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Supplemental Material

A Roadmap to the Structure-Related Metabolism Pathways of Per- and Polyfluoroalkyl Substances in the Early Life Stages of Zebrafish (*Danio rerio*)

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Table S1. Chemical information and structures of 74 PFAS from the U.S. EPA PFASs screening library.

Table S2. Among 74 PFAS, 36 ionizable PFAS were subjected for BCF analysis and 25 PFAS were detectable in zebrafish larvae. 31 PFAS from five structural categories prone to metabolism were selected for metabolite profiling with non-targeted analysis.

Table S3. Summary of 28 PFAS that were detectable in zebrafish larvae, including concentrations in medium (μM), recovery (%) and method detection limit (MDL) (ng/g).

Table S4. Bioaccumulations and toxicities of 74 PFAS in early life stage (ELS) of zebrafish. Bioconcentration factors (BCFs, L/kg) are shown for PFAS at both $0.5\mu\text{M}$ and $5\mu\text{M}$. Mortality was only shown for higher concentrations of $5\mu\text{M}$, $N=12-20$.

Table S5. Information of nine PFAS considered as high affinity liver fatty acid binding protein (L-FABP) ligands ($K_d < 20\mu\text{M}$) (Yang et al. 2020).

Table S6. Peak intensities of metabolites detected in zebrafish larvae exposed to 15 fluorotelomer alcohols (FTOHs) at $5\mu\text{M}$ and 120 hpf.

Figure S1. Relationships between BCFs and exposure concentrations. (A) Comparison of the BCFs of PFAS in zebrafish larvae at 120 hpf, at $0.5\mu\text{M}$ and $5\mu\text{M}$. Dots represent mean \pm SD ($n=3$). (B) The ratios of BCFs at $5\mu\text{M}$ to $0.5\mu\text{M}$. Notes: PFAS, per- and polyfluoroalkyl substances; BCFs, bioconcentration factor. The summary data can be found in Table S3. The lines from panel A represent stepwise linear trends.

Figure S2. Metabolites of NMe-FOSA and NEt-FOSA. Nontargeted detection of metabolites in zebrafish larvae exposed to 5 μ M of NMe-FOSA (top panel) and NEt-FOSA (bottom panel) was accomplished using the R scripts as detailed in method section. The sizes and colors of the dots are proportional to intensities. PFOS (46) and PFOSA (60) were confirmed with authentic standards (confidence level 1). Notes: NMe-FOSA, N-Methylperfluorooctanesulfonamide; NEt-FOSA, N-Ethylperfluorooctanesulfonamide; PFOSA, perfluorooctanesulfonamide; PFOS, perfluorooctanesulfonic acid.

Figure S3. Metabolites of perfluorooctane sulfonamide quaternary ammonium salt (PFOSAmS, 5). Tentative identification of metabolites from PFOSAmS was accomplished by interpreting the high-resolution MS² spectra of the three most abundant metabolite.

Figure S4. Representative chromatograms of shorter-chain PFCAs in zebrafish larvae metabolized from perfluoroalkyl carboxamides. (A) The chromatograms of 1H-PFPeA metabolized from amide 38 (top), and 1H-PFBA (bottom) directly exposed to zebrafish larvae. (B) The chromatograms of PFPeA metabolized from amide 19 (top), and PFHxA directly exposed to zebrafish larvae. (C) The chromatograms of PFBA metabolized from amide 38 (top), and PFBA directly exposed to zebrafish larvae. 1H-PFBA (4), PFHxA (20), PFBA (12) and PFPeA were confirmed with authentic standards (confidence level 1). The authentic standard of 1H-PFPeA was not available (confidence level 3). Note: PFBA, perfluorobutanoic acid; PFHxA, perfluorohexanoic acid; PFPeA, perfluoropentanoic Acid.

Figure S5. Time courses of carboxamides and polyfluoroalkyl carboxylic acids metabolites. Parent compound and carboxylic acids metabolites of 19, 31 and 38 were monitored separately across development in zebrafish exposed to 5 μ M of each parent carboxamides. The peak areas of polyfluoroalkyl carboxylic acid metabolites were plotted against the left y-axis, while the peak area of carboxamide 38 was plotted against the right y-axis. Perfluorobutanoic acid (PFBA, 12) and perfluoropentanoic acid (PFPeA) were confirmed with authentic standards (confidence level 1). The authentic standard of 1H-PFPeA was not available (confidence level 3). The summary data can be found in Excel Table S8.

Figure S6. Hydrolysis products of 8:2 FTOH. Representative metabolites of 8:2 FTOH were detected in zebrafish larvae exposed to 5 μ M of 8:2 FTOH after 120h exposure. (A) Chromatograms of representative metabolites; (B) Proposed metabolism pathways. PFOA (74) was confirmed with authentic standards (confidence level 1). The authentic standards of 7:3 FTUCA, 8:2 FTUCA, 8:2 FTOH sulfate, 8:2 FTOH Gluc, 7:3 FTUCA cysteine were not available (confidence level 3). Note: FTOHs, fluorotelomer alcohols; FTUCA, fluorotelomer unsaturated carboxylic acids; PFOA, perfluorooctanoic acid.

Figure S7. Metabolites of two FTOH. Metabolites detected in zebrafish larvae exposed to H-6:1 FTOH (67) and 4:4 FTOH (40) were plotted versus the intensities of metabolites from 8:2 FTOH. Blue dots represent metabolites showing significantly higher fold-changes (FC>20, p <0.05, student's t-test) relative to 8:2 FTOH. The authentic standards of 1H-PFHxA, 6:1 FTOH sulfate and 6:1 FTOH glucuronide were not available (confidence level 3). Note: FTOHs, fluorotelomer alcohols; PFHxA, perfluorohexanoic acid.

Figure S8. Taurine metabolites of PFAS 49, 30, and 56 in zebrafish. Taurine conjugates from PFAS were identified by employing high-resolution MS² spectra. The authentic standards of metabolites were not available, so they were tentatively identified at confidence level 3.

Figure S9. Metabolite of 6:2 FTMAC. 6:2 FTOH sulfate was detected in zebrafish larvae exposed to 5 µM of compound 2. The chromatograms of 6:2 FTOH sulfate from compound 2 and the control are shown in the top and bottom panels. Note that minor background contamination was detected from the control. The proposed metabolism pathway of compound 2 is shown on the right. 6:2 FTOH (70) was confirmed with authentic standards (confidence level 1). The authentic standards of 6:2 FTOH sulfate was not available (confidence level 3). Note: FTOHs, fluorotelomer alcohols.

Figure S10. Hydrolysis of polyfluoroalkyl carboxamide 19 by hCES1. Reaction mixtures contained various concentrations 19 and 100 µg/L human recombinant human liver carboxylesterase 1 (*hCES1*) was incubated with 100 µL phosphate buffer at 37 °C for 1 hour, N=3. (A) The percentages of parent PFAS hydrolyzed by *hCES1*. (B) The concentrations of corresponding hydrolysis product perfluoropentanoic acid (PFPeA) detected. One-way ANOVA with Dunnett test was employed for the statistical test. Asterisk indicates *0.01 < p < 0.05, **0.01 < p < 0.001 and ***p < 0.0001. Bars represent mean ± SD (n=3). Notes: The summary data can be found in Excel Tables S9 and S10.

Figure S11. Structures of hydrolysis products detected in human recombinant human liver carboxylesterase 1 (*hCES1*) hydrolysis assay. Perfluorobutanoic acid (PFBA, 12) and perfluoropentanoic acid (PFPeA) were confirmed with authentic standards, the confidence level was assigned to level 1. The authentic standards of 1H-PFPeA and 4:3 fluorotelomer carboxylic acid (FTCA) were not available, so they were confirmed with high resolution MS¹ and MS² spectra at confidence level 3.

References

Additional File- Excel Document