Looking Beyond TEQs for Association Between Thyrotoxicity and Behavioral Effects

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Abstract

Thyroid and nervous system effects caused by TCDD and PCBs may appear to be independent. Attempts to correlate both of these effects with TCDD equivalents distort relationships because they fail to consider non-Ah receptor agonists. They also fail to consider the varied mechanisms that cause thyroid dysfunction. Ah receptor-independent thyrotoxic effects and compensatory responses are very rapid. Examining larger and more complete biological as well as chemical data bases is necessary.

Introduction

TCDD and similar coplanar halogenated aromatics are potent toxicants which cause a well-defined spectrum of effects mediated through the Ah receptor [1,2]. Potencies for these effects can be estimated for individual compounds as well as mixtures based on the equivalence to TCDD in one or more parameters. These relative potencies represent "toxic equivalency quotients" (TEQs) [2,3]. TCDD TEQs have poor predictability for actions not mediated through the Ah receptor [3].

Key proteins induced by Ah receptor agonists are CYP 1A monooxygenases and UDP-glucuronyl transferases (UDPGTs) [2,4]. Coplanar PCBs and PCB mixtures [5,6] have long been known to disrupt thyroid hormone homeostasis though enhanced UDP-Glucuronic acid conjugation and excretion, among other mechanisms. According to a detailed model, UDPGT induction is the major mode of TCDD-associated hypothyroidism [7]. Perinatal hypothyroidism is known to affect nervous system development [8], but TCDD-associated behavioral changes were not associated with hypothyroidism in rats [9]. Likewise, prenatal and neonatal human exposure to dioxins and PCBs (as TCDD TEQs) caused both hypothyroidism [10] and mild neurodevelopmental deficits [11], but there was no relation between thyroid hormones and neurologic deficits [11].

Previous studies in fish-eating birds had also shown positive correlations between TCDD TEQs and embryo mortality and deformities, but not between TEQs and behavioral changes [12]. Yet, there was a positive correlation between total PCBs and behavioral changes [12].

There are multiple mechanisms for disruption of thyroid hormone homeostasis by PCBs [6] and PCBs have a much broader spectrum of biological effects than do pure Ah receptor agonists[3,13]. Halogenated aromatic-induced hypothyroidism may, indeed, not be of adequate magnitude and duration to influence neurodevelopment; however, preoccupation with TCDD-TEQs has resulted in censored analytical data so that any correlations between non-Ah receptor associated hypothyroidism and neurobehavioral changes cannot be determined from the data sets being analyzed. We have attempted to broaden the data base being considered to avoid premature and/or erroneous conclusions.

Experimental Methods

Concern regarding the lack of comparative data for PCB effects not associated with the Ah receptor [13,14] prompted exploratory studies which were eventually published [15]. Thyroid effects [16] and enzyme parameters [17] which indicate, as well as influence, responses were measured simultaneously in the same animals. A basic protocol was developed in order to systematically compare the actions of several relatively uninvestigated PCBs. This multi-system bioassay has been referred to as the female rat integrated endocrine disruption assay (FRIEDA) [17-22].

The FRIEDA uses weanling female Sprague-Dawley rats which receive 2 to 4 intraperitoneal (ip) injections on consecutive days and are then terminated the day after the last injection. The ip route was chosen to avoid complexities of less direct dose delivery. Weanling animals are used to make the data set more relevant to development, but still permit using litter mates as comparable experimental units. Microsomal P450 activities are assayed as O-dealkylation of 7-alkoxyresorufins. Ethoxy (EROD) being an indicator of CYP1A, pentoxy (PROD) indicates CYP2B1 and benzoxy (BROD) reflects both 2B1 and 3A23 activity.

Results and Discussion

The 2-dose duration of FRIEDA does not permit detection of certain well-documented endpoints such as vaginal cornification responses to xenoestrogens and thymic involution responses to Ah receptor agonists. In addition, hepatic enzymes are not fully induced during this time.

This protocol, however, has demonstrated that thyroid effects occur very rapidly and partial compensation may actually take place before the time of sacrifice. The hypothalamo-pituitary-thyroid axis responds to low circulating T4 levels by thyrotropin-mediated release of thyroid stimulating hormone (TSH) which then acts on the thyroid follicle to initiate mobilization of stored T4 [6]. The TSH response results in elongation of the follicular epithelial cells and a decrease in the volume of the colloid area. Longer *in utero* [16] or subchronic exposure [23] to PCBs may cause additional thyroid changes.

Following a 2-day exposure to a high TEQ landfill soil extract [19,24,25], morphometric changes indicating T4 mobilization occur at doses below those at which T4 depletion is significant[26]. These data also indicate that the follicular changes were maximal at the lower dose; thus, serum T4 declined at higher doses when the capacity for immediate response was surpassed.

In most cases, T4 mobilization has been demonstrated prior to probable enhanced excretion of T4-glucuronide. UDPGT induction lags behind induction of P450s and is marginal when the follicular response is apparent (Figure 1). The T4 mobilization following short-term dosing may even result in an elevation of serum T4 at lower doses of (a) a low TEQ PCB air extract [19], (b) 2,3,3',4',6-pentaCB (CB 110) [27] or (c) even acrylamide [28] which does not induce UDPGT. Thus, nonTCDD-like compounds affect thyroid hormones by mechanisms other than or in addition to UDPGT induction.

The rapid TSH increase indicates either an undetected earlier decrease in T4 or a direct effect on the hypothalamus and/or pituitary gland. Aroclor 1242 has previously been shown to sensitize pituitary cell suspensions to GnRH-mediated LH release [15]. At a total dose of 160 mg/kg Aroclor 1242, TSH returns to control values within a day while TSH remains elevated for at least 3 days following a total dose of 240 mg/kg Aroclor 1242 [21].

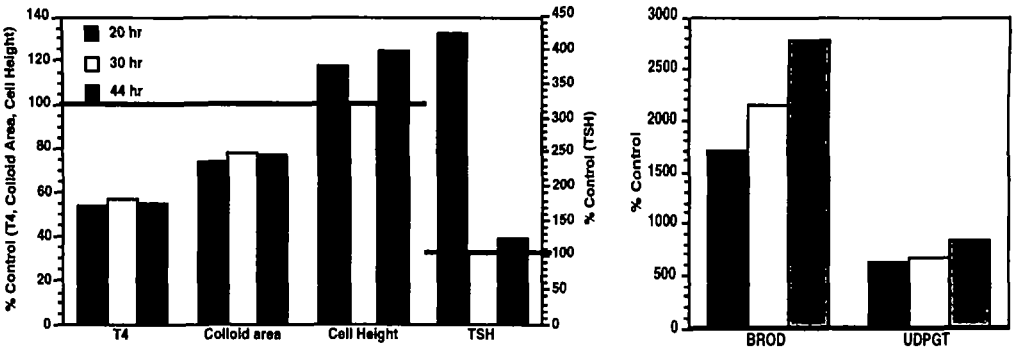

Figure 1. Time-dependent thyroid and enzyme responses to 2 X 80 mg/kg Aroclor 1242 in the FRIEDA [21].

At total doses of 120 mg/kg Aroclor 1242, serum T4 is lower if the dose is administered as 2 X 60 mg/kg on days 23 and 24 but the morphometric change is less than if dosing was started on day 20 (Table 1) [22].

Table 1. Total serum T4 and thyroid follicle morphometry in the FRIEDA when 120 mg/kg Aroclor 1242 doses are divided and administered on different days.

Aroclor 1242 (da x mg/kg)	Treatment Days					Thyroid Response (% Control)		
						Serum T4	Cell Height	Colloid Area
3 x 0	20		22		24	100	100	100
5 x 24	20	21	22	23	24	51*	152*	74*
3 x 40	20		22		24	42*	169*	73*
2 x 60				23	24	35*	161*	83

*Significantly different from controls ($p < .05$)

Thus, it appears that compensation for the initial disruption in T4 metabolism is partially effective within 2-3 days, even with continued exposure. Thyroid changes due to equilibrium exposure to environmental PCB levels would probably be undetectable. Even transient disruptions in thyroid hormone homeostasis is probably not of significance to adults. Very high continuous exposures to cumulative (PCBs + other agents) thyroid insults, however, may eventually lead to hypertrophic or hyperplastic thyroid diseases [6].

Pulses of high exposure (e.g. occupational, atmospheric, fish meal) superimposed on an already "vibrating" endocrine system may have more deleterious effects. This is especially true for immature animals where the thyroid disruption may occur during a critical window of development. Both PTU- and PCB-induced hypothyroidism in neonatal rats may cause such diverse manifestations as hearing impairment [29] and enlarged testes [30].

Structure:Activity relationships are limited. Many environmental toxicants can disrupt thyroid hormone homeostasis. For chlorinated aromatic compounds, the emphasis has been on Ah

receptor agonists because of their high potency for other effects. Aroclor 1254 has a high TCDD TEQ and is about twice as potent as Aroclor 1242 in causing hypothyroidism in Sprague-Dawley rats [30]. The landfill soil and dust extracts containing high PCB [24] and PCDF [25] levels were equivalent in decreasing serum T4 whether based on TCDD TEQ or total PCB [19]; the mixture of airborne PCBs over the landfill, however, was more potent than the TCDD TEQ would have predicted. Filtering the soil extract over activated charcoal greatly decreased the TCDD TEQ as well as P450 1A1 and UDPGT induction potential, but had little effect on T4 depletion [20]. Another difference between the Aroclors is that Aroclor 1254 has a greater proportion of more persistent congeners, but this difference was not great between the air and soil extracts.

Surprisingly, the PCB profiles of the soil and air extracts were very similar, being dominated by tri- and tetraCBs; the persistent CB 28 (2,4,4'-triCB) was the major congener in all 3 extracts [19,24]. At best, CB 28 has a mild thyroid effect when administered *in utero* [16]. The highly persistent CB 153 (2,2',4,4',5,5'-hexaCB also proved to have a mild thyroid effect in the FRIEDA [17] and a moderate effect following *in utero* exposure [16]. CB 47 (2,2',4,4'-tetraCB) had a moderate thyroid effect in FRIEDA when the weaning age was 21 days rather than 20 days [22]. The more labile congeners, CB 95 (2,2'3,5',6-pentaCB) and CB 110 (2,3,3',4',6-pentaCB) are major components of Aroclor 1254 and are more effective at depleting T4 than are persistent CBs 99 (2,2',4,4',5-pentaCB) and 153 [17,27,31].

Because of multiple mechanisms, independent criteria favoring thyroid hormone disruption by chlorinated aromatics include (a) high TCDD TEQs, (b) high proportion of lower chlorinated PCB congeners, and/or (c) PCBs with the labile 2,3,6-chlorination pattern. Enzyme induction has been used to categorize effects [2-4]. CBs 47, 99, 110 and 153 are good inducers of P450 2B (PROD and reflected in BROD) while CBs 47 [32] and 99 [31] are good inducers of P450 3A (reflected in BROD activity). CB 95 is a poor inducer of BROD and PROD [31] and none of these induce EROD as do Ah receptor agonists. Therefore, a pure SAR for thyroid hormone disruption is not possible with the limited data available. For certain, multiple mechanisms must be involved so that different PCB groups will have distinct SARs even though the net effect on serum T4 may be the same.

The effects of labile congeners on T4 may be regarded as trivial since they are not considered to be important components of the food chain. On the contrary, 2,3,6-chlorinated PCBs 84, 95, 110 and 149 are found to accumulate in fish [33,34], especially in pelagic fish [35]. Multi-ortho congeners also tend to be more volatile and constitute significant proportions of airborne PCBs [24]. Labile congeners such as CB 18 (2,2'5-triCB) are also high in fish and airborne mixes as well as in children [36] and accidentally exposed adults [37].

None of these congeners remain in humans very long, but the pulse of exposure may provide an endocrine insult which will be manifest at a later stage of development when records of exposure (i.e. residues) are gone. In addition, the emphasis on persistence and TCDD-like properties has censored reports of most of these congeners from residue studies; thus, the impression is created that they are not present even if they did present a hazard. It is precisely these labile and nonTCDD-like congeners which are also most active in the various neurochemical studies presented. Perhaps the thyroid insult, alone, is inadequate to cause neurodevelopmental deficits, but the combined pulses of simultaneous endocrine disruption and neurochemical dysfunction may be adequate. Certainly trying to force correlations between TCDD TEQs and neurotoxicity will not reveal whether or not these pulses are detrimental. We can only take a step backward and try to broaden the data base as well as work with uncensored analytical data.

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