Title: Identifying the role of mitochondrial carrier protein in PFOS-led toxicity

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Per/polyfluoroalkyl substances (PFAS) are synthetic organo-fluorine chemicals used as flame-retardants, stain repellants, and coating additives in nonstick cookware and the food packaging industry. Our research is mainly focused on elucidating the mechanism of PFAS exposure mediated neurotoxicity. Previous studies have shown the selective vulnerability of dopaminergic neurons in Caenorhabditis elegans (25 µM) along with loss of mitochondrial viability observed at far lower doses (~ 2 µM). Here, we investigated the effect of PFOS on mitochondria by exposing them to different concentrations of PFOS (10 to 500 µM for 15 minutes). Acute PFOS treatment in isolated mitochondria showed inhibition of complex II (succinate dehydrogenase) and complex III (cytochrome c reductase); inhibitory effect on complex III activity being relatively severe. There is currently a poor understanding of how PFOS enters mitochondria. By utilizing inhibitors specific to individual mitochondrial carrier proteins, we identified a significant role of oxoglutarate carrier protein in the uptake of PFOS and a lesser role of dicarboxylate carrier protein. Notably, these carrier proteins are also responsible for the GSH uptake. Previous studies exhibited ameliorative effects of GSH co-administration. As a next step, we identified that gamma-glutamylcysteine is critical to ameliorating PFOS-induced neurotoxicity. While a possible reduced GSH biosynthesis has been identified, an additional mechanism pertaining to the competitive effect on the uptake of GSH by mitochondria given that the same carrier proteins are utilized by both GSH and PFOS. Overall, our studies identify a novel mechanism of PFOS neurotoxicity, specifically as it relates to carrier protein mediated mitochondrial uptake.