Perfluoroalkyl substances (PFAS) are synthetic compounds that are used in food packaging products, firefighting materials, electronics, cookware, carpets, furniture, clothing, and many other applications. PFAS are composed of a fluorinated carbon chain. PFAS are persistent in the environment and bioaccumulative in organisms. The concerns of PFAS toxicity led to voluntarily phasing out of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) by their manufacturer. PFOA and PFOS are both composed of 8 carbon chain (C8). Shorter chain chemicals (such as perfluorobutyrate (PFBA, C4) and perfluorobutane sulfonate (K-PFBS, C4)) and compounds with chemical modifications (such as GenX, C6) were used as a replacement to the long chain PFAS, (>C7 for COO− containing PFAS or >C6 for SO3− containing PFAS), in order to increase their degradability potential. In this study, we compared toxicity of five PFAS in order to assess the role of chain length, functional group and chemical structure in their toxicity. We compared the toxicity of K-PFOS, PFOA, K-PFBS, PFBA and GenX using Zebrafish (Danio rerio). To determine LC50 of each chemical, zebrafish embryos were exposed to a range of concentrations within 1-hour post fertilization (hpf) for 120 hpf. The toxicity of these compounds was assessed by monitoring the survivability every 24 hours through 120 hpf. 120hpf-LC50 were determined using GraphPad 8.0 software. In addition, behavioral analysis using a visual motor response test was performed. For behavioral analysis, we used concentrations of 0, 4, 40, 400, and 4000 part per billion (ppb). The exposure was terminated at 72hpf and the test was done at 120hpf. Results showed that the 120hpf-LC50s are 23 part per million (ppm) for PFOS, 566.5 ppm for PFOA, 2470 ppm for PFBS, >10000 ppm for PFBA, and 8617 ppm for GenX. Toxicity ranking is K-PFOS > PFOA > K-PFBS >GenX > PFBA. Based on these results, we can conclude that toxicity increases with increasing the chain length. Also, presence of sulfonate group increased toxicity for PFAS of a given chain length. Behavioral analysis showed that embryonic exposure to K-PFOS, K-PFBS, PFBA or GenX induced changes in the locomotor activities in larvae, while PFOA didn’t cause any changes. Future work will focus on identifying the mechanism behind the observed behavioral changes.