ADVERSE EFFECTS OF *IN UTERO* AND LACTATIONAL EXPOSURE TO 3,3',4,4',5-PENTACHLOROBIPHENYL (PCB 126) ON THE FIRST OVULATION IN RATS

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Introduction

In rodent species, vaginal opening (VO) is synchronized with the first ovulation, since these events are commenced by estradiol-17β (E_2) from developing follicles in the prepubertal ovaries. While an increased amount of E_2 triggers the hypothalamohypophysial axis to initiate gonadotropin (GTH) surges for the first ovulation, E_2 promotes development of the uterus as well as cornifies the vaginal epithelium. *In utero* and lactational exposure to any of several halogenated xenobiotics, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and coplanar polychlorinated biphenyls (PCBs), has been reported to delay VO in female rats[1,2,3]. Since each of these compounds exerts anti-estrogenic effects via aryl hydrocarbon receptor, a delay in VO may not be accompanied by the first ovulation. In the present study, to examine whether VO of offspring exposed *in utero* and lactationally to 3,3',4,4',5-pentachlorobiphenyl (PCB 126), a congener of coplanar PCBs, synchronizes with the first ovulation, we examined for ovulation at VO of rat offspring from dams, which were daily administered with PCB 126 from mating to weaning after spontaneous parturition (Exp. I), or were single-administered on gestational day 15 (Exp. II). Furthermore, equine chorionic gonadotropin (eCG) or eCG and human chorionic gonadotropin (hCG) was injected to the offspring in Exp. II, to examine maturity of the hypothalamohypophysial/gonadal axis during the prepubertal period.

Methods and Materials

PCB 126 (AccuStandard Inc., New Haven, CT, USA) was dissolved in corn oil to a constant volume of 2 ml/kg body weight. eCG (Sigma, 2830 IU/mg) and hCG (Sankyo Zoki, 2200 IU/mg) were dissolved in saline to 5 IU/0.2 ml and 10 IU/0.2 ml, respectively.

Adult female Sprague-Dawley rats and their mating partners of the same strain were purchased from Charles River Japan, and were maintained under a lighting condition (lights on 08:00-20:00) in an animal husbandry facility. The animals were housed in a plastic cage with bedding materials, pellet chow (CE-2, Clea Japan Inc.), and water (tap water) *ad libitum*.

In Exp. I, 4 or 8 and 3 female rats copulated and conceived after daily oral administration for 2 weeks of either corn oil or 1 and 3 μg/kg/day of PCB 126, respectively. Daily administration was continued until 20 days after delivery through the gestational period. In this experiment, cumulative maternal exposure to PCB 126 until weaning was estimated to be 57-68 μg/kg in the 1μg/kg/day-exposed group and 171-186 μg/kg/day in the 3μg/kg/day-exposed group.

In Exp. II, 5 or 6 and 7 of timed pregnant females were single-administered orally either corn oil or 10 and 100 μg/kg of PCB 126 on gestational day 15 (GD 15, day 0=day of sperm positive).

In both experiments, dams were allowed to deliver spontaneously, and the size of each litter was adjusted to eight pups on one day after birth (day 1). On day 21, the offspring were weaned from the dam for the following examinations: 1) one female from each dam was sacrificed on day 24 to measure ovarian and uterine weights in both experiments; 2) in Exp. II, half of the remaining females in each dam were administered s.c. with 5 IU of eCG on day 25 to develop ovarian follicles. Among these, about half were further administered s.c. with 10 IU of hCG at 56 hrs after eCG treatment to confirm the number of the ovulatory follicles in the eCG-primed ovary. After more than 72 hrs of eCG-treatment, these females were sacrificed to examine freshly ovulated oocytes in the ampullulae, and their ovaries and uterus were weighed; 3) VO was monitored from day 28 in both experiments. In Exp. I, 1-2 offspring from each dam were examined for ovulation on the day of VO. In Exp. II, about half of the remaining females of each dam were examined for ovulation on the day of VO, and the others were examined on one day after VO. The number of the corpus lutea (CL) was counted in both experiments.

Results and Discussion

As shown in the Table, single injection of 10 or 100 μg/kg of PCB 126 on GD15 to the dams delayed VO of their offspring significantly. In contrast, daily exposure to PCB 126 delayed VO only at a dose of 3μg/kg/day, although cumulative doses of PCB 126 until GD 15 in the 1μg/kg/day-group were estimated as much larger than 10μg/kg. In addition, while a single injection of PCB 126 reduced the number of CL, daily exposure to 1μg/kg/day did not alter it. These findings indicate that daily exposure may diminish effects of PCB 126 on female offspring as compared to single injection on GD15.

Table. Ovulation and the number of corpus lutea (CL) at vaginal opening (VO)

Group	Age of days at vaginal opening (N)	Number of CL at VO or one day after VO (N)	Incidence of offspring at VO with		
			old oocytes	fresh oocytes	none
Experiment I (daily injection)					
Corn oil 2ml/kg/day	30.5±1.7 (4)	12.0±1.0 (3)	1/4	2/4	1/4
PCB 126 1μg/kg/day	31.3±0.6 (10)	12.6±1.0 (8)	3/10	5/10	2/10
PCB 126 3μg/kg/day	34.5±0.5 (4)**	10.5 (2)	2/4	0/4	2/4
Experiment II (single injection)					
Corn oil 2ml/kg	31.4±0.3 (20)	12.7±0.4 (19)	4/10	5/10	1/10
PCB 126 10μg/kg	33.3±0.4 (24)**	11.4±0.4 (20)*	3/12	7/12	2/12
PCB 126 100μg/kg	35.9±0.5 (21)**	9.3±0.8 (17)**	7/10	2/10	1/10

* and **: significant difference from oil-exposed offspring at $p<0.05$ and $p<0.01$, respectively

When offspring were examined for ovulation on the day of VO, the incidence of offspring with fresh oocytes was similar between oil- and 10 μg/kg-exposed groups in Exp. II, indicating that *in utero* and lactational exposure to 10 μg/kg PCB 126 delays the first ovulation with a reduction in number of oocytes shed. In contrast, most offspring exposed to 100 μg/kg in Exp. II showed old oocytes at VO, indicating that VO might be unsynchronized with the first ovulation in these offspring. Whether or not the timing of the first ovulation was differed between doses in Exp. II was not established from the present study; however, no formation of CL in two

cases of 100 μg/kg-exposed groups on days 36 and 37 suggested a later occurrence of the first ovulation than with the 10 μg/kg-exposed groups.

Since single injection of 5 IU eCG on day 25 induced ovulation to all offspring examined, maturity of the hypothalamohypophysial axis might not be involved with the delay in the first ovulation. As found in the spontaneous first ovulation, the number of oocytes shed in induced ovulation decreased in the 100 μg/kg-exposed group, indicating that ovarian ability to develop follicles for the first ovulation was reduced. Uterus weight was decreased in both doses of exposure after eCG-hCG treatment. Since prepubertal (on day 24) uterus weight was not significantly reduced in either experiment, PCB 126 might exert its anti-estrogenic effect on GTH-stimulated cascades.

In conclusion, *in utero* and lactational exposure to PCB 126 delays the first ovulation at a dose of 10 μg/kg on GD15 with a slight reduction in the number of the oocytes shed, and disrupts synchronization of VO with the first ovulation at that of 100 μg/kg. These abnormalities in female puberty may be caused by adverse effects of PCB 126 on the ovary and external genitalia including vagina, but may not on the maturity of the hypothalamohypophysial axis.

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