EFFECTS OF 2,3',4,4',5-PENTACHLOROBIPHENYL (PCB 118) IN SD RATS: TESTICULAR TOXICITY IN F_3 OFFSPRING

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Introduction

Polychlorinated biphenyls (PCBs) are a mixture of 209 congeners with different chlorine substitutions and they are potentially hazardous compounds in the environment for human beings[1]. The cogener 2,3',4,4',5-pentachlorobiphenyl (PCB 118), a mono-*ortho* substituted PCB, is one of the most persistent congeners found in human tissues and is regularly detected in human breast milk and adipose tissue[2]. Several studies indicate that prenatal exposure to PCBs might induce adverse reproductive effects, including in utero exposure can alter reproductive function in sexually mature men[3] and rat offspring in adulthood[4]. However, it remains unclear whether such effects may be transmitted to subsequent generations. The specific objective is to evaluate the transgenerational effects of exposure to PCB 118 on reproductive functions in F_3 male offspring.

Materials and methods

Animals and treatment. Fisher rat dams were treated by gavage on gestation day (GD) 8 to GD 15 with low dose of 380 μg PCB 118/kg based on the 100-fold higher than the concentration in human milk reported from the general population in Taiwan and other industrial countries[5]. An outcross experiment was performed to determine whether the transgenerational phenotype is tranfmitted through the male germ line. The F_1 male rats were mated with PCB 118-unexposed females on postnatal day (PND) 93. Male adult F_2 offspring were divided to control group (control F_1 fathers + control F_1 mothers) and PCB 118-exposed group (treated F_1 fathers + control F_1 motrs). The resulting F_2 males from control and PCB 118-exposed groups were bred with unexposed females to produce F_3 offspring.

Reproductive outcomes measurement. At PND 112, the male F_3 offspring were anesthetized by CO_2 and the reproductive organ were removed and weighed. Relative organ weight was calculated by calculating the ratio between organ weight and body weight. Epididymal sperm count, motility, sperm chromatin DNA structure, and reactive oxygen species (ROS) generation were measured. The fluorescence intensity of DNA content of the testis cells was measured using a FACScan flow cytometry (FCM). PI fluorescent emissions were monitored using a 620 nm band-pass filter. A total of 2×10^4 cells were collected for each sample. Based on the DNA content, main germ cell peaks can be classified into four categories: (1) mature haploid (elongated spermatids; stages XXIV), (2) immature haploid (round and elongating spermatids; stages I-IX), (3) diploid (spermatogonia, secondary spermatocytes, tissue somatic cells), and (4) tetraploid (mostly primary spermatocytes). The region between the diploid and tetraploid peaks, called the S-phase, is comprised of cells that actively synthesize DNA[6]. The relative proportions of haploid, diploid, S-phase and tetraploid cell types were calculated.

Data analysis. Data were expressed as means ± standard deviation. Parameters of body and organ weights and sperm functions were compared between the CC group and HC group using Student's *t* statistic test. All statistical analyses were performed on JMP 5.0. A p value of < 0.05 was considered statistically significant.

Results and discussion

Body and reproductive organ weights of male offspring:

We found no significant differences between the PCB 118-exposed group and the controls in male F_3 offspring with regard to body weight, relative weights of testis, epididymis, and seminal vesicle (Table 1).

Sperm count, motility, chromatin structure assay (SCSA,) and ROS generation analysis:

As can be seen in Table 2, a summary of average sperm count and motility for each of the 380 μg PCB 118/kg-treated F_3 offspring and their controls, there were no significant differences in sperm count and motility between

the PCB 118-exposed and control groups. Measurement of sperm chromatin structure after in situ denaturation by flow cytometric methods represents a relatively stable and sensitive indicator of conventional sperm quality characteristics. Epididymal sperm was analyzed by FCM SCSA to investigate whether there was any induced change in F_3 sperm chromatin DNA integrity after exposure to PCB 118 in F_0. We found no significant change in the two indicators of sperm chromatin DNA damage, αT and DFI, between the 380 μg of PCB 118/kg/day group and the control group of male F_3 generation offspring (Table 2). To investigate sperm oxidation stress, we next studied sperm H_2O_2 and $O_2^{-\bullet}$ generation. There were no significant alterations of the percentage and intensity of sperm with excessive sperm H_2O_2 and $O_2^{-\bullet}$ generation in PCB 118-exposed group compared to the control group (Table 2).

DNA content analysis of testis cells:

The cell frequencies of various testis subpopulations were expressed as percentage of total histogram area (Fig. 1). We found a significant decrease in testis DNA content of tetraploid and diploid cells, while a significant increase of elongated spermatids in PCB 118-exposed group compared to the control group ($p < 0.05$)(Table 2). However, there were no significant changes in testis DNA content of round spermatids and S-phase cells between the 2 groups.

Discussion. To our knowledge, this is the first study reporting long-lasting reproductive effects of low-dose exposure of PCB 118 spanning three generations in mammals. Fetal germ cells are particularly sensitive to cytotoxicants. In mice and rats, prenatal exposure to cytotoxic drugs or toxic substances induces permanent reductions in the number of germ cells in the offspring without affecting phenotypic sex differentiation. Many studies have investigated that F_1 offspring prenatally exposed to PCBs might induce the decrease of sperm count[4,7]. Recently, an animal study indicated that exposure to PCB mixture (101 and 118) at the time of gonadal sex determination perturbed, significantly, the reproductive physiology of male and female offspring in adulthood. Male reproductive deficiencies may be observed in at least two further generations[8]. These findings have significant implications for reproductive health and fertility of animals and humans prenatal exposure of PCBs on offspring. The observed effect of PCBs on sperm production is thought to be due to a thyroid-dependent mechanism, since goitrogen-induced hypothyroidism in the neonatal rat has also been shown to increase adult testis weight and daily sperm production[9]. It was expected that male offspring prenatally exposed to PCBs would exhibit an increase in adult testis size and sperm production, but reduced fertility. However, differences in species, timing of exposure, dosage, and the age when males are examined might influence the observed effects. Because the F_3 generation is the first generation not directly exposed to the environmental toxicants, it is the minimal requirement to evaluate the transgenerational effects on the F_3 generation[10, 11]. Environmental toxicants such as vinclozolin[10, 12] and the plastic component bisphenol A (BPA) promote transgenerational disease. The plasticizer BPA also promotes testicular disease from F_1 to F_3 generations in rats[13]. Different mechanisms have been postulated to explain multigenerational reproductive adverse phenotype, including epigenetic changes in the germ-line[14]. Some studies have addressed that the ability of an external agent to induce a transgenerational phenotype requires a genetic or an epigenetic phenomenon mediated through the germ line[15, 16]. In this study, PCB 118 has been shown to influence the spermatogenesis in male offspring of F_3 generation. Whethet PCBs affect male F_3 generation offspring via epigenetic alteration through the germ line is needed to clarify in the future.

Conclusion. This is the first study to demonstrate the transgenerational effects of PCB 118 on spermatogenesis of testicular function, including alterations of tetraploid, diploid cells, and elongated spermatids in male F_3 rat offspring.

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References

1. Giesy JP, Kannan K. (1998); *Crit Rev Toxicol*. 28(6): 511-69
2. WHO—Environmental Health in Europe No. 3, (1996). Denmark, pp. 1–117
3. Guo YL, Hsu PC, Hsu CC, Lambert GH. (2000); *Lancet*. 356(9237): 1240-1
4. Hsu PC, Pan MH, Li LA, Chen CJ, Tsai SS, Guo YL. (2007); *Toxicol Appl Pharmacol*. 221(1): 68-75
5. Wang SH, Lin CY, Guo YL, Lin LY, Chou WL, Chang LW. (2004); *Chemosphere* 54(10): 1459-73
6. Span`o M., Bartoleschi C, Cordelli E, Leter G, Segre L, Mantovani A. (1996);. J Toxicol Environ. Health 47(5): 101–119

7. (6). Bird DM, Tucker PH, Fox GA, LaguE PC. (1983); *Arch Environ Contam Toxicol*. 12(6): 633-9
8. Pocar P, Fiandanese N, Secchi C, Berrini A, Fischer B, Schmidt J-S, Schaedlich K, Rhind SM, Zhang Z, Borromeo V. (2012); *Toxicol Sci.* 126(1): 213-226
9. Cooke PS, Meisami E.(1991); *Endocrinology*. 129(1): 237- 43
10. Anway MD, Cupp AS, Uzumcu M, Skinner MK. (2005); *Science*. 308(5727): 1466-9
11. Campbell JH, Perkins P. (1988); *Prog Brain Res*. 73:535-53
12. Schneider S, Kaufmann W, Buesen R, van Ravenzwaay B. (2008) *Reprod Toxicol*. 25(3): 352-60
13. Salian S, Doshi T, Vanage G. (2009). *Life Sci*. 85(1–2): 11-8
14. Anway MD, Leathers C, Skinner MK. (2006); *Endocrinology* 147(12): 5515-23
15. Barber R, Plumb MA, Boulton E, Roux I, Dubrova YE. (2002). *Proc Natl Acad Sci USA* 99(10):6877-82
16. Dubrova YE. (2003). *Oncogene* 22: 7087-93

Table 1. Body and organ weights for male control and PCB 118-exposed F_3 offspring.

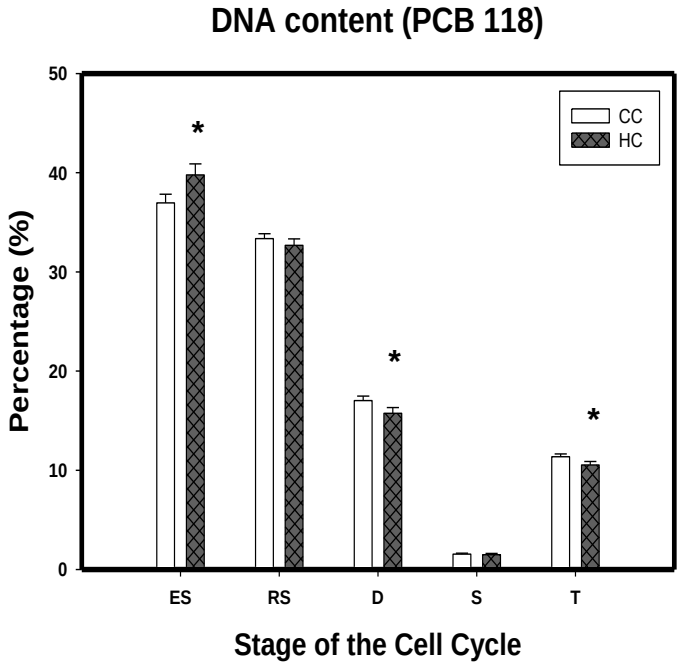
Parameters	Control (n = 12)	PCB 118-exposed (n = 12)
Body weight (kg)	0.4 ± 0.0	0.4 ± 0.0
Relative left testis (g/kg of B.W.)	4.2 ± 0.4	4.3 ± 0.3
Relative right testis (g/kg of B.W.)	4.2 ± 0.4	4.4 ± 0.3
Relative left epididymis (g/kg of B.W.)	1.2 ± 0.1	1.2 ± 0.1
Relative right epididymis (g/kg of B.W.)	1.3 ± 0.1	1.3 ± 0.1
Relative seminal vesicle (g/kg of B.W.)	3.2 ± 0.3	3.4 ± 0.5

n: Numbers of sample

Table 2. Sperm quality, sperm chromatin structure assay (SCSA), and reactive oxygen species (ROS) generation for male control and PCB 118-exposed F_3 offspring.

Parameters	Control (n = 12)	PCB 118-exposed (n = 12)
Sperm count (10^6/mL)	16.3 ± 4.1	16.3 ± 4.1
Sperm motility (%)	28.5 ± 11.2	23.3 ± 10.5
$\alpha T^{a, b}$ (arbitrary units)	284.2 ± 11.1	277.1 ± 10.7
DNA fragmentation index [c] (DFI, %)	0.7 ± 0.4	0.6 ± 0.4
Sperm $O_2^{-\bullet}$ generation[b] (arbitrary units)	122.0 ± 24.6	117.7 ± 24.7
Percentage of sperm with excessive $O_2^{-\bullet}$ generation (%)	2.3 ± 3.4	1.2 ± 0.8
Sperm H_2O_2 generation[b] (arbitrary units)	20.4 ± 9.4	19.1 ± 12.2
Percentage of sperm with excessive H_2O_2 generation (%)	0.2 ± 0.2	0.1 ± 0.1

n: Numbers of sample; [a]: Fluorescence intensity; [b] αT is the level of sperm with DNA damage.
[c] DNA fragmentation index (DFI) is the % of sperm with chromatin DNA damage.



ES: elongated spermatids; RS: round spermatids; D: diploid; S: S-phase;
T: tetraploid; *: significant difference compared with control group ($p < 0.05$)

Figure 1. Relative fractions (means ± S.E.M.) of the various testis subpopulations, calculated as percentage of the histogram area between mice postnatally exposed to PCB 118 and unexposed controls.