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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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Figure S2. Metabolites of NMe-FOSA and NEt-FOSA. Nontargeted detection of metabolites in zebrafish larvae exposed to 5 μM 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 MS^2 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 μM 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 5μM 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 MS^2 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 (*h*CES1) was incubated with 100 μL phosphate buffer at 37 °C for 1 hour, N=3. (A) The percentages of parent PFAS hydrolyzed by *h*CES1. (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 (*h*CES1) 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 MS^1 and MS^2 spectra at confidence level 3.

References

Additional File- Excel Document