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Original Article

In utero exposure to 2,2',4,4',5,5'-hexachlorobiphenyl accelerates the onset of eye opening in rat offspring

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ABSTRACT — Prenatal exposure to 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) and coplanar polychlorinated biphenyl (PCB) congeners has been reported to accelerate the onset of eye opening of rodents, but the effects of exposure to non-coplanar PCB congeners on the onset of eye opening remain unknown. TCDD binds to the cytosolic ligand-activated transcription factor aryl hydrocarbon receptor (AhR), leading to adverse effects through the alteration of AhR target gene expression. In contrast, non-coplanar PCB congeners show little or no binding to AhR. We examined whether *in utero* exposure to 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), a di-*ortho*-substituted non-coplanar PCB congener, affects the onset of postnatal eye opening of rat offspring. Pregnant rats were given PCB 153 (0, 16, or 64 mg/kg/day) orally from gestational day 10 to 16, and somatic growth and eye opening were assessed in pups. Body weight, body length, and tail length measurements in the PCB 153 groups were lower than the values measured for the control group on postnatal days 1 to 21. However, PCB 153-exposed pups exhibited dose-dependent acceleration of eye opening. These findings suggest that *in utero* exposure to PCB 153 delays postnatal development but induces unexpected acceleration of eye opening that might not occur through interaction with AhR.

Key words: 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), Postnatal growth, Eye opening, Rat

INTRODUCTION

Polychlorinated biphenyls (PCBs) are among the most widespread, persistent, and ubiquitous environmental contaminants. A large number of PCB congeners have been produced and used for the manufacture of industrial materials and domestic products worldwide (Tanabe, 1988). Environmental levels of PCBs are unlikely to decline in the near future because of the even greater quantities of PCBs still in use compared with the quantities that have already escaped into the environment. PCBs have been found in almost all compartments of the biosphere, including animal and human tissues and body fluids (Brouwer *et al.,* 1995). The toxicological properties of PCBs are often congener specific with considerable dependence on the number and position of chlorine substitutions (McKinney and Waller, 1994). PCBs cause a decrease in circulating thyroid hormone levels, and the mechanisms by which PCBs affect thyroid status are thought to differ among congeners. Several PCBs can increase the expression of thyroid hormone-responsive genes, indicating that PCBs can interfere directly with thyroid hormone signaling (Gauger *et al.,* 2004). The effects of PCBs on circulating thyroid status appear to be complicated, and the details of the toxicological mechanisms of each congener remain unclear.

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Experimental studies indicate that exposure to PCBs might affect development and growth. Although some studies on PCB mixtures in laboratory animals have been reported, detailed toxicity profiles of individual PCB congeners are not yet available. In this regard, PCB 153 has been detected in wildlife as well as in human plasma and breast milk, and thus it can serve as an appropriate biomarker for exposure to PCBs (Axmon *et al.,* 2001). The effects of industrial chemicals on developmental landmarks have been examined in toxicity studies. Notably, commercial PCB mixtures reportedly have the potential to accelerate the onset of eye opening in rat pups (Bowers *et al.,* 2004). In contrast, however, other researchers have failed to find this accelerating effect (Crofton *et al.,* 2000; Sugawara *et al.,* 2004; Steinberg *et al.,* 2008). Previous studies with rats have shown that prenatal exposure to PCBs causes a decrease in thyroid hormone level in combination with growth retardation of offspring (Bowers *et al.,* 2004). Following exposure to PCB 153 at gestational day (GD) 10–16 (16–64 mg/kg/day), circulating thyroxine (T_4) levels in rat offspring were found to significantly decrease in a dose-dependent manner (Ness *et al.,* 1993; Kobayashi *et al.,* 2008). The onset of eye opening, however, was not examined in those studies. Therefore, the relationship between PCB 153-induced growth retardation and the onset of eye opening has not been assessed. In this study, we evaluated the toxicological effects of *in utero* exposure to PCB 153 on eye opening in F_1 pups.

MATERIALS AND METHODS

Chemicals and experimental animals

PCB 153 was obtained from AccuStandard, Inc. (New Haven, CT, USA, Lot#: 070203MT-AC, the purity [100%] was reported using gas chromatography-mass spectrometry analysis according to the manufacturer's certificate of analysis), and corn oil was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). A total of 35 pregnant (GD 3) female rats (Crj: CD (SD) IGS strain, 9 weeks of age) were purchased from Charles River Japan, Inc. (Kanagawa, Japan). The presence of a copulatory plug defined GD 0. Rats were acclimated during GD 3–9; they were housed individually and maintained under controlled temperature (23 ± 1°C) and humidity (55 ± 5%) with a 12-hr light (08:00–20:00)/12-hr dark cycle throughout the study. A standard laboratory diet (CE-2, Clea Japan, Inc., Tokyo, Japan) and drinking water were available *ad libitum*. All experimental procedures using animals were approved by the Institutional Animal Care and Use Committee of the National Institute of Occupational Safety and Health, Japan (JNIOSH) and were in accordance with JNIOSH Guidelines for the Care and Use of Laboratory Animals.

Experimental design

Dams were randomly divided into three groups: 11 dams for the control group, 12 dams for the 16 mg/kg/day group, and 12 dams for the 64 mg/kg/day group. Each dam was weighed from GD 3 through postnatal day (PND) 21. The PCB 153-exposed groups were orally administered PCB 153 (16 or 64 mg/kg/day) in corn oil vehicle (4 mL/kg of body weight); PCB 153 was given between 08:30 and 09:30 from GD 10 through GD 16, and the control group (0 mg/kg/day) was given the same amount of corn oil during the same period. During the exposure period, we recorded maternal body weights and noted any clinical signs or abnormal behavior that may have resulted from toxic effects. For each dam, body weight during gestation and lactation and the duration of gestation were recorded. Dams were allowed to deliver naturally. Dams were checked for delivery until 10:00 on each day; the day on which pups were first observed was designated as PND 0. On PND 1, all live births were counted and sexed. Body weight, body length (nose–anus length), and tail length of all live pups on PND 1 were included in the analysis. The litter size was then standardized to eight (four males and four females when possible) between 10:00 and 11:00 on PND 1. Litters with a total of eight or fewer pups were not culled regardless of the sex ratio. After culling, one male and one female pup (when possible) from each dam were randomly selected for necropsy on PND 1, 7, 14, and 21 and subjected to organ studies not reported here. Body length and tail length were measured with a digital caliper (Mitutoyo, Kanagawa, Japan). Pups were checked for eye opening until 10:00 on each day; the day on which bilateral eye opening occurred was designated as "PND at eye opening". The percentage of pups with open eyes was calculated on PND 10–16.

Statistical analysis

The gestational period, the number of live births per litter on PND 1, body weight, body length, tail length, and PND at eye opening were analyzed as follows. First, Bartlett's test was performed to determine whether the samples exhibited a homogeneous distribution. If the Bartlett's test was insignificant ($P > 0.05$), an analysis of variance was performed. The differences with respect to the corresponding control group were analyzed by analysis of variance followed by Dunnett's test. If the Bartlett's test was significant ($P < 0.05$), a Kruskal-Wallis test was

performed, followed by a Steel's test. A Cochran-Armitage test was performed for a dose-response trend of the onset of eye opening. Statistical significance was set at 0.05 for all analyses.

RESULTS

Table 1 shows the number of dams and their offspring used for examination in each group. Of the 12 dams in the 64 mg/kg/day group, 3 were excluded from further analysis. One dam was found dead on GD 20. Another dam was found dead on GD 15 owing to a technical failure of the dosing procedure (accidental endobronchial intubation when dosing PCB 153). All pups of one dam were found dead by PND 5. The cause of death of each of these dams and pups was not determined.

No significant differences were observed between the control group and the PCB 153-treated groups in terms of the duration of the gestational period or the number of live births per litter on PND 1 (Table 1). Maternal body weight changes during gestation and lactation are shown in Table 2. Body weight changes among groups were similar during both the gestation and lactation periods. Although a slight decrease in body weight was apparent for the 64 mg/kg/day group during gestation (especially from GD 13), there were no statistically significant differences in dam weight between the control and PCB 153 groups from GD 10 through GD 21 or between the control and PCB 153 groups during the lactation period (Table 2).

Figure 1 presents data regarding the somatic growth of the male and female offspring. Significant dose-dependent decreases in body weight, body length, and tail length of both males and females on PND 1, 7, 14, and 21 were noted for the PCB 153 groups. Both the 16 mg/kg/day and 64 mg/kg/day groups exhibited significantly accelerated eye opening compared with the control group (Table 3). The effects of PCB 153 on the percentage of male and female pups with open eyes were significantly dose dependent from PND 12 through PND 14 (Table 4).

DISCUSSION

In a previous study, we found that 16–64 mg/kg/day PCB 153 decreased circulating thyroid hormone concentrations but did not affect body weight in F_1 rat pups (Kobayashi *et al.,* 2008). Here, we mimicked the experimental design of that study as much as possible to further evaluate the potential toxicological impact of *in utero* exposure to PCB 153 on rat offspring. Our previous study

Table 1. Reproductive outcomes of PCB 153-exposed F_0 dams.

	PCB 153 dose (mg/kg/day)		
	0	16	64
Pregnant females (n)	11	12	12
Used dams (n)	11	12	9[a]
Gestation period (days)	21.2 ± 0.4[b]	21.4 ± 0.5	21.4 ± 0.5
Live births/litter on PND 1	10.4 ± 2.0	12.1 ± 2.7	11.2 ± 1.6

[a] Three of 12 dams in the 64 mg/kg/day group were excluded from further analysis (see Materials and Methods).
[b] Values are mean ± S.D.
PCB153, 2,2',4,4',5,5'-hexachlorobiphenyl.
PND, postnatal day.

Table 2. Maternal body weight (g) during gestation and lactation.

	PCB 153 dose (mg/kg/day)		
	0	16	64
GD 3	223 ± 12[a]	227 ± 15	225 ± 12
GD 6	256 ± 14	258 ± 17	259 ± 12
GD 10	276 ± 18	278 ± 18	279 ± 12
GD 13	302 ± 21	296 ± 23	271 ± 17
GD 16	320 ± 22	316 ± 24	282 ± 22
GD 21	390 ± 31	387 ± 28	360 ± 23
PND 1	304 ± 16	292 ± 22	283 ± 22
PND 7	338 ± 22	331 ± 19	318 ± 21
PND 14	342 ± 22	336 ± 23	333 ± 20
PND 21	319 ± 23	316 ± 23	319 ± 22

[a] Values are mean ± S.D.
GD, gestational day.

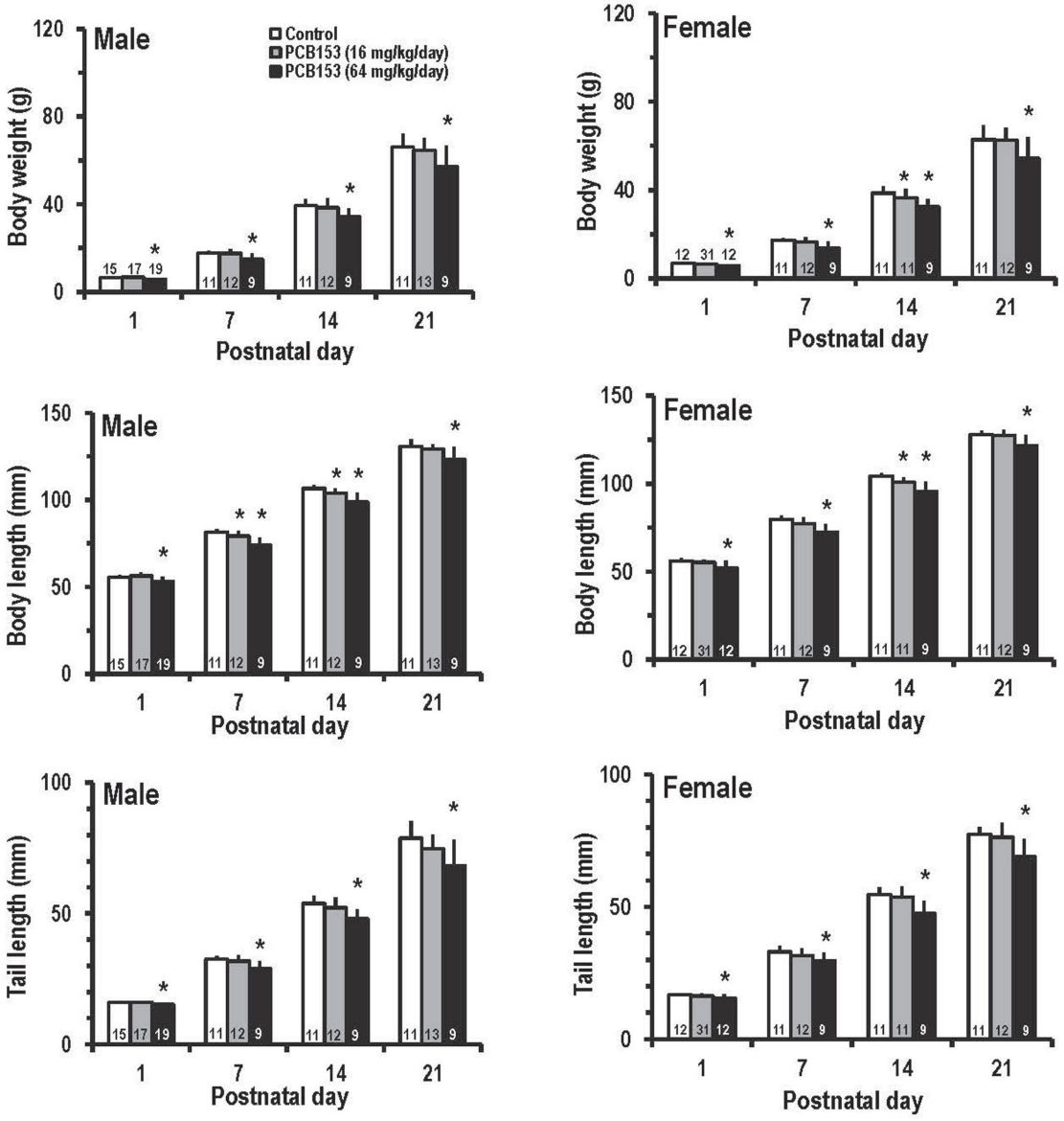

Fig. 1. Body weight (upper panel), body length (middle panel), and tail length (lower panel) of male and female offspring. Values are the mean ± S.D. of the number of pups shown in each column. *Significantly different from the control group ($P < 0.05$).

did not assess the effect of PCB 153 on the time of eye opening of the newborn offspring. In our current study, however, we found that *in utero* exposure to PCB 153 induced paradoxically unexpected earlier eye opening despite somatic growth suppression (Fig. 1, Table 3); the dose dependency of PCB 153 on eye opening was confirmed by the Cochran-Armitage trend test (Table 4).

Genetically congenital hypothyroid (*hyt*) mice (Adams *et al.,* 1989; Anthony *et al.,* 1993; Sprenkle *et al.,* 2001) have delayed eye opening. Prenatal and neonatal induction of hypothyroidism using propylthiouracil results in significantly delayed eye opening in rat offspring (Brosvic *et al.,* 2002; Zertashia *et al.,* 2002; Sui and Gilvert, 2003). These studies also demonstrate that prenatal/perinatal thyroid hormone deficiency produces a diminution in weight following a delay in the onset of eye opening. In contrast, rat pups born to dams with hyperthyroidism induced by daily injection of T_4 exhibit accel-

Table 3. PND at eye opening of PCB 153-exposed F_1 pups.

	PCB 153 dose (mg/kg/day)		
	0	16	64
Males	14.2 ± 0.8 (23)[a]	13.5 ± 0.7 (32)*	12.9 ± 1.0 (24)*
Females	14.1 ± 0.7 (19)	13.5 ± 0.6 (34)*	12.7 ± 0.9 (27)*

[a] Values are mean ± S.D. for the number of pups in parentheses. *Significantly different from the control group (0 mg/kg/day) ($P < 0.05$).

Table 4. Effect of PCB 153 on percentage of F_1 pups with open eyes.

		PCB 153 dose (mg/kg/day)						
		0		16		64		P value
PND 10	Male	0[a] /34[b]	(0%)[c]	0 /34	(0%)	1 /25	(4.0%)	0.175[d]
	Female	0 /30	(0%)	0 /36	(0%)	0 /28	(0%)	—
PND 11	Male	0 /34	(0%)	0 /34	(0%)	2 /25	(8.0%)	0.054
	Female	0 /30	(0%)	0 /36	(0%)	2 /28	(7.1%)	0.065
PND 12	Male	1 /34	(2.9%)	3 /34	(8.8%)	5 /25	(20.0%)	0.032
	Female	0 /30	(0%)	1 /36	(2.8%)	11 /28	(39.3%)	< 0.001
PND 13	Male	4 /34	(11.8%)	13 /34	(38.2%)	18 /25	(72.0%)	< 0.001
	Female	2 /30	(6.7%)	17 /36	(47.2%)	21 /28	(75.0%)	< 0.001
PND 14	Male	14 /34	(41.2%)	32 /34	(94.1%)	24 /25	(96.0%)	< 0.001
	Female	16 /30	(53.3%)	34 /36	(94.4%)	27 /28	(96.4%)	< 0.001
PND 15[e]	Male	23 /23	(100%)	22 /22	(100%)	15 /15	(100%)	—
	Female	18 /19	(94.7%)	25 /25	(100%)	18 /18	(100%)	0.197
PND 16	Male	23 /23	(100%)	22 /22	(100%)	15 /15	(100%)	—
	Female	19 /19	(100%)	25 /25	(100%)	17 /17	(100%)	—

[a] Number of pups with open eyes.

[b] Number of live pups.

[c] Percentage of pups with open eyes (%) [(Number of pups with open eyes /number of live pups) × 100].

[d] P values were derived from Cochran-Armitage trend test.

[e] After measuring body parameters on PND 14, randomly selected pups were then sacrificed for organ studies (not reported here). Consequently, the number of pups from PND 15 onward was reduced compared with that on PND 14 (see Materials and Methods).

erated eye opening (Brosvic *et al.,* 2002).

In our previous study, we examined the effect of *in utero* exposure to PCB 153 in rats under experimental conditions almost identical to those used in the current study (GD 10–16, 16–64 mg/kg/day) (Kobayashi *et al.,* 2008). The previous results suggested that *in utero* exposure to PCB 153 decreases neonatal thyroid hormone levels in rat offspring to some extent but does not affect somatic growth or its related hormonal parameters (Kobayashi *et al.,* 2008). In our previous study, body weight, body length, and tail length were not affected up to PND 21 in the PCB 153 groups compared with the control group (Kobayashi *et al.,* 2008). There were several differences in the methods used between our previous work (Kobayashi *et al.,* 2008) and the current study; indeed, the difference in the culling day (PND 7 *vs*. PND 1) and the number of lactating pups (10 pups *vs*. 8 pups) may have contributed to the discrepancy in the results. The growth of pups is influenced by litter size and is thus the rationale for culling of rodent litters (Agnish and Keller, 1997; Rödel *et al.,* 2008). Also, the batch numbers of PCB 153 (980924R-AC *vs*. 070203MT-AC) were different between the studies. The difference in the effects of PCB 153 on postnatal weight observed in the two studies might be due to the small difference in the PCB 153 purity (99.6% *vs*. 100%). A previous study reported that postnatal exposure to PCB 153 does not affect the onset of eye opening in rat pups (Holene *et al.,* 1998, 1999).

Coplanar PCB congeners and 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) can bind to the aryl hydrocarbon receptor (AhR) (Brouwer *et al.,* 1995). Exposure to TCDD accelerates the onset of eye opening in rats. The phenomenon has been postulated to be a consequence of AhR activation and subsequent changes in epidermal growth factor (EGF) signaling cascades (Madhukar *et al.,* 1988). EGF activation induces early eye opening (Tsutsumi *et al.,* 1986; Smart *et al.,* 1989), whereas treatment of neonatal mice with an antibody against EGF reduces weight gain and delays eye opening (Tsutsumi *et al.,* 1986; Zschiesche, 1989). Non-coplanar

congeners, including PCB 153, show little or no binding to AhR (Brouwer *et al.,* 1995); hence, one plausible hypothesis is that the accelerated eye opening after exposure to PCB 153 may be regulated by an AhR-independent pathway. In our previous study, developmental parameters (body weight, body length, and tail length) and thyroid-related parameters (circulating levels of T_4, T_3, and thyroid-stimulating hormone) were normal in offspring following exposure of dams to lower doses of PCB 153 (1–4 mg/kg/day) (Kobayashi *et al.,* 2009). In that experiment, we did not examine the onset of eye opening. It has not been determined whether eye opening following exposure to PCB 153 is accelerated even when thyroid function is impaired. The magnitude of the effect of PCB 153 on accelerated eye opening may be greater than that of the hypothyroxinemia-induced delay in eye opening. Thus, unknown AhR-independent mechanisms may be involved in the acceleration of eye opening by PCB 153 observed in the current study. PCB metabolites exert remarkable changes in endocrine function of laboratory animals. For example, hydroxy-PCB metabolites decrease vitamin A and thyroid hormone levels in female rats (Brouwer and van den Berg, 1986) but exhibit some estrogenic activity in the uterus of ovariectomized mice (Korach *et al.,* 1988). If a PCB 153 metabolite can bind to AhR, it may induce EGF activation and hasten the onset of eye opening. Additionally, it was reported that PCB 153 itself can function as a TCDD antagonist by interfering with TCDD-induced *CYP1A1* mRNA expression (Suh *et al.,* 2003). An investigation of PCB 153 effects in combination with AhR agonists (*e.g.* TCDD) may elucidate the AhR-associated biological changes including the occurrence of eye opening.

In conclusion, the results of this present study suggest that *in utero* exposure to PCB 153 induces postnatal growth retardation and early onset of eye opening in rat offspring. These findings suggest that hypothyroxinemia is likely to be involved in the *in utero* PCB 153-induced early postnatal growth retardation, but the mechanism of early-onset eye opening following exposure to PCB 153 appears to differ from that of somatic growth retardation. How PCB 153 accelerates eye opening and its toxicological significance under the experimental conditions of this study remain unknown.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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