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Supplemental Material

Metabolic Signatures of Youth Exposure to Mixtures of Per- and Polyfluoroalkyl Substances: A Multi-Cohort Study

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Table S1. PFAS concentrations ($\mu\text{g/L}$) for the low and high counterfactual exposure profiles used to calculate the overall mixture effect for the metabolome wide association study. The geometric mean and 95% confidence intervals are provided for additional context. In this study, the low exposure profile is defined as setting each log-transformed and standardized PFAS at a z-score of -0.5 ($\chi^* = -0.5$) and the high exposure profile is defined as setting each log-transformed and standardized PFAS at a z-score of 0.5 (z-score of 0.5 ; $\chi^* = 0.5$).

Table S2. Geometric mean and 95% confidence intervals of PFAS concentrations ($\mu\text{g/L}$) in overweight and obese adolescents from the SOLAR cohort, young adults from the CHS cohort, compared to PFAS levels in young persons aged 12-19 years old from the National Health and Nutrition Examination Survey (NHANES) years 2007-2008 and 2017-2018.

Table S3. Metabolic pathways associated with exposure to a mixture of six PFAS in adolescents from the SOLAR cohort ($n = 312$) and young adults from the CHS cohort ($n = 137$). Meta-analysis p values are provided for pathways associated with PFAS in both cohorts. Pathway enrichment was performed using MetaboAnalyst version 5.0. The number of significant empirical compounds may not match the number of metabolites presented in figures 2-5 (main text) because MetaboAnalyst can annotate individual empirical compounds to multiple pathways and because individual LC-MS features may map to multiple empirical compounds.

Table S4. Effect estimates of individual annotated metabolites associated with exposure to a mixture of six PFAS in overweight and obese adolescents from the SOLAR cohort (n = 312), in young adults from the CHS cohort (n = 137), and in a pooled analysis with both the SOLAR and CHS cohorts. Effect estimates for PFAS mixture (ψ) and the 95% Bayesian credible interval (BCI) estimate the change in metabolite levels (SD of the log transformed feature intensity) when increasing all PFAS in the mixture from the 30th percentile to the 70th percentile. This estimate is also equivalent to a standardized mean difference calculated between a hypothetical group of individuals with all PFAS at the ~70th percentile versus a hypothetical group of individuals with all PFAS at the ~30th percentile.

Table S5. Metabolic pathways associated with exposure to a mixture of six PFAS in the pooled analysis of adolescents from the SOLAR cohort (n = 312) and young adults from the CHS cohort (n = 137). Pathway enrichment was performed using MetaboAnalyst version 5.0.

Figure S1. Directed Acyclic Graph (DAG) showing the covariates included in the models between PFAS exposure and metabolites.

Figure S2. Correlation between plasma PFAS concentrations in A) adolescents from the SOLAR cohort (n = 312) and B) young adults from the CHS cohort (n = 137). The upper triangle shows the pairwise spearman correlation coefficient for all PFAS, the lower triangle shows a scatter plot between each pair of PFAS, and the diagonal shows a density plot of PFAS concentrations in each cohort.

Figure S3. Heatmap showing the posterior inclusion probabilities (PIPs) between individual PFAS and metabolites associated with the metabolism of aromatic amino acids in A) adolescents from the SOLAR cohort (n = 312) and B) young adults from the CHS cohort (n = 137). The PIP is the posterior probability that the coefficient is non-zero, and higher PIPs suggest that the specific PFAS congener is more likely to have a causal effect in the true model. PIPs greater than 1/6 (~0.167) indicate a greater likelihood that the individual PFAS has a non-zero effect on the overall mixture; PIPs > 1/6 are labeled with text. Metabolites are grouped by aromatic amino acid metabolism sub pathways, indicated on the right of the plot.

Figure S4. Heatmap showing the posterior inclusion probabilities (PIPs) between individual PFAS and metabolites associated with lipid metabolism pathways in A) adolescents from the SOLAR cohort (n = 312) and B) young adults from the CHS cohort (n = 137). The PIP is the posterior probability that the coefficient is non-zero, and higher PIPs suggest that the specific PFAS congener is more likely to have a causal effect in the true model. PIPs greater than 1/6 (~0.167) indicate a greater likelihood that the individual PFAS has a non-zero effect on the overall mixture; PIPs > 1/6 are labeled with text.

Figure S5. Heatmap showing the posterior inclusion probabilities (PIPs) between individual PFAS and metabolites associated with the metabolism of non-aromatic amino acids in A) adolescents from the SOLAR cohort (n = 312) and B) young adults from the CHS cohort (n = 137). The PIP is the posterior probability that the coefficient is non-zero, and higher PIPs suggest that the specific PFAS congener is more likely to have a causal effect in the true model. PIPs greater than 1/6 (~0.167) indicate a greater likelihood that the individual PFAS has a non-zero effect on the overall mixture; PIPs > 1/6 are labeled with text. Metabolites are grouped by non-aromatic amino acid metabolism pathways, indicated on the right of the plot.

Figure S6. Heatmap showing the posterior inclusion probabilities (PIPs) between individual PFAS and metabolites associated with metabolism of cofactors in adolescents from the SOLAR cohort (n = 312). No significant associations were observed in the CHS cohort. The PIP is the posterior probability that the coefficient is non-zero, and higher PIPs suggest that the specific PFAS congener is more likely to have a causal effect in the true model. PIPs greater than 1/6 (~0.167) indicate a greater likelihood that the individual PFAS has a non-zero effect on the overall mixture; PIPs > 1/6 are labeled with text.

Supplemental Code. R code used to generate the function for the Bayesian Hierarchical Regression Model with g-computation (BHRM-g). The complete code for this analysis can be found at github.com/chatzilab/PFAS_metabolomics_EHP_2022.

Additional File- Excel Document