The Mechanism of Dioxin Toxicity: Relationship to Risk Assessment

Linda S. Birnbaum
U.S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, North Carolina

Risk characterization involves hazard identification, determination of dose-response relationships, and exposure assessment. Improvement of the risk assessment process requires inclusion of the best available science. Recent findings in the area of dioxin toxicity have led to a major effort to reassess its risk. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), commonly referred to as "dioxin," is the most toxic member of a class of related chemicals including the polyhalogenated dibenzo-p-dioxins, dibenzofurans, biphenyls, naphthalenes, azo- and azoxy-benzenes, whose toxicities can be expressed as fractional equivalencies of TCDD. These chemicals exert their effects through interaction with a specific intracellular protein, the Ah receptor. While binding to the receptor is necessary, it is not sufficient to bring about a chain of events leading to various responses including enzyme induction, immunotoxicity, reproductive and endocrine effects, developmental toxicity, chloracne, tumor promotion, etc. Some of these responses appear to be linear at low doses. Immunotoxicity and effects on the reproductive system appear to be among the most sensitive responses. The Ah receptor functions as a transcriptional enhancer, interacting with a number of other regulatory proteins (heat shock proteins, kinases, translocases, DNA binding species). Interaction with specific base sequences in the DNA appear to be modulated by the presence of other growth factors, hormones, and their receptors as well as other regulatory proteins. Thus, dioxin appears to function as a hormone, initiating a cascade of events that is dependent upon the environment of each cell and tissue. While Ah receptor variants exist, all vertebrates examined have demonstrated such a protein with similar numbers of receptors and binding affinity for TCDD. Most species respond similarly to dioxin and related compounds. While a given species may be an outlier for a given response, it will behave like other animals for other responses. For both in vivo and in vitro end points where animal and human data exist, such as enzyme induction, chloracne, immunotoxicity, developmental toxicity, and cancer, the sensitivity of humans appears similar to that of experimental animals. Current levels of environmental exposure to this class of chemicals may be resulting in subtle responses in populations at special risk such as subsistence fisherman and the developing infant, as well as in the general population. Increased understanding of the mechanism of dioxin's effects as well as elucidation of exposure-dose relationships is leading to the development of a biologically based dose-response model in the ongoing process of incorporating the best science into the risk assessment of TCDD and related compounds.—Environ Health Perspect 102(Suppl 9):157-167(1994)

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Introduction

Risk assessment is a scientific process that can be divided into several stages. These phases involve exposure assessment, hazard identification, and elucidation of dose-response relationships. Integration of these various activities results in scientific risk characterization. These assessments can then be put in the context of economic considerations, societal benefits, policy considerations, etc., when formulating a risk management decision. Thus, while risk assessment is an integral component of risk management, this scientific exercise is only part of the decision-making process.

Improvement of the risk assessment process requires the incorporation of the best and most current scientific thinking. In place of default positions which make certain assumptions, dosimetric and mechanistic information can be incorporated to reduce the uncertainties involved in risk characterization.

Recent findings in the field of dioxin toxicity have led to a major effort to improve the assessment of its risk. This was in response to a meeting of international experts held under the auspices of the Banbury Center (I) in the fall of 1990. While this was not a consensus conference, general agreement was reached on several issues: a) as far as is known at this time, all dioxin effects are mediated through the Ah receptor; b) people have sensitivity similar to animals to dioxin effects and c) compounds which are related in structure to dioxin and have the same mechanism of action need to be considered as part of the dioxin problem. Therefore, having a better understanding of the hazard and the mechanism of dioxin effects, a more biologically based risk assessment should be achievable.

There was not general agreement on what a "safe" level of dioxin would be, although there was a hypothesis suggested that if a threshold for receptor activation existed, then there would be an exposure level at which no responses could occur. Research needs were identified to address this crucial question.

The focus of this manuscript is to address the issue of the mechanism of dioxin toxicity and how this information can improve the risk assessment process.

Background

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD, dioxin) is the most toxic member of a class of planar, halogenated chemicals (Figure 1). It has no known industrial or commercial use and has been produced as an unwanted byproduct of certain industrial processes and combustion. TCDD has been produced during the production of certain chlorinated phenols and their derivatives, and as a result of high temperature pyrolysis and combustion of organic compounds containing halogens. Chlorine bleaching of paper pulp has also led to the
production of dioxin in paper products. Dioxin is extremely stable, both to envi-
ronmental and biological breakdown, leading to its persistence in the environment and its bioaccumulation in the food chain. Because of its high lipophilicity and water insolubility, dioxin concentrates in sedi-
ments and is incorporated into the fatty tis-
sue of fish, birds, reptiles, and mammals. Much of its presence in plants is due to atmospheric transport on particles, result-
ning in settling on the leafy tissues of plants. Dioxin can also be found in consumer products such as chlorinated herbicides, chlorinated phenol-containing products, and contaminated paper goods (2).

**Biological Responses to TCDD**

Dioxin causes a broad range of effects, some of which are species specific (3–5). Dioxin is often described as the most toxic man-
made chemical because of the low doses which cause lethality in certain animal species such as the guinea pig. Dioxin causes delayed lethality, the time to death being dependent on the species in question, not on the doses (6). For example, the time to death varies from 1 to 2 weeks in the guinea pig, 2 to 3 weeks in the rat, 3 to 4 weeks in the mouse, and 6 to 8 weeks in the monkey. Death is usually preceded by a severe loss of body mass, called the wasting syndrome. Laboratory animals often lose from one third to one half of their body weight prior to death. This process is noticeable within days of a lethal dose. Nonlethal, but highly toxic, doses may also result in severe wasting (7). Atrophy of the lymphoid tissues, such as the thymus and spleen, and of the testes occurs at acutely toxic doses in adult animals.

Hyperplastic and/or metaplastic changes are also characteristic of certain epithelial tissues. The liver is a dioxin tar-
gel organ in many species (8). Increase in liver size can occur at relatively low doses, reflecting not only enzyme induction but also changes in lipid content. Necrosis and fatty changes occur at higher doses. In the guinea pig, effects on the liver can be observed at the ultrastructural level (9). No effects on liver function have been observed in highly exposed humans (10).

Table 1. Biochemical effects of TCDD.

| Effect                        | TCDD-sensitive Species |
|-------------------------------|------------------------|
| A. Enzyme Induction           |                        |
| CYP1A1 (3)                    |                        |
| COP1A2 (3)                    |                        |
| DT-diaphorase (3)             |                        |
| UDP-glucuronitransferase (3)  |                        |
| Glutathione-S-transferase (3) |                        |
| Aldehyde dehydrogenase (3)   |                        |
| Ornithine decarboxylase (3)   |                        |
| Tyrosine kinase (130,131)     |                        |
| Terminal deoxynucleotidyltransfease (16) |   |
| Phosphoenolpyruvate carboxykinase (132) |   |
| Plasminogen activator inhibitor-2 (189) |   |
| B. Modulation of hormones and receptors |   |
| Altered homeostasis           |                        |
| Androgens (188,133,87)        |                        |
| Estrogens (3)                 |                        |
| Glucocorticoids (3,136)       |                        |
| Glucocorticoid receptor (73)  |                        |
| Insulin (132,138)             |                        |
| Gastrin (139)                 |                        |
| Thyroid hormones (140,141)    |                        |
| Melatonin (142)               |                        |
| C. Modulation of growth factors and receptors |   |
| Altered growth and differentiation |   |
| Vitamin A (3)                |                        |
| EGF (143)                     |                        |
| TGFα (31,142,144,30)          |                        |
| EGF receptor (191,28,31)      |                        |
| TGFβ (31,143,144)             |                        |
| TNFα (145)                    |                        |
| IL1β (68)                     |                        |
| c-Ras (146)                   |                        |
| c-EBB (147)                   |                        |

Hyperplasia has also been reported in the gastric mucosa, and the bile duct and urinary bladder epithelia. Squamous meta-
plosia occurs in the Meibomian glands of the eyelid, resulting in blepharitis, and in the ceruminous glands of the ears, leading in both cases to waxy exudates.

Chloracne has been called the "hall-
mark of dioxin toxicity" (11). This is a severe form of cystic acne involving both hyperplastic and hyperkeratotic changes in the skin, as well as altered pigmentation. Chloracne occurs following either dermal or systemic exposure in sensitive species, which include man, monkeys, hairless mice, and rabbits. The condition is extremely persistent, in some cases lasting over 30 years following the initial exposure. Dioxin and related compounds cause a generalized ecdermal dysplasia (12), resulting in alterations in the teeth and nails in both humans and monkeys, as well as effects on the nails of hairless mice (13). Chloracne is a relatively high-dose response to dioxin, occurring in mice and monkeys at doses where effects such as thymic atrophy and some wasting are noted. In humans, it is a reliable indicator of heavy dioxin exposure (14).

TCDD is a developmental toxin in all species examined. However, it appears to induce major structural abnormalities following prenatal exposure only in the mouse (15) where it causes hydronephrosis and cleft palate at doses which are not fatal or maternally toxic. This characteristic syndrome has been used to categorize chemicals as to whether or not they are dioxinlike. Prenatal exposure of the develop-

ing mouse fetuses also has effects on the developing immune system, leading to altered differentiation of lymphocytes (16). Recent studies by Peterson and co-

workers (17–19) have demonstrated that in utero exposure to the developing male rat pup leads to persistent demasculiniza-
tion and feminization. Embryo/fetal toxic-
ity occurs at similar maternal doses in the guinea pig, rat, and hamster (20).

Dioxin is highly immunotoxic in the mouse (21). One of the most sensitive responses is the suppression of the primary antibody response, an integrated response requiring the combined action of B-cells, T-cells, and macrophages. In addition, dioxin appears to compromise the host defenses of the mouse as shown by enhanced sensitivity to influenza virus (22), and mutes the response to trichinella (148). In vitro studies have suggested that mouse, monkey, and human lymphocytes are responsive to dioxin effects. Recent studies have demonstrated, however, that the rat is relatively resistant to the immunosuppressive effects of TCDD, with doses that cause mild thymic atrophy actu-
ally resulting in enhancement of the primary antibody response (R Smialowicz, personal communication). Similar doses result in enhanced sensitivity in the rat to influenza virus (G Burleson, personal communication).

The carcinogenicity of TCDD has been examined in 17 studies in laboratory ani-

mals (23). All of these studies demon-

strated that dioxin is a positive animal carcinogen in the rat, mouse, and hamster. It causes tumors at multiple sites in both sexes. In addition, recent studies with fish have demonstrated that dioxin is a multi-
site, multisex carcinogen in Medaka (24). While a number of inconclusive epidemi-

ological studies have been conducted (25), three recent mortality studies (26–28) involving occupational exposure, validated by serum TCDD levels in a subset of the exposed cohort, have demonstrated an increased risk for all cancers after dioxin exposure.
The biochemical effects of TCDD may be difficult to classify as toxic or adverse responses, but they clearly represent a molecular and/or cellular response to that chemical. These effects can be grouped into three classes (Table 1): a) altered metabolism resulting from changes in enzyme levels; b) altered homeostasis resulting from changes in hormones and their receptors; and c) altered growth and differentiation resulting from changes in growth factors and their receptors. Not all of these effects occur in all species, and many are tissue specific. The mechanism is also not understood for many of these effects. However, changes in the drug-metabolizing enzymes involve transcriptional control. Some of the effects on hormone levels such as estrogen and thyroid hormones may involve increased metabolism of these hormones as a result of the induction of the drug-metabolizing enzymes. In contrast, the basis for the changes in the receptors for both the hormones and growth factors is not understood. The tissue and stage specificity of these effects must be emphasized. For example, while dioxin results in a decrease in the level of hepatic EGF receptor (29), and an increase in its ligand TGFe in human keratinocytes (30), the inverse is true in the developing palate (31). No change has been noted in the EGF receptor in hairless mouse skin undergoing a chloracnergic response (32).

**Mechanism of Action**

How does dioxin cause its biological effects? There is general agreement that all the effects of TCDD are mediated through the action of a cellular protein known as the *Ab* receptor (33–36). This is a high-affinity binding protein, present in low numbers per cell. It has been found in most tissues, although the number varies (37,38). The binding affinity appears to be similar for a large number of species, including humans (39). The general scheme for the action of the *Ab* receptor is shown in Figure 2. This cartoon is analogous to that developed for several of the steroid receptors, and it has been hypothesized that the *Ab* receptor may belong to the steroid receptor superfamily (40–42). However, recent cloning of the *Ab* receptor (43,44) has revealed no sequence homology between the ligand binding subunit of the *Ab* receptor and the steroid receptor superfamily. These two groups have found that the *Ab* receptor has a basic helix-loop-helix domain that allows interaction with DNA, and, as will be discussed, interacts with DNA in the form of a heterodimer with another basic helix-loop-helix protein (45).

Like any kind of hormone which acts as a second messenger in the cell, TCDD action can be thought of as involving three separate steps: a) recognition of the signal; b) transduction of the signal; and c) response. The first step of signal recognition involves binding of the ligand, TCDD, or a related compound, to the *Ab* receptor. This interaction is highly specific; detailed structure/activity relationships have been developed for this interaction, which appears to involve not only the necessity of lateral halogenation and polarizability, but planarity and stacking interactions as well (34,46,47). Recent studies have shown that the form of the ligand binding subunit of the *Ab* receptor that binds to TCDD is not an isolated peptide, but part of a multimeric complex. Based on results using immunoprecipitation of the complex with either antibodies to the ligand binding subunit (48) or to HSP90 (49,50), it has been suggested that two molecules of HSP90 are involved in this ligand-binding complex. Recent studies by Perdew (51) have indicated that the cytosolic form of the receptor is a tetramer involving two molecules of HSP90, the ligand binding subunit, and a molecule of p50. However, the presence of two hsp90 molecules it still tentative since recent studies using high stringency immunoprecipitation of the ligand binding subunit with monoclonal antibodies fails to bring down the stress proteins (52). Nevertheless, it is clear that binding of the ligand to the *Ab* receptor involves a multimeric protein complex.

Once TCDD is bound to the receptor, the other proteins dissociate. It is not clear whether this occurs prior to nuclear translocation. Perdew (53) has shown that the tetrameric species can be found both in the cytosol and in the nucleus. Other investigators have demonstrated that the physical behavior of the ligand-bound receptor is different in the cytosol and the nucleus (54). The predominant nuclear form of the receptor appears to be a heterodimer. Using wild-type and mutant mouse hepatoma lines, Hankinson and coworkers (55) had demonstrated that translocation of the ligand-binding subunit into the nucleus required interaction with a protein called "arnit" (aryl hydrocarbon receptor nuclear translocating protein). More recent work by this group (45) has shown that the arnt protein actually dimerizes with the ligand binding subunit to form the DNA-binding species.

Activation of the receptor requires more than ligand binding, dissociation of the multimeric complex, translocation into the nucleus, and dimerization. In addition, activation of the heterodimer appears to be required. Treatment of the ligand/receptor complex with RNase appears to block its DNA binding ability (56), suggesting the potential involvement of RNA in the receptor action. Phosphorylation also appears to be required for an active DNA-binding species to be formed. Phosphatase treatment blocks DNA binding (57), and DNA binding can be facilitated by treatment with protein kinase C (58), suggesting that serine/threonine phosphorylation plays an essential role in putting the receptor in a DNA binding form. However, other data suggests that dephosphorylation, possibly at another site, may also be a necessary step in the activation process since the active form of the receptor has a higher pI than the dimeric form which is unable to bind to DNA (53). The sequential nature of phosphorylation and dephosphorylation steps in the activation of the receptor is also suggested by *in vivo* data suggesting that phorbol esters, which activate protein kinase C, can block the action of TCDD at the level of the receptor (59).

The activated receptor-ligand complex binds to specific sites on DNA, and appears to function as a transcriptional enhancer (60). This interaction has been best described for control of the CYP1A1 gene, resulting in induction of the synthesis of this cytochrome not only in the liver, but in extrahepatic tissues. The dioxin responsive enhancer (DRE; xenobiotic responsive enhancer, XRE) is located in the 5' region upstream of the structural gene for the cytochrome. It involves a core heptanucleotide sequence, TXGCGTG, surrounded by flanking regions and occurs in multiple copies upstream of the transcription start site (61–63). Within the core sequence, several nucleotides are absolutely essential for binding; mutational analysis
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Figure 3. Possible mechanism of gene specificity.

has demonstrated that mutation of these bases eliminates binding (64). Use of reporter constructs has also shown that there appears to be cooperativity in the control of expression of CYP1A1 by the DRE (65). Presence of two consensus sequences within the DRE results in a synergistic enhancement of transcription as compared to the presence of only one consensus sequence. It is not yet clear whether the presence of the third, or even a fourth, consensus sequence within the DRE leads to additional cooperativity. Inhibitory regions have also been suggested to be present in the regulatory region, which may block the enhancer action of the receptor (66).

A note of caution should be exercised when the mechanism of action of TCDD and the receptor complex is described. Almost all of the studies have involved the control of the CYP1A1 gene in either liver or hepatoma cells. Analysis of control of CYP1A2 has failed to reveal the presence of a consensus sequence or even the exact CYP1A1 core in the region upstream of the CYP1A2 gene (67). Sequence analysis of the regulatory region of PAI-2, which has been shown to be transcriptionally regulated in human keratinocytes (68), has also failed to reveal the presence of a functional CYP1A1 DRE (C Corton, personal communication). The same caution should be exercised in the understanding of the heteromeric nature of the DNA binding form of the receptor. While the arnt protein is involved in binding to the receptor in regulating the expression of CYP1AI, it is possible that it is but one of a family of proteins which bind to the ligand-binding subunit (Figure 3). The presence of multiple “arnts” has been suggested by recent studies of Tukey and co-workers (69), indicating that a second protein may be involved in binding to the regulatory region of CYP1A2. It is possible that it is the interaction with this second protein that controls both the DNA binding, which is the interaction with specific and unique genes, and the tissue specificity of dioxin’s effects.

The question that is frequently asked is whether or not the Ah receptor controls all the effects of dioxin. The discussion above makes it clear that this is too simplistic a question. What is meant by the Ah receptor? Are we talking about the ligand-binding subunit? If so, the answer appears to be yes. If one is talking about the tetrameric cytosolic species, which probably modulates the specificity of the ligand binding, or about the activated heteromer which is involved in interaction with DNA, the answer may be more complicated. Two lines of evidence have been used to address the role of the receptor in dioxin’s effects. The first involves structure-activity studies in which there is an apparent rank order in the relationship between compounds that can bind to the receptor and their effects. Chemicals with higher affinity binding are more potent in their effects (70).

The second approach involves the use of mouse strains which have different alleles coding for the ligand binding subunit of the receptor (71). The prototypic strains are the C57BL/6, which has a high-affinity receptor, and the DBA/2, which has a low-affinity receptor. The difference in this receptor makes these two strains relatively more sensitive to TCDD and responsive to the effects of 3-methylcholanthrene, or resistant to TCDD and nonresponsive to 3MC. Poland and Glover (71) demonstrated that a number of the effects of TCDD—lethality, thymic atrophy, induction of cleft palate, enzyme induction— segregated with the Ah allele, with those animals having the responsive allele being more sensitive. Induction of chloracne and tumor promotion in hairless mice was also shown to segregate with the responsive allele (72). Using mice congenic at the Ah locus, Birnbaum and co-workers (6) demonstrated that effects on LD₅₀ values, organ weights, and clinical chemistry measures segregated with the Ah allele.

Induction of CYP1A1, as measured by hepatic ethoxyresorufin-O-deethylase activity, was allele-dependent as was decreased binding to the estrogen receptor (73). The responsive congenic mice were also more sensitive to the effects of TCDD on the EGF receptor than were the nonresponsive congenic mice (29).

Several recent studies have suggested that binding to the Ah receptor may not be required for dioxin’s effects. One of these studies involves the use of the two unrelated strains of mice and effects on developmental tissues from these animals (74). It is well known from studies of the steroid receptors that during development, receptor function may be differentially controlled. If the ability of the ligand binding subunit to bind TCDD is controlled by its interaction with other proteins, such as HSP90 or p50, changes in these proteins during development may alter the ligand binding specificity. The second series of studies which have been interpreted to suggest that the Ah receptor may not be essential for dioxin’s effects involve some of the immunotoxic effects of TCDD in mice (75). In vitro, the lack of structure activity relationships may be a reflection of the need for serum in the culture conditions (76). Dioxin’s effects are easily modulated by the presence of serum and other factors in the growth media. Of greater interest is the apparent lack of difference in immunotoxic responses (specifically the response to sheep red blood cells) between C57BL/6 and DBA/2 mice when TCDD is given over a 2-week period in divided doses (77). Dosimetric differences between these two strains may play a role in the apparent lack of Ah-mediated response (78). While even at low divided doses the hepatic binding protein which sequesters TCDD in the liver would be induced in the responsive strain, it would not be induced in the nonresponsive strain, leaving more dioxin available to target the cells of the immune system. Until such a pharmacokinetic study is conducted it will be difficult to determine whether this immunotoxic response is really an outlier in terms of the general understanding that all the responses of dioxin require binding to the Ah receptor. However, it is clear that while binding to the receptor is necessary, it is not sufficient. Interaction of dioxin with the ligand binding species and subsequent interaction of the ligand-receptor complex with regulatory sequences on DNA is controlled by a host of other proteins and regulatory steps.

Species Homology

One of the major questions concerning the risk assessment of dioxin is the issue of whether or not humans are a sensitive species to the toxic effects of TCDD. The issues of species differences in sensitivity to dioxin is often discussed, but little understood. Table 2 lists the approximate oral LD₅₀ for a variety of animal species following a single exposure to dioxin. While there is a difference of more than three orders of magnitude in the oral dose needed to kill a guinea pig from that needed to kill a hamster, most of the laboratory species will die with a dose that is within one order of mag-
nitude of 100 mg/kg. The dose can be modulated by developmental stage and body composition (79). In certain species, there appears to be a sex difference in the LD₅₀ dose, but there is no consistency as to whether males or females are more sensitive. While the guinea pig and hamster appear to be outliers in terms of their sensitivity to dioxin’s acutely lethal effects, they differ by only an order of magnitude in respect to the sensitivity of their developing pups to TCDD-induced developmental toxicity (20). Using organ cultures of the developing palate from the human, rat, and mouse, Abbott and Birnbaum (80) demonstrated that while the sensitivity of the rat and human was the same, the developing mouse palate was approximately 1000 times more sensitive to the teratogenic effects of TCDD. This in vitro difference between the rat and mouse is reflected in the teratogenicity of TCDD in the mouse but not in the rat where the only time cleft palate occurs is at doses which are both maternally and fetally toxic. Thus, the mouse is uniquely sensitive to the teratogenic effects of TCDD. However, other studies have indicated that the doses which are embryot/etotoxic in the mouse are similar to those in the guinea pig, rat, and hamster.

Chloracne has been examined as another end point for species similarity in sensitivity. Ryan et al. (81) have estimated the dose of dioxin-related compounds which resulted in chloracne in a population in Taiwan poisoned by contaminated rice oil. The necessary dose was very similar to that dose which causes chloracne in hairless mice, rabbit, ears, and monkeys. It should also be pointed out that the pathology of the lesion is very similar in all these species. This is most interesting given the innate human variability which is seen in the chloracne response in Seveso (82). While below an estimated initial body burden of <10,000 ppt no chloracne was observed, and above an estimated body burden of >60,000 ppt chloracne was always observed, there was a wide range of serum levels where the occurrence of chloracne was sporadic.

No studies have yet examined the issue of immunotoxic effects directly in exposed people although standard measures of immune dysfunction, such as changes in lymphocyte numbers or proportions have not revealed any effects. The most sensitive response in mice to dioxin appears to be suppression of the primary antibody response. If this were to occur in people, it could result in a low vaccination take rate in an affected population. Inuit women living along the Hudson Bay have elevated levels of dioxinlike chemicals in their breast milk, reflecting their diet of fatty sea mammals (83,84). The young children in this population appear to have a high rate of infections and a low rate of successful primary vaccinations (E Dewalley, personal communication). Human tonsillar lymphocytes appear to have similar sensitivity to TCDD in vitro as expressed by mouse splenocytes (85). Changes in the subpopulations of both marmoset and human lymphocytes have also been reported following exposure in vitro to TCDD (86). These data might suggest that humans have similar sensitivity in regard to their immune system as do mice and monkeys. In contrast, the rat appears to be relatively resistant to the immunotoxic effects of TCDD. This species may be an outlier for this toxic end point, as the mouse is for teratogenesis, and the guinea pig and hamster are for lethality.

Hormonal and growth factor changes have been reported in humans as well as experimental animals. A recent epidemiological study has reported that occupation-

Table 2. Acute toxicity of TCDD.a

| Species      | LD₅₀, µg/kg, po |
|--------------|----------------|
| Guinea pig   | 0.6–2.5        |
| Mink         | 4              |
| Rat          | 22–320         |
| Monkey       | <70            |
| Rabbit       | 115–275        |
| Mouse        | 114–280        |
| Dog          | >100–<3000     |
| Hamster      | 1150–5000      |

*Data from U.S. EPA (3).

As previously mentioned, dioxin has recently been demonstrated to be a carcino-

Because of the fact that dioxins and related compounds usually occur in complex mixtures, and the need to estimate the toxicity of such, the international community has come up with an approach involving toxic equivalency factors (TEFs) to address this issue (95–97). All of the dioxinlike com-

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require that compounds are structurally related and have the same mechanism of action (98). It is important that dose-response curves have a parallel slope in order that the relative toxicity can be assessed. Until now, this has only been done carefully for approximately ten isomers using the induction of cleft palate as an end point (99). Nevertheless, TEFs, which have been standardized for the polychlorinated dibenzo-p-dioxins and dioxins, are based in large part on in vitro studies involving enzyme induction and receptor binding. Some in vitro data exist for acute responses, such as enzyme induction, thymic atrophy, and lethality. For a limited number of these chemicals, information on dermal toxicity, teratogenicity, and carcinogenicity (including tumor promotion), exists. Short-term or in vitro measures of TEFs fail to take into account pharmacokinetic or species differences which exist. However, the use of the TEFs for the polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) has been quite successful in estimating the toxicity of several complex mixtures.

Only limited attention has been directed toward estimating TEFs for other classes of dioxinlike chemicals. It is clearly appropriate that they be developed for the small subset of the PCBs which are dioxinlike (98). Safe (70) has compiled the existing information on the relative potency of polychlorinated biphenyls (PCBs) and suggested conservative TEFs for the dioxinlike isomers. These values have been used by regulators and risk assessors in predicting the toxicity of complex mixtures of PCDDs, PCDFs, and PCBs. However, these values do not incorporate pharmacokinetic considerations as they are based largely on in vitro responses. Recent studies by Walker and Peterson (100) in fish and DeVito et al. (101) in mice indicate that Safe’s suggested TEFs for PCBs are overly conservative, in some cases by several orders of magnitude.

Although little information exists on the brominated congeners, the existing data suggest that in general they are only slightly less potent than the corresponding chlorinated molecules. One exception has been in the case of 2,3,7,8-tetrabromodibenzo-furan which appears to be more potent than the corresponding TCDF. This may reflect decreased metabolism of the brominated isomer (102). Limited data on the mixed chloro-bromo isomers suggest that they may be the most toxic (103).

Thus, general agreement has been reached in the dioxin community regarding three points needed to be considered in any risk assessment of TCDD. The first is that dioxin is the most potent growth dysregulator that is known and all of its responses appear to require binding to the Ah receptor as a necessary, but not sufficient, step. The second critical point of agreement is that people are sensitive to the effects of dioxin. The last point is that all dioxin-related molecules must be included in any assessment of the environmental risk from dioxin.

**Dose-Response Relationships**

Much of the concern involving human exposure to dioxin and related chemicals has concentrated on cancer. However, recent studies have indicated that cancer is a relatively high dose-response. In experimental animals, liver tumors only result at doses where acute toxicity is evident. For example in the Kociba study (92), at the high dose where there was a clear cut increase in liver tumors in the female rat, liver toxicity and weight loss were evident. Much of the regulatory focus has been on these liver tumors. However, it is important to remember that tumors were significantly increased at several other sites, including the nasal cavity and thyroid. In the males, lung tumors were present. It is interesting to note that the liver tumors are estrogen dependent in the female rat; ovarioectomy abolished the liver tumor response (104). In contrast, the lung tumors appear to be blocked by estrogen as they are only present in the intact male rats (92) and in the ovarioctomized females (105). In contrast, male mice appear to be more sensitive to liver tumors than are female mice (106). Squamous tumors and lesions of the respiratory tract have been noted in several animal studies. The human epidemiological studies suggest that lung tumors may be increased by exposure to dioxin (27).

While cancer appears to require relatively high body burdens to be detected, lower doses result in other serious adverse responses. In a multigenerational study, Murray et al. (107) observed that the daily dose needed to result in reproductive effects (10 ng/kg/day) was an order of magnitude lower than that resulting in hepatocellular carcinomas in rats (108). Recent studies by Peterson and co-workers (17–19) have demonstrated that a single prenatal exposure of pregnant rats to 64 ng dioxin/kg bw can result in permanent effects not only in sexual behavior of the male offspring but also lead to decreased levels of androgens and spermatogenesis.

Immunotoxic responses have been detected in the marmoset and in the mouse at similar low doses. Single doses to mice of as little as 100 ng/kg caused enhanced viral mortality (22). A single exposure of 10 ng TCDD/kg resulted in altered patterns of lymphocyte subsets in the marmoset (109). However, at weekly doses of 0.3 ng/kg, no clear-cut change occurred in the total lymphocyte population (110). The ED50 for the suppression of the primary antibody response to sheep red blood cells in mice is consistently lower than that for the induction of EROD or AhH, markers for the activity of CYP1A1 (75, 111). Detection of the increase in mRNA for CYP1A1 by quantitative PCR techniques has demonstrated that increases in the message occurs at doses 100 times lower than what can be detected either enzymatically, immunologically, or by Northern blot analysis (112). Recent studies have demonstrated that a significant increase in CYP1A1 mRNA can be noted in rats following a single dose of 100 pg/kg. Given a half-life of roughly 30 days, this would be roughly equivalent to a daily dose of 1 to 3 pg/kg/day in the rat.

Enzyme induction, immunotoxicity, and reproductive effects all seem to occur at similar low doses. Since binding to the receptor is necessary before any of these effects can occur, it is clear that ligand-binding occurs at very low doses. What is the shape of the dose-response curve in this region? The only responses for which good data exist are the induction of cytochromes P4501A1 and 1A2 in the liver of both mice and rats. Recent data from the laboratory of Lucier and coworkers (113) has shown that
the increase in the mRNA and protein for CYP1A1/1A2 gives no evidence of nonlinear-earity in the low dose region. Likewise, no evidence for a threshold in the induction of these two enzymatic activities was observed in mice (114). Both of these studies involved repeated exposures at levels of approximately 1 to 5 ng/kg/day. Lower exposures are currently being conducted. The critical conclusion that can be drawn from these studies is that there is no evidence for a threshold in relatively simple, Ah-receptor-mediated responses. That is not to say that other responses may not exhibit nonlinear behavior at low doses. However, it is clear that not all responses are nonlinear.

Therefore, there cannot be a dose which, by definition, will have no response.

Recent studies have also demonstrated that the pharmacokinetics of TCDD and related compounds is dose-dependent. Distribution to the liver is nonlinear, with the relative concentration increasing with increasing dose (115). This appears to be associated with the induction of a liver-specific binding protein which has been tenta-
vitively identified as cytochrome P450IA2 (116,117). Hepatic sequestration results in decreased distribution to extrahepatic tissues with increasing dose. Many of the experimental studies have been conducted at doses where this sequestration was occurring, leading to the false assumption that humans and animals behave differently in how TCDD distributes. Physiologically based pharmacokinetic models have been developed for both TCDD (118) and the related brominated congener, 2,3,7,8-tetrabromodibenzo-p-dioxin (TBDD) (119) which incorporate the induction of this binding protein and the dose-dependent distribution. These models accurately predict the effect of dose on absorption, distribution, and elimination in rats. Carrier (120) has used human data on the blood and adipose levels of PCDFs to develop a pharmacokinetic model to describe the behavior of these compounds which also predicts nonlinearity in disposition. These models raise some concern about the estimation of half-life in humans assuming a one-compartment elimination (121).

Pharmacokinetic studies have also been conducted comparing different relevant routes of human exposure. For a number of dioxin-related compounds, both oral and dermal absorption has been shown to be dose-dependent (122,123,124). Relative absorption decreases as the dose is increased. At relatively low experimental doses (~1 mol/kg), oral exposure results in nearly complete absorption as compared to an intravenous exposure. Intratracheal instillation, an approximation of pul-
monary absorption, results in similar absorption to that observed orally. Dermal absorption is always more limited, being maximal for TCDD from an organic sol-
vent of approximately 50% of an applied dose. Maximal dermal absorption, how-
ever, can be predicted from the octanol water partition coefficient (125).

Risk from Current Exposures
Where does current environmental exposure place us today? In the industrialized world, adults have approximately 6 ppt TCDD per ml serum, on a lipid adjusted basis (126). In nonindustrialized areas of the Third World which have not been exposed to heavy herbicide use, body bur-
dens are several times lower (127). If the total toxic equivalency of all the chlori-
nated dioxins and furans is included, the body burden is approximately 30 ppt. This value is further increased if the toxicity of the dioxinlike PCBs is included.

What are daily exposure levels? Dietary exposure accounts for the major source of the human body burden. Estimates are that daily exposure to TCDD is approximately 0.1 to 0.3 pg TCDD/kg/day, equivalent to approximately 1 to 3 pg TEQ/kg/day (128). If the PCBs are included in this esti-
mate, the daily dose is 3 to 10 pg/kg/day. It is important to note that there are people within the population who have higher exposure than the average. For example, dioxin and related chemicals are so lipophilic, they are mobilized from the adipose tissue during lactation and are elimi-
nated through the milk. Therefore, nursing infants can have daily exposures 10 to 20 times higher than the background population. Subsistence fishermen also have elevated exposure due to the presence of these compounds in fish.

From the foregoing discussion, it should be clear than exposure to high levels of dioxin and related compounds has the potential to result in a host of biological responses. At high doses, some of these are clearly adverse and have been observed in the human population (e.g., depression of circulating testosterone levels, chloracne, cancer). These overtly toxic responses have been noted at body burdens many times higher than those occurring in the general population in industrialized countries. For people with levels higher than the general populace but lower than occupationally exposed cohorts or those poisoned in industrial accidents, recent reports have indicated alterations in lipid metabolism and elevated incidence of diabetes (129). Exposure to a complex mixture of PCBs and PCDFs resulted in clear evidence of developmental toxicity (12).

The question of greater import is what is the risk of current environmental exposure to the general population? Are the subtle effects detected in experimental ani-
malstoday? If so, are these adverse? Results in enzyme induction from both rats (113) and mice (114) would suggest that at current environmental levels (~1 to 10 TEQ pg/kg/day) people may be experiencing small, but significant, increases in these markers of response. Highly exposed populations may be at special risk. Since animal studies suggest that changes in hepatic enzyme induction occur at body burdens similar to those at which immunotoxicity in mice and permanent effects on the reproductive system occur in rats, it is reasonable to hypothesize that subtle effects on these parameters may be occurring in the human population. Epidemiological studies to examine this hypothesis should be undertaken.

REFERENCES
1. Gallo MA, Scheuplein RJ, Van Der Heijden KA, eds. In: Biological Basis for Risk Assessment of Dioxins and Related Com-
ounds Banbury Conference Report 35, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1991.
2. Travis CC, Hattemer-Frey HA. Human exposure to dioxin. Sci Total Environ 104:97–127 (1991).
3. Health assessment document for polychlorinated dibenzo-p-dioxins. EPA/600/3-84/014F. Cincinnati OH: Center for Environmental Research Information, 1985.
4. Toxicological profile for 2,3,7,8-tetrachlorodibenzo-plexodioxins, halogenated biphenyls, terphenyls, naphthalenes, dibenzo-p-dioxins and related products. In: Topics in Environmental Health, 2nd revised ed, vol 4, Amsterdam: Elsevier, 1989.
5. Kimbrough R, Jensen A, eds. Halogenated biphenyls, terphenyls, naphthalenes, dibenzo-p-dioxins and related products. In: Topics in Environmental Health, 2nd revised ed, vol 4, Amsterdam: Elsevier, 1989.
10. Calvert GM, Hornung RW, Sweeney MH, Fingerhut MA, Halperin WE. Hepatic and gastrointestinal effects in an occupational cohort exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. JAMA 267(16):2209–2214 (1992).

11. Suskind RR, Hertzberg VS. Human health effects of 2,4,5-T and its toxic contaminants. JAMA 251(18):2372–2380 (1984).

12. Rogan WJ, Gladen BC, Hung K-L, Koong S-L, Shih L-Y, Taylor JS, Wu Y-C, Yang D, Yang NB, Hsu C-C. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 241:334–336 (1988).

13. Hebert CD, Harris MW, Elwell MR, Birnbaum LS. Relative toxicity and tumor-promoting ability of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PCDF), and 2,3,4,7,8-hexachlorodibenzofuran (HCDF) in hairless mice. Toxicol Appl Pharmacol 102:362–377 (1990).

14. Neuberger M, Landvoigt W, Derntl F. Blood levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin in chemical workers after chloracne and in comparison groups. Arch Occup Environ Health 63:325–327 (1991).

15. Couture LA, Abbott BD, Birnbaum LS. A critical review of the developmental toxicity and teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin: recent advances toward understanding the mechanism. Teratology 42:619–627 (1990).

16. Fine JS, Gasiewicz TA, Silverstone AE. Lymphocyte stem cell alterations following perinatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Mol Pharmacol 35:18–25 (1988).

17. Mahly TA, Moore RW, Peterson RE. In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin. 1. Effects on androgen status. Toxicol Appl Pharmacol 114:97–107 (1992).

18. Mahly TA, Moore RW, Goy RW, Peterson RE. In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin. 2. Effects on sexual behavior and the regulation of luteinizing hormone secretion in adulthood. Toxicol Appl Pharmacol 114:108–117 (1992).

19. Mahly TA, Bjerke DL, Moore RW, Gendron-Fitzpatrick A, Peterson, RE. In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin. 3. Effects on spermatogenesis and reproductory capability. Toxicol Appl Pharmacol 114:118–126 (1992).

20. Olson JR, McGarrigle BP. Comparative developmental toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Chemosphere 25(1–2):71–74 (1992).

21. Hollisapple MP, Morris DL, Wood SC, Snyder NK. 2,3,7,8-Tetrachlorodibenzo-p-dioxin-induced changes in immunocompetence: possible mechanisms. Annu Rev Pharmacol Toxicol 31:73–100 (1991).

22. House RV, Lauer LD, Murray MJ, Thomas PT, Ehrlich JP, Burleson GR, Dean JH. Examination of immune parameters and host resistance mechanisms in B6C3F1 mice following adult exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. J Toxicol Environ Health 31:203–215 (1990).

23. Huff JE, Salmon AG, Hooper NK, Zeise L. Long-term carcinogenesis studies on 2,3,7,8-tetrachlorodibenzo-p-dioxin and hexachlorodibenzo-p-dioxins. Cell Biol Toxicol 7(1):67–94 (1991).

24. Johnson R, Tietje J, Botts S. Carcinogenicity of 2,3,7,8-TCDD to medaka. Toxicologist 12(1):476 (1992).

25. Johnson ES. Human exposure to 2,3,7,8-TCDD and risk of cancer. Crit Rev Toxicol 25:451–463 (1992).

26. Zober A, Messerer P, Huber P. Thirty-four-year mortality follow-up of BASF employees exposed to 2,3,7,8-TCDD after the 1953 accident. Int Arch Occup Environ Health 62:139–157 (1990).

27. Fingerhut MA, Halperin WE, Malrow DA, Piagenti LA, Honchar PA, Sweeney MH, Greife AL, Dill PA, Steenland K, Suruda AJ. Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. N Engl J Med 324(4):212–218 (1991).

28. Klafl A, Berger J, Dwyer JH, Fleisch Janss D, Nagel S, Waltsgott H. Cancer mortality among workers in chemical plant contaminated with dioxin. Lancet 338(8773):959–964 (1991).

29. Lin FH, Clark G, Birnbaum LS, Lucier GW, Goldstein JA. Influence of the Ah locus on the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the hepatic epidermal growth factor receptor. Mol Pharmacol 39:307–313 (1991).

30. Choi EJ, Toscano DG, Ryan JA, Riedel N, Toscano WA. Dioxin reduces transforming growth factor-alpha in human keratinocytes. J Biol Chem 266:9591–9597 (1991).

31. Abbott BD, Birnbaum LS. TCDD induced altered expression of growth factors may have a role in producing cleft palate and enhancing the incidence of defects after coadministration of retinoic acid and TCDD. Toxicol Appl Pharmacol 106:418–432 (1990).

32. Stohs SJ, Abbott BD, Lin FH, Birnbaum LS. Induction of ethoxyresoruvin-O-deethylation and inhibition of glucocorticoid receptor binding in skin and liver of haired and hairless HRS/J mice by topically applied 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicology 65:123–136 (1990).

33. Neuberger DW, Maashuis CL, Lang MA, Hjelmen LM, Eien HG. The Ah locus, a multigene family necessary for survival in a chemically adverse environment: comparison with the immune system. In: Advances in Genetics, vol 21, (Demerec M, ed). New York:Academic Press, 1982:1–52.

34. Poland A, Knutson JC. 2,3,7,8-Tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. In: Annual Review of Pharmacology and Toxicology, vol 22 (George R, Okun R, Cho AK, eds). Palo Alto:Annual Reviews Inc,1982:517–554.

35. Whitlock JP Jr. The regulation of gene expression by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Pharmacol Rev 39:147–161 (1987).

36. Whitlock JP Jr. Genetic and molecular aspects of 2,3,7,8-tetrachlorodibenzo-p-dioxin action. Annu Rev Pharmacol Toxicol 30:251–277 (1990).

37. Carlssted-Duke JMB. Tissue distribution of the receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Cancer Res 39:3172–3176 (1979).

38. Gasiewicz TA, Rucci G. Cytosolic receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin: evidence for a homologous nature among various mammalian species. Mol Pharmacol 26(1):90–98 (1984).

39. Harper PA, Prokipcak RD, Bush LE, Golas CL, Okey AB. Detection and characterization of the Ah-receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin in the human colon adenocarcinoma cell line LS180. Arch Biochem Biophys 290(1):27–36 (1991).

40. Evans RM. The steroid and thyroid hormone receptor superfam. Science 240(4854):889–895 (1988).

41. Cuthill S, Wilkinson A, Mason, GGF, Gillner M, Poellinger L, Gastfasson JA. The dioxin receptor: A comparison with the glucocorticoid receptor. J Steroid Biochem 30(1–6):277–280 (1988).

42. Fuller PJ. The steroid receptor superfam: mechanisms of divergence. FASEB J 5:3092–3098 (1991).

43. Ema M, Sogawa K, Watanabe N, Chuiyoh Y, Matsushita N, Gotoh O, Funae Y, Fujii-Kuriyama Y. cDNA cloning and structure of mouse putative Ah receptor. Biochem Biophys Res Comm 184(1):246–253 (1992).

44. Burbach KM, Poland A, Bradford CA. Cloning of the Ah-receptor cDNA. Toxicologist 12(1):709 (1992).

45. Ryu E, Reisz-Porszasz S, Hankinson O. Identification of the Ah receptor nuclear translocator protein (Arnt) as a component of the DNA binding form of the Ah receptor. Science 256:1193–1194 (1992).

46. Romkes M, Piskorska-Pliszcynska J, Keys B, Safe S, Fujita T. Quantitative structure-activity relationships: analysis of interactions of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2-substituted...
analogues with rat, mouse, guinea pig, and hamster cytosolic receptor. Cancer Res 47:5108–5111 (1987).

47. McKinney, JD. Multifunctional receptor model for dioxin and related compound toxic action: possible thyroid hormone-responsive effector-linked site. Environ Health Perspect 82:323–336 (1989).

48. Poland A, Glover E, Bradfield CA. Characterization of polyclonal antibodies to the Ah receptor prepared by immunization with a synthetic peptide hapten. Mol Pharmacol 39:20–26 (1991).

49. Denis M, Cuthill S, Wilkstrom A-C, Poellinger L, Gustafsson JA. Association of the dioxin receptor with the M, 90,000 heat shock protein: a structural kinship with the glucocorticoid receptor. Biochem Biophys Res Commun 155:801–807 (1988).

50. Perdew G. Association of the Ah receptor with 90KDa heat shock protein. J Biol Chem 263:13802–13805 (1988).

51. Perdew G. Chemical cross-linking of the cytosolic and nuclear forms of the Ah receptor in hepatoma cell line 1c1c7. Biochem Biophys Res Commun 182:55–62 (1992).

52. Eltom SE, Poland AP. Studies on the structure-function of the Ah receptor in mouse hepatoma cell line (HEPA-1): receptor subunits and subunits of the phosphorylation. Toxicologist 12(1):710 (1992).

53. Perdew G. Comparison of the nuclear and cytosolic forms of the Ah receptor from Hepa 1c1c7 cells: charge heterogeneity and ATP binding proteins. Arch Biochem Biophys 219:284–290 (1991).

54. Henry EC, Rucci G, Gasiwicz TA. Characterization of multiple forms of the Ah receptor: comparison of species and tissues. Biochem Biophys Res Commun 283:6464–6469 (1992).

55. Hoffman EC, Reyes H, Fong-Fong C, Sander F, Conley LH, Brooks BA, Hankinson O. Cloning of a factor required for activity of the Ah (dioxin) receptor. Science 252:954–958 (1991).

56. Henry EC, Hayden KA, Baumann PA, Gasiwicz TA. Ribonucleic inhibits Ah receptor transformation in vitro. Biochem J 279:689–694 (1991).

57. Pongraz I, Stromstedt PE, Mason GGF, Poellinger L. Inhibition of the specific DNA binding activity of the dioxin receptor by phosphatase treatment. J Biol Chem 266(25):16813–16817 (1991).

58. Carrier F, Owens RA, Nebert DL, Puga A. Dioxin-dependent activation of murine cyc1-1 gene transcription requires protein kinase c-dependent phosphorylation. Mol Cell Biol 15386–15383 (1992).

59. Okino ST, Pendurthi UR, Tukey RH. Phorbol esters inhibit the dioxin receptor-mediated transcriptional activation of the mouse cyc-1 and cyc-2 genes by 2,3,7,8-tetrachlorodibenzo-p-dioxin. J Biol Chem 267(10):6991–6998 (1992).

60. Jones PBC, Durrin LK, Galeazzi DR, Whistock JP Jr. Control of cytochrome P450 gene expression by a phospholipid of a dioxin-responsive enhancer system. Proc Natl Acad Sci USA 83:2802–2806 (1986).

61. Haggard J, Cuthill S, Denis M, Poellinger L, Gustafsson JA. Specific protein-DNA interactions at a xenobiotic-responsive element: copurification of dioxin receptor and DNA-binding activity. Proc Nat Acad Sci USA 86:60–64 (1989).

62. Denison MS, Fisher JM, Whistock JP Jr. Protein-DNA interactions at recognition sites for the dioxin-Ah receptor complex. J Biol Chem 264:16478–16482 (1989).

63. Wu L, Whistock JP Jr. Mechanism of dioxin action: Ah receptor mediated increase in promoter accessibility in vivo. Proc Natl Acad Sci USA 89:4811–4815 (1992).

64. Shen ES, Whistock JP Jr. Protein-DNA interactions at a dioxin-responsive enhancer. J Biol Chem 267:6815–6819 (1992).

65. Fisher JM, Wu L, Denison MS, Whistock JP Jr. Organization and function of a dioxin-responsive enhancer. J Biol Chem 265:9676–9681 (1990).

66. Haggard J, Cuthill S, Soderkist P, Wilkinson A, Pongraz I, Tukey RH, Johnson EF, Gustafsson JA, Poellinger L. Liver cells contain constitutive DNase I hypersensitive sites at the xenobiotic response elements 1 and 2 (XRE1 and -2) of the rat cytochrome P450IA1 gene and a constitutive, nuclear XRE-binding factor that is distinct from the dioxin receptor. Mol Cell Biol 11(9):4313–4323 (1991).

67. Quattrochi LC, Phillips LAP, Tukey RH. Tissue-specific regulation of the human CYP1A2 gene. Drug Metabolism Enzymes: Regulation and Toxicity. Proceedings of the VIII International Symposium on Microbes and Drug Oxidations, Stockholm, Sweden (1990).

68. Sutter TR, Guzman K, Dold KM, Greenlee W. Targets for dioxin: genes for plasmidogen activator inhibitor-2 and interleukin-1b. Science 254:415–418 (1991).

69. Okino ST, Pendurthi UR, Tukey RH. 2,3,7,8 Tetrachlorodibenzo-p-dioxin induces the nuclear translocation of two XRE binding proteins in mice. Pharmacogenetics 3(2):101–109 (1993).

70. Safe S. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit Rev Toxicol 21:51–88 (1990).

71. Poland A, Glover E, 2,3,7,8-Tetrachlorodibenzo-p-dioxin: segregation of toxicity with the Ah locus. Mol Pharmacol 17:86–94 (1980).

72. Poland A, Palen D, Glover E. Tumor promotion by TCDD in skin of HRS/1 hairless mice. Nature 300:271–273 (1982).

73. Lin FH, Stos SJ, Birnbaum LS, Clark G, Lucier GW, Goldstein JA. The effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the hepatic estrogen and glucocorticoid receptors in congenic strains of Ah responsive and Ah nonresponsive C57BL/J6 mice. Toxicol Appl Pharmacol 108:129–139 (1991).

74. Harper, PA, Golas, CL, Okey, AB. Ah receptor in mice genetically “nonresponsive” for cytochrome P4501A1 induction: Cytotoxic Ah receptor, transformation to the nuclear binding site, and induction of aryl hydrocarbon hydroxylase by halogenated and nonhalogenated aromatic hydrocarbons in embryonic tissues and cells. Mol Pharmacol 40:818–826 (1991).

75. Kerkvliet NI, Steppan LB, Brauner JA, Deyo JA, Henderson MC, Tomar RS, Buhler DR. Influence of the Ah locus on the humoral immunotoxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin: evidence for Ah-receptor-dependent and Ah-receptor-independent mechanisms of immunosuppression. Toxicol Appl Pharmacol 105:20–36 (1990).

76. Morris DL, Jordan SD, Holsapple MP. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on humoral immunity: 1. Similarities to Staphylococcus aureus Cowan Strain I (SAC) in the in vitro T-dependent antibody response. Immunopharmacology 21:159–170 (1991).

77. Morris DL, Snyder NK, Gokani V, Blair RE, Holsapple MP. Enhanced suppression of humoral immunity in DBA/2 mice following subchronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicol Appl Pharmacol 112:128–132 (1991).

78. Birnbaum LS. Distribution and excretion of 2,3,7,8-tetrachlorodibenzo-p-dioxin in congenic strains of mice which differ at the Ah Locus. Drug Metab Disp 14(1):34–40 (1986).

79. Geyer HJ, Scheuert I, Rapp K, Kettrup A, Korte F, Greim H, Rozman K. Correlation between acute toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and total body fat content in mammals. Toxicology 65:97–107 (1990).

80. Abbott BD, Birnbaum LS. TCDD exposure of human embryonic palatal shelves in organ culture alters the differentiation of medial epithelial cells. Teratology 43:119–112 (1991).

81. Ryan JJ, Gasiwicz TA, Brown JR Jr. Human body burden of polychlorinated dibenzo-p-dioxins associated with toxicity based on the Yusho and Yucheng incidents. Fundam Appl Pharmacol 15:72–731 (1991).

82. Moczarelli M, Marocchi A, Brambilla P, Gertthoux PM, Colombo L, Mondonico A, Mezza L. Effects of dioxin exposure in humans at Seveso, Italy. In: Biological basis for risk assessment of dioxins and related compounds, (Gallo MA, Scheupme RJ, Van Der Heijden KA, eds). Banbury Conference Report 35. Cold Spring Harbor, NY:Cold Spring Harbor Laboratory Press 1991:95–110.

83. Dewailly E, Namel A, Weber J-P, Meyer F. High levels of PCBs in breast milk of Inuit women from Arctic Quebec. Bull Environ Contam Toxicol 43:641–646 (1989).

84. Dewailly E, Weber J-P, Gingras S, Laliberte C. Coplanar PCBs in human milk in the province of Quebec, Canada: Are they more toxic than dioxin for breast fed infants? Bull Environ Contam Toxicol 47:491–498 (1991).

85. Wood SC, Karras JG, Holsapple MP. Integration of the human
lymphocyte into immunotoxicological investigations. Fundam
Appl Toxicol 18:450–459 (1992).

86. Birnbaum LS, Golor G, Stahlmann R, Helge H, Neubert D.
Polyhalogenated dibeno-p-dioxins and dibenzofurans and the
immune system. 2. In vitro effects of 2,3,7,8-tetrachlorodibenzo-p-
dioxin (TCDD) on lymphocytes of venous blood from man and
a non-human primate (Callithrix jacchus). Arch Toxicol
65:213–219 (1991).

87. Burgand G, Sweeney M, Fingerhut M, Halperin W, Wells K,
Schnorr T. Serum dioxin (2,3,7,8-TCDD) and total serum testo-
terone, and gonadotropin in occupationally exposed men. Am J
Epidemiol 136:1014 (1992).

88. Moore RW, Potter CL, Theobald HM, Robinson JA, Peterson
RA. Androgenic deficiency in male rats treated with 2,3,7,8-
tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol 79:99–111
(1985).

89. Ryan RP, Sunahara GI, Lucier GW, Birnbaum LS, Nelson KG.
Decreased ligand binding to the hepatic glucocorticoid and epider-
mal growth factor receptors after 2,3,7,8-penta- and 2,3,7,8-
hexachlorodibenzo-p-dioxin treatment of pregnant mice. Toxicol
Appl Pharmacol 98:454–464 (1989).

90. Lucier GW, Nelson KG, Eversen RB, Wong TK, Philpot RM,
Tiemann T, Taylor M, Sunahara GI. Placental markers of human
exposure to polychlorinated biphenyls and polychlorinated diben-
zofurans. Environ Health Perspect 76:79–87 (1987).

91. Madhukar BV, Brewster DW, Matsumura F. Effect of in vivo
administered 2,3,7,8-tetrachlorodibenzo-p-dioxin on receptor
binding of epidermal growth factor in the hepatic plasma mem-
brane of rats, guinea pig, mouse and hamster. Proc Nat Acad Sci
USA 81(23):7407–7411 (1984).

92. Kociba RJ, Keyes DG, Beyer JE, Carreon RM, Wade CE,
Dittember DA, Kalnis RP, Frauson LE, Park CN, Barnard SD,
Hummel RA, Humiston, CG. Results of a two-year chronic toxic-
ity and onogenicity study for 2,3,7,8-tetrachlorodibenzo-p-dioxin
in rats. Toxicol Appl Pharmacol 46:279–303 (1978).

93. Greenlee WF, Osborne O, Dold KM, Hudson LG, Young MJ,
Toscano WA Jr. Altered regulation of epidermal cell proliferation
and differentiation by 2,3,7,8-tetrachlorodibenzo-p-dioxin
(TCDD). In: Reviews in Biochemical Toxicology, Vol 8 (Hodgson
E, Bend JR, Philpot RM, eds), New York Elsevier, 1987:1–35.

94. Lucier GW. Humans are a sensitive species to some of the bio-
chemical effects of structural analogs of dioxin. Environ Toxicol
Chem 10:727–735 (1991).

95. Barnes D, Kurz F, Bottrimore D. Interim procedures for esti-
mating risks associated with exposure to mixtures of chlorinated diben-
zodioxins and dibenzofurans and 1989 update. Risk Assessment
Forum, EPA-625-R-89-016, Springfield, VA: National Technical
Information Service, 1989.

96. NATO/CCMS. International toxicity equivalency factor (I-TEF)
method of risk assessment for complex mixtures of dioxins and
related compounds. Report no. 176. North Atlantic Treaty
Organization, Committee on the Challenges of Modern Society.

97. NATO/CCMS. Scientific basis for the development of interna-
tional toxicity equivalency factor (I-TEF) method of risk assess-
ment for complex mixtures of dioxins and related compounds.
Report no. 178. North Atlantic Treaty Organization, Committee
on the Challenges of Modern Society.

98. Barnes D, Stevens AA, Birnbaum LS, Kurz FW, Wood W, Patton
D. The dioxin toxicity equivalency factor (I-TEF): Quality Assurance:
Good Practice, Regulation and Law 1(1):70–81 (1991).

99. Birnbaum LS. Developmental toxicity of TCDD and related
compounds: species sensitivities and differences. In: Biological Basis
for Risk Assessment of Dioxins and Related Compounds Banbury
Conference Report 35, (Gallo MA, Scheulepin RJ Van Der
Heijden KA, eds), Cold Spring Harbor, NY:Cold Spring Harbor
Laboratory Press, 1991:51–68.

100. Walker MK, Peterson RE. Potencies of polychlorinated dibeno-p-
dioxin, dibenzofuran and biphenyl congeners, relative to 2,3,7,8-
tetrachlorodibenzo-p-dioxin for producing early life stage morality
in rainbow trout (Oncorhynchus mykiss). Aquatic Toxicol
21:219–238 (1991).

101. DeVito MJ, Maiet WE, Diliberto JJ, Birnbaum LS. Comparative
ability of various PCBs, PCDFs and TCDD to induced cytchrome P450
1A1 and 1A2 activity following 4 weeks of treatment. Fundam
Appl Toxicol 20:125–130 (1993).

102. Birnbaum LS, RE Morrissey MW Harris. Teratogenic effects
of 2,3,7,8-tetrabromodibenzo-p-dioxin and three polybrominated
dibenzo-furans in C57BL/6N mice. Toxicol Appl Pharmacol
107:141–152 (1991).

103. Mason G, Zacharewski T, Denovme MM, Safe L, Safe S.
Polybrominated dibenzo-p-dioxins and related compounds: quanti-
tative in vivo and in vitro structure-activity relationships.
Toxicology 44:245–255 (1987).

104. Lucier GW, Tittscher AM, Goldsworthy T, Foley J, Clark G,
Goldstein J, Maronpot R. Ovarian hormones enhance TCDD-
mediated increased cell proliferation and preneoplastic foci in a two
stage model for rat hepatocarcinogenesis. Cancer Res 51:1391–1397
(1991).

105. Clark GC, Tittscher AM, Maronpot R, Foley J, Lucier G. Tumor
promotion by TCDD in female rats. In: Biological Basis for Risk
Assessment of Dioxins and Related Compounds Banbury
Conference Report 35, (Gallo MA, Scheulepin RJ Van Der
Heijden KA, eds), Cold Spring Harbor, NY:Cold Spring Harbor
Laboratory Press, 1991:389–404.

106. Carcinogenesis bioassy of 2,3,7,8-tetrachlorodibenzo-p-dioxin
in Osborne-Mendel rats and B6C3F1 mice (gavage study). NTP
Technical Report no. 209. Research Triangle Park, NC: National
Toxicology Program, 1982.

107. Murray FJ, Smith FA, Nitsche KD, Humiston CG, Kociba RJ,
Schweitzer BA. Three-generation reproduction study of rats given
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the diet. Toxicol
Appl Pharmacol 50:241–252 (1979).

108. Brown WR. Implications of the reexamination of the liver sections
from the TCDD chronic rat bioassy. In: Biological Basis for Risk
Assessment of Dioxins and Related Compounds Banbury
Conference Report 35, (Gallo MA, Scheulepin RJ Van Der
Heijden KA, eds), Cold Spring Harbor, NY:Cold Spring Harbor
Laboratory Press, 1991:13–26.

109. Neubert R, Jacob-Muller U, Stahlmann R, Helge H, Neubert D.
Polyhalogenated dibeno-p-dioxins and dibenzofurans and the
immune system. 1. Effects on peripheral lymphocyte subpopula-
tions of a non-human primate (Callithrix jacchus) after treatment
with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Arch Toxicol
64:345–359 (1990).

110. Neubert R, Golor G, Stahlmann R, Helge H, Neubert D.
Polyhalogenated dibeno-p-dioxins and dibenzofurans and the
immune system. 4. Effects of multiple-dose treatment with
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on peripheral
lymphocyte subpopulations of a non-human primate (Callithrix jac-
chus). Arch Toxicol 66:250–259 (1992).

111. Dickerson R, Howie L, Davis D, Safe S. The structure-dependent
effects of heptachlorodibenzofuran isomers in male C57BL/6 mice:
Immunotoxicity and monooxygenase enzyme induction. Fund am
Appl Toxicol 15:298–307 (1990).

112. Vanden Heuvel JP, Clark GC, Tittscher AM, Greenlee WF, Lucier
GW, Bell DA. Dioxin-responsive genes: examination of dioxin-
response relationships using quantitative reverse transcriptase-poly-
merase chain reaction. Cancer Res 54:62–68.

113. Tittscher AM, Goldstein JA, Portier CJ, McCoy Z, Clark GC,
Lucier GW. Dose-response relationships for chronic exposure to
2,3,7,8-tetrachlorodibenzo-p-dioxin in a rat tumor promotion
model: quantification and immunolocalization of CYP1A1 and
CYP1A2 in the liver. Cancer Res 52:3436–3442 (1992).

114. DeVito MJ, Diliberto JJ, Birnbaum LS. Comparative ability of
TCDD to induce hepatic and skin cytochrome P450 1A1 activity
following 13 weeks of treatment. In: Toxicology, Epidemiology,
Risk Assessment, and Management. Dioxin 92, 12th International
Symposium on Dioxins and Related Compounds, 8/24–28/92,
Tampere, Finland, 10:41 (1992).

115. Abraham K, Krowke R, Neubert D. Pharmacokinetics and biologi-
cal activity of 2,3,7,8-tetrachlorodibenzo-p-dioxin. 1. Dose-
dependent tissue distribution and induction of hepatic ethoxyresorufin
O-deethylase in rats following a single injection. Arch Toxicol
62:359–368 (1988).
116. Poland A, Teitelbaum P, Glover E. [125I]2-Iodo-2,3,7,8-tetrachlorodibenzo-p-dioxin-binding species in mouse liver induced by agonists for the Ah receptor: characterization and identification. Mol Pharmacol 36:113–120 (1989).

118. Voorman RA, Aust SD. Specific binding of polyhalogenated aromatic hydrocarbon inducers of cytochrome P450 to the cytochrome and inhibition of its estradiol 2-hydroxylase activity. Toxicol Appl Pharmacol 50:69–78 (1978).

119. Anderson ME, Mills JJ, Gargas ML, Kedders LB, Birnbaum LS, Neubert D, Greenlee WF. Modelling receptor-mediated processes with dioxin: implications for pharmacokinetics and risk assessment. Risk Anal 13(1):25–36 (1993).

121. Kedders LB, Mills J, Anderson M, Birnbaum LS. Physiologically-based pharmacokinetic model for 2,3,7,8-tetrabromodibenzo-p-dioxin (TBDD) in the rat: tissue distribution and CYP4A induction. Toxicol Appl Pharmacol 121(1):87–98 (1993).

125. Roegner R. Toxicol Appl Pharmacol 121(1):377–382 (1992).

129. Roegner R. Toxicol Appl Pharmacol 121(1):87–98 (1993).

129. Roegner R. Toxicol Appl Pharmacol 121(1):377–382 (1992).

132. Weber LWD, Lebofsky M, Greim H, Rozman K. Key enzymes of glucuronogenesis are dose-dependently reduced in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-treated rats. Arch Toxicol 65:119–123 (1991).

133. Moore RW, Peterson RE. Androgen cabalism and excretion in 2,3,7,8-tetrachlorodibenzo-p-dioxin treated rats. Biochem Pharmacol 37:560–562 (1988).

134. Umbreit TH, Gallo MA. Physiological implications of estrogen receptor modulation by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Lett 42:5–14 (1988).

135. Safe S, Astroff B, Harris M, Zacharewski T, Dickerson R, Romkes M, Biegel L, 2,3,7-Tetrachlorodibenzo-p-dioxins (TCDD), and related compounds as antiestrogens: characterization and mechanism of action. Pharmacol Toxicol 69:400–405 (1991).

136. Gorski JR, Rozman T, Greim H, Rozman K. Corticosterone modulated acute toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in male Sprague-Dawley rats. Fundam Appl Toxicol 11:494–502 (1988).

137. Weber LWD, Greim H, Rozman K. Metabolism and distribution of [14C]glucose in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). J Toxicol Environ Health 22:195–206 (1987).

138. Gorski JR, Rozman K. Dose-response and time course of hyperthyroxiemia and hypoinsulinaemia and characterization of insulin hypersensitivity in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-treated rats. Toxicology 44:297–307 (1987).

139. Theobald HM, Ingall GB, Mably TA, Peterson RE. Response of the antral mucosa of the rat stomach to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol 108:167–179 (1991).

140. Bastomsky CH. Enhanced thyroid metabolism and high uptake goiters in rats after a single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Endocrinology 101(1):292–296 (1977).

141. McKinney JD, Fannin R, Jordan S, Chae K, Rickenbacher U, Pederson L. Polychlorinated biphenyls and related compound interactions with specific binding sites for thyroxine in rat liver nuclear extracts. J Med Chem 79:86 (1987).

142. Linden J, Pohjanvirta R, Rahko T, Tuomisto J. TCDD decreases rapidly and persistently serum melatonin concentration without morphologically affecting the pineal gland in TCDD-resistant Han/Wistar rats. Pharmacol Toxicol 69:427–432 (1991).

143. Abbot RD, Harris MW, Birnbaum LS. Comparison of the effects of TCDD and hydrocortisone on growth factor expression provide insight into their interaction in the embryonic mouse palate. Teratology 43:35–53 (1992).

144. Gaido KW, Maness SC, Leonard LS, Greenlee WF. TCDD-dependent regulation of transforming growth factor-α and TGF-B2 expression in a human keratinocyte cell line involves both transcriptional and post-transcriptional control. J Biol Chem 267:24591–24595 (1992).

145. Clark GG, Lindstrom MJ, Trischer AM, Lucier G. Tumor necrosis factor involvement in 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol 11:422–431 (1991).

146. Tullis K, Olsen H, Bombick DW, Matsumura F, Jankun J. TCDD causes stimulation of c-rex expression in the hepatic plasma membranes in vivo and in vitro. J Biochem Toxicol 7:107–116 (1992).

147. Bombick DW, Jankun J, Tullis K, Matsumura F, 2,3,7,8-Tetrachlorodibenzo-p-dioxin causes increases in expression of c-erb-A and levels of protein-tyrosine kinases in selected tissues of responsive mouse strains. Proc Natl Acad Sci USA 85:4128–4132 (1988).

148. Luebke B. Assessment of host resistance to Trichinella spiralis in mice following preinfection exposure to 2,3,7,8-TCDD. Toxicol App Pharmacol 125:7–16 (1994).