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Hepatic Enzyme Induction and Acute Endocrine Effects of 2,3,3′,4′,6-Pentachlorobiphenyl in Prepubertal Female Rats

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Abstract. Polychlorinated biphenyls (PCBs) with the labile 2,3,6-substitution are important components of atmospheric and certain food chain exposures, but little is known about their biological activities. Chlorobiphenyl 110 (2,3,3′,4′,6-pentaCB) was investigated in weanling female rats dosed ip on days 21 and 22 and killed on day 23 of age. The initial preparation of CB 110 markedly induced 7-ethoxyresorufin O-dealkylase (EROD) activity and was found to be contaminated with coplanar 3,3′,4,4′,5-pentaCB (CB 126). The contaminated preparation (CB 110C) was purified with activated charcoal (CB 110P). The CB 110P induced pentoxyresorufin O-dealkylase (PROD), was weakly uterotropic and a modest depleter of serum thyroxine (T4). CB 110C caused increased liver weight, induced EROD, PROD, and UDP glucuronyl transferase activities and caused a greater depletion of serum T4; on the other hand, it suppressed the PROD induction and the uterotropic effect of CB 110P. Hepatic residues of CB 110 were a constant 2-3% of the dose while those of CB 126 (from CB 110C) increased with increasing dose to as much as 50% of the dose.

The different types of PCB congeners (chlorobiphenyls, CBs) have distinct biological activities and the ''toxicity'' reported is dependent on the parameters measured and the congeners selected (Hansen 1979). A few coplanar PCB congeners resemble 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in molecular configuration and some toxicities are mediated through the aryl hydrocarbon (Ah) receptor. The toxic potencies of these TCDD-like coplanar CBs can be predicted based on induction of hepatic 7-ethyoxyresorufin *O*-deethylase (EROD) as compared to TCDD as well as other parameters, and TCDD equivalent factors (TEFs) have been determined (Safe 1994). The majority of PCB congeners present in the environment are not coplanar.

The major portion of environmental PCB residues consists of congeners that have not been described in terms of relevant toxicities. Specific CBs with the labile 2,3,6-chlorine pattern on one phenyl ring and 2′,3′-(CB 84), 2′, 5′-(CB 95), 3′,4′-(CB 110) or 2′,4′,5′-(CB 149) on the other phenyl ring are major constituents of common Aroclors (Frame *et al.* 1996), yet little is known of their biological activities. The 2,3,6 pattern is more readily metabolized to methyl sulfonyl PCBs (Lund *et al.* 1997) and these congeners and/or their metabolites have long been known to accumulate in lung and liver tissues (Brandt *et al.* 1981; Brandt and Bergman 1987).

CB 84 suppressed microsomal P450 in hens after 10 weeks dietary exposure, but was as embryotoxic to chicks as CB 118 (2,3′,4,4′,5-pentachlorophenyl), a mono-*ortho* P450 inducer (reviewed in Hansen 1979, 1987). More recently, 2,2′,3,5′,6-pentachlorophenyl (CB 95) was found to be the most potent of several congeners in altering microsomal calcium ion transport by activating ryanodine receptor-binding sites (Wong and Pessah 1996). This suggests that the 2,3,6-substituted congeners have distinct biological activities and could present a profile of actions different from ortho-chlorinated congeners with para-chlorines as well as from coplanar congeners.

An initial study to examine both TCDD-like and non-TCDD–like effects of CB 110 revealed TCDD-like activity in a reportedly pure preparation of CB 110; this preparation was subsequently found to be contaminated with TCDD-like compounds that could be removed by activated charcoal (Li *et al.* 1994a). The initial objective, to establish biological actions of CB 110, was expanded. The toxic properties of a landfill soil extract had previously been shown to change following removal of coplanar Ah receptor agonists by similar charcoal filtration (Li and Hansen 1996a); therefore, the readily purified CB 110 preparation provided the opportunity to assess whether or not a single congener would parallel these changes when coplanar contaminants were removed by the same methods. Serum and liver residues of CBs 110 and 126 were determined to compare relative persistence and possible interactive effects on disposition.

Materials and Methods

Chemicals

CB 110 was synthesized by the Unit of Environmental Chemistry, University of Stockholm (Sweden). An aliquot of this congener

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preparation, as received, caused induction of EROD in prepubertal female rats, and this activity was removed by filtration through activated charcoal (Li *et al.* 1994a). Therefore, the original batch was separated into equivalent aliquots and half of it was purified over activated charcoal. Gas chromatography/mass spectrometry (GC/MS) of the CB 110 before purification and the toluene back-flush of the charcoal column revealed the presence of CB 126 and minor amounts of two other pentachlorobiphenyls (not identified). The charcoal-purified preparation contained less than detectable amounts of these impurities. The probable source was contamination of the 1,2,4-trichlorobenzene used in synthesis with a trace amount of 1,2,3-trichlorobenzene (Åke Bergman, personal communication). Contaminated CB 110 (without further purification) is designated by the suffix ''C'' (CB 110C), whereas CB 110 further purified through activated charcoal is designated by the suffix ''P'' (CB 110P). Dr. Bergman did not detect any CB 126 in the purified preparation (DL not given) and GC analyses of all corn oil dosing solutions used in this study revealed no detectable CB 126 (DL = 0.01%) in the CB 110P preparations and 0.5% CB 126 in CB 110C preparations.

Animals and Treatments

Sprague-Dawley breeder rats were obtained from Harlan (Indianapolis, IN). A colony of 6–12 females and 3–6 males was maintained with periodic replacement of animals (one male and two females every 2–6 months). Breeding/rearing as well as dosing/holding were conducted in the Veterinary Medicine Laboratory Animal facility (21 ± 1°C; 60 ± 5% relative humidity; 12:12 photoperiod). Litter sizes were adjusted to 8–10 by removing male pups on day 1 (birth day = day 0). Pups were weaned on day 21 at which time male pups, retained for a minimum litter size of eight, were euthanized. Female pups were injected intraperitoneally with CB 110P or CB 110C dissolved in 0.1 ml corn oil or corn oil alone between 1:00 and 2:00 PM on day 21 and day 22. Target doses (up to 96 mg/kg total to better define the dose-response curve) were based on a 0.05-kg rat with half of the dose delivered on each of two consecutive days. Actual doses were based on the average weight of individual rats during the dosing period and were <10% lower than target doses (Table 1). A negative (corn oil) control was included for each litter along with as many representative dose groups as the number of females would permit. A positive control for uterotropic response (10 µg/kg estradiol benzoate per day) was included in the larger litters.

Additional dose groups of CB 110P only (16 and 24 mg/kg) were added early in the study to better define the dose:uterotropic response relationship.

Necropsy and Tissue Processing

Rats were decapitated between 9:00 and 11:00 AM on day 23, 20 h after the second dose; blood was collected immediately and allowed to clot. The uterus was excised, trimmed of fat, cut at the cervical os and weighed to the nearest 0.01 mg. Livers were perfused *in situ* with ice-cold 0.05 M Tris-0.15 M KCl (pH 7.4), excised, blotted on tissue paper, and weighed followed by homogenization in 12 ml of the same Tris-KCl buffer as soon as the uteri were removed. An aliquot (100 µl) of the liver homogenate was removed for residue analysis and liver microsomes were then prepared as described in Li *et al.* (1994b). Thymus glands were also removed and weighed.

Enzyme Assays and Thyroid Hormone Analysis

EROD and 7-pentoxyresorufin O-dealkylation (PROD) were determined by fluorescent methods as previously described in Li *et al.* (1994b). UDP glucuronyltransferase (UDPGT) activity in the microsomal suspension was measured using 4-nitrophenol (4-NP) and phenolphthalein (PP) as substrates as described in Li and Hansen (1996a, 1996b). Serum total T4 was measured by using a radioimmunoassay (RIA) kit (Coat-A-Count) purchased from Diagnostic Products Corporation (Los Angeles, CA). The assay kit was validated for rats by the Clinical Pharmacology Lab. The detection limit of the assay was 0.25 µg/dl. All samples were run in duplicate.

Residue Analysis

A Hewlett Packard 5790 GLC with ECD equipped with a 60-m DB-5 capillary column was used to analyze the serum and/or liver concentrations of CB 110 and CB 126 in rats following treatment (Li *et al.* 1994b; Soontornchat *et al.* 1994). Serum (100 µl) or tissue homogenate (100 µl) was extracted by adding 1 ml of acetone followed by 1 ml of hexane; after thorough mixing, enough sodium sulfate was added in small amounts to adsorb all the water. Then 0.2 ml of the extract was added to 0.8 ml of hexane containing 50 ng/ml mirex as an internal standard. The diluted extract (1 µl) was analyzed as previously reported (Li *et al.* 1994b). Samples were quantified by comparing to known amounts of CB 110 and CB 126 standards purchased from AccuStandards (New Haven, CT). The extraction efficiency of control serum and homogenates spiked with CB 110 was 96 ± 4%.

Data Analysis

All data were expressed as means ± standard error (SE) and $p < 0.05$ was selected as the measure of significance. Different RIA kits were used at different times and there was a significant interassay variance; therefore, results for serum T4 were calculated by comparing the T4 value for each rat to its own control in the same litter since these assays were conducted simultaneously with the same kit. Bartlett's test was performed to test for variance homogeneity. In case of heterogeneity of variance ($p < 0.05$), rank transformations were employed (Conover and Iman 1981). A two-way analysis of variance (ANOVA) was performed on homogenous data or transformed data for all the endpoints measured in this study. Main effect means were treatments (CB 110P and CB 110C) and dose (8, 32, 48, and 96 mg/kg), and one interaction was tested (treatment × dose). If a significant result was found, the Tukey multiple comparison test was used to compare all pairs of dose groups.

Results

The high relative concentration (0.5%) of CB 126 in the preparation results in a ''toxic equivalency quotient'' (TEQ) ratio of 5×10^4 mg TEQ/mg PCB since the TEF of CB 126 has been established as 0.1 × TCDD. This is 20-fold higher than the TEQ of Aroclor 1254 and 10-fold higher than the TEQ of the extract of soil from the landfill that contained higher levels of chlorodibenzofurans (Li and Hansen 1996a, 1996b).

Organ Weight Change

Body weight changes during the 2-day dosing of rapidly growing animals were highly variable, but there was a tendency toward reduced gain in the estradiol and CB 110C groups (Table 1). There were no significant changes in thymus weight, but relative liver weight increased in a dose-dependent manner

Table 1. Body weight and organ weight changes in prepubertal female rats dosed on days 21 and 22 with CB 110 preparations and killed on day 23

Dose Group	n	Actual Dose (mg/kg)[a]	Weight Change (%)[b]	Liver Weight (% body weight)	Thymus Weight (% body weight)
Control	11	0	5.0 ± 1.2	4.20 ± 0.27	0.42 ± 0.02
17 β-estradiol	8	0.02	−0.5 ± 2.3	4.11 ± 0.31	0.36 ± 0.03
CB110P					
8	5	7.6 ± 0.7	2.9 ± 1.6	3.99 ± 0.35	0.47 ± 0.03
32	6	29.9 ± 2.2	3.8 ± 0.9	4.51 ± 0.24	0.45 ± 0.02
48	7	45.3 ± 2.4	4.3 ± 1.6	4.45 ± 0.16	0.39 ± 0.04
96	4	94.4 ± 8.5	5.5 ± 1.5	4.21 ± 0.47	0.43 ± 0.04
CB 110C					
4	5	4.3 ± 0.3	4.2 ± 2.2	4.39 ± 0.37	0.35 ± 0.03
8	5	7.8 ± 0.6	6.8 ± 2.7	5.14 ± 0.24*[c]	0.41 ± 0.03
32	5	27.8 ± 1.2	3.8 ± 2.5	5.52 ± 0.20*	0.37 ± 0.03
48	5	47.5 ± 2.4	3.6 ± 3.2	5.57 ± 0.43*	0.44 ± 0.03
96	4	92.4 ± 7.9	−2.8 ± 5.1	5.82 ± 0.63*	0.39 ± 0.04

[a] The total nominal dose was based on two doses delivered to a 50.0-g rat; the actual dose is determined by the mean weight during dosing; mean ± SE

[b] [(Weight day 23 − Weight day 21)/weight day 21] × (100%); mean ± SE

[c] Values within a column with asterisks are significantly different from unmarked values by Tukey's multiple comparison test ($p < 0.05$)

for the CB 110C group (Table 1). Liver weights of the CB 110P group did not change within the dose range used.

Uterotropic Effect

Two doses of 10 μg/kg estradiol increased relative uterine weights 240 ± 13%, consistent with previous studies using this protocol. CB 110P caused a modest increase in uterine wet weight relative to controls within the dose range of 8 mg/kg to 96 mg/kg, significantly greater than controls at 32 and 96 mg/kg (Figure 1). On the other hand, the uterotropic responses from CB 110C treatment groups were consistently weaker than those from CB 110P groups and no dose of CB 110C caused a uterotropic response significantly different from controls (Figure 1).

The greater relative uterine weights at the lowest doses prompted the insertion of two additional doses of CB 110P at 16 and 24 mg/kg. The intermediate doses of CB 110P supported the suggested multiphasic dose-response relationship, but peak activities for both mixtures may have been at doses lower than those tested (Figure 1).

Hepatic Enzyme Activities

CB 110P markedly induced PROD activity in a dose-dependent manner from 32 mg/kg (fourfold) to 96 mg/kg (7.6-fold) (Table 2). There was no effect on EROD, 4-NP UDPGT, or PP UDPGT activities. In contrast, CB 110C significantly increased EROD, PROD, and 4-NP UDPGT activities at all doses tested (Table 2). Interestingly, the induction of PROD by CB 110C was maximal at the lowest dose with no further increase, whereas CB 110P induced PROD in a dose-dependent manner.

Serum Total T4 Level

CB 110P significantly increased serum T4 12% at 8 mg/kg. Estradiol also increased T4 20%, but this increase was quite variable and not statistically significant. Decreases by both CB 110 preparations were significant at doses of 32 mg/kg or greater, but CB 110C reduced serum T4 to a greater extent at all doses tested (Figure 2).

Serum Residues and Tissue Deposition of CB 110 and CB 126

Both serum and liver residues of CB 110 increased in a dose-dependent manner for both CB 110P and CB 110C groups (Table 3). While both CB 126 and CB 110 were detected in the liver of CB 110C treatment groups, only CB 110P was detected in the serum. The relative liver deposition of CB 110 in animals treated with CB 110P or CB 110C was about 2 ± 1% of all doses in both treatments. On the other hand, the liver deposition of CB 126 from CB 110C groups was about 23% of the CB 126 dose at 8 mg/kg and 40–50% of the CB 126 dose at 32 mg/kg or above. The ratio of CBs 126:110 in the livers increased from 0.5% of the dose to 5–8% at the lower doses and to 12% at the highest dose (Table 3).

Summary of Effects of Treatment and/or Dose on Different Endpoints

Uterotropic responses were significantly different between CB 110C and CB 110P, but the dose effect for each treatment was not obvious (Table 4), paralleling the previously reported overall effect of the charcoal-filtered soil extract (Li and Hansen 1996a). Distinct from the soil extract, the two treatments also had different effects on serum T4 in a dose-dependent manner (Figure 2, Table 4). There were significant differences between treatments and among doses in the induction of enzymes and the increases in liver weight, which again paralleled the soil extract with and without charcoal filtration. The induction of PROD was similar for both treatments and dose-dependent; however, the dose-response curve for CB 110P was much steeper. On the other hand, the induction of EROD was

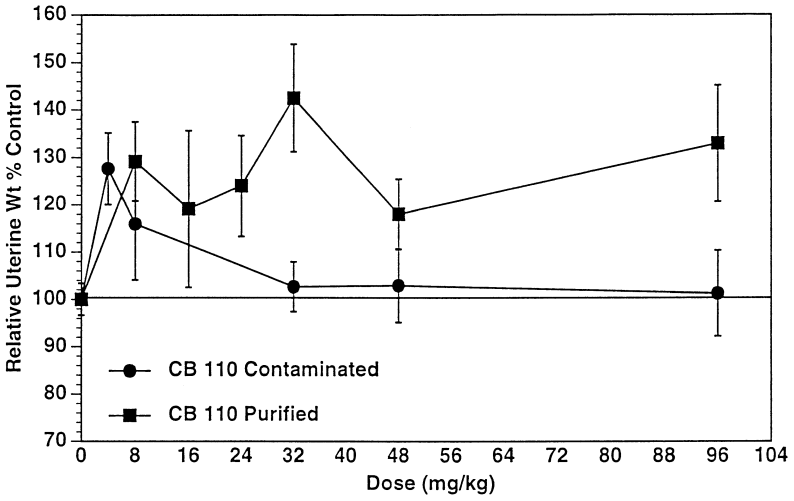

Fig. 1. Mild uterotropic activity of CB 110 purified to remove Ah-receptor agonists (CB 110P) compared to the preparation before removal of CB 126 and other Ah-receptor agonists (CB 110–contaminated). Values are mean ± SE. Only the doses of 32 and 96 mg/kg of CB 110P are significantly higher than controls ($p < 0.05$)

Table 2. Liver microsomal enzyme activities in prepubertal female rats administered CBs 110 and a preparation of CB 110 contaminated with 0.5% CB 126 (CB 110C)

Group	Dose (mg/kg)	n	Microsomal Enzyme Activities[a] EROD[b]	PROD[b]	4-NP UDPGT[c]	PP UDPGT[c]
Control		11	44 ± 5	4.3 ± 0.3	47 ± 6	34 ± 2
17 β-estradiol	0.02	8	47 ± 6	4.8 ± 0.7	46 ± 6	37 ± 7
CB 110P	8	5	52 ± 7	6.8 ± 1.2	52 ± 8	36 ± 2
	32	6	59 ± 6	17.1 ± 3.4*	41 ± 3	36 ± 3
	48	7	53 ± 3	23.1 ± 3.4*#	49 ± 6	43 ± 6
	96	4	79 ± 17	32.8 ± 4.5#	40 ± 4	36 ± 6
CB 110C	4	5	6,881 ± 530*[d]	21.8 ± 2.4*#	111 ± 14*	39 ± 8
	8	5	7,394 ± 962*	22.7 ± 1.9*#	90 ± 3*	38 ± 6
	32	5	8,257 ± 456*	21.8 ± 2.2*#	112 ± 8*	36 ± 4
	48	5	8,747 ± 908*	24.8 ± 1.4*#	123 ± 10*#	37 ± 4
	96	4	6,822 ± 579*	19.2 ± 0.4*#	157 ± 6#	42 ± 7

[a] Mean ± SE
[b] Activity expressed as pmol resorufin formed/minute/mg protein
[c] Activity expressed as nmol substrate disappearing/minute/mg protein
[d] Values within a column with different superscripts are significantly different by Tukey's multiple comparison test ($p < 0.05$)

significantly different between treatments and the flat dose-response for CB 110C resulted in no significant dose effect (Table 4).

Discussion

In order to expand the number and types of congeners assayed for relevant Ah receptor-independent as well as Ah receptor-dependent endpoints, a simple battery of enzyme and endocrine tests has been developed in immature female Sprague-Dawley rats (Li and Hansen 1995; Li *et al.* 1994b; Soontornchat *et al.* 1994). Hepatic enzyme, thyroid, and uterotropic endpoints are combined in a female rat integrated endocrine disruption assay (FRIEDA) (Li and Hansen 1996a, 1996b, 1997), which permits more uniform comparisons among congeners and between congeners and environmental mixtures. To our knowledge, this is the first report on CB 110.

Organ Weight and Endocrine Effects

The tendency for reduced body weight gain and increased liver weight in the CB 110C group indicated some responses not seen with CB 110P. The increase in relative liver weight was not due entirely to lower body weights because absolute liver weights also increased significantly. The coplanar-contaminated mixture resulted in increased relative liver weight with a 2-day NOEL of 4 mg/kg while the pure CB 110 did not increase liver weight even at 96 mg/kg. Liver weight increases in this 2-day dosing regimen have been limited to mixtures such as Aroclor 1242 (Soontornchat *et al.* 1994) or environmental mixtures containing Ah-receptor agonists (Li and Hansen 1996a, 1996b); di-*ortho* CBs 47 and 153 increased liver weight if there was a 5-day lag between the first dose and termination (Soontornchat *et al.* 1994).

CB 110P caused a greater uterotropic effect than CB 110C (Figure 1, Table 4). This is presumed to be due to removing Ah-receptor agonists (EROD inducers), which are generally

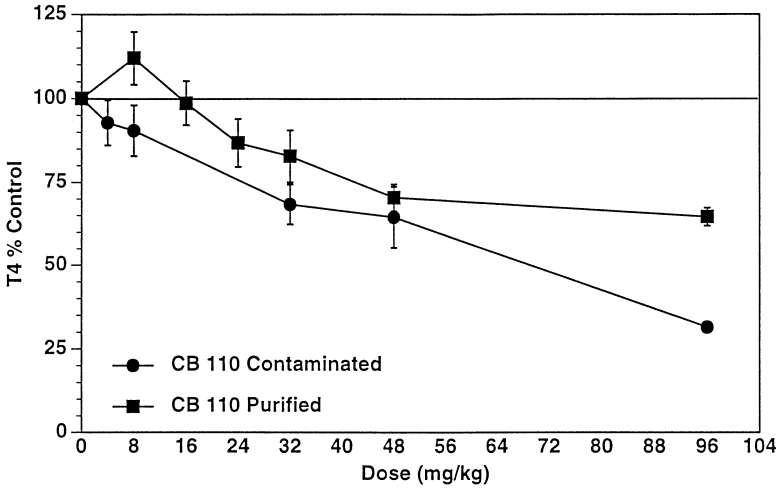

Fig. 2. Serum total T_4 level in prepubertal female rats dosed with CB 110 purified to remove Ah-receptor agonist (CB 110P) compared to the preparation before removal of Ah-receptor agonists (CB 110C). Values (mean ± SE) have been converted to percent of the same litter control. Control levels were 2.02 ± 0.12 µg/dcl or 100 ± 5.9%. T4 levels are significantly different ($p < 0.05$) between dose groups at 8 and 96 mg/kg and significantly lower than controls at 48 and 96 mg/kg

Table 3. Serum and liver residue levels (means ± SE) in prepubertal female rats 20 h after receiving the second of two doses of CB 110P (pure) or CB 110C (contaminated with 0.5% CB 126)

Treatment			Serum CB 110	Liver CB 110		Liver CB 126	
CB 110	CB 126	n	(ppb)	ng/g	% of the dose	ng/g	% of the dose
CB 110P							
8	0	5	159 ± 28	72 ± 12	2.0 ± 0.4	<1	
32	0	6	507 ± 70	246 ± 32	1.9 ± 0.2	<1	
48	0	7	618 ± 35	320 ± 75	1.6 ± 0.3	<1	
96	0	4	914 ± 191	538 ± 191	1.2 ± 0.4	<1	
CB 110C							
4	0.02	5	126 ± 9	34 ± 3	1.9 ± 0.2	<1	
8	0.04	5	149 ± 28	62 ± 8	2.3 ± 0.3	3 ± 1	22.9 ± 2.0
32	0.16	5	574 ± 81	278 ± 11	3.0 ± 0.2	21 ± 2	42.4 ± 4.4
48	0.24	5	913 ± 146	499 ± 63	3.2 ± 0.3	36 ± 4	44.6 ± 4.6
96	0.48	4	1,171 ± 97	674 ± 162	2.2 ± 0.6	82 ± 8	50.8 ± 3.8

antiestrogenic (Krishnan and Safe 1993). A similar interference of coplanar CB 77 with the uterotropic actions of both Aroclor 1242 and 17β-estradiol has been reported (Jansen *et al.* 1993). At lower doses, coplanar CB 77 is reported to have both estrogenic and antiestrogenic effects (Nesaretnam *et al.* 1996); the dose-response relationships were multiphasic for the uterotropic effect and CB 77 was additive and/or antagonistic with estradiol, depending on dose.

CB 126 caused a significant uterotropic response at a dose below significant liver and thyroid effects in this protocol (Li 1996). By extending the observation period to 3 days, a more dramatic effect for CB 126 at low doses has been recently confirmed (Seegal *et al.* 1997) and is similar to the response to coplanar CB 77. The downward trend in relative uterine weights in response to CB 110P between doses of 8–16 mg/kg may indicate a low-dose response that was not detected. The downward trend in relative uterine weights in response to CB 110C between doses of 4–32 mg/kg may be due to additive estrogenicity of CB 126 at lower doses and antiestrogenicity at higher doses. Li and Hansen (1996a) also found the weak uterotropic response was greater in rats receiving a charcoal-filtered extract of an environmental PCB mixture than in rats treated with the same extract without refining by alumina-charcoal–column chromatography, probably due to removal of antiestrogenic Ah-receptor agonists by the charcoal. A linear dose-response relationship was not obvious in either case.

Increase in relative uterine weight in immature or ovariectomized adult rodents is a reliable, but relatively insensitive indication of estrogenic potential (Kupfer 1987). Small changes in uterine weights may reflect biological significance but are difficult to confirm without adequate statistical power; nevertheless, PCBs consistently cause nonmonotonic uterine responses in the FRIEDA and retrospective insertion of additional dose groups has confirmed smooth inflexions rather than experimental inadequacy (Li *et al.* 1994b; Li and Hansen 1996a, 1996b; Figure 1). For broad-acting toxicants such as PCBs, smaller increments of doses may be necessary to detect subtle endocrine actions such as estrogenicity and thyroid hormone disruption.

The lack of a monotonic dose-response for PCB estrogenicity is undoubtedly influenced by other PCB effects on metabolizing enzymes and toxicokinetics (Li *et al.* 1994b; Soontornchat *et al.* 1994) as well as the interplay between estrogenicity and antiestrogenicity. PCBs induce their own metabolism and that of endogenous hormones. The metabolites have qualitatively and quantitatively different properties than the parent compounds and, in a short-term study, phase 2 enzymes such as UDPGT are not maximally induced within the same time frame

Table 4. Summary of significant differences in main effect means for treatment (CB 110P and CB 110C) and dose (8, 32, 48, and 96 mg/kg) by two-way ANOVA for all endpoints measured in this study

Response	Treatment	Dose	Treatment × Dose
Uterotropic response	$p = 0.03$	ns[a]	ns
Relative liver weight	$p \leq 0.001$	$p = 0.017$	ns
Relative thymus weight	ns	ns	ns
Serum T_4 level	$p = 0.002$	$p \leq 0.001$	ns
EROD	$p \leq 0.001$	ns	$p \leq 0.001$
PROD	ns	$p = 0.002$	ns
4-NP UDPGT	$p \leq 0.001$	$p = 0.002$	$p \leq 0.001$
PP UDPGT	ns	ns	ns

[a] Not significant

as phase 1 enzymes (Li and Hansen 1997; Hansen and Foley 1997).

Nonlinear dose-response relationships have also been observed for thyroid hormone status. Total T4 was significantly higher in serum from rats in the CB 110P low dose group, but T4 declined in a dose-dependent manner at higher doses (Figure 2). It is interesting that estradiol-treated rats also had elevated serum T4 ($120 \pm 26\%$ controls); although the increase was similar to that at 8 mg/kg CB 110P, it was more variable and not statistically significant. PCB depletion of circulating T4 is caused by multiple mechanisms, frequently independent of UDPGT induction (Capen *et al.* 1991; Hansen and Foley 1997; Li and Hansen 1997). Acute T4 mobilization at low doses has been demonstrated analytically and morphometrically in rats treated similarly with PCB mixtures (Hansen *et al.* 1995; Li and Hansen 1996b; Saeed and Hansen 1997). UDPGT was not induced by CB 110P (Table 2), eliminating one major route of T4 excretion, and this may have permitted the mobilized T4 to reach higher serum levels.

Enzyme Induction

The structure-activity relationships for PB-type CB congeners are less well defined than those for TCDD-like CB congeners. In this study, CB 110 markedly induced PROD, a putative PB-type activity, in a dose-dependent manner to 7.6 times control values at 96 mg/kg. Under similar treatment conditions, CB 153, a known strong PB inducer, caused 8.6-fold PROD induction in 22-day-old female rats at 102 mg/kg (Li *et al.* 1994b). Thus, surprisingly, the more labile CB 110 is a PB-type inducer with a potency similar to that of CB 153.

As with the uterotropic effect, different dose patterns for induction of hepatic enzymes by high TEQ (5×10^{-4} mg TEQ/mg PCB) and charcoal-filtered CB 110 paralleled the changes in patterns caused by charcoal filtering the landfill soil extract (6×10^{-5} mg TEQ/mg PCB) (Li and Hansen 1996a). In both studies, the contaminants were very potent inducers of EROD and 4-NP UDPGT. In the previous study, a similar maximum induction of EROD activity was observed for the environmental mixture within the same dose range while PROD activity was suppressed by Ah-receptor agonists, as evidenced by similar dose-dependent induction when these compounds were removed by activated charcoal (Li and Hansen 1996a). Ah-receptor agonists have also been shown to antagonize induction of PROD activity and/or other measures of CYP2B in mouse liver (De Jongh *et al.* 1995).

Residue Deposition

The increasing ratio of CBs 126/110 in liver residues is partly due to the labile 2,3,6-substituted ring (Bruhn *et al.* 1995; Hansen 1979, 1987) coupled with dramatic enzyme induction at even the lowest dose of CB 110C; however, this is further magnified by the propensity of coplanar chlorinated aromatics to accumulate in livers. The high liver deposition of TCDD and dioxin-like compounds has been reported in several studies (Curtis *et al.* 1990; Kedderis *et al.* 1991; De Jongh *et al.* 1995).

Conclusion

Brief exposure to readily metabolized PCBs may cause transient enzyme induction, estrogenic effects, and hyper- or hypothyroidism. These effects are probably of little consequence in healthy adults, but may be of significance in developing animals.

In this study, CB 110 was a PROD inducer (NOEL = 8 mg/kg), had biphasic effects on serum T4, and had weak estrogenic activity as measured by the uterotropic response in prepubertal rats. The NOEL for uterotropic response could not be determined since it was not a monotonic dose-response relationship. CB 110C contaminated with 0.5% CB 126 as well as two other pentaCBs had a TEQ/PCB ratio at least 10-fold higher than even TEQ-enriched environmental mixtures. This mixture significantly increased relative liver weight above 4 mg/kg and induced EROD, PROD, and 4-NP UDPGT activities at all doses. The contaminants decreased the estrogenic effect and PROD induction potential of CB 110 and potentiated the effect of T4 depletion by CB 110 alone. Forces that reduce the TEQ/PCB ratio of mixtures, such as selective volatilization and biotic *p*-dechlorination, may unmask other more subtle effects such as estrogenicity.

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