**Supplemental Information**

Adverse outcomes following exposure to perfluorooctanesulfonamide (PFOSA) in larval zebrafish (*Danio rerio*): A neurotoxic and behavioral perspective.

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**Supplemental Figure S1**. The distance moved in each of the light and dark zones (10-minute bins) of 7-day zebrafish larvae exposed to 0.1% DMSO, ERM, 0.1, 1, 10, or 100 µg/L PFOSA in 3 separate experiments. Mean values are depicted by the columns in each dark-light phase (mean ± S.D.) (N=8-12 fish/treatment/experiment).

**Supplemental Tables:**

**Supplemental Table S1.** Primers used for real-time PCR analysis.

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| --- | --- | --- | --- | --- |
| **Gene name** | **Gene Symbol** | **Forward (5' to 3')** | **Reverse (5' to 3')** | **Reference** |
| Acetylcholinesterase | *ache* | GCTAATGAGCAAAAGCATGTGGGC | TATCTGTGATGTTAAGCAGACGAGGCA | NM\_131846.2 |
| Beta-actin | *bactin* | CGAGCAGGAGATGGGAACC | CAACGGAAACGCTCATTGC | Wang et al. 2018 |
| BCL2 Associated X Apoptosis Regulator | *bax* | gacttgggagctgcacttct | tccgatctgctgcaaacact | Fang et al., 2024 |
| BCL2 Apoptosis Regulator | *bcl2* | AGGAAAATGGAGGTTGGGATG | TGTTAGGTATGAAAACGGGTGGA | Fang et al., 2024 |
| Catalase | *cat* | caaggtctggtcccataaa | tgactggtagttggaggtaa | Fang et al., 2024 |
| Caspase 3 | *caspase3* | CCGCTGCCCATCACTA | ATCCTTTCACGACCATCT | Fang et al., 2024 |
| ELAV Like RNA Binding Protein 3 | *elavl3* | AGACAAGATCACAGGCCAGAGCTT | TGGTCTGCAGTTTGAGACCGTTGA | Yang et al. 2023 |
| Growth Associated Protein 43 | *gap43* | TTAACGGAGGACCAGTGCAA | GTCCTGATCTCCAGCACACG | Dong et al., 2023 |
| Glial Fibrillary Acidic Protein | *gfap* | GGATGCAGCCAATCGTAAT | TTCCAGGTCACAGGTCAG | Dong et al., 2023 |
| Heat Shock Protein 70 | *hsp70* | GAAGACGGCATCTTTGAGGTGA | GGGCCCTCTTGTTCTGACTGAT | Hahn et al., 2014 |
| Mesencephalic Astrocyte Derived Neurotrophic Factor | *manf* | AGATGGAGAGTGTGAAGTCTGTGTG | CAATTGAGTCGCTGTCAAAACTTG | Yang et al., 2023 |
| Myelin Basic Protein | *mbp* | AATCAGCAGGTTCTTCGGAGGAGA | AAGAAATGCACGACAGGGTTGACG | Yang et al., 2023 |
| Nestin | *nestin* | ATGCTGGAGAAACATGCCATGCAG | AGGGTGTTTACTTGGGCCTGAAGA | Jiang et al., 2018 |
| Tumor Protein P53 | *p53* | CCCGGATGGAGATAACTTG | CACAGTTGTCCATTCAGCAC | Fang et al., 2024 |
| Ribosomal 18s | *rps18* | TCGCTAGTTGGCATCGTTTATG | CGGAGGTTCGAAGACGATCA | McCurley and Callard, 2008 |
| Sonic Hedgehog Signaling Molecule | *shha* | AGACCGAGACTCCACGACGC | TGCAGTCACTGGTGCGAACG | Guo et al., 2023 |
| Superoxide dismutase 1 | *sod1 (Cu/Zn SOD)* | CAACACAAACGGCTGCATCA | TTTGCAACACCACTGGCATC | Sarkar et al., 2014 |
| Superoxide dismutase 2 | *sod2 (Mn SOD)* | AGCGTGACTTTGGCTCATTT | ATGAGACCTGTGGTCCCTTG | Sarkar et al., 2014 |
| Synapsin IIa | *syn2a* | GTACCATGCCAGCATTTC | TGGTTCTCCACTTTCACCTT | Guo et al., 2023 |
| Tubulin 3 | *tubulin* | AATCACCAATGCTTGCTTCGAGCC | TTCACGTCTTTGGGTACCACGTCA | Wu et al., 2016 |

Dong, M., Wang, J., Liu, Y., He, Q., Sun, H., Xu, Z., ... & Gao, P. (2023). 3-Bromocarbazole-Induced Developmental Neurotoxicity and Effect Mechanisms in Zebrafish. *ACS ES&T Water*.

Fang, C., Di, S., Yu, Y., Qi, P., Wang, X., & Jin, Y. (2024). 6PPD induced cardiac dysfunction in zebrafish associated with mitochondrial damage and inhibition of autophagy processes. *Journal of hazardous materials*, *471*, 134357. https://doi.org/10.1016/j.jhazmat.2024.134357

Guo, Y., Fu, Y., & Sun, W. (2023). 50 Hz Magnetic Field Exposure Inhibited Spontaneous Movement of Zebrafish Larvae through ROS-Mediated syn2a Expression. *International journal of molecular sciences*, *24*(8), 7576. https://doi.org/10.3390/ijms24087576.

Hahn, M.E., McArthur, A.G., Karchner, S.I., Franks, D.G., Jenny, M.J., Timme-Laragy, A.R., Stegeman, J.J., Woodin, B.R., Cipriano, M.J. and Linney, E., 2014. The transcriptional response to oxidative stress during vertebrate development: effects of tert-butylhydroquinone and 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. PloS one, 9(11), p.e113158.

Jiang, F., Liu, J., Zeng, X., Yu, L., Liu, C. and Wang, J., 2018. Tris (2-butoxyethyl) phosphate affects motor behavior and axonal growth in zebrafish (Danio rerio) larvae. *Aquatic Toxicology*, *198*, pp.215-223.

McCurley AT, Callard GV. Characterization of housekeeping genes in zebrafish: male-female differences and effects of tissue type, developmental stage and chemical treatment. BMC Mol Biol. 2008 Nov 12;9:102. doi: 10.1186/1471-2199-9-102. PMID: 19014500; PMCID: PMC2588455.

Sarkar, S., Mukherjee, S., Chattopadhyay, A. and Bhattacharya, S., 2014. Low dose of arsenic trioxide triggers oxidative stress in zebrafish brain: expression of antioxidant genes. Ecotoxicology and environmental safety, 107, pp.1-8.

Wang, X.H., Souders 2nd, C.L., Zhao, Y.H., Martyniuk, C.J. 2018. Paraquat affects mitochondrial bioenergetics, dopamine system expression, and locomotor activity in zebrafish (Danio rerio). Chemosphere. 191, 106-117.

Wu, Q., Yan, W., Liu, C., Li, L., Yu, L., Zhao, S. and Li, G., 2016. Microcystin-LR exposure induces developmental neurotoxicity in zebrafish embryo. Environmental Pollution, 213, pp.793-800.

Yang Q, Deng P, Xing D, Liu H, Shi F, Hu L, Zou X, Nie H, Zuo J, Zhuang Z, Pan M, Chen J, Li G. Developmental Neurotoxicity of Difenoconazole in Zebrafish Embryos. Toxics. 2023 Apr 8;11(4):353. doi: 10.3390/toxics11040353. PMID: 37112580; PMCID: PMC10142703.