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Effects of acute postnatal exposure to 3,3′,4,4′-tetrachlorobiphenyl on sperm function and hormone levels in adult rats

Ping-Chi Hsu [a], Yueliang Leon Guo [b], Mei-Hui Li [c,*]

[a] *Department of Safety, Health and Environmental Engineering, National Kaohsiung First University of Science and Technology, 2 Juoyue Road, Nantsu, Kaohsiung 811, Taiwan, ROC*

[b] *Department of Environmental and Occupational Health, National Cheng Kung University Medical College, 61 Shiao Tong Road, Tainan 70428, Taiwan, ROC*

[c] *Environmental Toxicology Lab, Department of Geography, National Taiwan University, 1, Section 4, Roosevelt Road, Taipei 106, Taiwan, ROC*

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Abstract

Polychlorinated biphenyls (PCBs) are considered potential endocrine disruptors due to their ability to act as estrogens, antiestrogens and goitrogens. The aim of this study is to ascertain whether acute postnatal treatment with 3,3′,4,4′-tetrachlorobiphenyl (CB 77) affects sperm function and hormone levels in adult rats. Male Sprague–Dawley rats received CB 77 by *ip* injection of 2 or 20 mg/kg at day 21 and sacrificed at day 112. At day 112, right and left testis weights were significantly increased, whereas sperm count, motility, total motile sperm count, curvilinear velocity, average path velocity, straight-line velocity, and beat-cross frequency for motile sperm were significantly decreased in rats treated with 20 mg/kg CB 77. Sperm–oocyte penetration rate was significantly reduced in rats treated with either 2 or 20 mg/kg CB 77. There was high sperm acrosome reaction rate (ARR) in the 20 mg/kg CB 77-treated rats. There was a significant increase in thyroid-stimulating hormone level in the 20 mg/kg CB 77 group. However, no changes were seen in serum testosterone, thyroid hormones, or prolactin concentrations at day 112. In summary, this study showed that postnatal exposure to CB 77 might affect spermatogenesis, motility, ARR, and ability of fertilizing oocytes in mature rats. These results suggest that the sperm functions may be more susceptible or adapt less readily than the thyroid functions to endocrine disruption caused by dioxin-like PCB congeners.
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Keywords: PCBs; CB 77; Postnatal exposure; Sperm; Acrosome reaction; Hormone

1. Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous environmental pollutants with endocrine-disrupting effects. Among 209 possible PCB congeners, a small group of coplanar chlorobiphenyl (CB) congeners resembles 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in molecular configuration. The toxicities of these coplanar CB congeners are mediated through the aryl hydrocarbon (Ah) receptor, and the toxic potencies of these dioxin-like coplanar CB congeners can be predicted based on their induction of hepatic cytochrome P450 1A enzymes as compared to TCDD (Safe, 1994). Other dioxin-like effects of the coplanar CB congeners include thymic atrophy, teratogenicity, hepatic enzyme induction, reproductive and developmental toxicity, porphyria and immunotoxicity (Safe, 1994). In particular,

* Corresponding author. Tel.: +886-2-33665839; fax: +886-2-23622911.

E-mail address: meihuili@ccms.ntu.edu.tw (M.-H. Li).

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male reproductive toxicity related to dioxin-like compounds are reported to include altered regulation of luteinizing hormone (LH) secretion, reduced testicular steroidogenesis, reduced plasma androgen concentrations, reduced testis and accessory sex organ weights, abnormal testis morphology, decreased spermatogenesis, and reduced fertility (Peterson et al., 1993).

Information concerning the effects of PCBs on male reproduction is still limited and remains inconclusive. Most of the male reproductive effects have been observed following prenatal and/or postnatal exposure to 3,3′,4,4′-tetrachlorobiphenyl (CB 77) (Faqi et al., 1998a,b; Huang et al., 1998) and PCB mixtures (Sager, 1983; Sager et al., 1987, 1991; Cooke et al., 1996) in rodents. For example, an increase in testis weights was observed in mice (Huang et al., 1998) and in rats (Sager, 1983; Cooke et al., 1996; Faqi et al., 1998a), whereas a reduction in seminal vesicle and prostate weights was reported in rats (Sager, 1983; Faqi et al., 1998a) after neonatal exposure to PCB. In addition, sperm fertilizing ability in vitro was decreased in mice exposed to Aroclor 1242 (Fielden et al., 2001), but no effects observed in mice exposed to CB 77 (Rodriguez et al., 1997).

Humans are not exempt from the reproductive effects of dioxin-like compounds. There were indications that the concentrations of 2,2′,4,4′,5,5′- and 2,2′,3′,4,4′,5-hexachlorobiphenyl and 2,3′,4,4′,5,-pentachlorobiphenyl were negatively correlated with sperm motility in samples with a sperm count less than 20×10^6 cells/ml in 170 fertile men (Bush et al., 1986). We found that exposure to PCBs and polychlorinated dibenzofurans (PCDFs) in utero can alter reproductive function in the human male and they are compatible with animal studies, in which exposure in utero to similar toxic equivalency of TCDD reduced daily sperm production and increased percent of abnormal sperm while sexually mature (Guo et al., 2000). It is also likely that accidental exposure of PCBs and other related compounds occurs in human children; however, there is little information on the potential reproductive effects from postnatal exposure of PCBs in animal studies.

Although several studies reported effects of CB 77 on male reproduction, most of these studies only observed developmental and reproductive outcomes (Rodriguez et al., 1997; Faqi et al., 1998a,b), or examined sperm fertilizing ability (Kholkute et al., 1994; Huang et al., 1998). In this study, we use weanling male rats as an animal model to study acute postnatal exposure to CB 77 on the male reproduction system to further identify the toxicity and the hormonal pathways involved in dioxin-like CB congeners. Male pups were treated with CB 77 at day 21 and sacrificed at day 112. We examined sperm quality, sperm activities and functions, as well as both thyroid and sex hormone levels in male rats to investigate the possible effect of childhood-accidental exposure to PCBs on the male reproductive system in adults.

2. Materials and methods

2.1. Chemicals

CB 77 was purchased from AccuStandard (New Haven, CT; Purity >99%). Phosphate-buffered saline (PBS) and human tubule fluid (HTF) medium were from Gibco Life Technologies Ltd. (New York, USA). The medium had a pH of 7.4, an osmolarity of 330 mOsm/l when gassed with 5% CO_2 and air, and was prewarmed to a temperature of 34 or 37 °C. Triton X-100, and ammonium dihydrogen phosphate were purchased from Merck Co. (Darmstadt, Germany). All other reagents, corn oil and chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Radioimmunoassay (RIA) kits for determining serum hormone were purchased from Diagnostic Systems Laboratories, Inc. (Webster, TX, USA).

2.2. Animals and treatment regimens

Sprague–Dawley rats were purchased from the Animal Center of National Cheng Kung University Medical Center (Tainan, Taiwan) and were housed in a rodent vivarium under a 12-h light, 12-h dark cycle and controlled temperature. Laboratory Rodent Diet 5001 (LabDiet, Richmond, IN) and water were available ad libitum. Rats were weaned at 20 days of age. A total number of 22 male pups were divided into control and CB-treated groups. Male pups were injected intraperitoneally with 2 or 20 mg/kg of CB 77 or corn oil respectively at day 21 and sacrificed at day 112. The rationale of choosing the timing for treatment is to simulate the effect of childhood-accidental exposure to PCBs on the male reproductive system in adults.

2.3. Sperm suspension preparation

The right cauda epididymis was dissected from each male and transported in 1 ml 34 °C HTF buffer supplemented with 5 mg/ml bovine albumin. The cauda epididymis was removed from the transport buffer, cut, and placed in 1 ml HTF–albumin buffer, overlaid with mineral oil. A 1:10 dilution of spermatozoa was prepared and an epididymal sperm count done with a hemocytometer. The motile epididymal sperm count was obtained from multiplying the epididymal sperm count by percent motile spermatozoa.

2.4. Sperm motility analysis

Within 10 min of collection, sperm samples were held in a Makler chamber (Sefi Medical Instruments, Israel) for motility measurements. The motile epididymal sperm count was obtained from multiplying the epididymal sperm count by the percentage motile sperm. Computer-assisted sperm analysis (CASA) was obtained for motility indices with a Hamilton Thorn Research motility analyzer (version HTM-IVOS Specification, Beverly, MA, USA) at a temperature of 37 °C. CASA was gained for sperm motility parameters: curvilinear velocity (VCL, μm/s), average path velocity (VAP, μm/s), straight line velocity (VSL, μm/s), amplitude of lateral head displacement (ALH, μm), and beat-cross frequency (BCF, Hz). The velocity parameters were adjusted for evaluating the levels of all sperm performance.

2.5. Chlortetracycline (CTC) fluorescence assay

CTC was prepared freshly at a concentration of 1.5 mM in 20 mM tris buffer containing 130 mM NaCl and 5 mM L-cysteine. The final pH was adjusted to 7.8 and the solution was shielded from light at room temperature. A volume of 50 μl of sperm suspension was mixed with 50 μl of CTC solution. After 30 s, 8 μl of 12.5% (w/v) paraforamaldehyde in 0.5 mM tris buffer (pH 7.4) was added. A drop of 0.22 mM 1,4-diazabicyclo[2.2.2]octane in glycerol was introduced and mixed with the sperm suspension to retard fading of fluorescence. The slide was analyzed with an Olympus BX-60 microscope (Tokyo) equipped with phase-contrast and epifluorescence optics. A total of 200 cells were assessed and each sample was calculated in duplicate.

2.6. Measurement of sperm ROS generation

The sperm suspension was washed twice with phosphate buffered saline, pH 7.4, and resuspended in the same medium at a concentration of $45–50 \times 10^6$ sperm/ml. Then 50 μl of 1 mM luminol prepared in dimethyl sulfoxide and 100 μl of 30 mM $FeSO_4$ were added to 300 μl of the washed and vortexed sperm suspension. ROS levels were determined by measuring chemiluminescence with a luminometer (Autolumat-LB 953; EG&G Berthold Co., Bad Wildbad, Germany), with the counts being integrated over a 60-s period. ROS levels were converted from chemiluminescence counts per 10^6 sperm per second.

2.7. Sperm–oocyte penetration rate assay

Control female Sprague–Dawley rats were superovulated at age 63–70 d weighing 330–380 g. In the morning of day 1, the rats were injected with 25 IU of PMSG. On day 3, 52 h later, the animals were injected with 25 IU of hCG. Twenty hours later, the female rats were terminated and the oviducts dissected and placed into HTF–albumin buffer. The cumuli were dissected from the oviducts, collected in HTF–albumin buffer, and dissolved with 10 mg/ml of hyaluronidase. After adjusting to 10×10^6 sperm/ml with HTF–albumin buffer, 10 μl of epididymal sperm suspension was added to 100 μl of HTF–albumin buffer containing 10–15 zona-intact rat ova. Each culture well of the sperm–ova preparation was overlaid with mineral oil and incubated at 37 °C in 95% air/5% CO_2. After 48 h of insemination, the number of oocytes penetrated by sperm was determined by phase-contrast microscopy at 400× magnification. Sperm–oocyte penetration rate (SOPR) was used to evaluate the sperm–oocyte penetration capacity as described in the following equation:

$$\mathrm{SOPR}(\%) = [1 - (\text{number of not penetrated oocytes} / \text{number of total oocytes})] \times 100$$

2.8. Hormone assay

The concentrations of testosterone (TT), follicle-stimulating hormone (FSH), triiodothyronine (T_3), thyroxine (T_4), free T_3 (FT_3) and free T_4 (FT_4) in the serum were measured by RIA (International CIS RIA Kits). Rat luteinizing hormone (LH), prolactin (PRL), and thyroid-stimulating hormone (TSH) RIA kits were provided from the Pituitary Agency of National Institute of Arthritis, Diabetes and Digestive and Kidney Disease (NIAMDD), USA. Rat LH-I-5, PRL-I-5, and TSH-I-5 were iodinated with ^{125}I by a modification of the chloramines-T method of Greenwood et al. (1963). Rat LH-RP-1, PRL-RP-2, and TSH-RP-2 served as standards. The radioactivity in the precipitate was measured by automatic gamma counter. Each sample was assayed at one or two dilutions in duplicate.

2.9. Statistics

All the values are presented as means ± SD. Comparisons of body weights, testis weights, cauda epididymis weights, sperm count, motility, motile sperm count, motile velocity, sperm–oocyte penetration rate, acrosome reaction rate (ARR), sperm chemiluminescence counts, and serum hormone levels between CB 77-exposed and control groups were done by one-way analysis of variance (ANOVA), followed by the Tukey–Kramer Honestly Significant Difference (HSD). The relationships among motility, sperm counts, motile sperm counts, motile velocity and sperm–oocyte penetration rate were determined by using linear regression of the JMP statistical package (SAS Institute, Inc., Gary, NC).

3. Results

3.1. Body weights and tissue weights

Significant increases in left and right testis weights were observed in 20 mg/kg CB 77-treated postweaning male rats compared with 2 mg/kg CB 77-treated and control rats ($P < 0.05$ and 0.05, respectively). However, there were no statistical differences in body weights, relative total testes weights, and relative cauda epididymis weights between control and treated groups (Table 1).

3.2. Sperm quality

A summary of sperm count, sperm motility, total motile sperm count and parameters of velocity were presented in Table 2. Statistically significant ($P < 0.05$) decreases in sperm count, sperm motility, total motile sperm count and most of adjusted sperm motility parameters were found in the 20 mg/kg CB 77-treated postweaning male rats.

3.3. SOPR, ARR, and ROS levels

There were significant decreases in SOPR in CB 77-treated male rats at 2 mg/kg and 20 mg/kg ($P < 0.05$ and 0.05, respectively), and these penetration rate reductions were dose-related. Nevertheless, a significant ($P < 0.05$) increase in ARR was observed in the 20 mg/kg CB 77-treated male rats. There was no statistical difference in sperm ROS levels between control and treated groups (Table 3).

Table 1
Body and tissue weight between rats postnatally exposed to CB 77 (2 mg/kg or 20 mg/kg) and unexposed controls

Parameters	Control ($n = 10$)	Treatment ($n = 6$)	
		CB 77 (2 mg/kg)	CB 77 (20 mg/kg)
Body weight (g)	514.9 ± 21.4	512.5 ± 38.4	546.7 ± 27.9
Left testis weight (g)	1.60 ± 0.16	1.60 ± 0.15	1.90 ± 0.08*,#
Right testis weight (g)	1.64 ± 0.17	1.58 ± 0.15	1.88 ± 0.06*,#
Total testis weight (mg)[a]/body weight (100 g)	641.8 ± 44.3	622.2 ± 51.8	691.3 ± 28.5#
Left cauda epididymis weight (g)	0.24 ± 0.03	0.24 ± 0.06	0.23 ± 0.01
Right cauda epididymis weight (g)	0.24 ± 0.04	0.22 ± 0.05	0.23 ± 0.01
Total cauda epididymis weight[b] (mg)/body weight (100 g)	92.9 ± 12.6	90.4 ± 15.1	84.1 ± 6.0

* $P < 0.05$ as compared with control group.
# $P < 0.05$ as compared with CB 77 (2 mg/kg) group.
[a] Total testis weight = left testis weight + right testis weight.
[b] Total cauda epididymis weight = left cauda epididymis weight + right cauda epididymis weight.

Table 2
Sperm quality between rats postnatally exposed to CB 77 (2 mg/kg or 20 mg/kg) and unexposed controls

Parameters	Control ($n = 10$)	Treatment ($n = 6$)	
		CB 77 (2 mg/kg)	CB 77 (20 mg/kg)
Sperm count (10^6/ml)	250.0 ± 54.0	185.8 ± 89.8	148.3 ± 29.3*
Sperm motility (%)	64.8 ± 5.3	51.6 ± 25.7	41.5 ± 9.0*
Total motile sperm count[a] (10^6/ml)	161.9 ± 37.0	114.7 ± 58.2	61.8 ± 17.1*,#
VCL (μm/s)[b]	83.0 ± 19.2	75.8 ± 6.3	49.7 ± 5.7*,#
VAP (μm/s)[c]	59.2 ± 15.9	55.4 ± 6.0	36.0 ± 5.0*
VSL (μm/s)[d]	37.7 ± 12.1	37.9 ± 7.3	23.7 ± 2.9*
ALH (μm)[e]	2.6 ± 1.0	1.6 ± 1.0	1.9 ± 0.9
BCF (Hz)[f]	11.5 ± 2.6	8.6 ± 4.2	4.4 ± 2.9*

* $P < 0.05$ as compared with control group.
# $P < 0.05$ as compared with CB 77 (2 mg/kg) group.
[a] Total motile sperm count (10^6/ml) = Sperm count (10^6/ml) × motility (%).
[b] VCL: curvilinear velocity for all sperm after adjustment.
[c] VAP: average path velocity for all sperm after adjustment.
[d] VSL: straight-line velocity for all sperm after adjustment.
[e] ALH: amplitude of lateral displacement for all sperm after adjustment.
[f] BCF: beat frequency for all sperm after adjustment.

Table 3
Sperm–oocyte penetration rate, status of acrosome reaction, and levels of reactive oxygen species generation between rats postnatally exposed to CB 77 (2 mg/kg or 20 mg/kg) and unexposed controls

Parameters	Control ($n = 10$)	Treatment ($n = 6$)	
		CB 77 (2 mg/kg)	CB 77 (20 mg/kg)
Sperm–oocyte penetration rate (%)	61.5 ± 10.8	42.2 ± 13.5*	37.5 ± 5.1*
Acrosome reaction rate (%)	7.0 ± 3.4	3.4 ± 3.8	42.5 ± 25.5*,#
Sperm chemiluminescence level (count/sperm)	2.6 ± 0.1	2.6 ± 0.1	2.4 ± 0.1

* $P < 0.05$ as compared with control group.
# $P < 0.05$ as compared with CB 77 (2 mg/kg) group.

3.4. Relationships between motion analysis and SOPR

The correlations of sperm motion analysis and SOPR are fairly good (Table 4). The parameters of sperm motion analysis including motility, motile sperm count, VCL, BCF, and VAP were all positively associated with SOPR. Most of the parameters measured in sperm motion analysis were reduced in the 20 mg/kg CB 77-treated male rats. However, there were no significant correlations between ARR, ROS levels, and SOPR.

Table 4
Significant correlation parameters of sperm motion analysis and sperm–oocyte penetration rate

Correlation	*R* values	*P* values
Motility	0.6490	0.0015
Motile sperm count	0.6276	0.0023
VCL[a,b]	0.5880	0.0064
BCF[a,c]	0.5164	0.0197
VAP[a,d]	0.5067	0.0226

[a] Velocity parameters for all sperm after adjustment.
[b] VCL: curvilinear velocity for all sperm after adjustment.
[c] VAP: average path velocity for all sperm after adjustment.
[d] BCF: beat frequency for all sperm after adjustment.

3.5. Serum hormone levels

The concentration of FSH was significantly increased in the 20 mg/kg CB 77-treated group compared to the 2 mg/kg dose group (Table 5). There was a significant increase in TSH level in the 20 mg/kg CB 77-treated group compared to controls and 2 mg/kg dose group, respectively. On the other hand, there were no differences for serum TT, LH, PRL, T_3, T_4, FT_3, and FT_4 levels between the control and the treated groups at the termination of the study. Due to the limitation of serum volume, the LH level was not analyzed in 20 mg/kg CB 77-treated rats.

4. Discussion

In the present study, significant increases in left and right testis weights were observed in the 20 mg/kg CB 77-treated male rats compared to 2 mg/kg CB 77-treated rats and controls. However, the sperm count was decreased in the 20 mg/kg CB 77-treated rats compared to controls. In fact, increased testis weight was found in rats from postnatal exposure to CB 77 (Faqi et al.,

Table 5
Serum hormone levels between rats postnatally exposed to CB 77 (2 mg/kg or 20 mg/kg) and unexposed controls

Parameters	Control ($n = 10$)	Treatment ($n = 6$)	
		CB 77 (2 mg/kg)	CB 77 (20 mg/kg)
Testosterone (ng/ml)	4.3 ± 2.3	5.1 ± 6.2	4.1 ± 3.8
Follicle-stimulating hormone (ng/ml)	1.7 ± 0.4	1.5 ± 0.4	1.9 ± 0.2#
Luteinizing hormone (ng/ml)	0.7 ± 0.6	0.9 ± 0.3	–
Prolactin (ng/ml)	30.3 ± 20.1	39.4 ± 4.8	40.1 ± 12.0
Thyroid-stimulating hormone (μIU/ml)	7.7 ± 2.6	6.8 ± 3.1	11.4 ± 2.4*,#
Triiodothyronine (ng/dl)	232.0 ± 49.4	250.4 ± 28.9	198.0 ± 26.0
Thyroxine (μg/dl)	4.4 ± 0.8	4.4 ± 0.5	4.7 ± 0.6
Free T_3 (pg/ml)	1.9 ± 0.5	1.7 ± 0.3	1.7 ± 0.8
Free T_4 (pM)	20.0 ± 3.1	20.4 ± 2.0	23.0 ± 2.1

–: No data.
* $P < 0.05$ as compared with control group.
# $P < 0.05$ as compared with CB 77 (2 mg/kg) group.

1998b) and in male offspring after prenatal exposure of CB 77 (Faqi et al., 1998a). In a high dose group study, the relative testis and prostate weights were slightly increased, but sperm number per cauda epididymis was significantly reduced in the 60 mg/kg CB 77-exposed rats after 1 and 4 weeks of postnatal treatment (Faqi et al., 1998b). In another in utero study, increased testis weight was reported in male offspring on day 65 or day 140 of age exposed to CB 77 with dose of 0.1 mg/kg (Faqi et al., 1998a). Cooke et al. (1996) also reported that testis weight was significantly increased in rats neonatally dosed with 1.6 and 3.2 mg/day Aroclor 1242 doses as well as 0.4 and 1.6 mg/day Aroclor 1254 from birth to day 25. Exposure time may play an important role on the observed effects, however, our result did coincide with these observations from in utero or neonatal exposure.

The postnatal exposure to dioxin-like CB congeners might be associated with the decrease of sperm count in rats. Decreased spermatogenesis was suggested as among the most sensitive responses of the male rat reproductive system to perinatal TCDD exposure (Mably et al., 1992). A possible mechanism of decreased spermatogenesis is a decrease in FSH and/or TT levels. The homeostasis between FSH and TT is important for quantitatively normal spermatogenesis (Steinberger and Steinberger, 1989). We found there were no differences for serum TT or FSH levels between the control and the treated groups. However, we cannot rule out the possibility of early depression of FSH and TT in those rats during early stage of this study treatment. The possible mechanism of CB 77 on early development of male reproductive system remains to be investigated.

We found statistically significant decreases in sperm motility, total motile sperm count, most of adjusted sperm velocity parameters, and beat-cross frequency in the 20 mg/kg CB 77-treated rats compared to control rats. Our previous work also found that sperm motility is more susceptible to non-dioxin-like CBs after postnatal exposure (Hsu et al., 2003). In the present study, there was dose-related decreased in SOPR in CB 77-treated rats and the parameters of sperm motion analysis were significantly correlated with SOPR. In fact, parameters of sperm motility are important indexes for evaluating adverse effects on male fertility. Epidemiological study found that adverse effects on human reproduction have also been observed in young men prenatally exposed to PCBs/PCDFs, including sperm motility, velocity, beat-cross frequency and ability to bind and penetrate the hamster oocyte (Guo et al., 2000). A possible mechanism that might cause reduction of sperm motility and SOPR is damage by ROS generation. ROS were shown negatively associated with penetration and fertilization in humans (Aitken and Fisher, 1994) and rats (Hsu et al., 1997). In this study, there was no statistical difference in sperm ROS levels between control and treated groups at day 112 after 91 days of a single exposure to CB 77. However, we could not exclude the possibility of oxidative stress on sperm during early stage of this study treatment.

Another plausible explanation for the alteration of motility and SOPR is the role of sperm acrosome reaction. Spermatozoa are presumed to carry on their acrosome-reacted head a hydrolytic enzyme that causes digestion of the zona pellucida. It has been suggested that the acrosome reaction plays an important role in the fusion of sperm and oocyte (Guraya, 2000). These changes could have occurred during spermiogenesis in the gonad or sperm maturation in the epididymis. Our previous studies found that earlier exposure to chemicals capable of inducing premature epididymal sperm acrosome reaction, decrease of motility (Hsu et al., 1998), and reduction of fertilizing ability to zona pellucida (Hsu et al., 1999). In this study, we found that there was high percentage of epididymal sperm acrosome reaction in the 20 mg/kg CB 77-treated rats compared to control and 2 mg/kg CB 77-treated rats. Although there was no significant correlation between ARR and SOPR, the ARR was inversely correlated with sperm motility.

CB 77 is not a typical dioxin-like CB and can be rapidly metabolized. The failure to determine the thyroid hormone levels during early stage of this short-lived CB 77 treatment might limit our conclusion. We found that there was a rise of TSH levels at day 112 after 91 days of a single exposure to 20 mg/kg of CB 77. Serum T_3 and free T_3 were slightly lower in the 20 mg/kg CB 77-treated group compared to controls. PCB seldom affected TSH level even when T_4 is depressed, however, increased plasma TSH levels have been found in adult male rats exposed to Aroclor 1254 (Bastomsky, 1976) and 2,3,7,8-TCDD (Bastomsky, 1977). In addition, there were slightly lower serum free T_4 levels and slightly higher TSH levels in the dioxins and PCBs high-exposure group of neonates compared with the low-exposure group in 78 Dutch children (Koopman-Esseboom et al., 1994). Moreover, a significant negative correlation was found between some dioxin and PCB congeners in milk and plasma thyroid hormones in pregnant women, while newborn infants showed higher TSH at higher levels of dioxin exposure (Sauer et al., 1994).

In a rodent animal model, there was a decrease in circulating T_4 and an increase of serum TSH following chronic administration of TCDD. The altered TSH levels were attributed to the increased clearance of T_4 by the induced UDP-glucuronosyltransferase (GT) activities and the consequent modification of feedback control of hormone secretions (Kohn, 2000). UDP-GT inducers have been shown to lower plasma levels of T_4 by increasing its glucuronidation and elimination by the liver in rats postnally exposed to alpha-carbonitrile and PCBs (Barter and Klaassen, 1994; Vansell and Klaassen, 2002). However, there are no published literature which

provide direct evidences to explain possible mechanisms of postnatal exposure to CB 77 on increasing serum TSH levels in male rats. Aroclor 1254 and a persistent dioxin-like congener, CB 126 (3,3′,4,4′,5-pentachlorophenyl), were found to reduce the level of c-erbA mRNA which encodes for the thyroid hormone nuclear receptor in vitro (Hornhardt et al., 1994). It is possible that CB 77 and/or its metabolites interact with the nuclear T_3 or T_4 receptor in the pituitary and modify the response of TSH release. In this study, other pituitary hormones were not significantly affected in male rats at day 112, whether the changes of TSH secretion was caused by alteration of pituitary functions warrant further investigation.

In conclusion, our findings suggest that postnatal exposure to CB 77 might induce prematuration of acrosome reaction, adversely interfere with spermatogenesis, motility, and ability of fertilizing oocytes in mature rats. In addition, our results showed that there may be some alterations of sperm functions in adult rats after a single exposure of CB 77 during prepubertal periods. Future investigations should be carried out on the effects of different types of CB congeners exposure on the pituitary-gonadal axis in the fetal and pubertal stages of development.

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