Online Supplementary Material to: "Perfluoroalkyl Chemicals, Menstrual Cycle Length and Fecundity: Findings from a Prospective Pregnancy Study"

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eAppendix A. Model Specification and Assumptions

Here we present the details of modeling the relations between serum concentrations of perfluoroalkyl substances (PFASs) and both menstrual cycle length and the probability of pregnancy using a Bayesian joint model.[1] As an illustration of the use of our model, here we will focus on one PFAS: perfluorononanoate (PFNA).

A.1. Model for Menstrual Cycle Length

For the i^{th} $(i = 1, \ldots, n)$ woman, we modeled the length of the j^{th} $(j = 1, \ldots, n_i)$ menstrual cycle in relation to serum PFNA concentration (<0.1 ng/mL (reference), 0.1-1.4 ng/mL ('tertile 2'), $\geq$ 1.5 ng/mL ('tertile 3')) using a hierarchical accelerated failure time model

$$
\begin{aligned}
[Y_{ij} \mid \mathbf{v_i}^\top \boldsymbol{\eta}, W_i, \epsilon_{ij}] =& \exp(\mathbf{v_i}^\top \boldsymbol{\eta}) \times W_i \times \epsilon_{ij}, \\
\mathbf{v_i}^\top \boldsymbol{\eta} =& \eta_1 \text{PFNAtertile2}_i + \eta_2 \text{PFNAtertile3}_i + \eta_3 \text{Age}_i + \eta_4 \text{Smoke}_i \\
&+ \eta_5 \text{BMIcategory1}_i + \eta_6 \text{BMIcategory3}_i + \eta_7 \text{BMIcategory4}_i, \\
[W_i \mid \sigma_W] \sim& \text{Gamma}(\text{shape} = 1/\sigma_W^2, \text{rate} = 1/\sigma_W^2), \\
[\epsilon_{ij} \mid q, \mu_1, \sigma_1, \mu_2, \sigma_2] \sim& q f_1(\epsilon_{ij} \mid \mu_1, \sigma_1) + (1-q) f_2(\epsilon_{ij} \mid \mu_2, \sigma_2), \\
f_1(\epsilon \mid \mu_1, \sigma_1) =& \mathcal{N}(\mu_1, \sigma_1), \\
f_2(\epsilon \mid \mu_2, \sigma_2) =& \text{Gumbel}(\mu_2, \sigma_2) = \frac{\pi}{\sigma_2 \sqrt{6}} \exp(-z - e^{-z}), \\
z =& \frac{\epsilon - (\mu_2 - c\frac{\sigma_2\sqrt{6}}{\pi})}{\frac{\sigma_2\sqrt{6}}{\pi}};
\end{aligned} \tag{1}
$$

where the association between cycle length and PFNA is estimated by the acceleration factor (AF= $\exp(\eta_1), \exp(\eta_2)$) and adjusted for age (years), active smoking status based on serum cotinine (<10 ng/mL (reference), $\geq$10 ng/mL)[2], and body mass index (BMI,

<18.5 kg/m^2 ('category 1'), 18.5-24.9 kg/m^2 (reference), 25-29.9 kg/m^2 ('category 3'), ≥ 30 kg/m^2 ('category 4')). To avoid over-determination, we did not include an intercept in the fixed effects vector, $\mathbf{v_i}$. We included a latent woman-specific random effect, W_i, to account for within-woman correlation of cycle lengths and unexplained variability. We assumed the random effect is from a Gamma distribution with mean 1.0 and unknown standard deviation σ_W. To allow for the possibility of extremely short or long menstrual cycles we modeled the error variables, denoted ϵ_{ij}, using a mixture distribution comprised of a Gaussian distribution with mean μ_1 and standard deviation σ_1 and a Gumbel distribution with mean μ_2 and standard deviation σ_2 where c in (1) is Euler's constant.[1,3,4] Lum et al. have previously assessed the goodness of fit of this model (see Web Appendix C).[1] While there is a non-zero probability of a negative cycle length due to the Gaussian component of the model, this probability is virtually zero in our menstrual cycle length application where μ_1 is on the order of 29 days, σ_1 is approximately 2 days and q is about 0.80 (see e.g. Table 1 in Lum et al.).[1] An alternative to this Gaussian-Gumbel mixture error distribution is the log-normal error distribution as applied to cycle length data by Huang et al.;[5] however, we found that the Gaussian-Gumbel mixture provided a better fit than the log-normal to the menstrual cycle length data observed in the LIFE Study (see Web Appendix C).[1]

As fully described elsewhere,[1,4] we also accounted for length-bias in the enrollment cycle and right censoring of the length of the cycle in which the couple becomes pregnant. Briefly, couples were enrolled in the LIFE Study on an arbitrary day of the menstrual cycle. To account for length-bias, we model the length of the enrollment cycle, Y_{i1}, by

$$f_{Y_1}(y_{i1} \mid w_i, \mathbf{v_i}; q, \mu_1, \sigma_1^2, \mu_2, \sigma_2^2, \eta) = \frac{y_{i1} f(y_{i1} \mid w_i, \mathbf{v_i}; q, \mu_1, \sigma_1^2, \mu_2, \sigma_2^2, \eta)}{E(Y_i \mid W_i, e^{\mathbf{v_i}^\top \boldsymbol{\eta}}, q, \mu_1, \mu_2)}$$

where f is the density of $Y_{ij}, j > 1$ and

$$E(Y_i \mid W_i, e^{\mathbf{v_i}^\top \boldsymbol{\eta}}, q, \mu_1, \mu_2) = W_i e^{\mathbf{v_i}^\top \boldsymbol{\eta}} \{q\mu_1 + (1-q)\mu_2\}.$$

To account for right-censoring of the length of the cycle in which the couple becomes pregnant, we let τ_{in_i} denote the time (in days) from the first day of the n_ith cycle to censoring at the ovulation day detected by the fertility monitor. The contribution to the likelihood for post-enrollment cycles is $\{1 - F(\tau_{in_i} \mid w_i, \mathbf{v_i}; q, \mu_1, \sigma_1^2, \mu_2, \sigma_2^2, \eta)\}$; whereas for enrollment cycles, to account for both length-bias and right-censoring, the contribution is

$$\frac{1 - F(\tau_{in_i} \mid w_i, \mathbf{v_i}; q, \mu_1, \sigma_1^2, \mu_2, \sigma_2^2, \eta)}{E(Y_i \mid W_i, e^{\mathbf{v_i}^\top \boldsymbol{\eta}}, q, \mu_1, \mu_2)}.$$

Here, F is the CDF of $Y_{ij}, j > 1$.

Let $\phi_Y = (\eta_1, \ldots, \eta_7, \sigma_W, q, \mu_1, \sigma_1, \mu_2, \sigma_2)$ denote the parameters of the menstrual cycle length model. We assumed each of the parameters are independent *a priori*, such that $[\boldsymbol{\phi_Y}] = [\sigma_W][q][\mu_1][\sigma_1][\mu_2][\sigma_2] \prod_{r=1}^{7} [\eta_r]$. We selected uniform priors for each of the components of ϕ_Y with hyperparameters scaled to determine vague priors.

Using the model in (1) we estimated the woman's typical cycle length, denoted Y_i^*, from the woman-specific posterior predictive distribution of cycle length conditional on the woman's observed cycle lengths, baseline covariates, random effect, priors, and hyper-priors. We then included Y_i^* in the pregnancy model to adjust for cycle length when modeling the etiologic relation between PFNA and the probability of pregnancy.

A.2. Model for the Probability of Pregnancy

For the ij^{th} cycle, we let A_{ij} denote the pregnancy indicator with $A_{ij} \equiv 0, j < n_i$ and $\mathbf{z_i}$ denote a vector of covariates of interest including the PFNA indicator variables and potential confounders. We let x_{ijk} denote the intercourse indicator on day k of the j^{th} cycle for $(k = 1, \ldots, \ddot{Y}_{ij})$ with $\ddot{Y}_{ij}$ the greatest integer function of Y_{ij}. Let d_{ijk} denote the time elapsed (days) from intercourse day to ovulation day. We modeled the relation between PFNA and the probability of pregnancy adjusted for cycle length and intercourse using the

following hierarchical model

$$\rho_{ijk}(Y_i^*, \mathbf{z_i}, d_{ijk}) = \Pr(\text{Pregnancy by intercourse on day } k \mid \text{not previously}, Y_i^*, \mathbf{z_i}, x_{ijk} = 1, d_{ijk}),$$

$$\Pr(A_{ij} = 1 \mid \text{not pregnant in previous cycles}, Y_i^*) = 1 - \prod_{l=1}^{\ddot{Y}_{ij}} \{1 - \rho_{ijl}(Y_i^*, \mathbf{z_i}, d_{ijl})\}^{x_{ijl}},$$

$$\begin{aligned} \text{logit}\{\rho_{ijk}(Y_i^*, \mathbf{z_i}, d_{ijk})\} =& \mathbf{z_i}^\top \boldsymbol{\gamma} + \beta_1 Y_i^* + \beta_2 (Y_i^*)^2 + g(d_{ijk}), \qquad (2) \\ \mathbf{z_i}^\top \boldsymbol{\gamma} =& \gamma_1 \text{PFNAcategory1}_i + \gamma_2 \text{PFNAcategory2}_i \\ & + \gamma_3 \text{Age}_i + \gamma_4 \text{Smoke}_i \\ & + \gamma_5 \text{BMIcategory1}_i + \gamma_6 \text{BMIcategory3}_i + \gamma_7 \text{BMIcategory4}_i; \end{aligned}$$

where we adjusted for cycle length using linear and quadratic terms of Y_{ij}^* and for intercourse timing using a smooth function $g(d_{ijk})$ estimated by $\hat{g}(\cdot) = \alpha_0 + \sum_{l=1}^{L} \alpha_l B_l(\cdot)$ for which α_0 is the intercept and $\{B_1(\cdot), \ldots, B_L(\cdot)\}$ are the B-spline basis functions for a natural cubic spline with 8 knots placed at locations based on percentiles of day of intercourse. We also adjusted for female age, active smoking status and BMI category at enrollment as was done in the menstrual cycle length model.

We incorporated Y_i^* in (2) by mixing over the posterior predictive distribution for Y_i^* given the woman's observed cycle lengths, baseline covariates, random effect, priors, and hyper-priors. This joint modeling approach accounts for the uncertainty in estimating the woman's typical cycle length.

Let $\boldsymbol{\phi_A} = (\boldsymbol{\beta}, \boldsymbol{\gamma}, \boldsymbol{\alpha})$ denote the parameters of the pregnancy model. We assumed the components of $\boldsymbol{\phi_A}$ are independent *a priori* and are also independent of $\boldsymbol{\phi_Y}$. We completed the model specification by choosing noninformative uniform priors for each parameter in $\boldsymbol{\phi_A}$. For each coefficient in $\boldsymbol{\beta}$ and $\boldsymbol{\gamma}$, we found that a uniform prior distribution of $\mathcal{U}(-2, 2)$ was sufficiently wide. For each of the α components, we specified a much larger interval for the prior distributions: $\mathcal{U}(-1000, 1000)$.

A.3. Extension of Model for the Probability of Pregnancy

In this section we present an extension of the model for the probability of pregnancy to allow for an additional couple-specific random effect (recall the model in eAppendix A.2 included a single random effect, Y_i^*). We consider the following augmentation of the model in equation (2) of eAppendix A.2

$$\text{logit}[\rho_{ijk}\{U_i, Y_i^*, \mathbf{z_i}, d_{ijk}\}] = U_i + \mathbf{z_i}^\top \boldsymbol{\gamma} + \beta_1 Y_i^* + \beta_2 (Y_i^*)^2 + g(d_{ijk}),$$
$$U_i \sim \mathcal{N}(0, \sigma_u);$$

where U_i is the additional couple-specific random effect with mean 0 and σ_u is its unknown standard deviation. We assume U_i and Y_i^* are independent *a priori* and choose a non-informative uniform prior on the standard deviation: $\sigma_u \sim \mathcal{U}(0, 10)$.

eTable 1 displays results for this model, with a side by side comparison with the single random effect model presented in the main paper. Overall, the estimates from the models are very similar. The main finding from the multiple PFAS model of a negative association between PFNA and the probability of pregnancy (OR=0.64 [0.35,1.00]) when comparing women in the second (but not third) tertile versus first tertile was also observed in the model with the second random effect (0.58 [0.29,0.98]). In addition, using the model with two random effects, we observed a significant negative association with the probability of pregnancy comparing women with PFOSA concentration above the LOD to those with PFOSA concentration below the LOD (single PFAS model: 0.55 [0.30,0.99], multiple PFAS model: 0.50 [0.23,0.91]).

eTable 1. Associations between serum PFAS and day-specific probability of pregnancy with adjustment for cycle length, estimated using joint model with one or two random effects, LIFE Study, 2005-2009. Single PFAS models were fit separately for each PFAS while the multiple PFAS model included all PFASs in one model. All associations are adjusted for couple intercourse pattern, menstrual cycle length, female age, BMI, active smoking at enrollment.

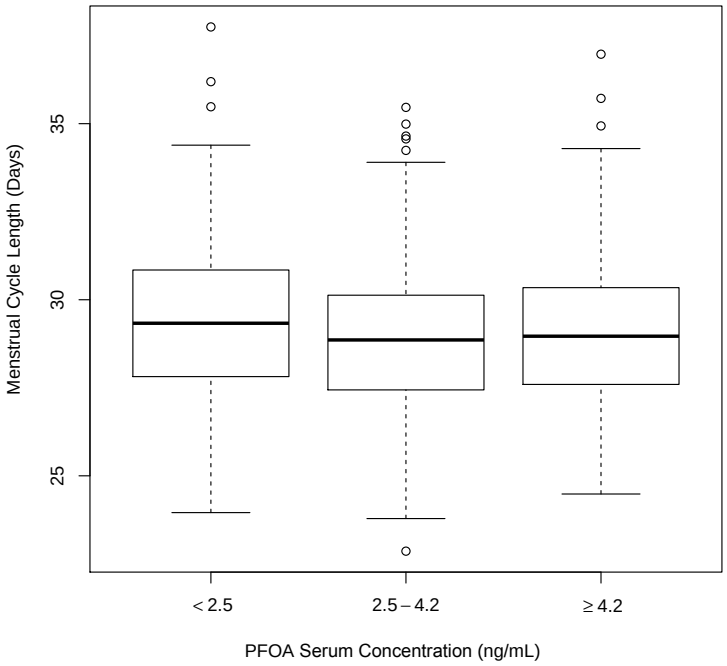
	Single PFAS Model		Multiple PFAS Model	
	One Random Effect	Two Random Effects	One Random Effect	Two Random Effects
	Adjusted	Adjusted	Adjusted	Adjusted
Covariate	OR (95% CrI)	OR (95% CrI)	OR (95% CrI)	OR (95% CrI)
Cycle Length				
Linear (days)	1.40 (0.99, 1.92)	1.33 (0.91, 1.93)	1.54 (1.10, 2.17)	1.21 (0.86, 1.73)
Quadratic (days^2)	0.71 (0.56, 0.87)	0.77 (0.58, 0.99)	0.76 (0.57, 0.94)	0.90 (0.70, 1.07)
PFAS (ng/mL)				
PFOSA				
LOD+ ($\geq$ 0.10)	0.63 (0.35, 1.12)	0.55 (0.30, 0.99)	0.59 (0.27, 1.03)	0.50 (0.23, 0.91)
Et-PFOSA-AcOH				
Q3+ ($\geq$0.06)	0.88 (0.60, 1.29)	0.84 (0.57, 1.27)	0.91 (0.57, 1.32)	0.89 (0.55, 1.33)
Me-PFOSA-AcOH				
Tertile 2 (0.02-0.03)	1.09 (0.74, 1.68)	1.07 (0.68, 1.69)	1.12 (0.70, 1.63)	1.13 (0.66, 1.74)
Tertile 3 ($\geq$ 0.04)	0.98 (0.65, 1.44)	0.93 (0.60, 1.45)	1.03 (0.62, 1.53)	1.05 (0.58, 1.62)

eTable 1 (continued). Associations between serum PFAS and day-specific probability of pregnancy with adjustment for cycle length, estimated using joint model with one or two random effects, LIFE Study, 2005-2009. Single PFAS models were fit separately for each PFAS while the multiple PFAS model included all PFASs in one model. All associations are adjusted for couple intercourse pattern, menstrual cycle length, female age, BMI, active smoking at enrollment.

	Single PFAS Model		Multiple PFAS Model	
	One Random Effect	Two Random Effects	One Random Effect	Two Random Effects
	Adjusted	Adjusted	Adjusted	Adjusted
PFAS (ng/mL)	OR (95% CrI)	OR (95% CrI)	OR (95% CrI)	OR (95% CrI)
PFDeA				
Tertile 2 (0.03-0.04)	0.71 (0.47, 1.06)	0.76 (0.49, 1.21)	0.89 (0.49, 1.40)	1.00 (0.51, 1.66)
Tertile 3 ($\geq$ 0.05)	0.90 (0.60, 1.34)	1.03 (0.66, 1.63)	1.40 (0.68, 2.47)	1.65 (0.66, 3.08)
PFNA				
Tertile 2 (0.10-1.40)	0.69 (0.46, 1.03)	0.68 (0.45, 1.05)	0.64 (0.35, 1.00)	0.58 (0.29, 0.98)
Tertile 3 ($\geq$ 1.50)	0.81 (0.56, 1.15)	0.88 (0.59, 1.32)	0.67 (0.32, 1.14)	0.67 (0.27, 1.24)
PFOS				
Tertile 2 (9.50-15.10)	1.01 (0.69, 1.52)	0.96 (0.63, 1.46)	1.24 (0.76, 1.84)	1.23 (0.69, 1.90)
Tertile 3 ($\geq$ 15.20)	0.90 (0.61, 1.30)	0.91 (0.60, 1.37)	1.14 (0.65, 1.82)	1.17 (0.56, 1.91)
PFOA				
Tertile 2 (2.50-4.10)	1.00 (0.68, 1.51)	1.01 (0.66, 1.57)	1.11(0.65, 1.69)	1.05 (0.57, 1.67)
Tertile 3 ($\geq$ 4.20)	0.72 (0.50, 1.06)	0.76 (0.50, 1.15)	0.75 (0.42, 1.19)	0.70 (0.35, 1.18)

eTable 2. Pearson product-moment correlation coefficients between serum concentrations of PFASs, LIFE Study, 2005-2009.

	Me-PFOSA-AcOH	PFDeA	PFNA	PFOSA	PFOS	PFOA
Et-PFOSA-AcOH	0.03	0.04	0.05	0.03	0.06	0.05
Me-PFOSA-AcOH		0.03	0.10	0.46	0.24	0.09
PFDeA			0.73	-0.03	0.56	0.55
PFNA				-0.06	0.60	0.60
PFOSA					0.13	-0.06
PFOS						0.45



eFigure 1. Boxplots of menstrual cycle length (days) by tertile of serum PFOA concentration (ng/mL), LIFE Study, 2005-2009.

References

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