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Title: The effect of prenatal perfluorinated chemicals exposures on pediatric atopy

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Abstract: Background: The role of perfluorinated compounds (PFCs) in the immune system and allergic diseases is not well-known. This study examined the effects of pre-natal exposure to PFCs on immunoglobulin E (IgE) levels and atopic dermatitis (AD).
Methods: In Taiwan Birth Panel cohort study, newborns with cord blood and peri-natal factors (i.e. birth body weight, weeks of gestation, and type of delivery) gathered at birth were evaluated. At age 2 years, information on the development of AD, environmental exposures, and serum total IgE were collected. The AD and non-AD children were compared for the concentration of cord blood serum PFCs measured by Ultra-performance liquid chromatography/triple-quadrupole mass (UPLC-MS/MS). Correlations among cord blood IgE, serum total IgE at 2 years of age, and cord blood PFC levels were made.
Results: Of 244 children who completed the follow-up and specimen collections, 43 (17.6%) developed AD. Concentrations of cord blood serum perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS) were median (range) 1.71 (0.75-17.40), 5.50 (0.11-48.36), 2.30 (0.38-63.87), and 0.035 (0.035-0.420) ng/mL, respectively. PFOA and PFOS levels positively correlated with cord blood IgE levels (per ln-unit: β=0.134 KU/l, p=0.047 for PFOA; β=0.161 KU/l, p=0.017 for PFOS). Analyses stratified by gender revealed that PFOA and PFOS levels positively correlated with cord blood IgE levels only in boys (per ln-unit: β=0.206 KU/l, p=0.025 for PFOA; β=0.175 KU/l, p=0.053 for PFOS). When dividing cord blood serum PFCs into quartiles in the fully adjusted models, AD had no significant association with PFOS.
Conclusions: Pre-natal PFOA and PFOS exposures positively correlated with cord blood IgE levels.

Dear Editor-in-Chief, February 7, 2011

Thank you for your precious commentary. We have made several changes in this manuscript as demanded by the reviewers. A revised version of our manuscript has been taken into account the comments of the referees with great efforts and please consider for eventual publication of this manuscript. Please do let us know if any further amendment should be made to fulfill the requirement for publication. Thank you for your kind consideration and critical review. All of us look forward to hearing from you.

With best wishes,

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Title: The effect of prenatal perfluorinated chemicals exposures on pediatric atopy Ms. No.: ER-10-580

Dear Reviewers,

Thank you for your precious commentary. We have made several changes in this manuscript as requested carefully and hope it will fulfill your requirement. Detailed corrections are listed below point by point and manuscript revisions are clearly underlined. A revised version of our manuscript has been taken into account the comments with great efforts and please consider for eventual publication of this manuscript.

Reply to Reviewer 1

General comments:

Question

1)The methods and language are confusing in several respects, including:

a)The methods state that total serum IgE concentrations were determined. Were these IgE serum concentrations determined from serum separated from cord blood or from blood collected from two year olds?

b)The results, including Tables 2 and 3, mention both serum IgE and cord blood IgE. What is the difference between "serum IgE" and "cord blood IgE?" Is it a methodological difference as far as analysis or is it a time-point difference (newborns versus two year olds)? The methods, results, and discussion should clearly indicate the type and source of the sample being discussed.

c)Similarly, the language associated with PFC concentration is confusing. The methods indicate that plasma samples (Section 2.3.2. line 1) of cord blood were stored for later analysis but when analyzed, over 90% of the PFCs were below the LOQ (Section 2.3.2. lines 2-3). The methods then go on to state that "Therefore

plasma samples of PFOA, PFOS, PFNA, and PFHxS were used for analysis" (Section 2.3.2 lines 3-4). These statements directly contradict each other. Further, PFCs generally partition to serum rather than plasma. In addition, the results and discussion use the language "cord blood." They should clearly indicate "plasma from cord blood" or "serum from cord blood."

Answer

Thank you for your suggestion. We have re-written the methods and language.

(a) The IgE concentrations were determined from cord blood and from serum collected at two year olds. To avoid misunderstanding, we have added “Cord blood serum IgE” to the sentence. See (Section 2.3.1 lines 1).

(b) The difference between "cord blood IgE” and "serum total IgE" and is a time-point difference (newborns versus two year olds). We have clearly indicated the type and source of the sample as “cord blood serum IgE levels” and “serum total IgE concentrations at 2 years of age “ in the methods, results, and discussion. (See underlined area in the methods, results, and discussion).

(c)We apologize for the confusion with the statement. We try to re-written the sentence as follows.

1.Serum samples of cord blood were stored at -80°C before analysis. Twelve PFCs were analyzed. However, for most PFCs, over 90% were below the limits of quantitation. Therefore, serum samples of PFOA, PFOS, PFNA, and PFHxS were used for analysis. (See section 2.3.2. lines 1-4).

2.The language "Serum from cord blood" has been clearly indicated in the results and discussion.

Question

2) The value of cord blood IgE as a predictor of atopic disease in offspring is somewhat questionable, especially given atopy in mothers. It does not appear that the health questionnaire provided to the study participants asked about atopic dermatitis in the parents. Could these data be obtained and included in the analysis?

Answer

Thank you for your suggestion. These data have been obtained in the questionnaire survey and have been included in the analysis.

1.See section 2.2. Questionnaire survey.

The parents were asked by home interview questionnaires for parental history of atopic diseases (i.e. atopic dermatitis, allergic rhinitis, or asthma).

2.Also see Table 4 Association of atopic dermatitis across quartiles of cord blood PFCs by univariate and multivariate logistic regression.

There was no significant relationship between PFCs exposure during pregnancy and atopic dermatitis after adjusting for gender, gestational age, maternal age, "maternal history of atopy", duration of breast feeding, and pre-natal ETS exposure.

Question

3) Throughout the manuscript, the authors refer to a review paper by Lau et al. (2007). This is undoubtedly one of the most comprehensive reviews on PFC toxicity to date. However, relying on a review to support speculations about data is inappropriate. This reviewer strongly suggests that the authors rely on the primary studies rather than on a review to support their findings.

Answer

Thank you for your suggestion. We have cited the references from the primary studies instead of the review paper by Lau et al. (2007).

See reference section.

Kemper, R. A.,Nabb, D. L, 2005. In vitro studies in microsomes from rat and human liver, kidney, and intestine suggest that perfluorooctanoic acid is not a substrate for microsomal UDP-glucuronosyltransferases. Drug Chem. Toxicol. 28, 281–287.

Kemper, R. A., and Jepson, G. W., 2003. Pharmacokinetics of perfluorooctanoic acid in male and female rats. Toxicologist 72(1-S), 148.

Emmett, E. A., Shofer, F. S., Zhang, H., Freeman, D., Desai, C., and Shaw, L. M., 2006. Community exposure to perfluorooctanoate: Relationships between serum concentrations and exposure sources. J. Occup. Environ. Med. 48, 759–770.

Fairley, K.J., Purdy, R., Kearns, S., Anderson, S.E., 2007. Meade BJ. Exposure to the immunosuppressant, perfluorooctanoic acid, enhances the murine IgE and airway hyperreactivity response to ovalbumin. Toxicol Sci 97, 375-383.

Houde, M., Wells, R. S., Fair, P. A., Bossart, G. D., Hohn, A. A.,Rowles, T. K., Sweeney, J. C., Solomon, K. R., and Muir, D. C. G.**,** 2005. Polyfluoroalkyl compounds in free-ranging bottlenose dolphins (Tursiops truncatus) from the Gulf of Mexico and the Atlantic Ocean. Environ. Sci. Technol. 39, 6591–6598.

Environ. Sci. Technol. 39, 6591–6598Vanden Huevel, J. P., Thompson, J. T., Frame, S. R., Gillies, P. J., 2006. Differential activation of nuclear receptors by perfluorinated fatty acid analogs and natural fatty acid: A comparison of human, mouse, and rat peroxisome proliferator-activated receptor-a, -b, and -c, liver X receptor-b, and retinoid X receptor-a. Toxicol. Sci. 92, 476–489.

Lovett-Racke, A.E., Hussain, R.Z., Northrop, S., Choy, J., Rocchini, A., Matthes, L., Chavis, J.A., Diab, A., Drew, P.D., Racke, M.K., 2004. Peroxisome proliferator-activated receptor alpha agonists as therapy for autoimmune disease. J Immunol 172:5790-5798.

Yang, Q., Abedi-Valugerdi, M., Xie, Y., Zhao, X., Moller, G., Nelson, B. D., DePierre, J. W., 2002. Potent suppression of the adaptive immune response in mice upon dietary exposure to the potent peroxisome proliferator, perfluorooctanoic acid. Int. Immunopharmacol. 2, 289–397.

Question

4)This reviewer suggests that a rewrite of the entire discussion is necessary.

a)Page 12, paragraph 2. The authors discuss the signaling pathway of the peroxisome proliferator activated receptor alpha (PPARa) as a potential mechanism for explaining the correlation of atopic disorders with PFCs. However, the data do not support PPARa signaling as a primary mechanism for several types of immunotoxicity; the study cited by the Lau et al. (2007) review paper, (Yang et al., 2002) has several methodological problems that calls their conclusions into question. First, the Yang et al. (2002) paper compared a C57BL/6 wild type to a PPARa null mouse on an Sv129 background; Sv129 mice appear to be less sensitive to PFCs than C57BL/6 mice (DeWitt et al., 2007). Second, the Yang et al.

(2002) paper did not report a LACK of effects of PFOA on lymphocytes, just a reduced effect. It is recommended that the authors evaluate the Yang et al. (2002) paper rather than relying on the Lau et al. (2002) review article.

b) Page 14, paragraph 1. The authors assert that differences observed between males and females in the current study can be explained with sex differences observed in elimination rates of PFCs reported for rats. Female rats appear to possess transporters that rapidly eliminate PFCs via urinary excretion. The applicability of the rat data to other species is highly debatable, especially given some of the earlier studies with primates (Butenhoff et al., 2004).

c)Additional citations are necessary. Page 12, paragraph 2, line 11, "Other PPAR-a agonists, including gemfibrozil,…" (no citation). Page 15, paragraph 1, lines 2-3, values for U.S. and Germany need citations.

d)The material from page 15, paragraph 3 through page 16, paragraph 1, can probably be reduced or included into the methods section.

Answer

Thank you for your suggestion. We have rewritten of the entire discussion.

(a)We have evaluated the Yang et al. (2002) and DeWitt et al (2007) papers rather than relying on the Lau et al. (2002) review article. We have rewritten of the discussion as follows:

In this study, there was a positive correlation between pre-natal PFOA and PFOS exposure and cord blood IgE levels. Until now, there has been no definite mechanism of altered immune responses and development of atopy for PFC exposure in humans. Peroxisome proliferator-activated receptor-alpha (PPAR-α) signaling pathway has been suggested to be related with this phenomenon. As peroxisome proliferators, PFOA and PFOS are able to induce PPAR-α (Vanden Huevel et al., 2006). PPAR-α agonists have been shown to promote IL-4 levels and inhibit interferon-γ in human T-cell lines (Lovett-Racke et al., 2004). Further, Fairley et al. (2007) examined the effects of PFOA dermal exposure on hypersensitivity response to ovalbumin (OVA) in mice and demonstrated increased IgE in co-administered PFOA and OVA. OVA-specific airway hyper-reactivity was increased significantly in these animals with enhanced pleiotropic cell response characterized by eosinophilia and mucin production. Grasty et al. (2005) examined critical periods of exposure by treating rats

with high dose PFOS during pregnancy. There was significant histologic and morphometric differences between control and PFOS-treated lungs in newborns, suggesting that PFOS may inhibit or delay peri-natal lung development. These results indicated that PFC exposure can potentially augment Th2 response followed by subsequent airway hyper-reactivity to OVA and environmental allergens through the PPAR mechanism (Fairley et al., 2007). However, Yang et al. (2002) demonstrated that reductions in the number of thymocytes and splenocytes caused by PFOA in the wild type mice was not observed in PPAR-α knock out mice. This suggested PPAR-α independence of some immune effects. Since human PPAR-α expression is less than that of rodents, potential PPAR-α independence implies that further studies to explore PFC action mechanisms, including PPAR-α-dependent and -independent pathways, is necessary (DeWitt et al., 2007).

(b) Thank you for suggestion. We have added the reference from (Butenhoff et al., 2004) and have rewritten this paragraph.

1.Butenhoff JL, Kennedy GL Jr, Hinderliter PM, Lieder PH, Jung R, Hansen KJ, Gorman GS, Noker PE, Thomford PJ. 2004. Pharmacokinetics of perfluorooctanoate in cynomolgus monkeys. Toxicol Sci 82:394-406.

2. The two possible explanations can be that in males, PFOA has longer half lives and lower renal clearance. Similar differences were reported in rats exposed to PFOA. Estimated half lives were longer in males than females in a variety of rat strains (Kudo and Kawashima, 2003). Differences in blood PFOS level between genders have been observed with higher levels in male donors (Fromme et al., 2007; Calafat et al., 2007; Holzer et al., 2008). However, Butenhoff et al.(2004) found that female monkeys had longer terminal half-life of PFOA in serum. Because of the inconsistent results, further studies are needed to clarify any gender-related influence on *in utero* PFC exposure and atopy.

(c) Additional citations have been added.

Page 12, paragraph 2. " PPAR-α agonists … ” Reference: (Lovett-Racke et al., 2004)

Page 15, paragraph 1. The values for U.S. and Germany” Reference: (Apelberg et al., 2007b; Midasch et al.,2007).

(d) We have reduced the length of the material from 335 to 250 words. See page 15, paragraph 3 through page 16, paragraph 1.

Specific comments

Question

Abstract

1)Methods. Please clarify "peri-natal factors."

Answer

Thank you for your suggestion. We have clarify "peri-natal factors."(See abstract method).

Peri-natal factors (i.e. birth body weight, weeks of gestation, and type of delivery) gathered at birth were evaluated.

Question

Introduction

1)Page 3, paragraph 1, line 1. Recommend changing "a kind of endocrine disruptor" to "multisystem toxicants."

2)Page 3, paragraph 1, line 5. Recommend putting a period at the end of the sentence.

3)Page 4, paragraph 3, line 1. Recommend inserting "a" before "paucity."

Answer

Thank you for your suggestion.

1)We have changed "a kind of endocrine disruptor" to "multisystem toxicants." See paragraph 1, line 1.

2)We have put a period at the end of the sentence. See paragraph 1, line 5.

3)We have inserted "a" before "paucity." See paragraph 3, line 1.

Question

Methods

1)Page 7, paragraph 1, line 4. Recommend changing "like" to "including."

2)Page 7, paragraph 3, lines 1 and 2. Recommend changing "Twelve kinds of PFCs were analyzed." To "Twelve PFCs were analyzed."

3)Page 7, paragraph 3, line 2. Recommend changing "However, in most kinds,…" to "However, for most PFCs, over 90% were below…"

4)Page 7, paragraph 3, line 6. Recommend changing "Plasma sample 100 uL…" to "A 100 uL plasma sample contained in a polypropylene centrifuge tube…"

Answer

Thank you for your suggestion.

1) We have changed "like" to "including." See paragraph 1, line 4.

2) We have changed "Twelve kinds of PFCs were analyzed." To "Twelve PFCs were analyzed." See paragraph 3, lines 1 and 2.

3) We have changed "However, in most kinds,…" to "However, for most PFCs, over 90% were below…" See paragraph 3, line 2.

4) We have changed "Plasma sample 100 uL…" to "A 100 uL plasma sample contained in a polypropylene centrifuge tube…" See paragraph 3, line 6.

Question

Results

1)Throughout the manuscript, "PFCs" is used consistently. Please use "PFC" when using as a modifier. For example, it is appropriate to say "PFCs were associated…" or "PFC concentrations were associated," but not "PFCs concentrations." This is especially prevalent in this section.

Answer

Thank you for your suggestion. We have replaced "PFCs" with "PFC" when using as a modifier in the manuscript,.

Reply to Reviewer 2

Question

1. The author did not mention the level of PFC at 2 years of age and also the immune status.

Answer

Thank you for your suggestion.

a. We did not mention the level of PFCs at 2 years of age because the objective of this study is to examine the effects of "pre-natal exposure to PFCs" on IgE levels and atopic dermatitis.

b. Since the source of exposure in pre- and post-natal period within the same family was be relatively constant and the half-lives of PFCs were long, post-natal PFC exposure was not likely to significantly confound the results.

c. We have mentioned immune status (total serum IgE level at 2 years of age) as our outcome and calculated its relationship with pre-natal PFC exposure (See Table 2 and Table 3).

Question

2. In the figure legend, a brief description about the experiment is necessary.

Answer

Thank you for your suggestion. We have added a brief description about the experiment in the figure legend.

Fig. 1. Box and whisker plots of cord blood serum PFC concentrations measured by

Ultra-performance liquid chromatography/triple-quadrupole mass. Horizontal lines inside boxes: median; boxes: inter-quartile range; whiskers: the most extreme data points <1.5 times the interquartile range from the ends of the box; circles: outliers.

Research highlights

1. Prenatal exposures to PFOA and PFOS positively correlated with cord blood IgE levels.
2. Analyses stratified by gender revealed that only boys had this positive correlation.
3. Further large-scale follow-up studies are required to determine whether the findings are replicable.

The effect of prenatal perfluorinated chemicals exposures on pediatric atopy

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Background: The role of perfluorinated compounds (PFCs) in the immune system and allergic diseases is not well-known. This study examined the effects of pre-natal exposure to PFCs on immunoglobulin E (IgE) levels and atopic dermatitis (AD).

***Methods*:** In Taiwan Birth Panel cohort study, newborns with cord blood and peri-natal factors (i.e. birth body weight, weeks of gestation, and type of delivery) gathered at birth were evaluated. At age 2 years, information on the development of AD, environmental exposures, and serum total IgE were collected. The AD and non-AD children were compared for the concentration of cord blood serum PFCs measured by Ultra-performance liquid chromatography/triple-quadrupole mass (UPLC-MS/MS). Correlations among cord blood IgE, serum total IgE at 2 years of age, ~~cord blood IgE,~~ and cord blood PFC levels were made.

***Results*:** Of 244 children who completed the follow-up and specimen collections, 43 (17.6%) developed AD. Concentrations of cord blood serum perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS) were median (range) 1.71 (0.75-17.40), 5.50 (0.11-48.36), 2.30 (0.38-63.87), and 0.035 (0.035-0.420) ng/mL, respectively. PFOA and PFOS levels positively correlated with cord blood IgE levels (per ln-unit: β=0.134 KU/l, p=0.047 for PFOA; β=0.161 KU/l, p=0.017 for PFOS). Analyses stratified by gender revealed that PFOA and PFOS levels positively correlated with cord blood IgE levels only in boys (per ln-unit: β=0.206 KU/l, p=0.025 for PFOA; β=0.175 KU/l, p=0.053 for PFOS). When dividing cord blood serum PFCs into quartiles in the fully adjusted models, AD had no significant association with PFOS.

***Conclusions*:** Pre-natal PFOA and PFOS exposures positively correlated with cord blood IgE levels.

Key words: *cord blood, perfluorinated compounds, IgE, atopic dermatitis*

1. Introduction

Perfluorinated chemicals (PFCs), a kind of multisystem toxicants~~endocrine disruptor~~, are of toxicologic concern because of their persistence in the environmental and their potential to accumulate in organisms and biomagnificate in the food chain (Fromme et al., 2008). Previous epidemiologic studies find no consistent association between serum fluorochemical levels and adverse health effects (Lau et al., 2007)~~.~~, In general, PFCs are known to be well absorbed orally but poorly eliminated and undergo extensive uptake from the entero-hepatic circulation (Kemper et al.,2005~~Lau et al., 2007~~). Therefore, the European Union, Canada, and USA have all adopted new regulations and restrictions on the use and release of all PFOS and related chemicals (Fromme et al., 2008). However, despite potential toxicity and negative health effects, the semiconductor and electronics industries continue to use PFOS (Lin et al., 2009). Taiwan, with its rapid industrialization and as the home to a world-class semiconductor industry, is most likely to have PFC bioaccumulation in its downstream rivers (Lin et al., 2009). Health effects of concern include hepatotoxicity, developmental toxicity, hormonal effects, immuno-toxicity, and carcinogenic potency (Fromme et al., 2008).

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The two most widely known and commonly studied PFCs – perfluorooctanoic acid (PFOA) and perfluorooctane sulfate (PFOS) – are especially stable, can resist direct and indirect photolysis, and have hydrolytic half-lives greater than 41 years (Lin et al., 2009). Both are used in a wide range of applications like oil and water repellant coatings for carpets, textiles, leather, paper, cardboard, and food packing materials; electronic and photographic devices; and surfactants in diverse cleaning agents, cosmetics, and fire-fighting foams (Fromme et al., 2008). Contaminated drinking water, dust, food, food packaging, and non-stick cookware are some of the potential routes of human exposure (Lin et al., 2010). Children may be exposed to PFCs either through dermal or

hand-to-mouth contact (Fairley et al., 2007).

Children's internal exposures to PFCs have not been fully evaluated to date. Previous findings from studies of pesticides and other chemicals reveal that toxicity is both age- and compound-dependent. The younger and the more immature the subject, the more different its response is from that of an adult. In addition, substantial anatomical, biochemical, and physiologic changes that occur throughout infancy, childhood, and adolescence can affect the absorption, distribution, metabolism, and elimination of chemicals (Bruckner, 2000). Pregnancy is arguably the most critical period of developmental programming (Prescott and Clifton, 2009). Compared to adults, fetuses are much more likely to be vulnerable to potential harmful substances of chemicals. Epidemiologic studies on children's pre-natal exposure to PFCs are urgently needed. A hospital-based study of 293 subjects in the United States reveal a small negative association between cord blood PFOS and PFOA concentrations and birth weight and size (Apelberg et al., 2007a). A Danish national birth cohort study suggests that fetal exposure to PFOA during the period of organ development may affect the growth of organs and skeleton (Fei et al., 2008). Moreover, a prospective cohort study in Japan indicates that *in utero* PFOS exposure is negatively correlated with birth weight (Washino et al., 2009).

However, there is still a paucity of data on the potential impacts of PFC exposure on the immune system and allergic diseases in children. Although the pharmacokinetic properties of PFOS and PFOA have been studied in some detail, many toxicology studies are done only on animals ~~(Lau et al., 2007)~~ (Kemper et al.,2003). It is difficult to extrapolate health risks from rat models due to dramatic variabilities in PFC toxic effects. The prevalence of allergic diseases appears to have increased dramatically over the past decade and *in utero* exposure to PFCs is ubiquitous in babies (Apelberg et al.,

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2007a). Therefore, this study aimed to examine the effects of pre-natal PFC exposures on the immune function and development of atopy in children.

2. Materials and Methods

2.1. Study population

Considering potential environmental exposures and nationwide representation, subjects were recruited from medical centers, regional hospitals, local hospitals, and clinics in Taiwan in 2004. Pregnant women in their 3rd trimester of pregnancy who had pre-natal examination in selected hospitals were invited to join. Cord blood was collected during delivery. The study protocol was as described previously in the Taiwan Birth Panel cohort study (Wang et al., 2008) and was approved by the Joint Institution Review Board in Taiwan. All study subjects provided informed consents.

Cases of AD were defined by the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire through three questions: “Has your child ever had an itchy rash which was coming and going for at least six months at any time?”, “Has the itchy rash been coming and going over elbows, knees, face, wrists, or generalized (4 or more localizations)?”, and “Has your child ever had atopic dermatitis diagnosed by a doctor?”. A dermatologist examined a sub-group of the participating young children and the combination of answers that resulted in the highest sum of sensitivity and specificity was determined (Chan et al., 2001; Benn et al., 2003). Exclusion criteria included multiple gestation (e.g. twins, triplets), inability to answer questions in Chinese, and plan to move out of the area before delivery. At the age of 2 years, those who developed AD were gathered. The concentrations of cord blood serum PFCs in AD cases were compared to those of non-AD cases.

2.2. Questionnaire survey

The parents were asked by home interview questionnaires for birth year, parental education levels and occupation, family income, parental history of atopic diseases (i.e.

AD, allergic rhinitis, or asthma), alcohol and drug use, diets and supplements during pregnancy, history of smoke exposure, including ETS exposure, and maternal active smoking during pregnancy. From records of the cooperating hospitals, the neonate's health data at birth, including ~~like~~ head circumference, birth body weight, height, weeks of gestation, and type of delivery, were obtained. At 2 years of age, the ISAAC questionnaire was performed and some post-natal exposures like duration of breast feeding, early consumption of egg, wheat, soy bean, or shrimp before 1 year of age, number of older siblings, furry pets or carpets at home, fungi at house walls, incensing at home, and post-natal ETS exposure were evaluated.

2.3. Laboratory method

2.3.1 IgE antibody analysis

Cord blood serum IgE levels and serum total IgE concentrations at 2 years of age ~~Total serum IgE concentrations~~ were determined using Pharmacia UniCap IgE assay test system (Pharmacia Diagnostics, Uppsala, Sweden). IgE levels were considered increased at values >100 kU/L. Concentrations <0.35 kU/L were defined as absent or undetectable IgE.

2.3.2 Measurement of PFC~~s~~ concentration

~~Plasma~~ Serum samples of cord blood were stored at -80°C before analysis. ~~Twelve kinds of PFCs were analyzed~~ Twelve PFCs were analyzed. ~~However, in most kinds,~~However, for most PFCs, over 90% were below the limits of quantitation. Therefore, serum ~~plasma~~ samples of PFOA, PFOS, PFNA, and PFHxS were used for analysis. A brief summary of the PFC~~s~~ analytical method was as follows: First, the frozen samples were thawed at room temperature and then vortex mixed for 30 sec to ensure homogeneity. A 100 uL serum sample contained in a polypropylene centrifuge tube ~~Plasma Serum sample 100 µL in polypropylene centrifuge tube~~ was vortexed with

100 μL of 1% formic acid (pH 2.8) for 30 sec. Then 80 μL of methanol and 20 μL of 0.375 ng/mL internal standard solution ($^{13}C_8$-PFOA) were added to each sample before the second vortex. The mixture was sonicated for 20 min and then centrifuged at 14,000 rpm for 20 min. The supernatant was collected (~150 μL) and then filtered through 0.22-μm PVDF syringe filter into a 2.0 mL auto-sampler vial.

The calibration standard solution was prepared in 100 μL of bovine serum and went through sample preparations under the same procedures. The final concentrations for all analytes were 0.05 to 100 ng/mL containing a fixed amount of internal standard (25 ng/mL) in bovine serum samples. The separation and detection were performed on Waters Acquity UHPLC system coupled with a Waters Quattro Premier XE triple-quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA). The instrument was operated in selected reaction monitoring (SRM) with an electro-spray negative ionization mode. An Acquity UHPLC BEH C_{18} column (2.1 × 50 mm, 1.7 μm) was used at flow rate of 0.5 mL/min. The limits of quantitation for PFOA, PFOS, PFNA, and PFHxS were 1.5, 0.22, 0.75, and 0.24 ng/ml, respectively. For concentrations below the detection limits, a value of half the lower limit of quantitation was assigned. All results were duplicate analysis.

2.4. Statistical analysis

Socio-demographic data of children were compared in terms of cord blood serum PFC levels using the geometric mean with standard error. Because of the skewed distributions, a log transformation was performed for cord blood IgE, serum total IgE, and cord blood serum PFC~~s~~ levels before further analyses. All log-transformed data in the study had a normal distribution and no significant outliers are found. Linear regression was performed to estimate the relationship between log-cord blood IgE and log-serum total IgE at 2 years of age, and a unit increase in log-PFC~~s~~ concentrations.

Cord blood serum PFCs concentrations were further analyzed as categorical variables after division into quartiles, with the lowest quartile used as the reference category.

The association of cord blood serum PFCs levels and AD were analyzed by univariate and multivariate logistic regression. Potential confounders from literature review, including infant gender, gestational age, parity, mode of delivery, maternal age, maternal education and occupation, alcohol consumption or smoking during pregnancy, diets and supplements during pregnancy, family income, parental history of atopy, duration of breast feeding, postnatal tobacco smoke exposure, incensing and carpets at home, and fungi on house walls were all taken into consideration. Only those with 10% change in point estimate were included in the final model. All hypothesis testing was two-sided at the significance level of 0.05 and performed with the SAS software version 9.1 (SAS Institute, Inc., Cary, NC).

3. Results

Cord blood specimens were obtained from 483 children, of which 155 had insufficient volume for laboratory analyses and were excluded from this study. In addition, 84 were excluded due to missing data and loss to follow-up.

There was no significant difference between those who lost to follow-up and those who completed the follow-up. Of the 244 study participants, 43 (17.6%) developed AD. The median (range) concentrations of PFOA, PFOS, PFNA, and PFHxS were 1.71 (0.75-17.40), 5.50 (0.11-48.36), 2.30 (0.38-63.87), and 0.035 (0.035-0.420) ng/mL, respectively. Figure 1 showed the box and whisker plots of PFC~~s~~ concentrations in serum from cord blood. PFOA, PFOS, and PFNA were detected in most cord blood samples (66.0%, 99.6%, and 59.4%, respectively). However, PFHxS levels were below the detection limit in 89.3% of samples.

A summary of geometric means of cord blood serum PFOA, PFOS, PFNA, and PFHxS concentrations in relation to the characteristics of mothers and infants was listed in Table 1. There were 133 male infants (54.5%) and 111 female infants (45.5%), with boys having a higher average concentration of PFCs than girls. Low birth weight and decreased gestational age was also associated with high PFC~~s~~ levels. However, the aforementioned factors failed to reach statistical significance. Other demographic characteristics, including maternal age, education, occupation, parity, and family income were not significantly associated with cord blood serum PFC concentrations.

The regression coefficients β (s.e.) for log-IgE levels (serum total IgE levels at 2 years of age and cord blood IgE levels) according to log-cord blood serum PFC~~s~~ concentrations were shown in Table 2. There was no significant correlation between cord blood PFCs and serum total IgE levels at 2 years of age. However, PFOA and PFOS levels positively correlated with cord blood IgE levels (per ln-unit: β=0.134 KU/l,

95% CI=0.003–0.458, *p*=0.047 for PFOA; β=0.161 KU/l, 95% CI=0.064–0.642, *p*=0.017 for PFOS) even after adjusting for potential confounders.

Analyses stratified by gender was further performed (Table 3). There was still no significant correlation between PFCs and serum total IgE levels at 2 years of age. However, there was a significantly positive correlation between PFOA and PFOS levels and cord blood IgE levels in boys. After adjusting for gestational age, parity, maternal age, and prenatal ETS exposure, PFOA and PFOS levels remained significantly correlated with cord blood IgE levels in boys (per ln-unit: β=0.206 KU/l, 95% CI=0.047–0.702, *p*=0.025 for PFOA; β=0.175 KU/l, 95% CI=0.004–0.704, *p*=0.053 for PFOS). There was no association between levels of PFCs and cord blood IgE in girls.

Table 4 presented the association of AD across quartiles of cord blood serum PFCs by univariate and multivariate logistic regression. When dividing serum PFCs into quartiles in the crude model, AD was significantly associated with PFOS at a concentration >2.775 ng/mL. However, in the fully adjusted models, AD failed to reach statistically significant association with PFOS. There was no significant relationship between PFOA and PFNA exposure during pregnancy and AD.

4. Discussion

This study contributes to the literature on the potential association between prenatal PFCs exposures and pediatric atopy. By current knowledge, the effect of pre-natal PFC exposure on the development of atopy in the offspring has not been previously studied. We found that pre-natal exposures to PFOA and PFOS positively correlated with cord blood IgE levels, particularly in boys. However, there was no statistically significant association between AD and PFCs.

In this study, there was a positive correlation between pre-natal PFOA and PFOS exposure and cord blood IgE levels. Although Until now, there has been was no definite mechanism evidence of altered immune responses and development of atopy for PFC exposure in humans., there were theoretical r Peroxisome easons to suspect a correlation of atopic disorders with PFCs. proliferator-activated receptor-alpha (PPAR-α) signaling pathway has been suggested to be related with this phenomenon. As peroxisome proliferators, PFOA and PFOS are able to induce PPAR-α (Vanden Huevel et al., 2006)Lau et al., 2007). PFOA was known to have immunomodulating induce immuno toxic effects in mice and the effect was most likely to be mediated by the PPAR-α signaling pathway.PPAR-α agonists, including gemfibrozil, ciprofibrate, and fenofibrate, have been shown to promote IL-4 levels and inhibit interferon-γindirectly in human T-cell lines (Lovett-Racke et al., 2004). Further, Fairley et al. (2007) examined the effects of PFOA dermal exposure on hypersensitivity response to ovalbumin (OVA) in mice and demonstrated increased IgE in co-administered PFOA and OVA. OVA-specific airway hyper-reactivity was increased significantly in these animals with enhanced pleiotropic cell response characterized by eosinophilia and mucin production. Grasty et al. (2005) examined critical periods of exposure by treating rats with high dose PFOS during pregnancy. There was significant histologic and

morphometric differences between control and PFOS-treated lungs in newborns, suggesting that PFOS may inhibit or delay peri-natal lung development. These results indicated that PFC exposure can potentially augment Th2 response followed by subsequent airway hyper-reactivity to OVA and environmental allergens through the PPAR mechanism (Fairley et al., 2007). However, Yang et al. (2002) demonstrated that reductions in the number of thymocytes and splenocytes caused by PFOA in the wild type mice was not observed in PPAR-α knock out mice. This suggested PPAR-α independence of some immune effects. Since human PPAR-α expression is less than that of rodents, potential PPAR-α independence implies that further studies to explore PFC action mechanisms, including PPAR-α-dependent and -independent pathways, is necessary (DeWitt et al., 2007).

Since PFC exposure during pregnancy positively correlated with cord blood IgE levels in this study, how did PFCs accumulate in the fetal body? Like polychlorinated biphenyls, organochlorine pesticides, and polybrominated diphenyl ethers, PFOS and PFOA may cross the placental barrier in order to enter fetal circulation (Covaci et al., 2002; Mazdai et al. 2003; Midasch et al., 2007). Measurement taken from paired maternal and umbilical cord blood samples was utilized to establish the amount of PFC transfer in humans (Geary et al., 2009). Recent studies reinforced the importance of *in utero* exposures (including dietary nutrients, allergens, cigarette smoking, pollutants, endocrine disruptor chemicals, and medication) in fetal immunologic development and in programming of susceptibility to allergic diseases (Prescott and Clifton, 2009). Exposure to environmental risk factors in adults is independent from *in utero* exposure to epigenetically active chemicals can induce health problems that may occur later in life (Gluckman et al., 2008). In addition, Wu et al. (2009) reported that pre-natal but not late post-natal exposure of mice to tobacco smoke increased airway

hyper-responsiveness later in life. Above findings indicated that pre-natal exposures played a dominant role in influencing later susceptibility to diseases.

In accordance with an earlier NHANES study (Calafat et al., 2007), our results indicated that boys had higher average concentrations of PFCs than girls but not statistically significant (Table 1). Analyses stratified by gender also revealed that PFOA and PFOS levels positively correlated with cord blood IgE levels only in boys (Table 3). The two possible explanations can be that in males, PFOA has longer half lives and lower renal clearance. Similar differences were reported in rats exposed to PFOA. Estimated half lives were longer in males than females in a variety of rat strains (Kudo and Kawashima, 2003). Differences in blood PFOS level between genders have been observed with higher levels in male donors (Fromme et al., 2007; Calafat et al., 2007; Holzer et al., 2008). However, Butenhoff et al.(2004) found that female monkeys had longer terminal half-life of PFOA in serum. Because of the inconsistent results, further studies are needed to clarify any gender-related influence on *in utero* PFCs exposure and atopy.

Even if there is no occupational exposure, pregnant women are still likely to be exposed to PFCs through various sources. Using mean intake data, dietary intake seemed to be the primary pathway responsible for 91% (PFOS) and 99% (PFOA) out of the total intake of the general population (Fromme et al., 2008). This result was consistent with previously published findings. A simple one compartment toxicokinetic model showed that the dietary intake corresponded well with the plasma serumlevel of the same population (Fromme et al., 2007c). Specifically, the primary environmental compartment where PFOS and PFOA were found was expected to be water (,Emmett, et al.,2006Lau et al., 2007). In addition, consumption of contaminated fish and seafood may be another intake route of concern for humans (Fromme et al., 2008). In general,

the highest concentrations of PFCs were found in the livers of fish-eating animals living near more industrialized areas (Houde et. al., 2005~~Lau et al., 2007~~). Taiwan was most likely to have PFC bioaccumulation in its downstream rivers of semiconductor factories. Other dietary routes that contribute to PFCs human body burden was food contaminated from food-packing or non-stick cookware (Fromme et al., 2008). Post-natal exposure may come largely from breast feeding (Fromme et al., 2007c). Of note, we found that PFC exposures in Taiwan were close to those in the United States and lower than those in Germany [the median (range) was 1.71 (0.75-17.40) vs. 1.6 (<0.3-7.1) and 3.4 (1.5-4.6) ng/mL for cord blood serum PFOA; 5.50 (0.11-48.36) vs. 4.9 (<LOD-34.8) and 7.3 (3.3-9.5) ng/mL for cord blood serum PFOS] (Apelberg et al., 2007b; Midasch et al., 2007). However, higher than those in Japan [5.50 (0.11-48.36) vs. 2.5 (1.6-5.3) ng/mL for PFOS] (Inoue et al., 2004). The inconsistencies between these results cannot be fully explained with respect to differences in PFC~~s~~ concentrations. Different geographic areas and diet habits of various populations, different exposure routes, duration, concentrations, and individual differences in rates or patterns of metabolism or excretion may account for these inconsistencies.

We found that pre-natal PFOA and PFOS exposure positively correlated with cord blood IgE levels. Interestingly, no statistically significant association was found between AD and PFCs. A possible explanation was that various stronger risk factors, such as food- and aero-allergens, may contribute to AD than pre-natal PFC exposure (Wang et al., 2007; Worth et al., 2010). After adjusting for gender, gestational age, maternal age, maternal history of atopy, duration of breast feeding, and pre-natal ETS exposure, there was no significant association between AD and PFCs. Moreover, the magnitude of change in cord blood IgE levels did not correlate well to the magnitude of AD development (Hansen et al., 1992).

The population based prospective cohort design and valid laboratory methods were the strengths of this study. This was the first to link cord blood PFC levels to cord blood IgE levels and serum total IgE concentrations at 2 years of age using a birth cohort study. The advantage of conducting a prospective cohort design is that causality can be established. In addition, a prospective cohort study minimizes recall bias. Instead of maternal self-report of relevant information, peri-natal information like birth weight and gestational age were collected from medical records, adding data reliability. As to the objectivity of PFC~~s~~ measurements, PFCs were analyzed using UPLC-MS/MS, which had excellent validity. ~~Furthermore, all subjects were measured in duplicate with good concordance.~~ Therefore, the exposure assessment is reliable. There were some potential limitations to this study. ~~and results should be cautiously interpreted.~~ One was that PFC~~s~~ measurements using serum from cord blood was a single opportunistic measurement ~~at delivery~~. However, PFCs ~~have long half-lives and their~~ concentrations ~~are relatively stable over time. They are most likely to~~ remain fairly constant and are at near steady-state values throughout pregnancy (Lin et al., 2009). ~~Furthermore, i~~If measurement error did ~~actually~~ occur, the result would lean toward the null and the effect of exposure was likely to be underestimated. Another limitation was that post-natal PFC exposure – blood PFC levels at 2 years of age – was probably a confounding factor for AD development, which we failed to check. Since the source of exposure in pre- and post-natal period within the same family was be relatively constant and the half-lives of PFCs were long, post-natal PFC exposure was not likely to significantly confound the results (So et al., 2006; Lin et al., 2009). ~~There may be potential selection bias when non-participants differ in socio-demographics or smoking status. However, the statistics of those lost to follow-up and those who completed the follow-up were not significantly different. Therefore, selection bias was not a~~

~~significant factor.~~

In conclusion, pre-natal exposures to PFOA and PFOS positively correlated with cord blood IgE levels. Although there is no significant association between PFCs and AD, future large-scale follow-up researches are warranted to elucidate this relationship.

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Table 1 Basic demographics of the study population in terms of geometric means (s.e.)of cord blood serum PFC concentrations

Characteristics	No.	Mean PFOA (ng/ml)	Mean PFOS (ng/ml)	Mean PFNA (ng/ml)	Mean PFHxS (ng/ml)
Mother					
Maternal age (years)					
< 28	15	1.45(0.53)	7.11 (1.09)	1.98 (1.52)	0.035 (0)
≧28	227	1.77 (1.66)	5.51 (0.37)	1.98 (0.60)	0.043 (0.0043)
Maternal education					
High school and below	108	1.69 (0.19)	6.04 (0.63)	2.05 (0.94)	0.044 (0.0064)
College and above	133	1.78 (0.24)	5.23 (0.40)	1.90 (0.70)	0.041 (0.0053)
Maternal occupation					
House wives	65	1.57 (0.33)	5.39 (0.43)	1.95 (0.68)	0.043 (0.0083)
Non-house wives	173	1.81 (0.18)	6.08 (0.65)	2.03 (1.09)	0.043 (0.0048)
Maternal history of atopy					
Yes	62	2.01 (0.28)	5.48 (0.56)	2.21 (1.12)	0.045 (0.0090)
No	182	1.68 (0.19)	5.72(0.45)	1.90 (0.65)	0.042 (0.0045)
Children					
Gender					
Male	133	1.88 (0.27)	5.84 (0.57)	2.22 (0.99)	0.045 (0.0058)
Female	111	1.67 (0.19)	5.45 (0.42)	1.79 (0.61)	0.040 (0.0056)
Birth weight (gm)					
< 2500	16	1.77 (0.17)	7.69 (2.24)	2.04 (0.59)	0.039 (0.0103)
≧2500	218	1.46 (0.37)	5.47 (0.35)	1.26 (1.75)	0.043 (0.0043)
Gestational age (<37weeks)					
Yes	40	1.76 (0.18)	6.73 (1.00)	2.06 (0.63)	0.040 (0.0076)
No	202	1.67 (0.28)	5.40 (0.38)	1.60 (1.32)	0.043 (0.0047)
Parity					
< 2	104	2.01 (0.22)	5.37 (0.89)	2.32 (1.06)	0.044 (0.0072)
≧2	137	1.56 (0.22)	5.78 (0.55)	1.74 (0.59)	0.041 (0.0046)
Environmental factors					
Duration of breast feeding					
< 6 months	116	1.68 (0.32)	5.30 (0.41)	1.73 (1.04)	0.045 (0.0064)
≧6 months	73	1.83 (0.22)	5.42 (0.46)	1.97 (0.83)	0.040 (0.0060)
Incensing at home					
Yes	108	1.90 (0.24)	5.87 (0.47)	2.25 (0.65)	0.042 (0.0061)

No	125	1.58 (0.21)	5.36 (0.56)	1.63 (0.99)	0.043 (0.0055)
Prenatal ETS exposure					
Yes	82	1.75 (0.21)	6.19 (0.70)	2.18 (0.84)	0.043 (0.0062)
No	151	1.70 (0.24)	5.28 (0.42)	1.80 (0.76)	0.042 (0.0054)
Family income per year (NT dollars)					
< 600,000	43	1.43 (0.22)	6.09 (1.03)	2.15 (1.66)	0.044 (0.0105)
600,000-1,500,000	123	1.77 (0.26)	4.98 (0.38)	1.68 (0.67)	0.040 (0.0048)
> 1,500,000	57	1.79 (0.26)	6.43 (0.91)	2.20 (1.31)	0.050 (0.0104)

Abbreviations: ETS, environmental tobacco smoke; NT, New Taiwan dollars

*p<0.05

Table 2 Crude and adjusted regression coefficients β (s.e.) for log-cord blood IgE and log-serum total IgE at 2 years of age according to log-cord blood PFC concentrations

	PFOA (ng/ml)	P	PFOS (ng/ml)	P	PFNA (ng/ml)	P
Log-serum total IgE (KU/l)						
Crude β (s.e.)	0.032 (0.069)	0.629	0.012 (0.010)	0.859	-0.047 (0.133)	0.470
Adjusted β (s.e.)[#]	0.027 (0.244)	0.870	0.251 (0.179)	0.147	0.039 (0.471)	0.837
Log-cord blood IgE (KU/l)						
Crude β (s.e.)	0.150 (0.038)	0.023*	0.155 (0.030)	0.019*	0.006 (0.075)	0.927
Adjusted β (s.e.)[#]	0.134 (0.115)	0.047*	0.161 (0.147)	0.017*	0.024 (0.154)	0.908

*$p<0.05$

[#]adjusted for gender, gestational age, parity, maternal age, and pre-natal ETS exposure

@cord blood PFHxS levels failed to appear in the table because 89.3% PFHxS levels were lower than the detect limits

Table 3 Crude and adjusted regression coefficients β (s.e.) for log-cord blood IgE and log-serum total IgE at 2 years of age according to log-cord blood PFC concentrations stratified by gender

Girls (n = 111)	**PFOA (ng/ml)**	**P**	**PFOS (ng/ml)**	**P**	**PFNA (ng/ml)**	**P**
Log-serum total IgE (KU/l)						
Crude β (s.e.)	0.120 (0.099)	0.222	0.013 (0.062)	0.892	0.050 (0.189)	0.612
Adjusted β (s.e.)#	0.001 (0.452)	0.998	0.095 (0.325)	0.723	0.101 (0.874)	0.737
Log-cord blood IgE (KU/l)						
Crude β (s.e.)	0.088 (0.062)	0.380	0.160 (0.039)	0.109	0.027 (0.119)	0.788
Adjusted β (s.e.)#	0.067 (0.231)	0.823	0.151 (0.165)	0.616	0.110 (0.438)	0.740
Boys (n = 133)	**PFOA (ng/ml)**	**P**	**PFOS (ng/ml)**	**P**	**PFNA (ng/ml)**	**P**
Log-serum total IgE (KU/l)						
Crude β (s.e.)	0.034(0.101)	0.700	0.045(0.092)	0.609	-0.120(0.197)	0.177
Adjusted β (s.e.)#	0.097(0.345)	0.710	0.359(0.255)	0.238	0.072(0.637)	0.808
Log-cord blood IgE (KU/l)						
Crude β (s.e.)	0.205(0.048)	0.021*	0.157(0.044)	0.077	0.037(0.097)	0.678
Adjusted β (s.e.)#	0.206(0.165)	0.025*	0.175(0.179)	0.053*	0.015(0.169)	0.959

*$p<0.05$

#adjusted for gender, gestational age, parity, maternal age, and pre-natal ETS exposure

Table 4 Association of atopic dermatitis across quartiles of cord blood serum PFCs by univariate and multivariate logistic regression

	PFOA levels (ng/mL)			
	<0.085 (n=61)	**0.085 to 0.5700 (n=62)**	**0.5700 to 1.085 (n=60)**	**>1.085 (n=61)**
Crude OR (95% CI)	1.00	0.81(0.29-2.26)	0.97(0.41-2.27)	0.85(0.36-2.02)
Adjusted OR (95% CI)[#]	1.00	0.84(0.28-2.48)	1.03(0.42-2.56)	0.58(0.22-1.58)
	PFOS levels (ng/mL)			
	<1.250 (n=62)	**1.250 to 1.835 (n=60)**	**1.835 to 2.775 (n=61)**	**>2.775 (n=61)**
Crude OR (95% CI)	1.00	0.71(0.21-2.39)	2.56(0.96–6.82)	2.79(1.06-7.38)*
Adjusted OR (95% CI)[#]	1.00	0.68(0.20-2.30)	2.34(0.86-6.41)	2.19(0.78-6.17)
	PFNA levels (ng/mL)			
	<0.140 (n=101)	**0.140 to 0.765 (n=21)**	**0.765 to 2.515 (n=61)**	**>2.515 (n=61)**
Crude OR (95% CI)	1.00	1.18(0.35-3.95)	1.52(0.67-3.45)	1.23(0.53-2.89)
Adjusted OR (95% CI)[#]	1.00	1.46 (0.35-6.07)	1.53(0.59-3.93)	0.72(0.23-2.21)

Abbreviations: OR, odds ratio; CI, confidence interval

*$p<0.05$

[#]adjusted for gender, gestational age, maternal age, maternal history of atopy, duration of breast feeding, and pre-natal ETS exposure

Figure Legends:

Fig. 1. Box and whisker plots of cord blood serum PFC concentrations ~~.~~measured by Ultra-performance liquid chromatography/triple-quadrupole mass. Horizontal lines inside boxes: median; boxes: inter-quartile range; whiskers: the most extreme data points <1.5 times the interquartile range from the ends of the box; circles: outliers.

Figure

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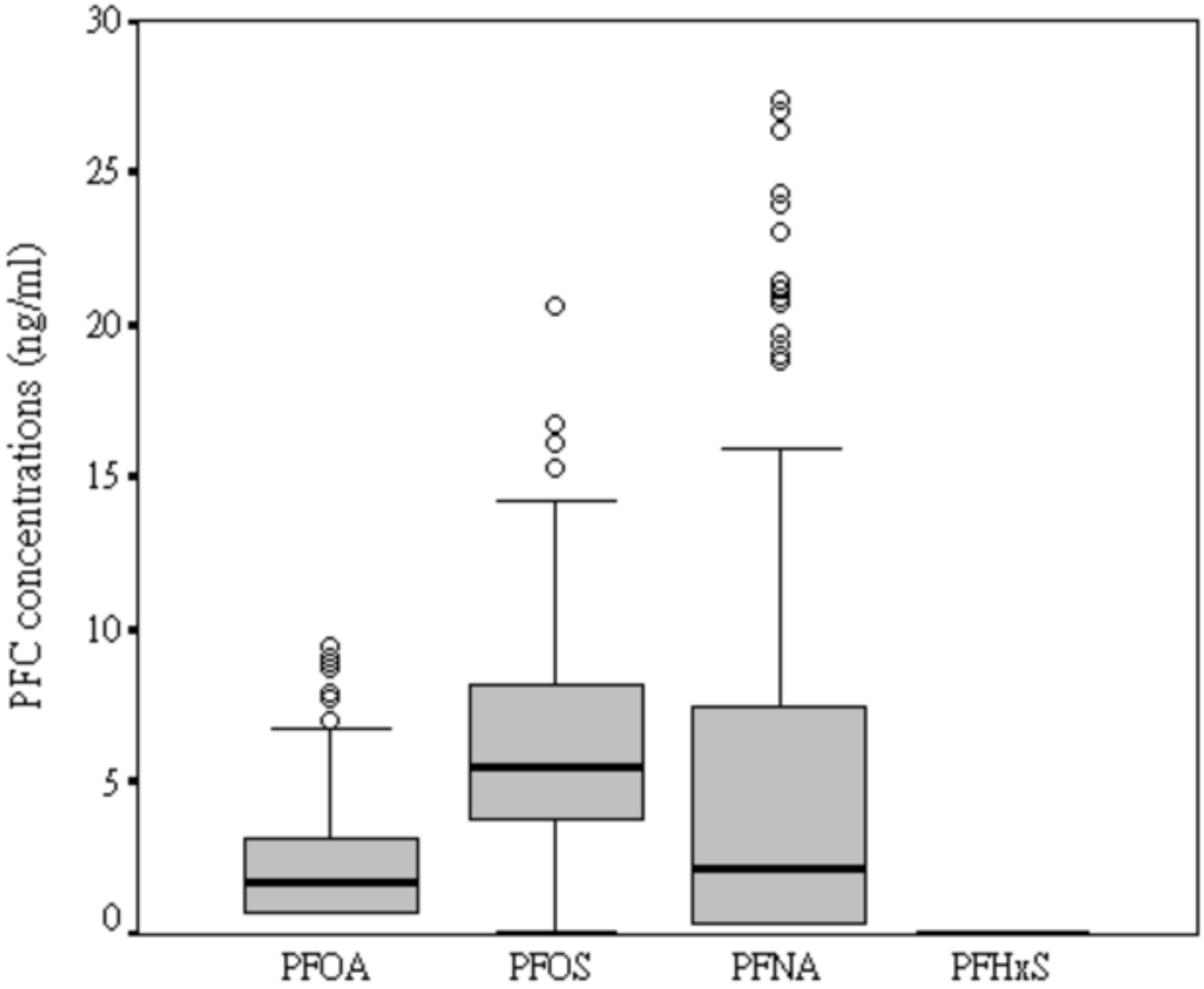

Supplementary Material

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