

## Supplementary Materials

Mixture effects of PFAS on embryonic and larval sheepshead minnows (*Cyprinodon variegatus*)

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*There are 5 pages with 1 Supplementary Figure and 3 Supplementary Tables.*

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Table S1. Primer sequences used for qPCR. All forward primers are 5' to 3' and reverse primers are 3' to 5'.

| Gene                            | Direction | Sequence                  | Amplicon | Accession number |
|---------------------------------|-----------|---------------------------|----------|------------------|
| <i><math>\beta</math>-actin</i> | Forward   | AACACTGTGCTGTCTGGAGGTA    | 219      | XM_015378596.1   |
|                                 | Reverse   | AGACGGAGTATTTACGCTCTGG    |          |                  |
| <i>ppara</i>                    | Forward   | GACCATCCGTCTGAAGCTGGAGTAT | 100      | XM_015389442.1   |
|                                 | Reverse   | GCACTTCTGGAAGCGGCAGTATT   |          |                  |
| <i>apoa4</i>                    | Forward   | AGACTCAGGCTGCTCCTCTCA     | 99       | XM_015391647.1   |
|                                 | Reverse   | CCAAAGCGGTCAAATCGTTCTCCA  |          |                  |

Table S2. PFAS analytes, acronyms, diagnostic ions used in the determination of the targeted analytes, depolarization energy (DP), Entrance Potential (EP), collision energy for the product and qualifier ions (CE M<sub>2</sub>, CE M<sub>3</sub>), and Collision Cell Exit Potential (CXP).

| Analyte  | Acronym    | Selected diagnostic ions <i>m/z</i> |                        |                          | DP  | EP  | CE M <sub>2</sub> | EXP | CE M <sub>3</sub> |
|--|------------|-------------------------------------|------------------------|--------------------------|-----|-----|-------------------|-----|-------------------|
|  |            | Precursor M <sub>1</sub>            | Product M <sub>2</sub> | Qualifier M <sub>3</sub> |     |     |                   |     |                   |
| Perfluoro-n-butanoic acid  | PFBA       | 213                                 | 169                    | N/A                      | -70 | -10 | -20               | -16 | N/A               |
| Perfluoro-n-hexanoic acid  | PFHxA      | 313                                 | 269                    | 119                      | -70 | -10 | -10               | -16 | -10               |
| Perfluoro-n-octanoic acid  | PFOA       | 413                                 | 369                    | 169                      | -70 | -10 | -20               | -16 | -30               |
| Perfluoro-1-octanesulfonate  | PFOS       | 499                                 | 80                     | 99                       | -60 | -10 | -60               | -16 | -60               |
| Perfluoro-n-[ <sup>13</sup> C <sub>4</sub> ]-butanoic acid           | 13C4-PFBA  | 217                                 | 172                    | N/A                      | -70 | -10 | -20               | -16 | N/A               |
| Perfluoro-n-[1,2,3,4,6- <sup>13</sup> C <sub>5</sub> ]-hexanoic acid | 13C5-PFHxA | 318                                 | 273                    | N/A                      | -70 | -10 | -10               | -16 | N/A               |
| Perfluoro-n-[ <sup>13</sup> C <sub>8</sub> ]-octanoic acid           | 13C8-PFOA  | 421                                 | 376                    | N/A                      | -70 | -10 | -20               | -16 | N/A               |
| Perfluoro-1-[1,2,3- <sup>13</sup> C <sub>3</sub> ]-hexanesulfonate   | 13C8-PFOS  | 507                                 | 99                     | N/A                      | -60 | -10 | -60               | -16 | N/A               |

Table S3. PFAS concentrations in exposure solutions measured at t=0 and t=24. Percent (%) nominal represents measured concentrations relative to nominal concentrations as a percentage after 0 or 24 h exposure.

| Chemical | Nominal<br>Concentration<br>(mg/L) | Concentration<br>t=0 (mg/L) | % Nominal<br>t=0 | Concentration<br>t=24 (mg/L) | % Nominal<br>t=24 |
|----------|------------------------------------|-----------------------------|------------------|------------------------------|-------------------|
| PFOS     | 1.25                               | 1.42                        | 113              | 1.22                         | 97                |
|          | 2                                  | 1.71                        | 86               | 1.42                         | 71                |
|          | 2.5                                | 1.91                        | 76               | 2.14                         | 85                |
|          | 5                                  | 3.98                        | 80               | 3.38                         | 68                |
|          | 10                                 | 6.40                        | 64               | 7.03                         | 70                |
| PFOA     | 6.25                               | 6.90                        | 110              | 7.82                         | 125               |
|          | 12.5                               | 14.09                       | 113              | 13.8                         | 110               |
|          | 25                                 | 25.55                       | 102              | 21.21                        | 85                |
|          | 50                                 | 46.81                       | 94               | 46.86                        | 94                |
|          | 100                                | 97.87                       | 98               | 88.55                        | 89                |
| PFHxA    | 100                                | 128.2                       | 128              | 111.8                        | 112               |
| PFBA     | 100                                | 126.7                       | 127              | 146.9                        | 147               |

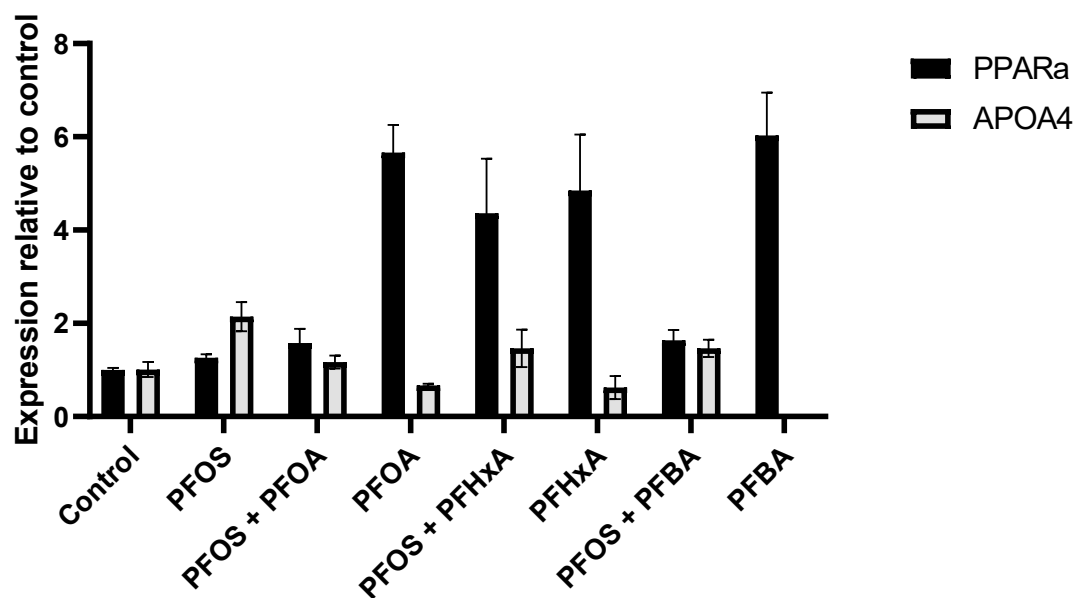


Figure S1. Relative gene expression of *ppara* and *apoa4* following 96 h exposure of larval sheepshead minnows to various PFAS mixtures. PFOS = 2 mg/L and PFOA, PFHxA, and PFBA = 100 mg/L. Values represent the mean of three replicates  $\pm$  SEM.