

DOCTORAL THESIS

The risk of exposure and the mechanistic actions of perfluorinated compounds on male infertility and metabolic disorders

Wan, Hin Ting

Date of Award:
2013

[Link to publication](#)

General rights

Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

- Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
- Users cannot further distribute the material or use it for any profit-making activity or commercial gain
- To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent URL assigned to the publication

**The Risk of Exposure and the Mechanistic Actions of Perfluorinated
Compounds on Male Infertility and Metabolic Disorders.**

WAN Hin Ting

**A thesis submitted in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy**

**Principal Supervisor: Prof. Chris K.C. Wong
Hong Kong Baptist University**

Aug 2013

Abstract

Endocrine disrupting chemicals (EDCs) are ubiquitous in our environment. The risk of the exposure and their effects on ecological and human health has raised public concerns recently. Over the past 20 years, significant levels of EDC contamination have been detected in both abiotic samples (i.e. air, soil and water) and biotic samples (i.e. wildlife and humans) at different geographical regions. Dietary consumption, inhalation dermal absorption are believed to be the major routes of the exposure to EDCs. Pathways of exposure to EDCs can be diverse; the identification of all possible exogenous exposure is not yet a feasible task. There is a need for a transformational change in the approach to contaminants in which more emphasis be placed on correlating population-based data to reveal human-environment interactions. In chapter 2, we analyzed a dataset of human blood samples in order to provide a framework of accumulated concentrations of EDCs. Evidence for the presence of PFCs, BPA and phthalates in the blood samples of most Hong Kong samples is provided. The observed characterizations of the contamination profile in human blood samples suggest a general exposure route to these contaminants. In the subsequent experimental chapters, one of the detected EDCs, PFOS which belongs to a family of synthetic fluorinated hydrocarbons (C_4-C_{14}) with the charged functional moiety of carboxylate, sulfonate or phosphonate will be studied. Because of its unique hydrophobic and oleophobic properties, they have been extensively used in various industrial and consumer products. The carbon-fluoride bonds render PFOS to be non-biodegradable, leading to their persistence in the environment and lengthy serum elimination half-life in animals. The manufacture of PFOS has been banned in most of the countries; however it is still produced

in many developing countries like China. Being geographically closed to China, we are at great risk of exposure to PFOS.

Previous studies demonstrated that PFOS is hepatotoxicity. However, the underlying mechanism and the clinical significance of PFOS-induced biochemical changes in livers are not known. Herein, in chapter 3 a murine model was used to study the mechanistic effects of PFOS-induced hepatotoxicity. A time- and dose-dependent effect of PFOS exposure on hepatic lipid accumulation, resulting from the inhibition of mitochondrial β -oxidation and the disturbance on hepatic lipid transport were demonstrated. The data reveal the similar hallmark features as compared with the development of NAFLD (non-alcoholic fatty liver disease). Of special interest is the fact that PFOS has been suggested to act on PPARs to modulate energy homeostasis and listed as one of the risk factor in the alternation of development programming for metabolic diseases in life. Maternal transfer of PFOS across the human placenta has been reported, however toxicological information regarding the perinatal PFOS exposure to susceptibility of metabolic disorders in adult offspring is not known. In chapter 4, we investigated the effects of perinatal exposure to PFOS on glucose metabolism in animal offspring and whether these effects would be exacerbated under different diets. The effects of the environmental equivalent dose of PFOS exposure on the disturbance of hepatic lipid metabolism and glucose metabolism in pups and adults were demonstrated in F₁ at PND 21 and 63. The phenotypes of insulin resistance and glucose tolerance were evident (i.e. HOMA-IR index and glucose AUC) in the F₁ adults. The metabolic disturbance effects were exacerbated under high-fat diet during postnatal growth, highlighting the synergistic action of dietary fat content and PFOS on the development of metabolic disorders.

In addition to hepatotoxicity, negative effects of PFOS exposure on male fertility have been reported in both *in vitro* and *in vivo* animal models. A considerable number studies demonstrated the inhibitory effects of PFOS on testosterone synthesis and spermatogenesis. Nevertheless, the underlying molecular mechanism has not been fully elucidated. Here in chapter 5, by using an *in vitro* primary Sertoli cell model that mimics BTB *in vivo*, PFOS disturbed the organization of F-actin in Sertoli cells was first demonstrated. The localization of actin regulatory and adhesion proteins at the cell-cell interface which are essential to maintain BTB integrity, were disrupted. In addition, PFOS was found to perturb Sertoli-Sertoli cell gap junction (GJ) communication, by down-regulating the expression of the major GJ integral membrane protein, connexin 43. Intriguingly an overexpression of phosphorylated FAK-Tyr⁴⁰⁷ was found to protect, at least in part, the PFOS-induced destruction in BTB integrity. Collectively the study highlighted the mechanistic actions of PFOS on steatosis, impairment of glucose metabolisms and reproductive system, particularly in male.

Table of Contents

Declaration	i
Abstract	ii
Acknowledgements	v
Table of Contents	vi
List of Tables	xiv
List of Figures	xvi
List of Abbreviations	xix
Chapter 1 Literature review	
1.1 Endocrine Disrupting Chemicals	1
1.1.1 Perfluorinated compounds (PFCs)	2
1.1.2 Perflurooctane sulfonate (PFOS)	2
1.2 Effects of PFOS in experimental studies	5
1.2.1 Effects of PFOS exposure to liver	5
1.2.1.1 PFOS act as weak PPARα agonist	6
1.2.1.2 Activation of lipid metabolic enzymes	6
1.2.1.3 PFOS lead to hepatic steatosis	7
1.2.1.3.1 Fatty acid metabolism	7
1.2.1.3.2 Glucose metabolism	8

1.2.2 Effects of EDCs on reproduction system	11
1.2.2.1 Impact of EDCs on hormone dialogue – from HPG axis to testosterone action in the testis	13
1.2.2.2 Multiple steroidogenesis regulatory pathways in Leydig cells are the target of EDCs	14
1.2.2.3 Impact of EDCs on sperm production in the testis via their effects of Sertoli and germ cells	17
1.2.2.4 EDC-induced disruption of the local hormone/autocrine/paracrine microenvironment in the testis	18
1.2.2.4.1 The somatotropic GH/IGF axis	18
1.2.2.4.2 Sertoli cell peptide hormones	18
1.2.2.4.3 Impact of EDCs on induction of reaction oxygen species	22
1.2.2.4.4 Impact of EDCs on the blood-testis-barrier	23
Working Hypothesis	44

Chapter 2 Blood plasma concentrations of endocrine disrupting chemicals in Hong Kong populations

2.1	Introduction	46
2.2	Materials and methods	47
2.2.1	Samples collections	47
2.2.2	Chemical materials for instrumental analysis	48
2.2.3	Determination of PFCs	48
2.2.4	Determination of BPA	50
2.2.5	Determination of phthalates	50
2.2.6	Statistical analysis	52
2.3	Results	53
2.3.1	Levels of PFCs were detected in blood samples	53
2.3.2	Contaminations of plasticizers were also found in blood samples	53
2.4	Discussion	55
2.4.1	The trend of PFCs exposure profile towards male is consistent with other studies	55
2.4.2	Contamination of plasticizers were ubiquitous	56

2.5	Conclusion	59
------------	-------------------	-----------

Chapter 3 PFOS-induced hepatic steatosis, the mechanistic actions on β -oxidation and lipid transport

3.1	Introduction	71
3.2	Materials and Methods	73
3.2.1	Experimental animals and chemicals	73
3.2.2	Histological examination of the mouse livers	74
3.2.3	Liver lipid content determination	74
3.2.4	RNA isolation and quantitative PCR	74
3.2.5	Western blot analysis	75
3.2.6	Serum HDL and LDL/VLDL Assay	75
3.2.7	β-oxidation Assay	76
3.2.8	Statistical analysis	77
3.3	Results	78
3.3.1	Effects of PFOS exposure on body and liver weights	78
3.3.2	Histological and TG analyses on liver	78
3.3.3	Effects of PFOS exposure on the expression levels of fatty acid translocase and lipoprotein lipase in liver and adipose tissues	79

3.3.4	Effects of PFOS exposure on hepatic lipid export	79
3.3.5	Effects of PFOS exposure on oxidation of fatty acids in liver	79
3.4	Discussion	81
3.4.1	PFOS induce lipid accumulations in hepatocytes	81
3.4.2	Fatty acid oxidation	82
3.4.3	Hepatic fatty acid uptake	83
3.4.4	Hepatic export of lipids	83
3.5	Conclusion	84

Chapter 4 Perinatal exposure to PFOS affect glucose metabolism in adult life on a high fat diet

4.1	Introduction	100
4.2	Materials and Methods	103
4.2.1	Experimental animals and chemicals	103
4.2.2	Serum PFOS analyses	104
4.2.3	Liver PFOS analyses	105
4.2.4	RNA isolation and real-time PCR	106
4.2.5	Serum fasting glucose and insulin	106
4.2.6	Oral Glucose Tolerance Test (OGTT)	107
4.2.7	Statistical analysis	107

4.3	Results	107
4.3.1	Oral gavage exposure to PFOS interferes with maternal glucose metabolism	108
4.3.2	Maternal-fetal transfer and lactational exposure to PFOS affects glucose metabolism and hepatic gene expression of the pups at postnatal day 21	108
4.3.3	The elimination of body loading and the effects of PFOS on glucose metabolism of STD- and HFD-fed F₁ adult at PND 63	109
4.4	Discussion	111
4.4.1	Effects of PFOS exposure on glucose metabolism of maternal mice	112
4.4.2	Effects of maternal transfer and lactational exposure to PFOS on F₁ pups at PND 21	113
4.4.3	Effects of post-perinatal PFOS exposure on glucose metabolism of F₁ adult offspring at PND 63	115
4.5	Conclusion	117

Chapter 5 PFOS perturbs blood-testis barrier function by affecting gap junction via p-FAK-Tyr⁴⁰⁷

5.1	Introduction	132
5.2	Materials and Methods	134
5.2.1	Animals and antibodies	134
5.2.2	Toxicants	134
5.2.3	Isolation of Sertoli cells and treatment of cells with toxicants	134
5.2.4	Preparation of FAK Y407E phosphomimetic mutant cDNA construct	136
5.2.5	Assessing Sertoli cell TJ-permeability barrier function after transfection of Sertoli cells either with FAK Y407E phosphomimetic mutant for its overexpression, or miR-135b for FAK silencing	137
5.2.6	Dual-labeled immunofluorescence analysis	138
5.2.7	Assessment of GJ communications by dye-transfer assay	139
5.2.8	Cytotoxicity assay	140
5.2.9	Actin polymerization assay	141
5.2.10	Electron microscopy	142
5.2.11	RT-PCR and immunoblot analysis	143
5.2.12	Statistical analysis	143
5.3	Results	144
5.3.1	PFOS induces dose-dependent and reversible disruption of the	

Sertoli cell BTB in vitro mediated by down-regulating the expression of BTB-associated and regulatory proteins	144
5.3.2 PFOS perturbs F-actin organization at the BTB, impeding the localization and/or distribution of BTB-associated proteins at the Sertoli cell-cell interface	145
5.3.3 PFOS perturbs intercellular gap junction (GJ) communication between Sertoli cells at the BTB	146
5.3.4 Overexpression of p-FAK-Tyr⁴⁰⁷ via the use of a phosphomimetic mutant in Sertoli cells alleviates the disruptive effects of PFOS on Sertoli cell TJ barrier function	147
5.3.5 Silencing of FAK by FAK-specific miR-135b further sensitizes and worsens Sertoli cell BTB function to the disruptive effects of PFOS	148
5.4 Discussion	150
5.5 Conclusion	153
Chapter 6 Conclusions	168
References	171
Curriculum Vitae	196