidal, with an initial phase where little change in response is noted at increasing drug concentration. This fact has led many to believe that there must be a threshold for the biological effects of TCDD. However, when a simple relationship between receptor occupancy and biological response exists, there is no threshold. It is also important to note that not all receptor-mediated events share the same dose–response characteristics. As shown in Figure 4, basic pharmacological theory dictates that receptor-mediated effects on various target organs may differ in both efficacy and potency of response depending on many tissue-specific factors such as receptor concentration, ligand-binding characteristics, and recognition and binding to regulatory regions of DNA.

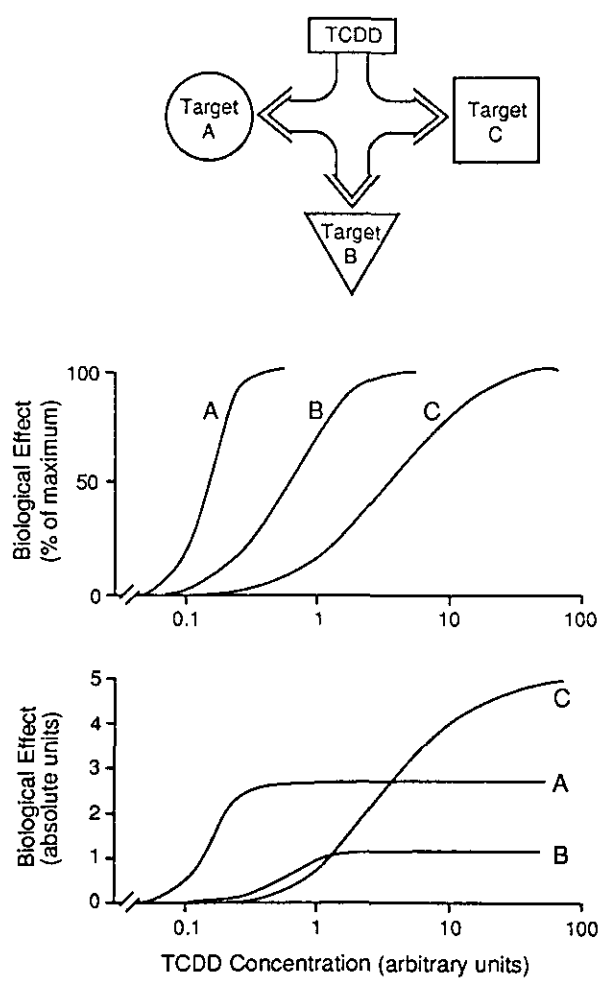

FIGURE 4. Target cell specificity of TCDD action.

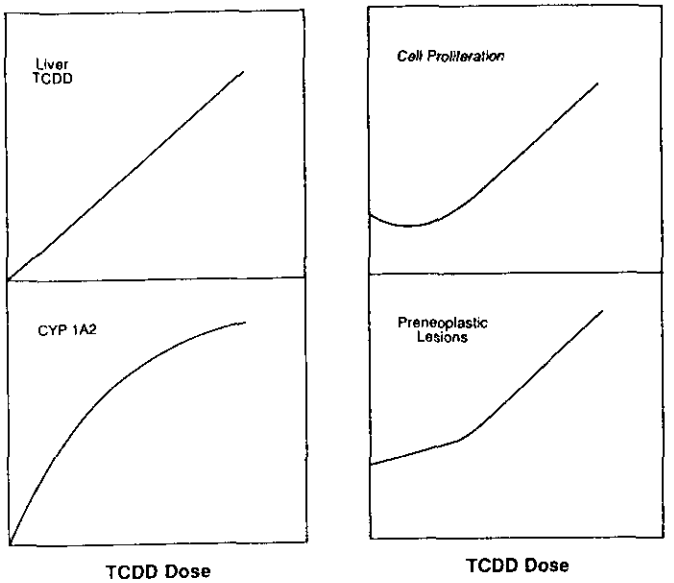

FIGURE 5. Representation of hepatic dose–response relationships for TCDD following chronic administration of (0–100 ng/kg/day) in diethylnitrosamine-initiated female rats. Adapted from Tritscher et al. (*68*).

As shown in Figure 5, the dose–response characteristics of chronic TCDD administration resemble those that may be predicted from the discussion above. That is, CYP1A1 and 1A2 induction are sigmoidal and show no evidence for a threshold (*68,69*). For more complex biological response, i.e., those requiring multiple events such as cell proliferation or formation of preneoplastic lesions, the dose–response curves show more variability and complexity. Therefore, if the induction of CYP1A1 were used to estimate the cancer risk posed by TCDD, then the acceptable daily intake (1 cancer in 10^6 individuals) would be similar to the current EPA standard of 0.006 pg/kg/day. However, increases in cell proliferation, possibly a better indicator of cancer risk, are only detected at much higher doses than those needed for enzyme induction. Using cell proliferation data, the acceptable daily intake may be higher than predicted by the U.S. EPA.

Interindividual Variability in Responses

An important aspect of extrapolating data to the whole of the human population is that the response to environmental contaminants is highly variable among individuals. Therefore, a certain population may be genetically more suceptible to the effects of PCDDs and PCDFs. The appearance of chloracne as a result of dioxin exposure in humans is clearly a response with considerable interindiviual variation. For example, in the Sevaso incident there were individuals who showed no chloracnogenic response despite significant exposure to TCDD, while cohorts with much lower exposure exhibited chloracne (*70*). Recently in our laboratory we have demonstrated that the induction of CYP1A1 activity in humam lymphocytes by TCDD *in vitro* falls into a bimodal distribution with high-responders and low-responders (*71*). This indicates that there may be genetic differences in the capacity of human cells to respond to TCDD. A high inducibity phenotype for CYP1A1 induction may be associated with increased susceptibility to lung cancer (*71–74*). Therefore, induction of CYP1A1 activity may be useful for phenotyping susceptible individuals. Interindividual differences in human CYP1A1 induction by TCDD may reflect a polymorphism in the *CYP1A1* gene as well as differences in the Ah receptor itself.

Future Considerations and Conclusions

The ubiquitous presence of PCDDs and PCDFs in the environment and in human tissues has been a major health concern for over 20 years. Significant advancement has been made in understanding the mechanism by which this group of chemicals produce their characteristic enzyme induction. However, the use of these relatively simple responses may be inappropriate for estimating the cancer risk to these compounds. A primary response such as enzyme induction may not be the mechansim by which these compounds cause cancer. The sequence in events initated by the Ah receptor interacting with dioxin-responsive genes and ending with altered patterns of differentiation and growth must be sought. In this manner other surrogates for cancer risk may be based on the mechanism of the tumorigenic response and not solely on enzyme induction. With a knowledge of the sequelae of events necessary to produce tumors, questions may be answered on tissue, species, sex, and interindividual variation in biological responses to dioxin.

Available results indicate that not only does the Ah receptor play an essential role in the toxicity of dioxin and related chemicals, but this receptor may also have a role in cellular differentiation (*16*) and possibly in wound healing (*75*). The pleiotropic response produced by TCDD–Ah receptor includes induction of xenobiotic metabolizing enzymes (P-450 and glutathione transferase) as well as causing altered differentiation of epithelial cells. To date no endogenous ligand has been described for the Ah receptor. Interestingly, several proteins that have been shown to be affected by TCDD have growth-regulatory functions including plasminogen activator inhibitor-2, tumor necrosis factor-α, epidermal growth factor receptor, interleukin 1β, and transforming growth factor-α. In addition, these proteins are secreted into the bloodstream, where they may exert effects on cells that do not contain the Ah receptor. Dissecting the mechanism of action of PCDDs and PCDFs on gene regulation may ultimately lead to a greater understanding of the regulation of cellular differentiation by these endogenous cytokines and lymphokines. Despite extensive research efforts, the effects of PCDDs and PCDFs on humans are not well characterized. However, available data indicates that in general there is good agreement between known effects of dioxin in laboratory animals and those described in epidemiological studies for effects in humans. Therefore, in all likelihood, dioxin and related compounds do pose a threat to human health. Although the debate over "safe" levels of PCDDs and PCDFs will probably continue, the lessons learned from these compounds may have impact on the regulation of other chemicals as well.

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