COMPARISON OF CHANGES OF TESTICULAR GENE EXPRESSION BETWEEN RATS NEONATALLY EXPOSED TO PLANAR (PCB 77) AND NONPLANAR (PCB 132) POLYCHLORINATED BIPHENYL CONGENERS

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Abstract
The purpose of this study is to explore the expression of apoptosis-related genes in the testis to compare the alterations of genes expression between rats neonatal exposure to 3,3',4,4'-tetrachlorobiphenyl (PCB 77) and 2,2',3,3',4,6'-hexachlorobiphenyls (PCB 132). Male rats received 2 or 20 mg/kg for PCB 77 and 9.6 or 96 mg/kg for PCB 132 at Day 21 and sacrificed at Day 112. Right testis and epidydimus weights were removed and weighted. Real time RT-PCR was used to detect testicular gene expression, including *Fas*, *Bax*, *Bcl-2*, and *p53*. In this study, expression of *Fas* and *Bax* was significantly increased, whereas *Bcl-2* and *p53* were not significantly changed in rats treated with 20 mg/kg PCB 77. However, there was no significant difference in apoptosis-related genes expression in PCB 132 exposed rats. This study showed that neonatal exposure to higher dose of PCB 77 might affect testicular *Fas* and *Bax* expression in adult rats. These results suggested that the decrease of spermatogenesis may be associated with the cellular apoptosis after exposure of planar PCB congeners. Further efforts are required for a more complete understanding of the underlying molecular mechanisms governing the pathway of apoptosis in different cells of the testis.

Introduction
Polychlorinated biphenyls (PCBs) occur in mixtures of multiple congeners that differ in the numbers and positions of chlorines around the biphenyl ring. Different congeners can exert distinct effects.[1] The halogenated aromatic hydrocarbons are of particular concern as hormone mimics since they often have similar molecular recognition factors, similar bulk physico-chemical properties controlling uptake and distribution in biological systems, and are relatively more resistant to metabolism and elimination.[2] Because of the broad range of toxic effects and their persistence in the biota, studies of the effects of PCB congeners and the subsequent upregulation or downregulation of physiological processes at critical stages of development have been discussed.[3] Most of the male reproductive problems have been observed in rodents with *in utero* and lactational exposure to TCDD or prenatal and/or postnatal exposure to dioxin-like CB congeners.[4-6] Our previous study showed that postnatal exposure to PCB 77, a planar PCB congener, might affect spermatogenesis, motility, acrosome reaction rate, and ability of fertilizing oocytes in mature rats.[7] PCB132 is stable non-dioxin like and nonplanar congener. Methylsulfonyl PCB 132 and its metabolites can be detected in human tissues.[8] We have demonstrated that postnatal exposure to PCB132 affects serum triiodothyronine (T_3) levels, and sperm motility, velocity and capability of penetrating oocytes in rats.[9] Although these data were suggestive of probable toxicity at the sperm function, it is not clear how PCB 77 and PCB132 affect testis function, and if so, through what mechanisms. Apoptosis is a physiological process that entails the programmed death of the cells. Although apoptosis has a functional role in normal development and tissue homeostasis, aberrant triggering of the process by toxicants may lead to abnormal function or disease. The objective of this study is to explore the expression of testicular apoptosis-related genes in the testis to compare the alterations of genes expression between rats neonatally exposed to PCB 77 and PCB 132.

Materials and Methods
Male Sprague-Dawley rats received either PCB 77 or PCB 132 by *ip* injection of 2 and 20 mg/kg for PCB 77 or 9.6 and 96 mg/kg for PCB 132 at Day 21 and sacrificed at Day 112. The rationale of choosing the timing for treatment is to simulate the effect of childhood accidental PCB exposure on later reproductive system in adulthood. Testis and epidydimus weights were removed and weighted. Real time RT-PCR was used to detect

testicular gene expression and western blot was measured to confirm the content of apoptosis-related protein in testis. The housekeeping gene beta-glucuronidase (*GUS*; genebank accession M13962) was used as a control to normalize expression of the genes concerned.[10] For quantitative analysis, *GUS* was co-amplified as an internal control. All PCR reactions were performed for 45-55 cycles in 4 mM $MgCl_2$. Gene-specific primer pairs were designed with Probe Design software (Roche). The primer sequences are listed in Table 1. All values are presented as mean ± SD. Comparisons between PCB-exposed and control groups were made by one-way analysis of variance (ANOVA), followed by the Tukey-Kramer Honestly Significant Difference (HSD) test with the JMP statistical package (SAS Institute, Inc., Gary, NC, USA).

Results and Discussion

To determine whether apoptosis in rat testis is induced or inhibited by neonatal exposure to PCB 77 or PCB 132, apoptosis-related gene expression was examined in frozen testicular tissue. In rats received 20 mg/kg PCB 77, the gene expression of *Fas* and *Bax* was significantly higher than in the control groups (Table 2). There were no statistical differences in expression of *Fas*, *Bax*, *bcl-2*, and *p53* between control and 2 mg/kg PCB 77-treated groups (Table 2). Moreover, the gene expression *Fas*, *Bax*, *bcl-2*, and *p53* were also not significantly changed in 9.6 or 96 mg/kg of PCB132 neonatal exposure (Table 2). In addition, we found that *bcl-2* was slightly lower in the 20 mg/kg of PCB 77-treated groups. In the present investigation, the relationships of testicular gene expression with PCB 77 or PCB 132 treatment are summarized in Figure 1. In the high-dose of PCB 77-treated group, *Fas* and *Bax* was significantly induced (Fig. 1A). The balance between germ cell proliferation, differentiation, and apoptosis is critical to the maintenance of spermatogenesis. During spermatogenesis, *Fas* has been localized to germ cells, and *Fas-L* to Sertoli cells, within the rat testis.[11] The *Fas* and *Fas-L* genes and their protein products have been shown to be up-regulated in rats exposed to Sertoli cell toxicants that induce apoptosis of the germ cells.[11] There are some clues for understanding the underlying molecular mechanisms governing germ cell death in the testis. For example, *bcl-2* transgenic mice, in which a human *bcl-2* transgene, an antiapoptotic gene, is overexpressed in spermatogonia, have overpopulated spermatogonia and a decreased incidence of germ cell apoptosis.[12] Targeted gene disruption of *Bax*, a proapoptotic gene, in mice revealed hyperplasia of spermatogonia as well as massive death of early spermatocytes, suggesting *Bax*-dependent and -independent apoptosis pathways.[13] In addition to physiologic germ cell apoptosis, massive increases in germ cell apoptosis are observed after exposure of laboratory animals to various toxicants,[14] raised testicular temperature,[15] or radiation.[16] Apoptosis can be raised through different pathways, the main intracellular effectors being the caspases, a family of cysteine proteases. These enzymes exist in cells as inactive zymogens and become activated through proteolysis when cells receive apoptotic signals. In apoptosis, caspases function both in cell disassembly and in initiating this disassembly. The first or the intrinsic pathway for apoptosis involves the release of cytochrome c into the cytosol where it binds to apoptotic protease activating factor-1 (Apaf-1). Once activated by the cytochrome c, Apaf-1 then binds to procaspase-9 via the caspase recruitment domain (CARD) at the amino terminus in the presence of dATP, resulting in activation of the initiator caspase-9 and the subsequent proteolytic activation of the executioner caspase-3, -6, and -7. The active executioners are then involved in the cleavage of a set of proteins, including poly(ADP) ribose polymerase (PARP), lamin, actin, and gelsolin, and this causes morphological changes to the cell and nucleus typical of apoptosis. Members of the *bcl-2* family of proteins play a major role in governing this mitochondria-dependent apoptotic pathway, with proteins such as *Bax* functioning as inducers, and proteins such as *bcl-2* as suppressors, of cell death.[17] In conclusion, this study demonstrated the effects of neonatal exposure to higher dose of PCB 77 on alterations of apoptosis-related gene expression. The data may provide insights into the mechanisms by which dioxin-like PCB congeners affects reproduction in rats. Further efforts are required for a more complete understanding of the underlying molecular mechanisms governing the pathway of apoptosis in different cells of the testis, and the role of endocrine disruptors in male reproductive disorders.

Acknowledgements

This study was supported by the National Science Council of Taiwan under grant NSC 91-2320-B-327-001 and NSC 92-2320-B-327-001.

References

1. Giesy JP, Kannan K. *Crit Rev Toxicol* 1998; 28:511-569.
2. McKinney JD, Waller CL. *J Toxicol Environ Health B Crit Rev* 1998; 1:27-58.
3. DeRosa C, Richter P, Pohl H, Jones DE. *J Toxicol Environ Health B Crit Rev* 1998; 1:3-26.
4. Mably TA, Bjerke DL, Moore RW. *Toxicol Appl Pharmacol* 1992; 114:118-126.
5. Faqi AS, Dalsenter PR, Merker HJ, Chahoud I. *Human Exp Toxicol* 1998; 17:365-372.
6. Huang A, Powell D, Chou K. *Arch Environ Contam Toxicol* 1998; 34:204-208.
7. Hsu PC, Guo YL, Li MH. *Chemosphere* 2004; 54:611-618.
8. Weistrand C, Noren K. *Environ Health Perspect* 1997; 105:644-649.
9. Hsu PC, Li MH, Guo YL. *Toxicology* 2003; 187:117-126.
10. Aerts JL, Gonzales MI, Topalian SL. *Biotechniques* 2004; 36:84-91.
11. Lee J, Richburg JH, Younkin SC, Boekelheide K. 1997. *Endocrinology* 1997; 138:2081-2088.
12. Furuchi T, Masuko K, Nishimune Y, Obinata M, Matsui Y. *Development* 1996; 122:1703-1709.
13. Knudson CM, Tung KSK, Tourtellotte WG., Brown GAJ, Korsmeyer SJ. *Science* 1995; 270:96-99.
14. Uzumcu M, Suzuki H, Skinner MK. *Reprod Toxicol* 2004; 18:765-774.
15. Yin Y, DeWolf WC, Morgentaler A. *Biol Reprod* 1998; 58:492-496.
16. Lee K, Park JS, Kim YJ, Lee YS, Hwang TS, Kim DJ, Park EM, Park YM. *Biochem Biophy Res Commun* 2002; 296:337-342.
17. Hengartner MO. *Nature* 2000; 407:770-776.

Table 1. Primers for apoptosis and housekeeping genes in real-time RT-PCR

Gene	Forward primer	Reverse primer
Fas	5'TGTCAACCGTGTCAGC 3'	5'GGTCACAGAGAGAAGC 3'
Bax	5'ATGATTGCTGACGTGG 3'	5'CCACAAAGATGGTCACT 3'
bcl-2	5'TGGACAACATCGCTCT 3'	5'ACTGCTTTAGTGAACCT 3'
p53	5'CCGTATGCTGAGTATCT 3'	5'ACAAACACGAACCTCAA 3'
Gusb	5'CTAAAGCTACGACTACCT 3'	5'CCTTAGCCGGTAACCA 3'

Table 2. Apoptosis-related genes expression between rats neonatally exposed to PCB 77 (2 mg/kg or 20 mg/kg), PCB 132 (9.6 mg/kg or 96 mg/kg), and their unexposed controls, respectively.

Parameters	Control	PCB 77 (mg/kg)		ANOVA	PCB 132 (mg/kg)		ANOVA
		2	20	*P*-value	9.6	96	*P*-value
Fas/*GUS*	1.09±0.10	1.36±0.06	1.52±0.06*	0.0248	1.13±0.14	1.29±0.14	0.531
Bax/*GUS*	1.48±0.17	1.6±0.27	2.35±0.10*	0.0203	1.50±0.27	1.62±0.24	0.901
bcl-2/*Gusb*	1.02±0.14	1.28±0.24	0.81±0.08	0.1933	0.61±0.12	0.99±0.12	0.299
p53/Gusb	0.65±0.08	0.81±0.04	0.65±0.11	0.3527	0.56±0.09	0.73±0.002	0.477

* $P < 0.05$ compared with control

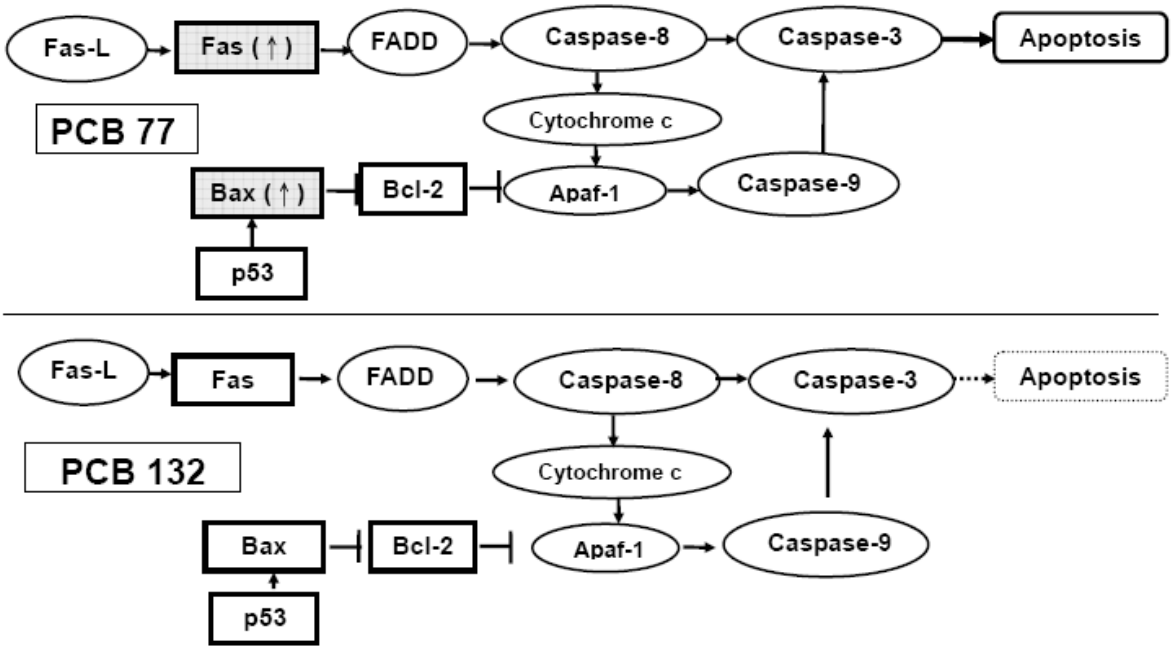

Figure 1. Key components of gene expression on pathway of apoptosis changed by dioxin-like congener, PCB 77 (A) or non-dioxin like congeners, PCB 132 (B). Comparison of rats neonatally exposed to PCB 77 or PCB 132 was conducted to determine whether altering expression of apoptosis-related genes including *Fas*, *Bax*, *bcl-2*, and *p53* in testis. Gene expression in square box was analyzed with real-time RT-PCR. Genes in grey square box were increased. In mammalian cells, the first pathway of apoptosis involves release of cytochrome c from mitochondria into the cytosol where it binds to Apaf-1, resulting in the activation of caspase-9 and the subsequent activation of the executioner caspase-3, -6, and -7. The second pathway involves ligation of *Fas* to *Fas-L*, resulting in the activation of a different set of initiator caspases, namely caspases-8 and -10, through interactions between death domains and death effector domains of an adopter molecule such as FADD and these caspases. The *bcl-2* family of proteins usually governs the first pathway for apoptosis. In addition, the ability of *p53* to serve as a transcriptional regulator may be associated with the expression of *Bax*. The third pathway involves the ER and caspase-12 (not shown in this figure). All these pathways eventually converge on caspase-3 and other executioner caspases that drive the terminal events of programmed cell death.