Exploring Potential Differences in Developmental Toxicology of Per- and Polyfluoroalkyl Substances (PFAS) Through Targeted Metabolomics

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Introduction

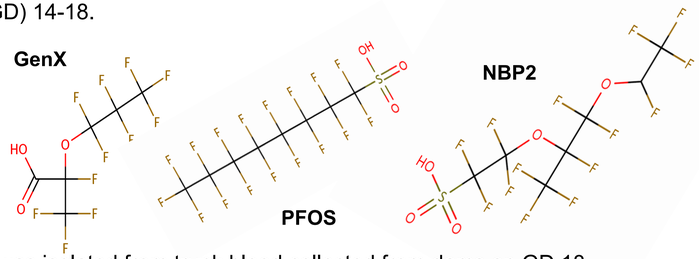
Per- and polyfluoroalkyl substances (PFAS) are:

- Synthetic chemicals with multiple, diverse uses: food packaging, stain-resistant coatings, fire-fighting foams, and outdoor fabrics.
- Pervasive, leading to human and environmental exposures, bioaccumulation, and potential adverse health effects.

The EPA is conducting PFAS toxicity assessments to understand potential risks that may threaten human health, especially the health of vulnerable populations. We investigated metabolomic changes from 5-day gestational exposure of rats to three different PFAS that may inform molecular mechanisms and key events associated with potential developmental toxicity.

Methods

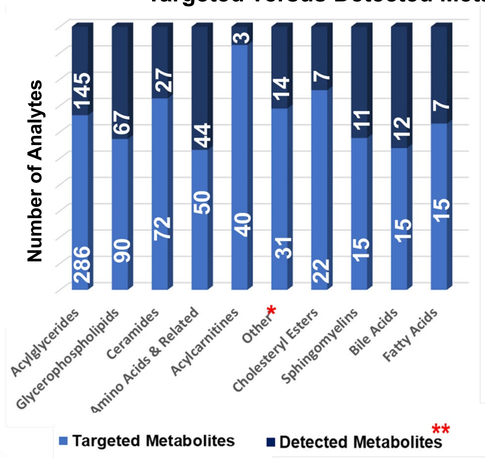
- Pregnant Sprague-Dawley rats were dosed via oral gavage with hexafluoro-propylene oxide-dimer acid (GenX) (Conley *et al.,* 2019), perfluorooctanesulfonic acid (PFOS) or Nafion byproduct-2 Nafion byproduct-2 (NBP2) over gestation days (GD) 14-18.



- Serum was isolated from trunk blood collected from dams on GD 18.
- Aliquots (10 µL) were prepared and analyzed using a Biocrates (Innsbruck, Austria) MxP Quant 500 kit.
- Data were acquired in positive and negative ion mode with electrospray ionization for > 600 metabolites on a Sciex (Framingham, MA) 6500+ Qtrap.
- Raw data were processed using Sciex MultiQuant 3.0.2 and Biocrates MetIDQ, Oxygen version.
- Analytes observed in ≥80% of samples were evaluated for changes relative to controls and one-way ANOVA analysis using GraphPad Prism and MetIDQ.

Results

Targeted versus Detected Metabolites



The table to the left shows the number of analytes targeted (light blue) and detected (dark blue) in columns by chemical class. Of the total 636 targeted metabolites, 337 (53%) were detected in at least 80% of the samples. The highest percent coverage was obtained for amino acids and related compounds (88% coverage (44 detected of 50 targeted)), glycerophospholipids (74% (67 of 90)), and sphingomyelins (73% (11 of 15)). All other metabolite classes gave 50% or lower detection coverage. Lowest coverage was for acylcarnitines (7.5% (3 of 40)).

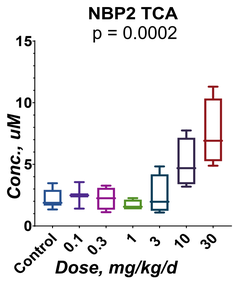
***: Hexoses, amine oxides, alkaloids, indoles, biogenic amines, vitamins, nucleobases, and hormones**

****: Metabolites detected in ≥ 80% of samples**

Metabolic Changes – NBP2

Significant ($p < 0.05$) increases or decreases were observed for **only a few metabolites**: elevated bile acids TCA (taurocholic acid) and TCDCA (taurochenodeoxycholic acid); and decreased 3-IPA (3-indolepropionic acid).

- Elevated bile acids, including TCA and TCDCA, may be biomarkers of intra-hepatic cholestasis of pregnancy, a common human gestational liver disease (Chen *et al*., 2013).
- 3-IPA is an anti-oxidant formed during tryptophan metabolism by gut microbiota.



Metabolic Changes – GenX and PFOS

Significant changes ($p < 0.05$) were observed for **many metabolites.**

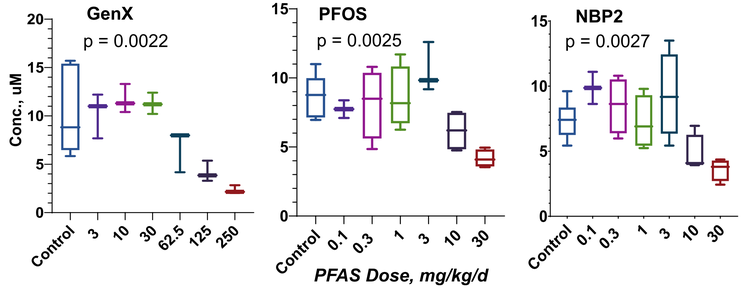
- GenX-exposed serum: decreased amino acids Arg, Thr, and Trp, bile acid TCDCA, indole derivatives 3-IAA (3-indoleacetic acid) and 3-IPA, several phosphatidylcholines (PCs), and others.
- PFOS-exposed serum: decreased Trp, TCDCA, 3-IAA, 3-IPA, several PCs, and others.

Metabolic Changes Common to GenX, PFOS, and NBP2

Changes were observed for several metabolites, but often not in the same direction. For example, bile acids increased for NBP2 and decreased for GenX and PFOS.

- 3-IPA , an anti-oxidant formed during tryptophan metabolism, decrease was consistent for the three PFAS.

3-IPA Response after PFAS Exposure



Conclusions

Metabolic changes observed here after PFAS exposure suggest dose-related impacts to bile acid, lipid, and amino acid metabolism. Additional research is needed to investigate health effects, if any, of these changes to pregnant rats.

References

Chen *et al*., (2013) Int J Gynecol Obstet **122**(1):5. doi: 10.1016/j.ijgo.2013.02.015.

Conley *et al.* (2019). Environ Health Perspect **127**(3): 37008. doi: 10.1289/EHP4372.