

COMPARATIVE TOXICOLOGY AND MECHANISM OF ACTION OF POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS

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INTRODUCTION

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are members of a chemical family (polyhalogenated aromatics) that also includes the polychlorinated biphenyls, naphthalenes, azobenzenes, and azoxybenzenes, and the polybrominated biphenyls. In contrast to the polychlorinated biphenyls and naphthalenes and the polybrominated biphenyls, the PCDDs and PCDFs are not primary industrial products. PCDFs are found as by-products (< 1 ppm) in commercial polychlorinated biphenyls and naphthalenes and are probably derived from dibenzofuran impurities in the industrial hydrocarbons that are subsequently chlorinated (1-3). There is also circumstantial evidence that the effects of heat or arcing may produce PCDFs from polychlorinated biphenyls during use (4). This is evidenced by the relatively high levels of PCDFs (ca 100 ppm) detected in the polychlorinated biphenyl-containing heat transfer fluids that were the toxic agents in the Yusho disasters in Japan and Taiwan (3, 5, 6). PCDDs and PCDFs are also found as impurities in chlorinated phenols and their derived products (3, 7, 8), and it is apparent that the combustion of chlorinated aromatics and diverse types of chemical, industrial, and municipal waste results in the formation and release of these toxic chemicals into the environment (9-14). The potential for the

formation of PCDDs and PCDFs from nonindustrial sources, i.e. energy-derived combustion and forest fires, led to the "trace chemistries of fire" hypothesis, which speculated that the origins of PCDDs and PCDFs in the environment were nonanthropogenic (15, 16). However, analysis of aquatic sediment cores from the Saginaw River and Bay and from Lake Huron does not support the trace chemistries of fire hypothesis (17, 18). The PCDD and PCDF congener composition of dated sediment cores demonstrates that the concentrations of these compounds in sediments have greatly increased since the 1940s and that "this historical increase is similar to trends for the production, use, and disposal of chlorinated organic compounds" (17, 18).

Human exposure to PCDDs and PCDFs has occurred via three major pathways: occupational, accidental, and environmental. Industrial workers engaged in the manufacture or use of polychlorinated biphenyls, chlorinated phenols, and their derived products are exposed to PCDDs and/or PCDFs in combination with their associated major commercial product (19–21). Accidents in which PCDDs have been released into the workplace or into the environment (e. g. the Seveso accident in Italy) have also resulted in human exposure to mixtures of the industrial chemicals and their PCDD/PCDF toxic contaminants (22–24). The Yusho poisoning in Japan and Taiwan involved the exposure of several thousand individuals to PCBs and their PCDF contaminants (5, 6, 25–27). The uptake of environmental residues of PCDDs and PCDFs into higher trophic levels of the food chain is only now being investigated, and trace levels (parts per trillion) have been detected in fish, wildlife, and human tissues (19, 28–32). In common with accidental and industrial exposures to PCDDs and PCDFs, exposure levels to these toxins represent only a small fraction of the total bioavailable lipophilic environmental pollutants. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is the major by-product formed from 2,4,5-trichlorophenol and its derived products; this highly toxic compound has been the focus of most biologic and toxic studies on the PCDDs. However, all other human and environmental exposures to PCDDs and PCDFs involve a complex mixture of isomers and congeners in combination with other chemicals.

The scientific, regulatory, and media attention focused on PCDDs, PCDFs, and particularly 2,3,7,8-TCDD has continued unabated; moreover, with the recent identification of trace levels of these toxins in human tissue, domestic animals, the environment, and toxic chemical waste dumpsites, the scientific and societal concern about this class of compounds will no doubt continue. It is apparent from the scientific literature that one member of this class of compounds, namely 2,3,7,8-TCDD, ranks with benzo[a]pyrene as one of the most thoroughly studied toxins. Unfortunately, the biologic and toxic effects of the remaining 74 PCDD and 135 PCDF congeners have not been thoroughly

investigated, and the interactive effects of these compounds or their activities in combination with polychlorinated biphenyls and chlorinated phenols have also not been addressed. This article (*a*) briefly reviews the species-dependent toxic and biologic effects of PCDDs and PCDFs, (*b*) demonstrates the parallel modes of action of PCDDs and PCDFs and endogenous cellular hormones, (*c*) summarizes the data that support the proposed receptor-mediated mechanism of action, including the structure-activity relationships (SARs) that have been developed for both PCDD and PCDF congeners, and (*d*) discusses the few interactive studies published.

TOXIC AND BIOLOGIC EFFECTS OF PCDDs AND PCDFs

Several review articles (33–45) have summarized the toxic and biologic effects elicited by PCDDs, PCDFs, and related toxic halogenated aryl hydrocarbons. The toxic effects resulting from exposure to this group of chemicals are dependent on a number of factors which include the dose of the toxin, and the age, strain, species, and sex of the animals used. The complete spectrum of toxicity is not usually observed in any single animal species; however, the limited data available indicate that the toxic PCDDs, PCDFs, and related compounds elicit the same qualitative pattern of responses within each species. The differences in species susceptibility to this group of chemicals are illustrated by the LD₅₀ values for 2,3,7,8-TCDD, which vary over 5000-fold (33, 34) from the highly sensitive guinea pig to the resistant hamster [LD₅₀s (μg/kg): guinea pig (0.6–2.0), rat (22–45), chicken (25–50), monkey (70), rabbit (115), dog (100–200), mouse (114–284), bullfrog (>1000), hamster (1157–5051)]. The quantitative differences in the toxicity of PCDF congeners have recently been demonstrated (47) for a series of ten congeners (see structure-activity section, below). The 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) ED₅₀ values for thymic atrophy and body weight loss in the rat were 0.21 and 1.04 μmol/kg; the 1,2,4,7,8-PeCDF isomer elicited the same toxic effects, but the ED₅₀ values were 220 and 47 times higher, respectively (47). The toxic responses observed in several animal species by PCDDs and PCDFs include dermal toxicity, teratogenicity, reproductive problems, body weight loss, hepatotoxicity, gastric lesions, lymphoid involution, immunotoxicity, and carcinogenicity. The two most characteristic toxic effects observed in all laboratory animals are lymphoid involution and/or immunotoxicity and body weight loss. Chloracne and related dermal lesions are the most frequently noted signs of PCDD and PCDF toxicosis in humans; dermal lesions are also observed in rhesus monkeys, hairless mice, and rabbits that have been exposed to this group of toxins. In contrast, rats, most strains of mice, guinea pigs, and hamsters do not develop chloracne and related dermal toxic lesions after



Figure 1 Structure of the polychlorinated dibenzofurans and dibenzo-p-dioxins.

exposure to 2,3,7,8-TCDD. Poland & Knutson (38) have noted that many of the observed toxic lesions are either hyperplastic/metaplastic or hypoplastic, and primarily affect epithelial tissues. The mechanisms by which PCDDs and PCDFs elicit this diverse group of species-dependent toxicities remain unexplained although several hypotheses have been advanced and are discussed in this review.

PCDDs and PCDFs cause diverse biological responses in mammals and mammalian cells in culture including the highly characteristic induction of microsomal benzo[a]pyrene hydroxylase (aryl hydrocarbon hydroxylase, AHH) and several related cytochrome P-450-dependent monooxygenases (37, 38, 44, 47–53). In the rat, these activities are associated with the preferential induction of cytochrome P-450c, P-450d, and P-450a, with the former isozyme responsible for most of the induced monooxygenase enzyme activities (54). In the mouse, 2,3,7,8-TCDD induces cytochromes P₁-450 and P₃-450 (55–57), and the former isozyme exhibits antigenic and enzymatic similarities with the rat cytochrome P-450c (58). 2,3,7,8-TCDD induces two cytochrome P-450 isozymes (forms 4 and 6) in the rabbit, and their inducibility is highly tissue-specific (59, 60). 2,3,7,8-TCDD and related compounds also induce glutathione S-transferases (61) and glucuronosyl transferase (62, 63) and several other enzymes including DT-diaphorase (64), ornithine decarboxylase (65), δ -aminolevulinic acid synthetase (48), epidermal transglutaminase (66), and hepatic DNA polymerase B (67). Detailed summaries of these and other biochemical effects of 2,3,7,8-TCDD have recently been reviewed (38, 42).

The remarkably broad spectrum of biologic and toxic responses observed in animals exposed to 2,3,7,8-TCDD and related toxic halogenated aromatics has stimulated research on the mechanism or mechanisms of action of these chemicals. Unlike many toxins, the most active halogenated aryl hydrocarbons do not appear to require metabolic activation into presumed "toxic" intermediates that alkylate specific cellular acceptors (e. g. DNA, RNA, and protein) or initiate cellular lipoperoxidation. On the contrary, the most toxic halogenated aryl hydrocarbons are highly resistant to oxidative metabolic degradation and exhibit minimal metabolically mediated alkylation of cellular macromolecules (68–70); moreover limited data suggest that PCDD and PCDF metabolites are much less toxic than their parent hydrocarbons (71). Many of the effects of this

class of environmental toxins are comparable to those associated with modulation of several hormone-mediated responses. For example, there are many similarities between animals that exhibit thyroid dysfunction and those treated with toxic halogenated aryl hydrocarbons. Daily injections of the active thyroid hormone, triiodothyronine (T_3) to male mice treated with a lethal dose (200 $\mu\text{g/kg}$) of 2,3,7,8-TCDD did increase their mean survival times; however, all the animals in the 2,3,7,8-TCDD and 2,3,7,8-TCDD + T_3 -treated groups died (43). More recent studies (71, 72) have demonstrated that thyroid hormones may play a more important role in modulating the toxicity of 2,3,7,8-TCDD; radiothyroidectomy protected rats against 2,3,7,8-TCDD-mediated T-cell immunotoxicity (as measured by the spleen anti-SRBC plaque-forming cell assay), mortality, and body weight loss (73). Like the glucocorticoids, PCDDs, PCDFs, and related toxic halogenated aryl hydrocarbons cause lymphoid involution (33, 34, 37, 38, 40, 41, 74, 75), are teratogens in mice (76–80), and induce cytochrome P-450-dependent monooxygenases (37, 38, 44, 46–54). However the mechanisms of action of glucocorticoids and 2,3,7,8-TCDD are not directly linked since the latter compound is toxic to adrenalectomized rats, does not bind to the glucocorticoid receptor, and does not induce tyrosine aminotransferase (43) or the dexamethasone-induced cytochrome P-450 isozyme (81). Other adrenal steroids resemble the toxic halogenated aryl hydrocarbons since they also induce several hepatic drug-metabolizing enzymes, including monooxygenases and glucuronosyl transferases (81–84).

It has been proposed that 2,3,7,8-TCDD and related toxic isostereomers, like the steroid hormones, elicit their responses via the initial noncovalent interaction with a cytosolic receptor protein in target tissues (37, 38, 75). The synthesis of radiolabelled [^3H]-2,3,7,8-TCDD with high specific activity (52.5 Ci/mmol) resulted in the identification of a specific binding protein in hepatic cytosol of responsive C57BL/6J mice, whereas minimal binding activity was observed in nonresponsive DBA/2J hepatic cytosol (85). The role of this Ah receptor protein in the mechanism of action of toxic halogenated aryl hydrocarbon has been thoroughly investigated and satisfies most of the specific criteria that support a receptor mediated cellular process. These criteria include: (a) the existence of a finite number of binding or receptor sites and therefore saturable binding, (b) high affinity ligand binding that is commensurate with the usually low levels of circulating hormones, (c) stereoselective binding capacity for the receptor, (d) tissue or organ response specificity for the receptor ligand, and (e) a correlation between binding affinities, receptor occupancy, and the magnitude of the response. This review focuses on research that supports the role of the Ah receptor in the mechanism of action of PCDDs and PCDFs and highlights the detailed structure activity relationships (SARs) that have been developed for this group of environmental and industrial toxins.

PCDD AND PCDF ACTIVITIES: EVIDENCE THAT SUPPORTS THE ROLE OF THE CYTOSOLIC Ah RECEPTOR PROTEIN

High Affinity Saturable Binding

The saturable binding of [^3H]-2,3,7,8-TCDD with hepatic and extrahepatic cytosolic receptor protein from several species has been demonstrated using the following receptor assay procedures: charcoal/dextran absorption, protamine sulfate precipitation, hydroxylapatite absorption, isoelectric focusing in polyacrylamide gels, gel permeation chromatography, sucrose density gradient centrifugation, and gel permeation high performance liquid chromatography (85–110). Scatchard plot analysis of [^3H]-2,3,7,8-TCDD specific binding to hepatic cytosolic receptor protein gives dissociation constants (K_D) that are dependent on a number of factors including the animal species and strain, the receptor binding assay used, and the age of the animal. The K_D values for responsive C57BL/6 mice and rats vary from 0.27–3.0 nM (85, 95) and 0.13–1.2 nM (91, 95, 102), respectively, and the value for cynomolgus monkeys was approximately 3 nM (108). The concentration of hepatic cytosolic receptor was also highly variable, but the upper limit for most studies was less than 110 fmol/mg cytosolic protein (85, 91, 95). One study demonstrated that hepatic receptor levels in the rat varied with age and that these levels were endocrine independent since hepatic receptor levels were virtually unaltered by orchietomy, ovariectomy, adrenalectomy, or hyposectomy (106). This observation was consistent with the inactivity of several steroid hormones as competitive ligands for this receptor protein (85, 86, 89, 99, 102).

Since certain polycyclic aromatic hydrocarbons resemble 2,3,7,8-TCDD in their mode of induction of AHH and related cytochrome P-450 isozymes in responsive strains of mice and mammalian cells in cultures, it is not surprising that many of these compounds competitively displace [^3H]-2,3,7,8-TCDD from the receptor protein. Moreover, [^3H]-3-methylcholanthrene, benzo[a]pyrene, and dibenzo[a,h]anthracene, three active AHH inducers, exhibit saturable binding with the rat hepatic receptor protein and the ligand-receptor complex sediments at 8-9 S under low ionic strength conditions using the sucrose density gradient technique (97, 105). The radioactive binding peaks were eliminated after competition with a 200-fold molar excess of 2,3,7,8-TCDD. Similar results were also observed with responsive C57BL/6 mouse hepatic cytosol. However, [^3H]-benzo[a]pyrene unexpectedly does not yield a radiolabelled ligand-receptor binding peak that is eliminated after competition with a 200-fold molar excess of unlabelled 2,3,7,8-TCDD.

Tissue/Organ, Strain, and Cell Culture Response Specificity

The 2,3,7,8-TCDD receptor levels in several organs and tissues in Sprague-Dawley rats, C57BL/6J and DBA/2J mice have been reported (96, 103, 104,

106). The cytosolic Ah receptor concentrations (fmol/mg cytosolic protein) in the C57BL/6J mice and Sprague-Dawley rat organs and tissues were: liver, 32 ± 1.5 and 39 ± 1.9 ; lung, 23 ± 6.4 and 47 ± 4.3 ; kidney, 10 ± 0.4 and 1.2 ± 0.9 ; intestine, 8 ± 2.3 and 15 ± 1.7 ; thymus, 8 ± 2.2 and 54 ± 3.9 (The Ah receptor was not detectable in the adrenals, heart, brain, skeletal muscle, and testis). Although the Ah receptor was not detected in the cytosol of DBA/2J mice, 18 hr after administration of [^3H]-2,3,7,8-TCDD to these animals levels of the receptor-ligand complex could be measured in nuclear protein extracts from liver (5.4 ± 0.3 fmol/mg), lung (7.4 ± 0.3 fmol/mg), and kidney (4.7 ± 0.1 fmol/mg). The appearance of hepatic nuclear radiolabeled ligand-receptor protein complexes and the elimination of this radioactivity by preinjection with a large excess of unlabeled 2,3,7,8-TCDD has been reported by several groups (89, 91, 96, 99, 102, 106); however, Mason & Okey (96) demonstrated that lung, liver, and kidney nuclear Ah receptor levels were higher in the responsive C57BL/6J mice than in the nonresponsive DBA/2J strain. This observation is consistent with the fact that 2,3,7,8-TCDD and other toxic halogenated aryl hydrocarbons elicit biologic and toxic responses in both strains of mice but at different dose levels. Unfortunately there are insufficient data available to correlate tissue/organ receptor levels with the magnitude of specific responses in these target sites.

The criteria for receptor response specificity are supported by numerous studies with genetically inbred responsive and nonresponsive strains of mice and with some mammalian cells in culture. For example, there is an excellent rank order correlation between the maximum AHH inducibility in several inbred strains of mice and F_1 hybrids and the number of Ah receptor molecules per liver cell (109). Nebert and co-workers have also shown a linear correlation ($r = 0.99$) between the amount of 2,3,7,8-TCDD-receptor complex appearing in hepatic nuclei of C57BL/6 and DBA/2 mice and the percentage of maximally induced cytochrome P_1 -450 mRNA (104). Hudson and co-workers have demonstrated that for several human squamous cell carcinoma lines, the relative amount of receptor measured in each cell line correlated well with the 7-ethoxycoumarin O-deethylase inducibility in these cells by 2,3,7,8-TCDD (110).

These data that support the receptor-mediated response specificity are in contrast to data in several other studies with animals and cell cultures. Hepatic 2,3,7,8-TCDD receptor levels in guinea pigs, rats, mice, hamsters, and nonhuman primates vary less than tenfold (10 – 100 fmol/mg cytosolic protein) and exhibit comparable K_D values for [^3H]-2,3,7,8-TCDD binding (93); these levels show no correlation between their maximal hepatic AHH inducibility or susceptibility to the toxic effects of 2,3,7,8-TCDD and related halogenated aryl hydrocarbons (93, 97, 108). For several mammalian cells in culture there is no correlation between receptor levels and their AHH inducibility (98, 99, 111–113). Recent studies by Whitlock and co-workers indicate “that transcription of

the cytochrome P₁-450 gene is under both positive and negative control by at least two trans-acting regulatory factors" (113). The factors that control cytochrome P₁-450 in variant mouse hepatoma cells may also play a role in some animal species and requires further investigation. It is apparent that response specificity to Ah receptor ligands is a highly complex process that depends not only on receptor levels but also on many other factors, an observation not unique to the Ah receptor protein (113, 114).

Structure-Activity Relationships

RECEPTOR BINDING AFFINITIES OF PCDDs AND PCDFs: SUBSTITUTION EFFECTS Poland, Glover & Kende first reported the relative binding affinities of 23 halogenated dibenzo-p-dioxins and dibenzofurans using the dextran charcoal receptor assay and [³H]-2,3,7,8-TCDD as the competing radioligand (85). This study included 10 PCDD congeners and 7 PCDF congeners that differed only with respect to their degree of chlorination and substitution pattern. Table 1 summarizes results from a more recent study of the effects of structure on the receptor binding affinities of 14 PCDDs and 14 PCDFs using rat hepatic cytosol and the sucrose density gradient assay procedure (47, 51; G. Mason, J. Pikorska-Pilaszczynska, B. Keys & S. Safe, unpublished results). 2,3,7,8-TCDD and 1,2,3,7,8-pentachlorodibenzo-p-dioxin were the most avid PCDD competitive binding ligands for displacement of [³H]-2,3,7,8-TCDD from the receptor protein, and their EC₅₀ values were 1.0×10^{-8} and 7.9×10^{-8} M, respectively. Inspection of these data clearly demonstrated the importance of the lateral Cl substituents in facilitating the interaction between the PCDD ligands and the cytosolic receptor protein. The relative receptor binding EC₅₀ values for a series of tetrachloro isomers were 2,3,7,8- > 2,3,6,7- > 1,3,7,8- > 1,2,3,4-, in the order of decreasing number of lateral substituents. The fivefold difference in the receptor binding activities of the 2,3,6,7- and 1,3,7,8-TCDD isomers illustrates a more subtle structural feature that affects binding. The increased affinity of the former compound must be due to the receptor binding site preference for a vicinal 6,7- (or, 1,2) group over a meta 1,3-dichloro functionality. The data also illustrate that the degree of chlorination of non-lateral sites is an important structural determinant for interaction with the receptor protein. The 2,3,7,8-tetra-, 1,2,3,7,8-penta-, 1,2,3,4,7,8-hexa-, and 1,2,3,4,6,7,8,9-octachlorodibenzo-p-dioxins all contain four lateral Cl substituents; however, there is a marked decrease in their receptor binding avidities with increasing Cl substitution at the nonlateral 1, 4, 6, and 9 positions. The stepwise addition of Cl groups at 1, 4, 6, and 9 would result in several structural changes in the more highly chlorinated PCDDs including increased molecular size and volume, increased lipophilicity, a possible decrease in PCDD coplanarity associated with steric crowding, and decreased

Table 1 The effects of structure on the rat hepatic cytosolic receptor binding affinities and AHH/EROD induction potencies of PCDDs and PCDFs

PCDD	In Vitro EC ₅₀ (M)			PCDF	In Vitro EC ₅₀ (M)		
	Receptor Binding	AHH	EROD		Receptor Binding	AHH	EROD
2,3,7,8-	1.0×10^{-8}	7.2×10^{-11}	1.9×10^{-10}	2,3,4,7,8-	1.5×10^{-8}	2.6×10^{-10}	1.3×10^{-10}
1,2,3,7,8-	7.9×10^{-8}	1.1×10^{-8}	1.7×10^{-8}	2,3,4,7-	2.5×10^{-8}	1.8×10^{-8}	1.5×10^{-8}
2,3,6,7-	1.6×10^{-7}	6.1×10^{-8}	1.1×10^{-8}	2,3,7,8-	4.1×10^{-8}	3.9×10^{-10}	2.0×10^{-10}
2,3,6-	2.2×10^{-7}	—	—	2,3,4,6,7,8-	4.7×10^{-8}	6.9×10^{-10}	5.8×10^{-10}
1,2,3,4,7,8-	2.8×10^{-7}	2.1×10^{-9}	4.1×10^{-9}	1,2,3,7,8-	7.5×10^{-8}	2.5×10^{-9}	3.1×10^{-8}
1,3,7,8-	7.9×10^{-7}	5.9×10^{-7}	3.2×10^{-7}	1,2,3,7-	1.1×10^{-7}	2.7×10^{-5}	6.3×10^{-5}
1,2,4,7,8-	1.1×10^{-6}	2.1×10^{-8}	1.1×10^{-8}	1,3,4,7,8-	2.0×10^{-7}	1.6×10^{-9}	1.4×10^{-9}
1,2,3,4-	1.3×10^{-6}	3.7×10^{-6}	2.4×10^{-6}	2,3,4,7,9-	2.0×10^{-7}	7.9×10^{-9}	5.8×10^{-9}
2,3,7-	7.1×10^{-8}	3.6×10^{-7}	1.4×10^{-7}	2,3,4,8-	2.0×10^{-7}	4.1×10^{-8}	3.8×10^{-8}
2,8-	3.2×10^{-6}	$>1.0 \times 10^{-4}$	$>1.0 \times 10^{-4}$	1,2,3,4,7,8-	2.3×10^{-7}	3.6×10^{-10}	3.8×10^{-10}
1,2,3,4,7-	6.4×10^{-6}	6.6×10^{-7}	8.2×10^{-7}	1,2,3,6,7,8-	2.7×10^{-7}	1.5×10^{-9}	1.2×10^{-9}
1,2,4-	1.3×10^{-5}	4.8×10^{-5}	2.2×10^{-6}	1,2,3,7,9-	4.0×10^{-7}	8.6×10^{-8}	8.6×10^{-8}
OCDD	$>1.0 \times 10^{-5}$	3.1×10^{-7}	7.0×10^{-7}	1,2,4,7,8-	1.3×10^{-6}	1.1×10^{-7}	1.5×10^{-7}
1-	$>1.0 \times 10^{-4}$	$>1.0 \times 10^{-4}$	$>1.0 \times 10^{-4}$	1,2,4,6,8-	3.1×10^{-6}	1.0×10^{-5}	1.2×10^{-5}

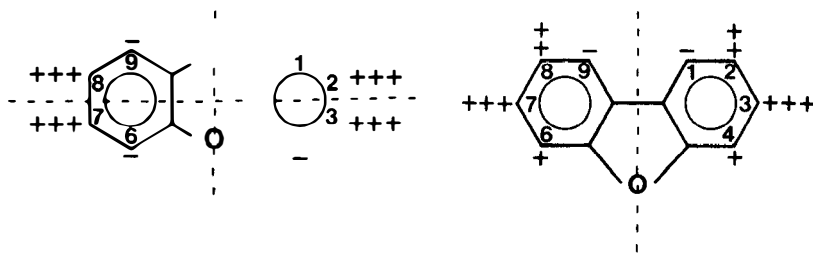


Figure 2 The differential effects of chlorine substituents at different positions in the dibenzo-p-dioxin and dibenzofuran rings on the relative receptor binding affinities of PCDD and PCDF congeners.

aromatic ring electron density (due to the additional electronegative Cl groups). One or more of these changes may be related to the decrease in binding affinities of the more highly chlorinated 2,3,7,8-substituted PCDDs. Figure 2 summarizes the differential effects of Cl substituents on the affinities of PCDDs for the cytosolic receptor protein and illustrates the importance of lateral chloro groups. It has also been suggested that the other critical structural factors that contribute to the high binding affinities of 2,3,7,8-TCDD include the planar ring structure and an ideal ligand area, $3 \times 10^4 \text{ \AA}^2$ (37, 38).

The direct binding of radiolabelled 2,3,7,8-tetrachlorodibenzofuran (TCDF) and other PCDFs to the Ah receptor protein has not been demonstrated. However, the competitive binding affinities of three 2,3,7,8-substituted compounds demonstrated their relatively high binding affinities for the receptor protein (85). The development of new procedures for the synthesis of PCDFs (115) has resulted in the preparation of over 40 congeners that have been used to develop detailed SARs for this series of halogenated aryl hydrocarbons (47, 51). The dibenzofuran ring system possesses a single axis of symmetry (Figure 2); therefore there are four geometrically different positions on each aromatic ring, namely C-1 (or C-9), C-2 (or C-8), C-3 (or C-7), and C-4 (or C-6). A complete SAR for PCDFs as ligands for the receptor protein must distinguish between the differential contributions of all four positions on the dibenzofuran ring. (See Table 1 for compounds selected for this study.) Inspection of these data confirms that the most active congeners, 2,3,4,7,8-penta-chlorodibenzofuran (PeCDF, $1.5 \times 10^{-8} \text{ M}$), 2,3,7,8-TCDF ($4.1 \times 10^{-8} \text{ M}$), 2,3,4,6,7,8-hexachlorodibenzofuran (HCDF, $4.7 \times 10^{-8} \text{ M}$), and 1,2,3,7,8-PeCDF ($7.5 \times 10^{-8} \text{ M}$) were all fully substituted in their lateral 2, 3, 7, and 8 positions. Moreover, a comparison of the receptor binding EC_{50} values for the 2,3,4,7,8-, 1,2,4,7,8-, and 1,2,4,6,8-PeCDF isomers demonstrates the importance of lateral chloro substituents since there is a decrease in receptor binding affinities with decreasing lateral substitution. Two pairs of PCDF isomers, namely 1,3,4,7,8- and 1,2,4,7,8-PeCDF, 2,3,4,7- and 2,3,4,8-TCDF, differ only with respect to their substitution of C-2 (or C-8) and C-3 (or

C-7). In both cases the C-3 (or C-7) substituted compounds were 6.5–8 times more active than the corresponding C-2 (or C-8) isomers as competitive ligands for the rat hepatic cytosolic receptor protein. A comparison of the relative binding affinities of a series of C-1 (or C-9) and C-4 (or C-6) isomer pairs illustrates the higher binding activities of the isomer that retains the C-4 (or C-6) substituent. For example the EC_{50} values for the 2,3,4,7-, 2,3,4,7,8-, 2,3,4,7,9-, and 2,3,4,6,7,8-substituted PCDFs were 2.5×10^{-8} M, 1.5×10^{-8} M, 2.0×10^{-7} , and 4.7×10^{-8} M whereas the values for the corresponding C-1 (or C-9) isomers (i.e. 1,2,3,7-TCDF, 1,2,3,7,8-, and 1,2,3,7,9-PeCDF, and 1,2,3,6,7,8-HCDF) were 1.1×10^{-7} M, 7.5×10^{-8} M, 3.4×10^{-7} M, and 2.7×10^{-7} M, respectively.

Figure 3 illustrates an overlay of 2,3,7,8-TCDD and 2,3,4,7,8-PeCDF, the two most active PCDD and PCDF ligands for the Ah receptor. The molecular areas and volumes of the dibenzofuran and dibenzo-p-dioxin ring systems are similar, but the spatial orientations of their substituents exhibit marked differences. The C-3 (or C-7) substituents occupy a position between the lateral 2,3 (or 7,8) groups in 2,3,7,8-TCDD and clearly occupy the dominant lateral position in the dibenzofuran ring system. The spatial orientations of the C-4 (and C-6) and C-2 (and C-8) substituents are comparable and exhibit less overlap with the lateral positions of 2,3,7,8-TCDD; the C-1 (or C-9) PCDF-substituents exhibit the least overlap with the lateral positions of 2,3,7,8-TCDD. These observations on the molecular orientations of the dibenzofuran Cl substituents are consistent with the observed SARs for PCDF receptor binding affinities and illustrate the stereospecific nature of the receptor protein-ligand interactions.

RECEPTOR BINDING AFFINITIES OF PCDDs AND PCDFs: A QSAR ANALYSIS The receptor binding avidities of PCDDs and PCDFs summarized in Table 1 and in other studies (37, 38, 47, 51) not only demonstrate the importance of Cl substitution patterns on ligand-receptor protein complex formation but also show that substituents are important structural determinants for these interactions. For example, the receptor binding EC_{50} values for 2,3,7-trichlorodibenzo-p-dioxin is 7.1×10^{-8} M; replacement of the 7-Cl substituent with H gives 2,3-dichlorodibenzo-p-dioxin, which exhibits a greatly diminished receptor binding EC_{50} value ($> 10^{-5}$ M). It is clear that these substituent effects at this lateral C-7 position must be related to differences in their physicochemical characteristics which in turn influence ligand-receptor avidities. A series of substituted PCDD, PCDF, and polychlorinated biphenyl analogs have been synthesized (Figure 4) as probes for delineating the effects of substituent structure on ligand-receptor binding affinities (116–118). Each series of analogs contains a variable substituent group at a single lateral position, and it is apparent that substituent structure has a remarkable effect on

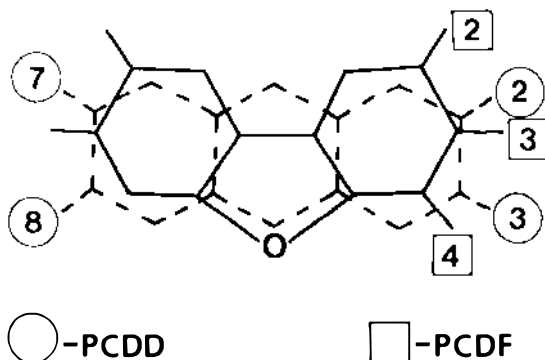


Figure 3 Overlay of 2,3,7,8-TCDD and 2,3,4,7,8-PeCDF structures.

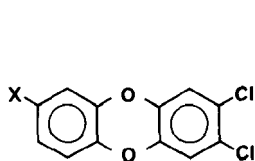
the receptor binding avidities of these compounds. For example the EC_{50} value for 7-trifluoromethyl-2,3-dichlorodibenzo-p-dioxin (1.95×10^{-8} M) was 1000 times lower than the value for 7-amino-2,3-dichlorodibenzo-p-dioxin (2.88×10^{-5} M). The effects of different substituents on the activity of a series of analogs can be analyzed quantitatively by correlating the differences in a biological effect (e.g. receptor binding) with known substituent physicochemical parameters (116–119), such as lipophilicity (π), electronegativity (σ), hydrogen bonding capacity (HB), and substituent width (ΔB_5). Multiparameter linear regression analysis of the receptor binding data for sixteen 7-substituted-2,3-dichlorodibenzo-p-dioxins gave the following equation (1):

$$\log (1/EC_{50}) = 1.24\pi + 6.11 \quad 1.$$

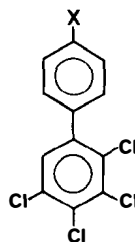
$$(n = 14, s = 0.29, r = 0.950),$$

where π is the substituent lipophilicity, s is the standard deviation, and r is the correlation coefficient. The only substituents treated as outliers for the derivation of this equation were the bulky C_6H_5 and $t-C_4H_9$ groups, which possess van der Waals volumes of 48.5 and 41.8 cm^3/mol , respectively. This suggests that substituent molecular volumes are also important structural determinants for determining ligand affinities for the receptor protein binding site. Previous studies with the 4'-substituted-2,3,4,5-tetrachlorobiphenyls indicated that the maximum molecular volume for lateral substituents was < 35 cm^3/mol (118). However it is apparent that if substituent molecular volume requirements are satisfied, the the receptor binding affinities of these analogs are directly related to the lipophilicity of the 7-substituents.

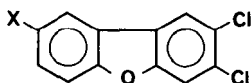
The competitive receptor binding affinities of a series of thirteen 8-substituted-2,3,4-trichlorodibenzofurans and ten 8-substituted-2,3-dichlorodibenzofurans (Figure 4) have also been determined, and the competi-



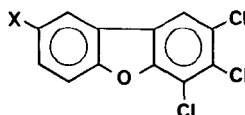
X = CF₃, Br, I, Cl, F, OH, CH₃,
OCH₃, NO₂, CN, NH₂



X = H, OH, CH₃, F, OCH₃, COCH₃, CN,
Cl, CH₂CH₃, Br, I, CH(CH₃)₂, CF₃



X = *t*-C₄H₉, F, Br, I, *i*-C₃H₇,
Cl, CH₃, OCH₃, OH, H



X = Cl, Br, CF₃, I, F, CH₃, *i*-C₃H₇,
C₂H₅, *t*-C₄H₉, H, OCH₃, OH, CH₂Br

Figure 4 Structures of substituted PCDDs, PCDFs, and polychlorinated biphenyls used for QSAR studies.

tive displacement binding data results have been analyzed by multiparameter linear regression analysis to give Equations 2 and 3, respectively.

$$\log (1/EC_{50}) = 1.09\pi + 5.77 \quad 2.$$

$$\log (1/EC_{50}) = 1.10\pi + 5.19 \quad 3.$$

For the 8-substituted-2,3,4-trichlorodibenzofurans, both the *t*-C₄H₉ and *i*-C₃H₇ were outliers, whereas only the *t*-C₄H₉ substituent was not included in the derivation of Equation 3. Analysis of the collective data for 33 substituted polychlorinated dibenzofurans and dibenzo-*p*-dioxins (Figure 5) has demonstrated the excellent linear correlation between the log (1/EC₅₀) receptor binding data and lipophilicity (π). Moreover the slopes and intercepts for Equations 1–3 were not significantly different. These data are consistent with a receptor protein that binds the PCDDs and PCDFs at a common binding site(s) on the protein; this site must accommodate the molecular area and volume encumbered by these ligands, and the QSAR results are consistent with a binding site that is highly hydrophobic.

The effects of substituent structure on the rat hepatic cytosolic receptor binding affinities of 4-substituted-2,3,4,5-tetrachlorobiphenyls have also been

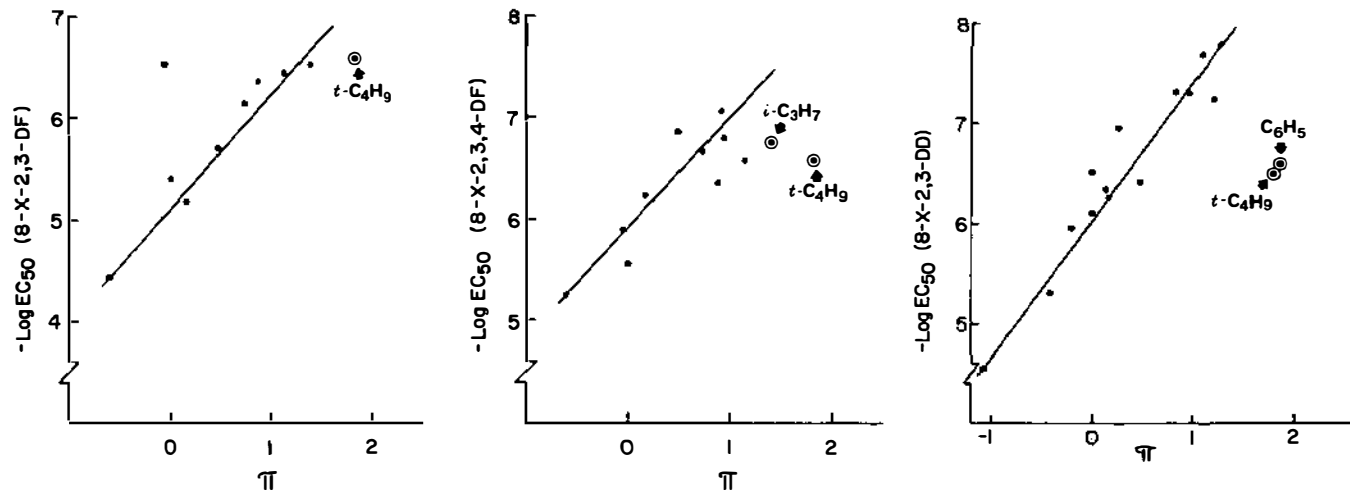


Figure 5 Correlation between receptor binding avidities for the substituted PCDDs and PCDFs vs the substituent lipophilicity (π) values.

reported (118). Multiparameter linear regression analysis of the results of these analogs gave Equation 4, which suggests that substituent lipophilicity, electronegativity (σ) and hydrogen bonding capacity (HB) are factors that influence receptor-ligand affinities:

$$\log (1/EC_{50}) = 1.39 \sigma + 1.31\pi + 1.12 \text{ HB} + 4.20 \quad 4.$$

The bulky $t\text{-C}_4\text{H}_9$ and C_6H_5 substituents were also treated as outliers for the derivation of Equation 4. These results demonstrate that substituent molecular volumes play a role in ligand-receptor interactions for the substituted PCBs, PCDDs, and PCDFs. However, the data analysis for the former group of analogs suggests that the critical 4'-substituent for the PCBs interacts with both polar and hydrophobic regions of the receptor binding site. The differences between Equations 1–3 and Equation 4 are somewhat paradoxical since molecular overlap of the four sets of substituted ligands does not indicate that there are major differences in their spatial orientation (Figure 4). Therefore it is likely that the differences observed for the substituted PCBs are due to the free rotation about the Ph-Ph bond and a limited population of the coplanar conformers. Chlorinated biphenylenes that possess a fixed coplanar ring structure exhibit binding affinities comparable to those of 2,3,7,8-TCDD and related isostereomers; this emphasizes the importance of a planar ring system. It is also possible that the receptor binding sites for the substituted PCBs and PCDDs/PCDF are not identical, a problem currently being investigated in my laboratory.

AHH INDUCTION ACTIVITIES OF PCDDs AND PCDFs The *in vivo* and *in vitro* SARs for PCDDs and PCDFs as inducers of hepatic and extrahepatic AHH have been reported by several groups (37, 38, 47–49, 51–53). The most active PCDDs were substituted in their 2,3,7, and 8 position; inspection of the data in Table 1 indicates that there were comparable SARs for PCDDs as ligands for the receptor protein and as AHH inducers; however, there is not a linear correlation between these two bioassays. SARs for several PCDF congeners as *in vitro* AHH inducers were comparable to those already discussed for receptor binding. Moreover, for the PCDFs summarized in Table 1 a comparison of *in vitro* EC_{50} values for AHH induction in rat hepatoma H-4-II E cells and *in vivo* ED_{50} s for AHH induction in male Wistar rats showed a linear correlation between these two values. Like the PCDD congeners, however, there was not a strong correlation between AHH induction potencies and receptor binding avidities for the PCDF congeners. A comparison of the AHH and EROD induction potencies of the 7-substituted-2,3-dichlorodibenzo-p-dioxins with their rat hepatic cytosolic receptor binding avidities also showed that there was not a linear correlation between the two *in vitro* activities for this series of

analogs (116). Multiple parameter linear regression analysis of the AHH induction results for these compounds gave the following equation:

$$\log (1/EC_{50})_{AHH} = 1.60 \pi - 0.33(\Delta B_5)^2 + 5.85 \quad 5.$$

Like the receptor binding avidities for these substituted PCDDs, their AHH induction potencies were dependent on substituent lipophilicity; however, a second parameter, STERIMOL (ΔB_5) has also been included in the derivation of this equation. The STERIMOL parameter (119) is a measure of the maximum width of the substituents (compared to H) from the axis connecting the 7-substituent to the rest of the molecule and has previously been used in QSAR studies that involve the interaction of substituted organic ligands and macromolecules. The dependence of AHH induction activities by the substituted PCDDs on both ΔB_5 and π suggests that substituent-dependent effects such as conformational changes in the ligand-receptor complex occur after the initial receptor-ligand binding process.

The AHH and EROD induction EC_{50} values for the 8-substituted-2,3-di- and 2,3,4-trichlorodibenzofurans in rat hepatoma H-4-II E cells were also subjected to multiple parameter linear regression analysis to give Equations 6 and 7, respectively (117).

$$\log (1/EC_{50})_{AHH} = 0.80\pi + 0.87\Delta B_5 - 0.35(\Delta B_5)^2 + 4.63 \quad 6.$$

$$\log (1/EC_{50})_{AHH} = 0.76\pi + 1.11\Delta B_5 + 2.23 \sigma_p + 6.78 \quad 7.$$

Both Equations 5 and 6 showed that AHH induction potencies for the 8-substituted-2,3-dichlorobenzofurans and 2,3-dichlorodibenzo-p-dioxins were dependent on substituent and ΔB_5 parameters. The correlation for the more highly chlorinated set of analogs, the 8-substituted-2,3,4-trichlorodibenzofurans, also includes a Hammett substituent parameter (σ_p); presumably the requirement for σ_p must be due to the effects of the C-4 chlorine group, which constitutes the only structural difference between the two sets of substituted PCDF analogs.

Equations 8–10 were developed from the AHH and EROD induction data for the 8-substituted-2,3-dichlorodibenzo-p-dioxins, -2,3-dichlorodibenzofurans, and -2,3,4-trichlorodibenzofurans, respectively.

$$\log (1/EC_{50})_{EROD} = 0.99 \log (1/EC_{50})_{AHH} - 0.07 \quad 8.$$

$$\log (1/EC_{50})_{EROD} = 0.90 \log (1/EC_{50})_{AHH} + 0.83 \quad 9.$$

$$\log (1/EC_{50})_{EROD} = 0.92 \log (1/EC_{50})_{AHH} + 0.28\pi + 0.27 \quad 10.$$

For a total of twenty-five 7-substituted-2,3-dichlorodibenzo-p-dioxins and 8-substituted-2,3-dichlorodibenzofurans and fifteen 4'-substituted-2,3,4,5-tetrachlorobiphenyls there was a linear correlation between the EC_{50} values for AHH and EROD induction, and the slopes for these equations were not significantly different from that of equation one. These data suggest that both ethoxyresorufin and benzo[a]pyrene are catalyzed by the same cytochrome P-450 isozyme(s). In contrast, Equation 10 required a π term to correlate the effects of the substituted 2,3,4-trichlorodibenzofurans; the rationale for these differences between the two sets of substituted PCDF analogs is unknown.

OTHER BIOLOGIC EFFECTS OF PCDDs AND PCDFs: STRUCTURE-ACTIVITY RELATIONSHIPS PCDDs and PCDFs elicit a broad spectrum of species-dependent biologic effects, but because pure standards have been unavailable few studies report qualitative or quantitative SARs for these compounds. The SARs for PCDDs as inducers of ALA synthetase were comparable to the effects of structure on their activities as AHH inducers (48). Knutson & Poland have used cultured XB cells derived from a mouse teratoma as an *in vitro* model for halogenated aryl hydrocarbon toxicity (120). 2,3,7,8-TCDD and related toxic isostereomers produce a dose-dependent keratinization response which in part resembles the *in vivo* dermal toxicity that develops in some animals after exposure to these toxins. The most active PCDD congeners in this *in vitro* assay possessed three or four lateral substituents, and the SARs were similar to those reported for their receptor binding affinities. 2,3,7,8-TCDD causes comparable dermal toxicity in cultures of newborn foreskin keratinocytes (121).

Several human squamous cell carcinoma (SCC) lines have been utilized as model systems for investigating the mechanism of action of toxic halogenated aryl hydrocarbons (110, 122). SCC cells possess variable Ah receptor levels, and the relative amount of receptor in several cell lines correlates with the maximal 7-ethoxycoumarin O-deethylase inducibility in these cell lines. 2,3,7,8-TCDD causes down-regulation of the epidermal growth factor (EGF) receptor in the SCC-12F cell line, and this effect is dose- and structure-dependent. Both 2,3,7,8-TCDD and 2,3,7,8-tetrabromodibenzofuran, which exhibit a high affinity for the Ah receptor, decrease EGF receptor binding, whereas 2,7-dichlorodibenzo-p-dioxin is inactive (123). Comparable results have been observed in keratinocyte strains derived from normal neonatal foreskin; the authors report that 2,3,7,8-TCDD acts (in part) through the Ah receptor in epidermal basal cells to enhance terminal differentiation (123). 2,3,7,8-TCDD also down-regulates EGF receptor activity in hepatic plasma membranes in several animal species and cultured mouse hepatoma cells (124, 125); however, structure-activity effects in the rat do not necessarily support the role of the Ah receptor in mediating this process (125).

TOXICOLOGY OF PCDDs AND PCDFs: STRUCTURE-ACTIVITY RELATIONSHIPS

The dose-response acute toxicities of nine PCDD isomers and congeners in the guinea pig and responsive mouse have been reported (33, 34, 126). The relative LD₅₀ values in both species were highly dependent on the number of lateral Cl substituents and the degree of substitution; their rank order of toxic potencies was similar to their in vitro receptor binding and AHH induction activities as discussed above. A comparative study (127) of the toxicity of 2,3,7,8-TCDF, 2,3,7,8-tetrabromodibenzofuran, and 2,3,4,7,8-pentachlorodibenzofuran in guinea pigs, mice, and rhesus monkeys confirms that these compounds elicit the characteristic broad spectrum of toxic effects observed for 2,3,7,8-TCDD and related isostereomers.

Poland & Glover (75) reported the effects of several PCDD congeners and other toxic halogenated aryl hydrocarbons on genetically inbred strains of mice. Although dose-response studies were carried out only with 2,3,7,8-TCDD, the relative toxicities (i. e. thymic atrophy) of these compounds correlated with their in vitro binding and induction activities. The dose-response toxicities (thymic atrophy and body weight loss) of the PCDFs listed in Table 1 have been determined in the immature male Wistar rat (47). Inspection of the ED₅₀ data for the toxic effects showed that the potencies of these congeners were structure-dependent and that the in vivo SARs for toxicity were identical to those observed for their in vitro AHH induction potencies. Figure 6 summarizes a plot of the $-\log ED_{50}$ values for thymic atrophy and body weight loss in immature male Wistar rats vs their in vitro AHH induction activities. The linear correlation constant (r) and slope for the plots of the reciprocal log values for AHH induction vs body weight loss were 0.96 and 1.30 (slope), respectively, and values of 0.88 (r) and 1.16 (slope) were obtained for the comparable plot of the reciprocal log values for AHH induction vs thymic atrophy. The linear correlation was observed only for those compounds that do not contain vicinal unsubstituted carbon atoms and are not significantly metabolized. For example, the toxicity of 1,2,3,7- or 2,3,4,8-TCDF in the rat was lower than predicted by the in vitro AHH (or EROD) induction data (not shown) owing to in vivo metabolism. Current research in my laboratory (S. H. Safe, unpublished results) indicates that for several PCDD isomers and congeners there is a linear correlation between $-\log EC_{50}$ (AHH induction) and $-\log ED_{50}$ (thymic atrophy and body weight loss in the rat). These results suggest that the rat hepatoma cell monooxygenase induction bioassay may serve as a short-term test system for predicting the toxicities of PCDDs, PCDFs, and related halogenated aryl hydrocarbons.

Poland and co-workers have investigated the effects of 2,3,7,8-TCDD, several PCDD congeners, and related halogenated aryl hydrocarbons in the skin of inbred HRS/J hairless mice segregating for the *hr* locus (128, 129). The homozygous *hr/hr* hairless and heterozygous *hr/+* haired mice exhibit identical

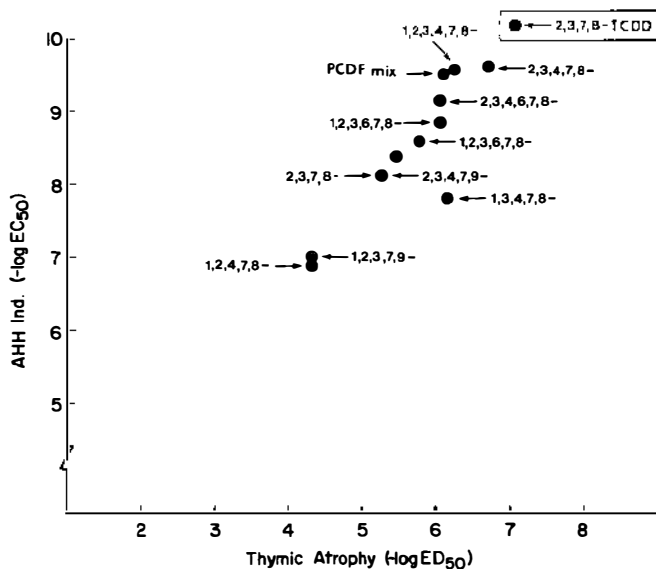
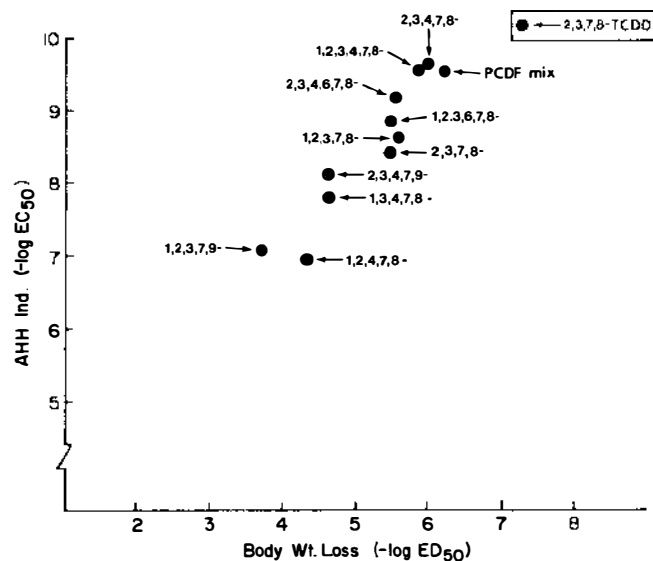


Figure 6 A plot of the $-\log EC_{50}$ values for in vitro AHH induction vs the $-\log ED_{50}$ values for thymic atrophy (right) and body weight loss (left) in the male Wistar rat for several PCDF congeners and 2,3,7,8-TCDD.

genetics except for one allele at the *hr* locus. Topical application of 2,3,7,8-TCDD to the dorsal skin of hairless mice resulted in epidermal hyperplasia, sebaceous gland metaplasia, and hyperkeratosis, but these histological lesions are not observed in *hr*/+ haired mice. The development of a graded epidermal response by several PCDD congeners, 2,3,7,8-TCDF, and related toxic aryl hydrocarbons was structure-dependent and correlated with rank order of receptor binding affinities for these compounds (128). For example, the total dose (nmol/mouse) required to produce a 2⁺ response was 0.36, 1.76, 1.2 > 360 and > 360 for 2,3,7,8-TCDD, 1,2,3,6,7,8-HCDF, 2,3,7,8-TCDD, 1,3,6,8-TCDD, and 2,7-DCDD, respectively. These results are consistent with the involvement of both the *Ah* and *hr* loci in the development of murine epidermal lesions after exposure to PCDDs, PCDFs, and related toxins. 2,3,7,8-TCDD can also act as a potent tumor promoter in HRS/J mice, and the results of this study confirm the segregation of the activity with the *Ah* and *hr* loci. A qualitative structure-activity study also suggests a possible role for the *Ah* receptor in mediating the tumor promotion activities of the toxic halogenated aryl hydrocarbons (130).

The SARs for PCDDs and PCDFs clearly support an *Ah* receptor-mediated mechanism of action for these compounds; comparable studies have been reported for other classes of halogenated aryl hydrocarbons. It is assumed that the persistent effects elicited by these toxins are related to a sustained receptor-ligand occupancy of nuclear binding sites, but this has not yet been demonstrated experimentally. The identity and role of any endogenous ligand(s) for the *Ah* receptor have not been determined; however, lumichrome, a riboflavin metabolite, does bind to the receptor (131).

Genetic Evidence

Pharmacogenetic studies with genetically inbred strains of mice typified by the *Ah*-responsive C57BL/6 and nonresponsive DBA/2 mice have provided strong evidence in support of the role of the *Ah* receptor in mediating the biologic and toxic effects of toxic halogenated aryl hydrocarbons. Nonresponsive DBA/2 mice contain relatively low levels of hepatic or extrahepatic cytosolic or nuclear *Ah* receptor (< 1 fmol/mg cytosolic protein), whereas much higher levels of the receptor are detected in responsive strains of mice. The ED₅₀ for 2,3,7,8-TCDD mediated hepatic microsomal AHH induction in C57BL/6J mice was 1 nmol/kg whereas this value is at least tenfold higher in DBA/2J mice. In genetic crosses and backcrosses between C57BL/6J and DBA/2J mice the trait or responsiveness to AHH induction is inherited in a simple autosomal mode (50, 132–134). The responsive backcross animals also had detectable hepatic receptor levels (85, 89, 97, 103, 109). The segregation of the toxicity of PCDDs and PCDFs with the *Ah* locus has been determined primarily with 2,3,7,8-TCDD using both responsive and nonresponsive genetically inbred mice and their

crosses and backcrosses. The results illustrate that several toxic effects including teratogenicity, porphyria and hepatotoxicity, immunotoxicity, and body weight loss segregate with the Ah locus (75–80, 135–139). Dermal toxic lesions appear to be dependent on the interaction between the Ah and hr locus as previously noted (128–130). It has also been suggested that additional genetic loci may also be involved in the hepatotoxic effects of 2,3,7,8-TCDD, however this observation requires further substantiation (140).

PCDDs, PCDFs, AND RELATED COMPOUNDS—INTERACTIVE EFFECTS

Although the SARs and toxicology of PCDDs and PCDFs have been extensively studied the interactive effects of PCDD/PCDF mixtures and related compounds are not well understood. 2,3,7,8-TCDD can act as a tumor promoter for several initiators in the rodent liver (141), mouse skin (130), and C3H/10T1/2 cells (142), and as a cocarcinogen causing 3-methylcholanthrene-initiated subcutaneous tumors in nonresponsive DBA/2 mice (143). In contrast, 2,3,7,8-TCDD exhibits anticarcinogen activity in female CD-1 mice (144). These effects are related to the agent's induction of drug-metabolizing enzymes that alter rates of metabolic activation of polynuclear aromatic hydrocarbon initiators. Several studies (51, 53, 120, 145, 146) report the application of *in vitro* bioassays as short-term tests for assessing the potential toxicity of PCDD/PCDF mixtures; a comparison of the *in vitro* AHH induction activity of a reconstituted mixture of PCDFs identified in Yusho patients (147) and the toxicity of this mixture (Figure 6) suggest that the effects of the individual PCDFs in this mixture are additive (148). In contrast, the immunotoxicity and AHH induction activity of 2,3,7,8-TCDD in C57BL/6 mice were decreased by coadministering a nontoxic or noninducing dose of 2,3,7,8-TCDF (10 µg/kg) (149). A rational explanation for the antagonistic effects of 2,3,7,8-TCDF is not apparent.

Birnbaum and co-workers (77) have reported that treatment of pregnant mice with a combination of 2,3,7,8-TCDD (3 µg/kg) and a nontoxic dose of 2,3,3',4,4',5-hexachlorobiphenyl (20 µg/kg) resulted in a tenfold increase in incidence in cleft palate compared to those animals receiving only 2,3,7,8-TCDD. A second PCB congener, 2,2',4,4',5,5'-hexachlorobiphenyl, at dose levels of 50 or 25 mg/kg in combination with 2,3,7,8-TCDD (3 µg/kg), did not effect the teratogenic potency of the latter compound. Although 2,3,3',4,4',5-hexachlorobiphenyl is less toxic than 2,3,7,8-TCDD, this monortho coplanar PCB congener elicits several receptor-mediated biologic and toxic effects (74). It is conceivable that the interactive effects of 2,3,3',4,4',5-hexachlorobiphenyl and 2,3,7,8-TCDD may be additive if the dose-response curve for the former compound is steep and if the 20 mg/kg dose level is just

below the minimum observable teratogenic dose. Current research in my laboratory has demonstrated that administration of several compounds, including several polychlorinated biphenyl congeners, increase hepatic 2,3,7,8-TCDD receptor levels in rats and C57BL/6 mice. Pretreatment of rats and mice with these receptor modulators followed by administration of 2,3,7,8-TCDD results in markedly increased hepatic AHH and EROD induction activities. Both positive and negative modulators and antagonists of the hepatic and extrahepatic cytosolic receptor protein are currently being investigated as probes for delineating the mechanism of action of PCDDs and PCDFs and the role of the receptor protein in mediating these effects. These interactive studies will also be important for assessing the effects of polyhalogenated aromatic environmental pollutant mixtures and their potential human health impact.

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