Human Reproduction, Vol.24, No.5 pp. 1200–1205, 2009
Advanced Access publication on January 28, 2009 doi:10.1093/humrep/den490

human reproduction

ORIGINAL ARTICLE *Reproductive epidemiology*

Maternal levels of perfluorinated chemicals and subfecundity

Chunyuan Fei[1,5], Joseph K. McLaughlin[2,3], Loren Lipworth[2,3], and Jørn Olsen[1,4]

[1]Department of Epidemiology, School of Public Health, University of California – Los Angeles, PO Box 951772, 71-254 CHS, 650 Charles E. Young Drive South, Los Angeles, CA 90095-1772, USA [2]International Epidemiology Institute, 1455 Research Boulevard, Rockville, MD 20850, USA [3]Vanderbilt University Medical Center, Vanderbilt-Ingram Cancer Center, Nashville, TN 37232, USA [4]Institute of Public Health, University of Aarhus, Vennelyst Boulevard 6, DK 8000 Aarhus C, Denmark

[5]Correspondence address. Tel: +1-310-825-5373; Fax: +1-310-206-6039; E-mail: cfei@ucla.edu

BACKGROUND: Perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) are ubiquitous man-made compounds that are possible hormonal disruptors. We examined whether exposure to these compounds may decrease fecundity in humans.

METHODS: Plasma levels of PFOS and PFOA were measured at weeks 4–14 of pregnancy among 1240 women from the Danish National Birth Cohort recruited from 1996 to 2002. For this pregnancy, women reported time to pregnancy (TTP) in five categories (<1, 1–2, 3–5, 6–12 and >12 months). Infertility was defined as having a TTP of >12 months or received infertility treatment to establish this pregnancy.

RESULTS: Longer TTP was associated with higher maternal levels of PFOA and PFOS ($P < 0.001$). Compared with women in the lowest exposure quartile, the adjusted odds of infertility increased by 70–134 and 60–154% among women in the higher three quartiles of PFOS and PFOA, respectively. Fecundity odds ratios (FORs) were also estimated using Cox discrete-time models. The adjusted FORs were virtually identical for women in the three highest exposure groups of PFOS (FOR = 0.70, 0.67 and 0.74, respectively) compared with the lowest quartile. A linear-like trend was observed for PFOA (FOR = 0.72, 0.73 and 0.60 for three highest quartiles versus lowest quartile). When all quartiles were included in a likelihood ratio test, the trends were significant for PFOS and PFOA ($P = 0.002$ and $P < 0.001$, respectively).

CONCLUSIONS: These findings suggest that PFOA and PFOS exposure at plasma levels seen in the general population may reduce fecundity; such exposure levels are common in developed countries.

Key words: maternal blood / time to pregnancy / fecundity / perfluorooctanoate / perfluorooctane sulfonate

Introduction

Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) belong to a class of perfluorinated chemicals (PFCs) that are widely used in many consumer products (paper wraps, fire-fighting foams, pesticides, textiles including clothing, upholstery, carpets and personal care products) and in manufacturing processes (industrial surfactants and emulsifiers). They are persistent in the environment and have been detected in wildlife and humans around the world (Giesy and Kannan, 2001; Kannan *et al*., 2004; Apelberg *et al*., 2007; Calafat *et al*., 2007; Fei *et al*., 2007). They were considered biologically inactive when first commercially introduced in the 1950's, but animal studies of PFOA and PFOS have since indicated toxic effects on the liver, immune system and developmental and reproductive organs (Kennedy *et al*., 2004; Lau *et al*., 2004, 2007). PFOA and PFOS may affect sex hormone homeostasis, and have been associated with increased incidence of fetal resorptions and pregnancy loss in animals (Case *et al*., 2001; Butenhoff *et al*., 2004; Lau *et al*., 2006; Wolf *et al*., 2007).

In recent decades, a remarkable decline in fertility rates has been observed in developed countries, which can largely be explained by social changes in desired family sizes and better contraceptive methods, but may also in part be attributed to reduced fecundity, the biological capacity to reproduce (Olsen and Rachootin, 2003). In the USA, 2% of women of reproductive age had an infertility-related medical appointment within the previous year, and 8% had an infertility-related medical visit at some point in the past (Centers for Disease Control and Prevention (CDC), 2008). Infertility is associated with psychological stress and anxiety for women and couples, and with adverse birth outcomes or diseases (Zhu *et al*., 2007; Jensen *et al*., 2008), and treatment carries financial burdens for individuals and for society.

Environmental pollutants, such as polychlorinated biphenyls, pesticides and other chemicals (Taskinen *et al*., 1999; Law *et al*., 2005),

have been linked to subfecundity. In a previous paper (Fei *et al*., 2007), we reported that maternal PFOS and PFOA levels were inversely related to parity, perhaps due to fetal uptake during pregnancy or excretion by breast milk, but this association could also be explained by a link between exposure to PFOS or PFOA and subfecundity. We used data from the Danish National Birth Cohort (DNBC) to assess whether maternal plasma PFOS and PFOA levels, measured in early pregnancy, were associated with a longer time to pregnancy (TTP), a measure that has been used to estimate fecundity in epidemiologic studies for more than 25 years (Rachootin and Olsen, 1982).

Materials and Methods

The women were enrolled into the DNBC, a nationwide follow-up study of almost 100 000 children and their mothers. More details about the cohort have been presented elsewhere (Olsen *et al*., 2001). Briefly, pregnant women were recruited through their general practitioners around weeks 6–12 of gestation. Approximately 50% of all general practitioners in Denmark participated in the study, and approximately 60% of invited women accepted the invitation to participate. Self-reported data were collected by computer-assisted telephone interviews twice during pregnancy and twice after birth. A study biobank was set up, consisting of two maternal blood samples taken during pregnancy, and one umbilical cord blood sample obtained shortly after birth.

We measured plasma PFOA and PFOS levels in a subset of DNBC participants. Among all participants ($n = 43\,045$) who provided the first maternal blood sample, gave birth to a single live born child without congenital malformation, and completed all four telephone interviews, we randomly selected 1400 women. Detailed information about sampling has been described elsewhere (Fei *et al*., 2007). We excluded 160 with unplanned pregnancies or unknown TTP. Altogether, 1240 women were included in our main analyses.

Written informed consent was obtained from all participants at recruitment. The UCLA Office for Protection of Research Subjects (Reference No. 06-08-023-01) and the Danish Data Protection Agency (Reference No. J.Nr 2006-41-6324) approved the study protocol.

Time to pregnancy

In the first interview (at approximately 12 weeks of gestation), women were asked if their pregnancy was planned, partly planned or not planned. All except those responding 'not planned' were further asked, 'For how long did you try to get pregnant before you succeeded?' followed by five fixed answering categories of TTP: got pregnant immediately (i.e. <1 months), 1–2, 3–5, 6–12 and >12months. Infertility was defined as having a TTP of >12 months or infertility treatment to establish the current pregnancy.

PFOA and PFOS exposure

We used the maternal blood samples taken at the first antenatal visit (weeks 4–14 of pregnancy) for this study. Concentrations of PFOS and PFOA in plasma were measured by high performance liquid chromatography/tandem mass spectrometry at the 3M Toxicology Laboratory (Ehresman *et al*., 2007). Stable labeled analogs of PFOS ($18O_2$ PFOS) and PFOA ($13C_2$ PFOA) were used in extraction procedures, and the extractions were performed using solid phase extraction techniques and based on 100 μl of plasma. All values were above LLOQ (the lower limit of quantification: 1 ng/ml), except one PFOA value that was assigned a value of half the LLOQ. Further details about the analysis methods were given in our earlier report (Fei *et al*., 2007). The laboratory was blinded to any information on the pregnant women.

Statistical analysis

PFOA and PFOS levels were analyzed as continuous variables and were also categorized a priori into quartiles using the lowest quartile as the reference group. We first used logistic regression to estimate the odds ratios (ORs) of infertility for women who were exposed to higher levels of PFOA or PFOS compared with the reference level. Fecundity odds ratios (FORs) were then estimated using the Cox model modified for discrete time data (Allison 1995). Approximate median numbers of months (i.e. 1, 2, 4 and 9) were assigned to the first four reported categories of TTP. TTP was censored at the 13th month if women had a TTP >12 months or received infertility treatment for this pregnancy ($n = 201$). In our study, FORs measure the odds of a successful conception for women who had higher levels of PFOA or PFOS compared with the reference levels within a given calendar month, given that the women did not become pregnant in the previous month. FORs <1 therefore indicate decreased fecundity and a longer TTP. A value of $P < 0.05$ was considered statistically significant.

Potential confounders included maternal age at delivery, parity, pre-pregnancy body mass index (BMI), maternal socio-occupational status, paternal education, paternal age and alcohol consumption before pregnancy. Age at menarche, irregular menstrual periods, history of spontaneous miscarriages, abdominal diseases (e.g. endometriosis, pain), paternal occupation and gestational weeks at blood drawing were also considered, but were not included in the final models as they had little effect on the estimated associations. Information on smoking and coffee consumption were only reported after pregnancy and women with TTP >12 months were more likely to have stopped smoking; furthermore, adjustment for these variables did not change the associations between PFOA or PFOS and the outcome, and were therefore not included in the models.

Results

Half of the women became pregnant within the first 2 months of trying, while 379 (30%) had a TTP of ≥6 months, 188 of whom had a TTP of >12 months. The average age of the women was 30.6 years and 15% were above 35 years of age at delivery. About half of the women were expecting their first child (Table I), and one-third were overweight or obese. Eighteen per cent had a history of spontaneous miscarriages and 14% reported experiencing irregular menstrual cycles prior to this pregnancy.

The median plasma PFOA and PFOS levels among the planned or partly planned pregnancies were 5.3 ng/ml [interquartile (IQR): 4.0, 7.0] and 33.7 ng/ml (IQR: 26.6, 43.5), respectively. As previously reported (Fei *et al*., 2007), PFOS and PFOA levels decreased with increasing age or parity and with decreasing pre-pregnancy BMI. PFOA levels were associated with irregular menstrual periods (9.0% in the lowest quartile versus 15.0% in the upper three quartiles), as well as PFOS (11.6% in the lowest quartile versus 14.2% in the upper three quartiles). Compared with women who got pregnant in the first 6 months of waiting time, women who had a longer TTP had higher PFOA and PFOS levels ($P < 0.001$ for both), and they were more likely to be older, of middle socio-occupational status, and to have a history of spontaneous miscarriages or irregular menstrual cycles (Table I). Those with younger partners or partners with higher education levels had a shorter TTP.

The proportion of women with TTP >12 months (infertility) was higher in the higher three quartiles of PFOA and PFOS versus the lowest quartile (Table II). We estimated that the odds of infertility

Table I Characteristics of study subjects and TTP among women with planned pregnancy[a]

	Women with planned pregnancy (n = 1240)%	<6 months (n = 861)%	6–12 months (n = 191)%	>12 months (n = 188)%	PFOA (ng/ml)[b]	PFOS (ng/ml)[b]
PFOS, ng/ml[b]	35.5 ± 12.8	34.6 ± 12.7	36.6 ± 12.5	38.3 ± 13.0	–	–
PFOA, ng/ml[b]	5.6 ± 2.6	5.4 ± 2.2	6.0 ± 3.4	6.3 ± 2.7	–	–
Maternal age at delivery						
<25 years	7.8	8.8	8.4	2.1	6.1 ± 1.9	38.0 ± 10.9
25–29 years	40.0	42.1	39.3	31.4	6.1 ± 2.8	37.1 ± 12.6
30–34 years	37.0	37.0	35.1	39.4	5.2 ± 2.3	34.2 ± 13.3
≥35 years	15.2	12.1	17.3	27.1	5.2 ± 2.4	33.2 ± 12.5
Parity						
0	44.7	41.9	49.2	54.8	6.7 ± 2.7	37.8 ± 12.7
1	36.3	39.4	32.5	33.0	4.8 ± 2.0	33.7 ± 12.7
≥2	19.0	18.7	18.3	12.2	4.6 ± 2.2	33.5 ± 12.3
Pre-pregnancy BMI						
<18.5 kg/m^2	4.2	3.8	5.9	4.3	5.2 ± 2.1	32.9 ± 13.8
18.5–24.9 kg/m^2	66.3	67.9	61.0	64.2	5.6 ± 2.7	35.0 ± 12.8
25.0–29.9 kg/m^2	22.3	21.9	24.6	21.4	5.7 ± 2.3	36.7 ± 11.8
≥30.0 kg/m^2	7.2	6.3	8.6	10.2	6.1 ± 2.6	38.8 ± 13.8
Maternal SES						
Higher	51.9	54.2	45.3	47.6	5.6 ± 2.4	34.2 ± 12.5
Middle	40.2	37.4	47.4	46.0	5.7 ± 2.8	37.1 ± 12.8
Lower	7.9	8.4	7.4	6.4	5.6 ± 2.2	36.9 ± 13.8
Alcohol consumption before pregnancy (drinks/week)						
0 to <1	22.0	20.8	23.7	25.7	5.3 ± 2.2	35.4 ± 12.4
1–1.5	24.0	22.7	22.6	31.6	5.8 ± 2.4	37.0 ± 12.8
2–3	22.0	23.3	20.5	17.6	5.5 ± 2.2	35.7 ± 13.1
>3	32.0	33.3	33.2	25.1	5.9 ± 3.1	34.4 ± 12.8
Smoking in early pregnancy						
Yes	22.7	22.5	27.2	19.0	5.6 ± 2.3	34.4 ± 11.7
No	77.3	77.5	72.8	81.0	5.6 ± 2.6	35.8 ± 13.1
History of spontaneous miscarriages						
No	82.0	85.2	74.9	74.3	5.7 ± 2.6	35.6 ± 12.7
Yes	18.0	14.8	25.1	25.7	5.3 ± 2.2	35.1 ± 13.4
Irregular menstrual periods						
No	86.4	88.2	87.9	77.1	5.6 ± 2.6	35.4 ± 13.0
Yes	13.6	11.8	12.1	22.9	5.8 ± 2.0	36.0 ± 11.6
Paternal age						
<30 years	29.4	32.3	28.4	17.1	6.2 ± 2.4	38.0 ± 12.3
30–34 years	39.3	41.0	35.8	35.3	5.5 ± 2.8	35.0 ± 12.9
35–39 years	23.1	20.1	24.7	35.3	5.3 ± 2.2	34.3 ± 13.4
≥40 years	8.2	6.7	11.0	12.3	5.2 ± 2.5	33.3 ± 11.3
Paternal education						
Lower	20.2	19.2	24.6	20.7	5.7 ± 2.1	38.2 ± 12.4
Middle	33.9	31.7	36.1	41.5	5.9 ± 3.0	37.5 ± 12.8
Higher	41.6	45.1	34.6	33.0	5.4 ± 2.4	32.8 ± 12.5
Other or unknown	4.3	4.1	4.7	4.8	5.7 ± 2.6	33.4 ± 12.9

[a]Missing data: maternal age at delivery (n = 1), pre-pregnancy BMI (n = 27), maternal socio-occupational status (SES) (n = 4), history of spontaneous miscarriages (n = 1), alcohol consumption before pregnancy (n = 3), paternal age (n = 11); [b]data are mean ± SD. PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

Table II Estimated OR for infertility (TTP >12 months) and FOR according to plasma concentrations of PFOS or PFOA (ng/ml) in early pregnancy[a]

Exposure	No. of planned pregnancy	Infertility		FOR (95% CI)[c]
		Per cent	OR (95% CI)[b]	
PFOS (ng/ml)				
6.4–26.0	293	10.6	1.00	1.00
26.1–33.3	305	15.4	1.70 (1.01, 2.86)	0.70 (0.56, 0.87)
33.4–43.2	317	19.5	2.34 (1.40, 3.89)	0.67 (0.53, 0.84)
≥43.3	317	18.6	1.77 (1.06, 2.95)	0.74 (0.58, 0.93)
P-value for trend[d]			0.025	0.002
PFOA (ng/ml)				
<LLOQ–3.91	293	8.9	1.00	1.00
3.91–5.20	308	18.2	2.06 (1.22, 3.51)	0.72 (0.57, 0.90)
5.21–6.96	315	15.5	1.60 (0.93, 2.78)	0.73 (0.58, 0.92)
≥6.97	316	21.5	2.54 (1.47, 4.39)	0.60 (0.47, 0.76)
P-value for trend[d]			0.006	<0.001

[a]The estimates were adjusted for maternal age at delivery, parity, pre-pregnancy BMI, maternal SES, alcohol consumption before pregnancy, paternal age, and paternal education; seven women (from a total of 1240) were excluded because of missing data on covariates; [b]Logistic regression; [c]Cox discrete-time model; [d]The *P*-values for trend tests of FORs were given for the four-quartile comparison of PFOA and PFOS levels using a likelihood ratio test. CI, confidence interval; FOR, fecundity odds ratios; LLOQ, lower limit of quantification.

increased by 70–134% for women in each of the higher exposure categories of PFOS, and 60–154% for women in each of the higher exposure categories of PFOA, compared with the lowest quartile. Women who were exposed to higher levels of PFOA or PFOS had a longer TTP; the adjusted FORs were 0.70, 0.67 and 0.74 for the top three quartiles of PFOS and 0.72, 0.73 and 0.60 for the top three quartiles of PFOA (versus the lowest quartile) (Table II). When all quartiles were included in a likelihood ratio test, the trends were significant for PFOS and PFOA ($P = 0.002$ and <0.001, respectively).

Women who were carrying unplanned pregnancies had lower PFC levels. The median level was 4.8 ng/ml (IQR: 3.5, 6.6) for PFOA and 30.0 ng/ml (IQR: 26.6, 43.5) for PFOS. They were of younger age (<25 years) or much older (≥35 years), more often parous and of lower socio-occupational status. They were less likely to take contraceptive pills and to drink alcohol before pregnancy, but more likely to smoke in early pregnancy. Including these women as having a TTP of '<1 month' strengthened the observed associations (data not shown). Only including women with regular menstrual periods slightly lessened the association (data not shown).

Discussion

To our knowledge, this is the first study to assess the associations between PFOA and PFOS plasma levels and TTP in humans. Higher maternal PFOA and PFOS levels measured in early pregnancy were found to be associated with longer TTP. The odds of a conception leading to a recognized pregnancy were reduced for women who were exposed to PFOS and PFOA above the lowest quartile, and the magnitude of the reduction in fecundity was similar for the three higher quartiles.

The exposure time window of interest is at the start of pregnancy planning, but our exposure data were taken at 4–14 weeks gestation. PFOA and PFOS levels were, however, rather stable over pregnancy (Fei *et al*., 2007). We assume that our exposure assessment reflects the exposure level during the entire pregnancy planning time period. Information on TTP was obtained during the first trimester when women should be able to recall this with reasonable accuracy (Joffe *et al*., 2005). We further provided five response categories in order to facilitate valid recall, at the expense of precision. Differential recall by women according to PFC levels is unlikely to be a problem in our study since the exposure levels were unknown to them.

Maternal age, paternal age and paternal education substantially changed the associations between PFOS and TTP, while parity and paternal education were the most influential confounders in the analysis of PFOA. We did not have information on some important determinants of TTP, including frequency and timing of intercourse, and sperm quality. Sperm quality could potentially contribute to the associations between maternal PFC levels and TTP, if these compounds impact sperm quality and if PFC levels in male and female partners are similar, which is likely to some extent since the couples may share some aspects of lifestyle and around 99% subjects in this subcohort had a spouse or partner. Adjustment for paternal occupation did not change the estimates.

There are several potential limitations of our study. First, we studied TTP of pregnancies which led to the birth of a child, limiting conclusions that can be drawn regarding women who were unable to get pregnant, even after infertility treatment. Only couples who planned or partly planned their pregnancy can report a TTP. Ninety per cent of the women planned or partly planned their pregnancy in our study. Selection bias resulting from exclusion of fertile women who did not plan their pregnancy is possible, and our data showed that the associations between PFC levels and longer TTP

were stronger after inclusion of unplanned pregnancies in the analyses.

The biological mechanism by which exposure to PFOS and PFOA may reduce fecundity is unknown. PFCs may interfere with hypothalamic–pituitary–ovarian regulation, possibly causing irregular menstrual cycles, delayed ovulation or early abortions not recognized by the mother. In this study, we found similarly higher proportions of women reporting irregular menstrual periods in the upper three quartiles of PFCs compared with the lowest. Similarly, exposure to PFOS can reduce the number of regular estrous cycles in rats (Austin *et al*., 2003). Although the doses administered to rats were higher than the levels found in our study, but close to those found in occupationally exposed human populations (Olsen *et al*., 1999), the findings of Austin *et al*. (2003) may not apply to man, since the rodent estrous cycle is not an ideal model for the human cycle and the half-lives of these chemicals in humans greatly differ from those in animals. Increased levels of estrogen and/or decreased levels of testosterone have also been found in male rats given oral doses of PFOA at 2 mg/kg/day or higher (Cook *et al*., 1992; Biegel *et al*., 1995; Liu *et al*., 1996; Martin *et al*., 2007). A dose of 3 mg/kg/day is approximately equivalent to 4000 ng/ml in rat serum, which is slightly higher than levels observed in occupationally exposed populations (Butenhoff *et al*., 2004), but female rats were not studied as often as male rats in the studies of PFOA. Abnormal hormone levels may have an impact on infertility, and results from several animal studies, although not all, showed that exposure to PFOA or PFOS increased the incidence of spontaneous miscarriages (Case *et al*., 2001; Butenhoff *et al*., 2004; Lau *et al*., 2006; Wolf *et al*., 2007).

In conclusion, our data suggest that exposure to PFOA and PFOS at levels found in the general population may increase TTP. Whether our results will add PFCs to the list of risk factors for subfecundity remains to be seen, but PFCs may explain some of the fertility differences seen among different populations in developed countries.

Authors' contributions

C.F. designed the study, analyzed and interpreted the data, drafted the paper. J.O. monitored each step of the study, designed the study, guided analysis and interpretation of the data, and revised the paper.J.K.M. designed the study, interpreted the data and revised the paper. L.L. interpreted the data and revised the paper.

Acknowledgements

Thanks are extended to the 3M Toxicology Laboratory led by David J. Ehresman. They performed all laboratory analyses.

Funding

The original study was supported by the International Epidemiology Institute (IEI), which received a funding grant from the 3M Company. The 3M Company has no control over the design, data analysis and interpretation or writing of this study. J.K.M. and L.L. were employed by IEI. J.O. and C.F. independently work at the University of California, Los Angeles and have no competing financial interest.

References

Allison PD. *Survival Analysis using SAS: A Practical Guide*. Cary, NC: SAS Institute Press, 1995, 134–136.

Apelberg BJ, Witter FR, Herbstman JB, Calafat AM, Halden RU, Needham LL, Goldman LR. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. *Environ Health Perspect* 2007; **115**:1670–1676.

Austin ME, Kasturi BS, Barber M, Kannan K, MohanKumar PS, MohanKumar SM. Neuroendocrine effects of perfluorooctane sulfonate in rats. *Environ Health Perspect* 2003;**111**:1485–1489.

Biegel LB, Liu RC, Hurtt ME, Cook JC. Effects of ammonium perfluorooctanoate on Leydig cell function: *in vitro*, *in vivo* and *ex vivo* studies. *Toxicol Appl Pharmacol* 1995;**134**:18–25.

Butenhoff JL, Kennedy GL Jr, Frame SR, O'Connor JC, York RG. The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. *Toxicology* 2004;**196**:95–116.

Calafat AM, Wong LY, Kuklenyik Z, Reidy JA, Needham LL. Polyfluoroalkyl chemicals in the U.S. population: data from the National Health and Nutrition Examination Survey (NHANES) 2003–2004 and comparisons to NHANES 1999–2000. *Environ Health Perspect* 2007;**115**:1596–1602.

Case MT, York RG, Christian MS. Rat and rabbit oral developmental toxicology studies with two perfluorinated compounds. *Int J Toxicol* 2001;**20**:101–109.

Centers for Disease Control and Prevention (CDC). *Assisted Reproductive Technology*. 2008; http://www.cdc.gov/ART/2008(date last accessed, 6 May 2008).

Cook JC, Murray SM, Frame SR, Hurtt ME. Induction of Leydig cell adenomas by ammonium perfluorooctanoate: a possible endocrine-related mechanism. *Toxicol Appl Pharmacol* 1992;**113**:209–217.

Ehresman DJ, Froehlich JW, Olsen GW, Chang SC, Butenhoff JL. Comparison of human whole blood, plasma, and serum matrices for the determination of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and other fluorochemicals. *Environ Res* 2007;**103**:176–184.

Fei CY, McLaughlin JK, Tarone RE, Olsen J. Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. *Environ Health Perspect* 2007;**115**:1677–1682.

Giesy JP, Kannan K. Global distribution of perfluorooctane sulfonate in wildlife. *Environ Sci Technol* 2001;**35**:1339–1342.

Jensen A, Sharif H, Olsen JH, Kjaer SK. Risk of breast cancer and gynecologic cancers in a large population of nearly 50,000 infertile Danish women. *Am J Epidemiol* 2008;**168**:49–57.

Joffe M, Key J, Best N, Keiding N, Scheike T, Jensen TK. Studying time to pregnancy by use of a retrospective design. *Am J Epidemiol*. 2005; **162**:115–124.

Kannan K, Corsolini S, Falandysz J, Fillmann G, Kumar KS, Loganathan BG, Mohd MA, Olivero J, Van Wouwe N, Yang JH *et al*. Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. *Environ Sci Technol* 2004;**38**:4489–4495.

Kennedy GL Jr, Butenhoff JL, Olsen GW, O'Connor JC, Seacat AM, Perkins RG, Biegel LB, Murphy SR, Farrar DG. The toxicology of perfluorooctanoate. *Crit Rev Toxicol* 2004;**34**:351–384.

Lau C, Butenhoff JL, Rogers JM. The developmental toxicity of perfluoroalkyl acids and their derivatives. *Toxicol Appl Pharmacol* 2004; **198**:231–241.

Lau C, Thibodeaux JR, Hanson RG, Narotsky MG, Rogers JM, Lindstrom AB, Strynar MJ. Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. *Toxicol Sci* 2006;**90**:510–518.

Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J. Perfluoroalkyl acids: a review of monitoring and toxicological findings. *Toxicol Sci* 2007;**99**:366–394.

Law DC, Klebanoff MA, Brock JW, Dunson DB, Longnecker MP. Maternal serum levels of polychlorinated biphenyls and 1,1-dichloro-2,2-bis (*p*-chlorophenyl)ethylene (DDE) and time to pregnancy. *Am J Epidemiol* 2005;**162**:523–532.

Liu RC, Hurtt ME, Cook JC, Biegel LB. Effect of the peroxisome proliferators, ammonium perfluorooctanoate (C8), on hepatic aromatase activity in adult male Crl: CD BR (CD) rats. *Fundam Appl Toxicol* 1996;**30**:220–228.

Martin MT, Brennan RJ, Hu W, Ayanoglu E, Lau C, Ren H, Wood CR, Corton JC, Kavlock RJ, Dix DJ. Toxicogenomic study of triazole fungicides and perfluoroalkyl acids in rat livers predicts toxicity and categorizes chemicals based on mechanisms of toxicity. *Toxicol Sci* 2007;**97**:595–613.

Olsen J, Rachootin P. Invited commentary: Monitoring fecundity over time—if we do it, then let's do it right. *Am J Epidemiol* 2003;**157**:94–97.

Olsen GW, Burris JM, Mandel JH, Zobel LR. Serum perfluorooctane sulfonate and hepatic and lipid clinical chemistry tests in fluorochemical production employees. *J Occup Environ Med* 1999; **41**:799–806.

Olsen J, Melbye M, Olsen SF, Sørensen TI, Aaby P, Andersen AM, Taxbøl D, Hansen KD, Juhl M, Schow TB *et al*. The Danish National Birth Cohort—its background, structure and aim. *Scand J Public Health* 2001;**29**:300–307.

Rachootin P, Olsen J. Prevalence and socioeconomic correlates of subfecundity and spontaneous abortion in Denmark. *Int J Epidemiol* 1982;**11**:245–249.

Taskinen HK, Kyyrönen P, Sallmén M, Virtanen SV, Liukkonen TA, Huida O, Lindbohm ML, Anttila A. Reduced fertility among female wood workers exposed to formaldehyde. *Am J Ind Med*. 1999; **36**:206–212.

Wolf CJ, Fenton SE, Schmid JE, Calafat AM, Kuklenyik Z, Bryant XA, Thibodeaux J, Das KP, White SS, Lau CS *et al*. Developmental toxicity of perfluorooctanoic acid in the CD-1 mouse after cross-foster and restricted gestational exposures. *Toxicol Sci* 2007; **95**:462–473.

Zhu JL, Obel C, Hammer Bech B, Olsen J, Basso O. Infertility, infertility treatment, and fetal growth restriction. *Obstet Gynecol* 2007; **110**:1326–1334.

Submitted on September 12, 2008; resubmitted on November 7, 2008; accepted on December 18, 2008