Supplemental Material

Plasma concentrations of perfluorooctane sulfonamide (PFOSA) and time to pregnancy among primiparous women

Kristina W. Whitworth, Line S. Haug, Azemira Sabaredzovic, Merete Eggesbo, Matthew P. Longnecker

Multiple Imputation procedure:

The MI procedure was conducted using IVEware v0.2 (University of Michigan, http://www.isr.umich.edu/src/smp/ive/). In each model, estimated PFOSA values were forced to be <LOQ and 10 iterations were run. The following were included as predictors in MI model #1: maternal age, BMI, education, annual income, pre-pregnancy smoking, shellfish, lean fish, and oily fish consumption, menstrual cycle regularity, oral contraceptive use in the previous 12 months, and serum albumin concentration (g/dL). We chose these variables based on their correlation with plasma PFAS concentrations in this population.[1] MI model #2 included the model #1 variables plus plasma concentrations of nine other PFASs (PFDA, PFDoDA, PFHpS, PFHxS, PFNA, PFOA, PFOS, PFTrDA, and PFUnDA). MI model #3 included the model #1 variables plus PFNA, which had the strongest correlation with PFOSA concentrations ($r=0.27$, $p<0.001$). For each MI model, we calculated age and BMI-adjusted FORs and 95% CIs for the association between each of the 10 iterations of the multiply-imputed PFOSA variable (divided by the IQD of the original variable). These 10 estimates were combined using PROC MI in SAS, resulting in one estimate of the association between PFOSA and TTP for each MI model.

eTable 1. Comparison of analyses of PFAS and time-to-pregnancy from the Norwegian Mother and Child Cohort Study (MoBa).

Study	Analysis	Sample Size	Treatment of Parity	PFASs Analyzed	Main Results
Whitworth et al.[2]	Logistic Regression	910 women	Stratified by parity	PFOS and PFOA	Increased odds of subfecundity among parous women but not primiparous women
Ding et al.[3]	Outcome Dependent Sampling Design	910 women	None	PFOS and PFOA	Decreased fecundability associated with PFOA concentrations among all women
Present Study	Discrete-Time Survival Analysis	451 women	Restricted to primiparous women	PFDA, PFDoDA, PFHpS, PFHxS, PFNA, PFOA, PFOS, PFOSA, PFTrDA, and PFUnDA	Limited evidence of decreased fecundability associated PFAS

eTable 1 summarizes relevant issues and results across the three analyses of PFAS and fecundity or fecundability that have been conducted in MoBa. It is important to note that the analysis by Ding et al.[3] used the PFAS data from the MoBa study, including data on parous women, in an example analysis to demonstrate an innovative statistical method. Their analysis showed improved efficiency of the estimator, but the results were not intended to have subject matter relevance. For results relevant to the subject matter, refer to Whitworth et al.[2] and results from the present analysis.

eTable 3. Demographics of 451 nulliparous women from a case-base study among the Norwegian Mother and Child Cohort (MoBa) Study, 2003-2004

	n	%
Age at Pregnancy Attempt		
<25 years	129	29
25-29 years	211	47
30-34 years	91	20
≥35 years	20	4
Prepregnancy BMI		
<18.50	14	3
18.50-24.99	274	61
25.00-29.99	107	24
≥30.00	56	12
Prepregnancy Smoking		
Daily	90	20
Sometimes	53	12
None	308	68
Maternal Education		
< High School	26	6
High School	135	30
Some College	200	44
4+ Years of College	89	20
Missing	1	0
Annual Maternal Income (NOK)*		
<150,000	46	10
150,000 - 299,999	225	50
>300,000	168	37
Missing	12	3
*1 NOK is approximately 0.12 USD		

eTable 2. Crude and adjusted fecundability odds ratios (FOR[a]) for the association between perfluoroalkyl substances (ng/ml) and time to pregnancy among 924 women[b] from a case-base study among the Norwegian Mother and Child Cohort (MoBa) Study, 2003-2004

					Crude			Adjusted[g]		
	n (%) >LOQ[c]	n[d]	Median A (IQR)[e]	Median B (IQR)[f]	FOR	Lower Limit	Upper Limit	FOR	Lower Limit	Upper Limit
Perfluorinated Sulfonamide										
PFOSA	353 (38.2)	482	0.04 (0.03, 0.04)	0.03 (0.01, 0.06)	0.98	0.86	1.13	0.85	0.83	1.09
Perfluorinated Carboxylates										
PFBA	2 (0.0)	--	--	--	---	---	---	---	---	---
PFHpA	108 (11.7)	--	--	--						
PFOA	924 (100.0)	924	2.25 (1.66, 3.04)	2.25 (1.66, 3.04)	0.79	0.69	0.89	0.80	0.70	0.90
PFNA	924 (100.0)	924	0.39 (0.29, 0.52)	0.39 (0.29, 0.52)	0.94	0.85	1.04	0.91	0.82	1.01
PFDA	642 (69.5)	866	0.09 (0.04, 0.15)	0.10 (0.05, 0.15)	0.96	0.87	1.05	0.92	0.83	1.02
PFUnDA	868 (93.9)	918	0.22 (0.13, 0.33)	0.22 (0.13, 0.33)	0.95	0.85	1.06	0.87	0.77	0.97
PFDoDA	203 (22.0)	840	0.04 (0.03, 0.05)	0.04 (0.02, 0.05)	0.93	0.83	1.04	0.87	0.76	0.99
PFTrDA	216 (23.4)	718	0.04 (0.03, 0.05)	0.04 (0.02, 0.06)	1.00	0.90	1.13	0.96	0.85	1.08
PFTeDA	6 (0.01)	--	--	--						
Perfluorinated Sulfonates										
PFHxS	922 (99.8)	923	0.60 (0.44, 0.86)	0.60 (0.44, 0.87)	0.94	0.88	1.01	0.94	0.88	1.01
PFHpS	811 (87.8)	911	0.13 (0.09, 0.19)	0.13 (0.09, 0.19)	0.85	0.76	0.95	0.86	0.77	0.96
PFOS	924 (100.0)	924	13.04 (10.30, 16.58)	13.04 (10.30, 16.58)	0.88	0.79	0.98	0.87	0.78	0.97

PFOSA: perfluorooctane sulfonamide; PFBA: perfluorobutanoic acid; PFHpA: Perfluoroheptanoic acide; PFOA: perlurooctanoic acid; PFNA: perfluorononanoic acid; PFDA: perfluorodecanoic acid; PFUNDA: perfluoroundecanoic acid; PFDoDA: perfluordodecanoic acid; PFTrDA: perfluorotridecanoic acid; PFTeDA: perfluorotridecanoic acid; PFHxS: perfluorohexane sulfonate; PFHpS: perfluoroheptane sulfonate; PFOS:

[a]FORs represent the odds of conception in a given month per interquartile increase in PFAS (ng/ml) concentration, based on the interquartile distance corresponding to Median B.

[b]Although these analyses include all women (primiparous and parous), we believe the results that include parous women are biased, and the reasoning is explained in the second paragraph of the Methods section of the paper and in eFigure 1.

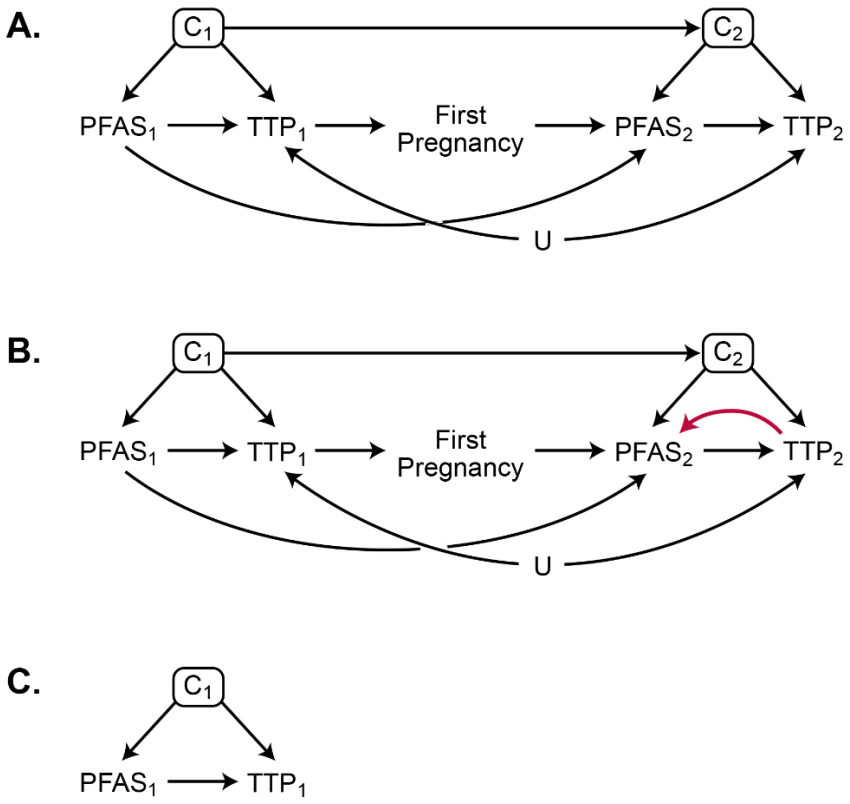
[c]The LOQ for PFBA was 0.1 ng/ml; the LOQ for all other compounds was 0.05 ng/ml.

[d]Indicates the number of observations included in the FOR analsis (i.e., this is the number of women with PFAS concentration >LOQ plus women with measured PFAS concentration <LOQ). We did not analyze PFBA, PFHpA, or PFTeDA for lack of observations.

[e]Medians were calculated among all 924 women, assigning a value equal to the LOQ/sqrt(2) for non-measured PFAS concentrations.

[f]Medians were calculated among women included in the FOR analysis (i.e., women with PFAS concentration >LOQ plus women with measured PFAS concentration <LOQ).

[g]Adjusted for maternal age at conception and pre-pregnancy BMI. The explanation for why we did not adjust for previous pregnancy in this analyis is found in eFigure 1.



eFigure 1. Graphs of the association between perfluoroalkyl substances (PFAS) and time-to-pregnancy (TTP). These graphs were modified from related ones in Bach et al.[4] and Howards et al.[5]

In Panel A, the directed acyclic graph (DAG) shows PFAS exposure before the first pregnancy ($PFAS_1$) causes longer time-to-first-pregnancy (TTP_1), and exposure before the second pregnancy ($PFAS_2$) causes longer time-to-second-pregnancy (TTP_2). In addition, TTP_1 causes the first pregnancy; this first pregnancy and $PFAS_1$ are causes of $PFAS_2$, a set of measured confounders (C_1 and C_2) acts on the PFAS→TTP causal paths, and TTP_1 and TTP_2 have an unmeasured shared cause (U). Note that the path $PFAS_2$←$PFAS_1$→TTP_1←U→TTP_2 is partially blocked because first pregnancy (i.e., parity) is a child of the collider TTP_1. By adjusting for parity, the path through the collider TTP_1 would be opened and an association between $PFAS_2$ and TTP_2 induced.

Panel B shows the same figure as in Panel A, but with the addition of the feedback loop pathway, by which TTP_2 increases $PFAS_2$ (see manuscript for an explanation, second paragraph in the Methods section). Unlike DAG A, which uses an informal x-axis for time (i.e., $PFAS_2$ is depicted to the left of TTP_2) and in which there is no violation of the DAG assumption of no feedback loops between an outcome and its cause, the figure in Panel B does violate this assumption. In other words, with parous women in the analysis, the $PFAS_2$→TTP_2 path has no causal interpretation. If data were available on time from delivery of the first pregnancy to the beginning of attempting the second pregnancy, and on breastfeeding during this period, the diagram could be redrawn as a more complicated time-dependent DAG. Measurement error for these two factors, however, might still bias the $PFAS_2$→TTP_2 relationship.

Panel C shows a DAG with the simpler relation among variables for primiparous women, which complies with the assumptions of causal graphs and in which the potential source of bias from previous pregnancies is no longer a problem.

References

1. Brantsaeter AL, Whitworth KW, Ydersbond TA, Haug LS, Haugen M, Knutsen HK, Thomsen C, Meltzer HM, Becher G, Sabaredzovic A, Hoppin JA, Eggesbo M, Longnecker MP. Determinants of plasma concentrations of perfluoroalkyl substances in pregnant Norwegian women. *Environ Int* 2013;**54**:74-84.
2. Whitworth KW, Haug LS, Baird DD, Becher G, Hoppin JA, Skjaerven R, Thomsen C, Eggesbo M, Travlos G, Wilson R, Longnecker MP. Perfluorinated compounds and subfecundity in pregnant women. *Epidemiology* 2012;**23**(2):257-63.
3. Ding J, Zhou H, Liu Y, Cai J, Longnecker MP. Estimating effect of environmental contaminants on women's subfecundity for the MoBa study data with an outcome-dependent sampling scheme. *Biostatistics* 2014;**15**(4):636-50.
4. Bach CC, Bech BH, Nohr EA, Olsen J, Matthiesen NB, Bossi R, Uldbjerg N, Bonefeld-Jorgensen EC, Henriksen TB. Serum perfluoroalkyl acids and time to pregnancy in nulliparous women. *Environ Res* 2015;**142**:535-41.
5. Howards PP, Schisterman EF, Poole C, Kaufman JS, Weinberg CR. "Toward a clearer definition of confounding" revisited with directed acyclic graphs. *Am J Epidemiol* 2012;**176**(6):506-11.