Linking Dioxins to Diabetes: Epidemiology and Biologic Plausibility

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Recent epidemiologic studies suggest a possible association between dioxin-like compounds (DLCs) and diabetes in human populations, although experimental links between DLCs and diabetes are lacking. The public health significance of such an association is that all populations are exposed to small but measurable levels of DLCs, chronic low-dose exposure to which may hasten the onset of adult-onset diabetes in susceptible individuals. In this article, we review the epidemiologic studies and propose biologically plausible connections between dioxins and diabetes. Specifically, we suggest that aryl hydrocarbon (Ah) receptor functions may antagonize peroxisome proliferator–activated receptor (PPAR) functions, and hence that the Ah receptor may promote diabetogenesis through a mechanism of PPAR antagonism. ***Key words:*** **Ah receptor, diabetes, non-insulin-dependent diabetes mellitus, peroxisome proliferator-activated receptors, PPARγ, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, type 2 diabetes.** ***Environ Health Perspect*** **110:853–858 (2002). [Online 17 July 2002]**

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The term *dioxin* can refer both specifically to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and more generally to other polychlorodibenzo-*p*-dioxin congeners, polychlorinated dibenzofurans, and coplanar polychlorinated biphenyls. Like other organochlorine compounds, dioxin-like compounds (DLCs) are persistent, lipophilic, and prone to bioaccumulation. Most toxicologic work has been carried out with TCDD, and the toxicologic behavior of other DLCs is usually assumed to be similar. The total dioxin concentration is estimated as its toxic equivalence (TEQ), where $TEQ = \Sigma(DLC)_i \times (TEF)_i$ and the TEFs are toxic equivalency factors that normalize the toxic potencies of the various DLCs to that of the reference toxicant TCDD, with TEF = 1 (*1*).

When the toxicity of TCDD was first discovered, attention was directed toward its acutely lethal effects (*2–4*) in highly sensitive rodents such as guinea pigs (*5*). The realization that human beings are less susceptible to these acute effects shifted the focus to low-level chronic effects such as carcinogenicity (*6*) and reproductive toxicity (*7,8*), both of which can be induced in animal models at concentrations close to those to which humans are exposed (*9*).

Humans are exposed to DLCs either occupationally or environmentally. Environmental sources of DLCs include combustion by-products that form whenever organic materials are burned in the presence of chlorine sources and, in the case of coplanar PCBs, improper disposal and accidental contamination (*9*). Occupations that formerly led to exposure included the manufacture and use of 2,4,5-trichlorophenol and associated products such as herbicides, and bleaching of wood pulp with chlorine (*10,11*). Releases of dioxins from these activities have greatly declined (*12*). In the general population, exposure comes mostly from fatty foods such as meat, fish, and dairy products, at the rate of some 3–6 pg of TEQ/kg body weight/day (*9*). Almost all human subjects have detectable body burdens of DLCs, mostly stored in adipose tissue, and because DLCs have long whole-body half-lives, levels in the general population have probably not yet kept pace with lower rates of release to the environment.

Low levels of dioxin exposure have recently become a focus of interest in the context of their possible link with the incidence of diabetes. Adult-onset diabetes (*13,14*), whose incidence continues to rise in Western countries, is a metabolic disorder involving abnormal energy metabolism (*15,16*). Perhaps significantly, lethal poisoning by dioxins also involves disruption of energy metabolism, characterized by depletion of lipid stores of the affected animal (the "wasting syndrome") (*17*). In rats, Rozman (*18*) demonstrated nearly identical dose–response behavior by TCDD for suppression of food intake and inhibition of the enzyme phosphoenolpyruvate carboxykinase (PEPCK) involved in gluconeogenesis [although later work (*19*) with a wider range of rodents did not consistently affirm this correlation]. Experimental evidence has also linked dioxin exposure to impaired glucose transport, leading to speculation that chronic low-level exposure to dioxins might be a risk factor for diabetes. In this article, we review the relevant epidemiologic studies concerning the strength of association between DLCs and adult-onset type 2 diabetes [non-insulin-dependent diabetes mellitus (NIDDM)] and consider mechanisms that attempt to provide biologic plausibility for this association. The focus is on biochemical similarities and differences among aryl hydrocarbon (Ah) receptor function, mechanisms for diabetogenesis, and peroxisome proliferator-activated receptor (PPAR) functions.

Review of Epidemiologic Studies

Several epidemiologic studies have linked high dioxin burdens to increased risk of diabetes or modified glucose metabolism (*20–25*). The following groups of exposed humans have been the subject of dioxin-related epidemiologic studies: German employees of factories where phenoxy herbicides were manufactured (*20*); U.S. employees of factories located in Newark, New Jersey, and Verona, Missouri, involved in the manufacture of 2,4,5-trichlorophenol and the associated herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T, no longer used in Europe or North America) (*21,22*); residents of Seveso, Italy, where a 1976 explosion at a 2,4,5-trichlorophenol factory exposed local residents to TCDD (*23*); and U.S. Air Force veterans involved in spraying defoliants during the Vietnam War (*24–27*).

A major difficulty is that exposure occurred many years before the epidemiologic studies. Dioxin levels at the time of exposure must be back-calculated from current concentrations in serum lipids, using the whole-body half-life of TCDD, which is quite variable in humans, 5.8–9.6 years (*27*). This variation probably reflects differences in body fat, with high body fat acting to retain TCDD, because mobilization from adipose tissue may be the rate-limiting step in its elimination (*28,29*). Moreover, although the study subjects were all exposed specifically to TCDD (a ubiquitous contaminant in 2,4,5-trichlorophenol and 2,4,5-T), they were also exposed to much larger amounts of phenols and phenoxy herbicides, and both they and the referents were also exposed to other DLCs. With one exception, the epidemiologic studies monitored only the TCDD concentrations.

Sweeney et al. (*21*) compared 281 living male U.S. workers, employed more than 15 years earlier in the production of chemicals contaminated with TCDD, with an unexposed group of 260 volunteers. The workers

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had a mean serum lipid adjusted level of 220 pg TCDD/g lipid ppt); that of the referents was 7 ppt. Exceptionally, these investigators measured serum lipid concentrations of other DLCs, which were similar in both workers and controls. The TEQ values for workers and referents, based on 1998 values of TEFs (*1*), were 256 and 35 ppt; that is, the ratio of exposed to unexposed concentrations fell from 31 based on TCDD alone to 7.3 based on TEQ. Sweeney et al. (*21*) found a slight increase in the risk of diabetes [odds ratio (OR) = 1.12, $p < 0.003$] and high fasting serum glucose ($p < 0.001$) with increasing serum concentrations of TCDD, but cautioned that age, weight, and family history are more important risk factors than TCDD exposure in the development of diabetes. Serum triglyceride levels increased with TCDD exposure, and testosterone levels were lower in the exposed group.

A follow-up study of the same cohort, but excluding individuals under treatment for diabetes, considered the OR for diabetes as a function of serum lipid TCDD concentration (*22*). When all subjects were included, irrespective of TCDD level, the OR for diabetes was 1.49 [95% confidence limit (CL), 0.77–2.91). However, no dose–response relationship was seen between (present) TCDD levels of the exposed workers and either diabetes incidence (Table 1) or mean glucose levels.

Somewhat parallel results were reported by Zober et al. (*20*) in the follow-up of workers exposed to TCDD in a 1953 accident in Germany. Development of chloracne was used as a surrogate for TCDD exposure at the time of the accident. As an exception, workers experiencing chloracne had a lower incidence of diabetes than did referents, as defined by the *International Classification of Diseases,* 9th revision (*30*). This inverse relationship was strongest for workers with the least chloracne. We discuss this study again further below.

Henriksen et al. (*24*) studied glucose levels, insulin levels, and diabetes prevalence in 989 U.S. veterans of Operation Ranch Hand who were exposed to Agent Orange in Vietnam. The referents were 1,276 other U.S. veterans who served in Southeast Asia during that period, but who were not involved with the defoliant program. Veterans with a history of diabetes before service were excluded from the study. An important difference from the studies of industrial workers discussed above is the much lower serum lipid concentrations of TCDD: mean values 12.2 ppt for Ranch Hand veterans and 4 ppt for referents at the time of study in 1992. Ranch Hand veterans had a higher incidence of serum glucose abnormalities [relative risk (RR), 1.4; 95% CL, 1.1–1.8], serum insulin abnormalities (RR, 3.4; 95% CL, 1.9–6.1), diabetes (RR, 1.5; 95% CL, 1.2–2.0), and the use of oral medications to control diabetes (RR, 2.3; 95% CL, 1.3–3.9). The Ranch Hand subjects were divided into three exposure groups: background (BG) with TCDD < 10 ppt in 1992, LO (extrapolated initial TCDD < 94 ppt), and HI (extrapolated initial TCDD > 94 ppt; Table 2). High TCDD concentrations correlated with increasing body fat and shorter time to onset of diabetes among diabetic veterans.

Michalek et al. (*25*) used the same definitions for the BG, LO, and HI exposure groups in a similar study of 871 Ranch Hand veterans and 1,121 referent Vietnam veterans. Table 3 shows abnormalities in the HI group for insulin levels (diabetic and nondiabetic subjects), fasting glucose concentrations (diabetics), and steroid hormone-binding globulin (SHBG) among diabetics.

Longnecker and Michalek (*26*) investigated whether the effects of prior exposure to TCDD persist even after levels returned to background, by restricting their investigation to Ranch Hand veterans in the BG group. Again, there was a relation between increased body mass index and higher serum lipid TCDD. Quartiles were established, and Q1 was defined as the reference group (Table 4). Although not all statistically significant, diabetes incidence and changes in serum insulin and blood glucose all showed a dose–response trend with increasing serum lipid TCDD.

Cranmer et al. (*31*) examined 69 people who lived within 25 miles of the Vertac/Hercules Superfund site in Jacksonville, Arkansas, restricting their study to those with normal glucose levels during glucose tolerance testing. Only subjects in the top decile for serum lipid TCDD concentration showed any statistically significant effects. Hence, the reference group was TCDD < 15 ppt (n = 62), and the high exposure group was TCDD > 15 ppt (n = 7). No differences between the groups were seen for blood glucose, after either fasting or a 75-g glucose challenge, but differences in insulin levels were observed (Table 5). Other known risk factors for hyperinsulinemia could not explain this finding, thus suggesting that high blood TCDD concentrations may cause insulin resistance.

A well-studied population is that of Seveso, Italy, where a 1976 explosion at a factory that produced 2,4,5-trichlorophenol exposed the general populace to TCDD. Bertazzi et al. (*23*) reported death rates among this population to 1991 in three zones

Table 1. Lack of dose–response relationship between present TCDD levels of the exposed workers and diabetes incidence.

TCDD (ppt)	< 20	20–75	75–238	> 238
OR (95% CL)	2.11 (0.77–5.75)	1.51 (0.53–4.27)	0.67 (0.17–2.57)	1.97 (0.79–4.90)

Diabetes defined as > 7.8 mmol/L glucose on 2 consecutive days after a 12-hr fast. Results were unchanged when reevaluated at 7.0 mmol/L glucose. Hyperglycemia = fasting serum glucose > 140 mg/dL. The same criteria were also used in Sweeney et al. (*21*). Data from Calvert et al. (*22*).

Table 2. Dose–response relationships between serum lipid TCDD and glucose metabolism parameters for Ranch Hand subjects.

Condition	BG [OR (95% CL)]	LO [OR (95% CL)]	HI [OR (95% CL)]
Diabetes	0.7 (0.5–1.0)	1.3 (1.0–1.7)	1.5 (1.2–2.0)
Fasting glucose	0.7 (0.5–1.0)	1.3 (0.9–1.7)	1.2 (0.9–1.7)
2 hr postglucose	0.9 (0.6–1.2)	1.2 (0.9–1.7)	1.6 (1.2–2.2)
High insulin[a]	0.8 (0.4–1.9)	1.0 (0.4–2.5)	3.4 (1.9–6.1)

Diabetes is defined as > 200 mg/dL 2 hr postprandial (or by medical diagnosis); hyperinsulemia is defined as serum insulin > 97th percentile of comparison group; for hyperglycemia, fasting glucose challenged with 100 g glucose measured 2 hr postprandial (> 115 mg/dL = high; 140–200 mg/dL = impaired; > 200 mg/dL = abnormal). Subjects were divided into three groups: background (BG: TCDD < 10 ppt in 1992), LO (extrapolated initial TCDD < 94 ppt) and HI (extrapolated initial TCDD > 94 ppt), exposure groups. Data from Henricksen et al. (*24*).
[a]Among nondiabetics.

Table 3. Abnormalities in the HI group for insulin levels (diabetic and nondiabetic subjects), fasting glucose concentrations (diabetics) and SHBG among diabetics for 871 Ranch Hand veterans and 1,121 referent Vietnam veterans (means).

Condition	Referents	BG	LO	HI
Body fat (%)	21.7	20.1	22.4	23.4
Insulin, nondiabetic (μIU/mL)	67.7	62.6	64.2	81.1
Insulin, diabetic (μIU/mL)	63.6	72.9	87.3	48.5
Fasting glucose, nondiabetic (mg/dL)	99.0	99.3	98.9	98.6
Fasting glucose, diabetic (mg/dL)	137.4	132.2	126.7	156.1
SHBG, nondiabetic (nmol/L)	28.5	29.9	31.4	30.0
SHBG, diabetic (nmol/L)	24.0	24.6	24.3	31.7

Exposure groups: BG, background (TCDD < 10 ppt in 1992); LO, extrapolated initial TCDD < 94 ppt; HI, extrapolated initial TCDD > 94 ppt. Diabetes is defined as > 200 mg/dL postprandial or by medical diagnosis; hyperinsulemia and hyperglycemia, by statistical comparison with referents. Data from Michalek et al. (*25*).

(labeled in decreasing order of exposure as A, B, and R) compared with the background Italian population. For both males and females, overall death rates were within Italian norms, but there were consistent slight increases in diabetes mortality among the exposed populations (Table 6). Unfortunately, no data on diabetes incidence were provided. All RRs are clearly small but point consistently in the direction of a slight enhancement of mortality from diabetes with dioxin exposure, especially for females.

Discussion of Epidemiologic Studies

Except for the chemical manufacturing workers (*20,22*), there is a rather consistent finding of a slight increase in diabetes incidence among subjects exhibiting elevated serum lipid TCDD concentrations, as well as abnormal glucose and/or insulin levels among subjects who were exposed to TCDD. No information is available to identify other substances to which the chemical manufacturing workers may have been exposed, although exposure to high levels of trichlorophenol and phenoxy herbicides may be inferred. Inevitably, there are differences in the statistical measures used in these studies, making direct comparisons difficult. Another possible confounding factor is the choice of referents (allegedly unexposed chemical workers rather than the general populations).

Leaving aside the studies involving industrial workers for the moment, several factors suggest underestimation of the RR parameters. First, and typical of studies involving xenobiotics, no completely "dioxin-free" control population is available; there is background exposure of both subjects and referents to TCDD; further, all subjects are exposed to other DLCs, usually to an unknown extent. This compresses the apparent OR for an adverse effect of TCDD (but by an unknown amount), just as if one compared the RR of lung cancer between heavy smokers and light smokers, rather than between heavy smokers and nonsmokers. In addition, present knowledge does not indicate the significance of chronic exposure to approximately 5 pg TCDD/g serum lipid (or 35 pg TEQ per gram of serum lipid) in the general population.

Second, the actual serum lipid TCDD concentrations at the time of exposure are always unknown and must be extrapolated back from the time of analysis, many years later. This compresses the range of TCDD levels between slightly and highly exposed subjects, because all concentrations are lower; also, one cannot be confident that the rank ordering of TCDD concentrations at the time of analysis matches the rank ordering at the time of exposure, because whole-body half-lives are variable. One cannot improve the statistical power of these studies by recruiting additional subjects, because all those willing to participate are already involved (*20*).

The justification for the study by Longnecker and Michalek (*26*) was the possibility (*23,32,33*) that prior dioxin exposure may have health consequences after dioxin levels have returned to the background range. The investigators found a slight correlation between diabetes incidence and serum lipid TCDD among Ranch Hand veterans whose TCDD concentrations had returned to the normal range by the time of the study, although the data did not necessarily indicate a causal relationship.

Biologic Plausibility between DLCs and Diabetes: Receptor-Mediated Mechanisms

DLCs are universally regarded as exerting their toxic effects through binding with the Ah receptor, a cytoplasmic multimeric protein complex whose structure and function were reviewed recently (*34*). The Ah receptor ligand-binding subunit, often abbreviated ALBS, is a ligand-activated transcription factor, whose identified heterodimerization partners include Arnt (*34*), the NFκB protein RelA (*35*), and retinoblastoma protein (*36*). ALBS/Arnt heterodimers also bind coregulators that influence the activity of various nuclear receptors, such as the estrogen receptor (*37,38*). Although the Ah receptor is believed to mediate all of the toxic effects of DLCs, the possibility exists that components of the multiprotein complex besides ALBS initiate some of the signaling pathways when the liganded complex dissociates.

In the context of glucose metabolism, PPARs, which are also nuclear receptors, are ligand-activated transcription factors that control lipid metabolism and homeostasis. PPARs have recently been linked to cellular proliferation, differentiation, and apoptosis, as well as to obesity, diabetes, atherosclerosis, inflammation, cancer, and aging (*39*). Among PPAR coregulatory proteins, a number have PPAR-binding LXXLL motifs in their primary sequences. The three transcription factors PPAR α, β (also called δ), and γ are expressed from related genes with varying degrees of homology and have isoforms that are splice variants (*40*).

Links between PPARγ and diabetes are its mediation of the antidiabetic effects of thiazolidinediones (see below) and the activated receptor's role in promoting differentiation of adipocytes, which mediate glucose and lipid homeostasis, and translation of the glucose transporter protein GLUT4 (*41,42*). Because DLCs inhibit preadipocyte differentiation (*43*), Ah receptor activation may therefore antagonize PPARγ function, perhaps suggesting that dioxin exposure might be a risk factor for diabetes through antagonism of PPARγ functions, which may lead to insulin resistance. Enan et al. (*44*) and Liu and

Table 4. Dose–response trend with increasing serum lipid TCDD for Ranch Hand veterans in the background group.

TCDD in 1992, ppt	Q1 < 2.8	Q2 2.8–4.0	Q3 4.0–5.2	Q4 > 5.2
Diabetes		0.93 (0.53–1.66)	2.51 (1.53–4.11)	2.63 (1.56–2.94)
Change in insulin (μIU/μL)		0.16 (0.02–0.30)	0.24 (0.09–0.38)	0.37 (0.23–0.51)
Change in fasting glucose (mg/dL)		1.3 (−0.3–2.8)	1.9 (0.3–3.4)	3.0 (1.5–4.6)
Change in postchallenge glucose (mg/dL)		4.8 (0.2–9.4)	6.2 (1.4–11)	10.2 (5.4–15)

Values are OR (95% CL). Diabetes, > 200 mg/dL postchallenge with 100 g glucose or medical diagnosis. Hyperglycemia was not measured. The response to a glucose challenge was compared with those of the reference group. Data from Longnecker and Michalek (*26*).

Table 5. Insulin levels, either fasting or after a 75-g glucose challenge, for residents living within 25 miles of the Vertac/Hercules Superfund site in Jacksonville, Arkansas.

	Insulin level (mIU/mL)		
	TCDD > 15 ppt	TCDD < 15 ppt	OR for high insulin
Fasting	7.0	2.0	8.5
30 min postglucose	412	79	7.0
60 min postglucose	325	100	12
120 min postglucose	294	65	56

The reference group was TCDD < 15 ppt (n = 62) and the high-exposure group was TCDD > 15 ppt (n = 7). Subjects had normal glucose tolerance in tests of 75 g glucose with blood sampled at 0, 30, 60, 120 min, with the amount of insulin released, calculated in mIU/mL/hr, statistically greater than referents. Data from Cranmer et al. (*31*).

Table 6. Deaths to 1991 ascribed to diabetes among residents of Seveso, Italy, who were exposed to TCDD after a 1976 explosion at a 2,4,5-trichlorophenol factory.

Zone	No., RR (95% CL)	
	Males	Females
A	None, NA	2, 1.8 (0.4–7.0)
B	6, 1.2 (0.5–2.7)	18, 1.8 (1.0–3.0)
R	38, 1.1 (0.8–1.5)	75, 1.2 (0.9–1.5)

The cause of death was obtained from death certificates. Zones A, B, and R had decreasing exposure to TCDD. Data from Bertazzi et al. (*23*).

Matsumura (*45*) have shown that adipocytes from dioxin-treated guinea pigs or mice exhibit reduced glucose transport activity. Consistent with antagonism of PPARγ functions, Liu and Matsumura (*45*) found that both the copy number of GLUT4 [which is expressed in adipose and striated muscle tissues (*46*)] and its mRNA were significantly reduced in adipose tissue of mice after TCDD treatment. This experiment involved a single exposure at high dose and long exposure time (116 mg/kg dioxin for 40 hr), in contrast with human populations, which experience years of exposure at much lower doses; nevertheless, it raises the possibility that dioxin exposure might lower GLUT4 expression in human subjects. Because the *in vivo* concentration of any protein reflects the balance between production and degradation, continuous exposure to DLCs might gradually reduce the production of glucose transporter proteins such as GLUT4, thereby causing progressive insulin resistance. An unknown factor, however, is how useful rodent experiments are in modeling human metabolism involving PPARs, because peroxisome proliferation is much more strongly induced in rodents.

A novel class of insulin-sensitizing drugs, thiazolidinediones (*47*), increases insulin-stimulated glucose import rates in peripheral tissues via increased expression of GLUT1 and GLUT4 (*48*). Thiazolidinediones bind to and activate PPARγ in adipocytes, stimulating the removal of glucose from the circulatory system and its transformation to lipids (*49*). Suzuki et al. (*50*) evaluated the effects of thiazolidinedione treatment on the expression of 42 diabetes-related genes. If DLCs are indeed diabetogens, their effect on the production of these mRNAs should antagonize that of thiazolidinediones, but this is not yet known.

Thiazolidinediones also inhibit the action of tumor necrosis factor α (TNF-α), a multifunctional cytokine that induces insulin resistance (*47*) and influences hyperlipidemia, nutrient-sensing pathways, body fat, and mortality after chronic infections. The TNF-α system is thought to provide survival advantage during periods of food shortage by inducing peripheral insulin resistance, thus saving glucose for the brain (*51*). Experiments carried out in the context of dioxin-induced immunotoxicity (*52–54*) and carcinogenesis (*55*) have revealed Ah receptor-dependent increases in TNF-α expression after TCDD exposure. A possible link to adult-onset diabetes is that TNF-α has been shown to reduce the production of PPARγ mRNA [see Greene et al. (*40*) and references therein], raising the possibility that chronic dioxin-induced TNF-α expression might cause adult-onset diabetes by inducing insulin resistance.

Compared with other members of the nuclear receptor superfamily, PPARs have a spacious ligand-binding pocket (*40*) that allows them to be activated by diverse ligands, some of which, like DLCs, are polycyclic and lipophilic. This suggests that certain ligands for the Ah receptor might bind PPARγ and interfere, positively or negatively, with its activation. Such a finding might refute the long-standing dogma that all the toxic effects of DLCs are mediated by the Ah receptor, although this idea has not yet been tested experimentally. A potentially relevant observation is that TCDD-induced cytochrome P450 (CYP)1A1 expression in the male guinea pig is saturated at three orders of magnitude lower than the amount of TCDD required for acute lethality (*16*), which demands different mechanisms for lethality and CYP1A1 induction. One hypothesis might have lethality involving DLC binding to PPARs, and CYP1A1 expression involving DLC binding to Ah receptor.

Another possible pathway for cross-talk between Ah receptor and PPARγ signaling is through LXXLL domains (nuclear receptor boxes), which are present on numerous coactivators and corepressors that bind to members of the nuclear receptor superfamily (*56–63*). Of these, SRC-1 was found to bind Ah receptor via LXXLL motifs (*64*), whereas RIP140 was shown to bind Ah receptor via an LXXLL-independent mechanism (*65*). Whereas these experiments explored the role of LXXLL domains in coregulator sequences in mediating binding to the Ah receptor, the possibility that ALBS contains LXXLL domains that can bind to and influence the activity of nuclear hormone receptors such as PPARγ has never been determined. In that case, ALBS might corepress PPAR transcription factors, besides being a transcription factor itself.

The amino acid sequences of 10 Ah receptors (zebrafish, rabbit, chicken, human, mouse, rat, two sequences from the killifish, and Ah receptor A and B of the rainbow trout) were downloaded from the Pubmed protein database (*66*) and the Prosite Web site (*67*). They were searched for LXXLL domains, including those in which L is replaced by V, M or I, which are chemically similar. The LXXLL domains located at positions 48–52 and 114–118 of the rainbow trout Ah receptor A sequence were conserved in all 10 sequences, and the domain located at 441–446 was present in 9 of the 10 sequences (Table 7). The conservation of these sequences, especially the one within the PAS domain, which mediates heterodimerization, suggests that the Ah receptor may bind receptors such as PPARγ, although the small size of this consensus sequence is also consistent with it having arisen by chance.

Another link between Ah receptor and PPAR functions is that both receptors influence PEPCK activity or expression [PPARs (*68–70*) and Ah receptor (*17,71*)]. Additionally, both the Ah receptor and PPARα have been connected to hyperlipidemia [Ah receptor (*72*) and PPAR (*73*)], and type 2 diabetes is associated with abnormal lipid metabolism (*74*). Another analogy is that PPARγ is involved with the action of interleukin-1 (IL-1), IL-6, and TNF-α induction of IL-1β (*40*), whereas Ah receptor functions influence or are influenced by IL-6 (*75*), IL-2 (*76*), and IL-1β (*53,77*).

As noted above, most of the extant epidemiologic studies involving highly exposed industrially exposed workers do not follow the pattern of a correlation between serum lipid TCDD concentrations and diabetes-related disorders (*20,22*). The workers were involved specifically in the production of phenoxy herbicides: 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-T are known peroxisome proliferators that act by binding to PPARs (*78,79*). Hence, we speculate that the apparent lack of a positive association between TCDD exposure and diabetes in the industrial workers may have been caused by antagonism between the diabetes-promoting action of TCDD and the possible antidiabetic actions of the phenoxy herbicides. However, although Ah receptor function may antagonize PPAR functions generally, its possible antagonism of PPARγ, which is implicated in diabetes, has not been studied. Conversely,

Table 7. Alignment of LXXLL and LXXLL-like domains in 10 Ah receptor primary amino acid sequences.

Species identifiers	Positions of conserved sequences		
	First	Second	Third
Rainbow trout (*Oncorhynchus mykiss*) Ahr A, AF065137[a]	48–51 LtgLL	114–118 LLqaL	441–446 (L)LgsL(M)
Rainbow trout (*Oncorhynchus mykiss*) Ahr B, T30557[a]	49–54 LtgLL	115–119 LLqaL	452–457 (L)LgsL(M)
Zebrafish (*Danio rerio*) AF0634461.1[a]	48–52 LtnLL	122–126 LLqaL	450–455 (L)LgsM(L)
Rabbit (*Oryctolagus cuniculus*) D382261.1[a]	48–54 LasLL	117–121 LLqaL	454–459 (L)LsaL(M)
Chicken (*Gallus gallus*) AAF70373[a]	47–51 LasLL	117–121 LLqaL	454–459 (L)LsaM(L)
Homo sapiens[b]	49–54 LasLL	118–122 LLqaL	456–460 LLaaM
Mus species[b]	49–54 LasLL	116–120 LLqaL	450–454 (L)MsaL(I)
Rat species[b]	49–53 LasLL	116–120 LLqaL	454–459 (L)Msa(L)
Killifish (*Fundulus heteroclitus*) AF024591.2[a]	42–46 LasLL	108–113 LLqaL	448–453 (L)LgaL(M)
Killifish (*Fundulus heteroclitus*) AAC59[a]	49–53 LmeLL	113–118 LLqaL	not present

Residues whose positions create an overlapping LXXLL domain are indicated in parentheses.
[a]Pubmed (*66*) sequences are identified with the ID number. [b]Data from Prosite (*67*).

although PPARγ is implicated in mediating antidiabetic actions by thiazolidinediones, possible contributions to antidiabetic actions from the other PPARs and their ligands are unknown, as is the ability of PPARγ to interact with peroxisome proliferators or other environmental agents (*80*).

We draw three major conclusions from this review. First, epidemiologic evidence indicates a weak association between low (ppt) concentrations of TCDD in serum lipid and diabetes. Although the RR is small, the prevalence of diabetes in Western society and the ubiquity of DLCs suggest that even a small increased risk of diabetes among susceptible members of society would have public health significance. Second, we propose biologic plausibility for a link between DLCs and diabetes through interaction between Ah receptor and PPARγ-mediated signaling pathways. Finally, peroxisome proliferation by phenoxy herbicides may explain the apparent contrast in effects of TCDD on highly exposed industrial workers. We believe that exploration of the interaction between Ah receptor and PPAR signaling will be a fruitful area for future research.

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