3758 Toxicity of Environmentally Relevant Concentration of PFAS Chemicals in *Lumbriculus variegatus* (Oligochaeta, Lumbriculidae): A Multi-Endpoint Study

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PFAS, or Per/Polyfluoroalkyl Substances, are a family of man-made chemicals found in a variety of products from non-stick cookware and food wrappers to fire retardants. PFAS chemicals are widely distributed in the environment and have notorious longevity, posing a threat to both human health and the ecosystem. PFAS can be classified into two categories, long chain chemicals with 6 or more carbons in the backbone, and short chain chemicals with less than 6 carbons which are proposed as a safer alternative. This study analyzed the impact of exposure to PFAS, including PFDA, PFOA, PFOS (all long chain) and PFHxA (short chain), on the physiology of the annelid *Lumbriculus variegatus* (ie, blackworms). L. *variegatus* lives in the benthic zone at the edges of freshwater bodies and is found throughout North America and Europe. It is prey for a variety of animals and contributes to organic material decomposition in the sediment. As such, it is a keystone species in shallow freshwater ecosystems. At an environmentally-relevant concentration of 1 µg/l, 12 day aqueous exposure to PFOA, PFOS, and PFDA, but not PFHxA, markedly slowed the pulse rate of the dorsal blood vessel in *L. Variegatus*, indicating a suppressive effect on blood circulation in the worms. The average pulse rate was reduced from 9.6 beats/minute to 6.2 and 7.0 beats/min in PFOA and PFOS, respectively ($P < 0.0001$). Further, PFOA, PFOS and PFDA, but not PFHxA reduced the escape responsiveness of *L. Variegatus* to physical stimulation of the head and tail. The percentage of worms showing normal escape behavior was reduced from 99.0% in control to 90.6% in the PFOS exposed group ($P < 0.01$). In a long term (4 week) growth study, aqueous and sediment exposure, but not sediment exposure alone, to PFOA, PFOS, and PFDA reduced the total dry weight as well as the number of worms, indicating a suppressive effect on both growth and reproduction. For instance, PFOA and PFDA reduced the total dry biomass by 26.3% and 28.5%, respectively, compared with vehicle control ($P < 0.05$). Again, PFHxA had no detectable effect on growth or reproduction. The mechanism of the toxicities of PFOA, PFOS and PFDA in blackworms likely involved an increase in oxidative stress. The levels of MDA, an indicator of reactive oxygen species (ROS), in the PFOA, PFOS, and PFDA exposure groups were significantly higher than those of the control and PFHxA groups, indicating increased ROS and oxidative stress. Our results demonstrate that environmental concentration of long chain PFAS chemicals have toxic effects on *L. Variegatus* as measured by multiple physiological endpoints including blood circulation, behavior, growth, and reproduction. Such toxicity may have a detrimental impact not only on *L. Variegatus* but also on the freshwater ecosystems in which *L. Variegatus* is a keystone species.

3759 Systemic Toxicity Induced by Topical Application of Per- and Polyfluoroalkyl Substances (PFAS) in a Murine Model

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Per- and polyfluoroalkyl substances (PFAS) are a class of over 5000 synthetic, structurally diverse chemicals incorporated into industrial and consumer products. These fluorinated chemicals are used in flame-retardant products, stain resistant textiles, water and grease repellent products, food packaging material, nonstick cookware, carpets, and firefighting foams due to their stability and resistance to degradation. Carboxylic PFAS are considered long-alkyl chain if they contain 7 or more carbons in their carbon chain and short-alkyl chain if they contain 6 or fewer. Sulfonic acid PFAS are considered short chain with 5 or fewer carbons. Due to health concerns, legacy PFAS (such as perfluorooctanoic acid (PFOA; C8) and perfluorooctane sulfate (PFOS; C8)) are being phased out. Alternative PFAS (such as heptafluorobutyric acid (PFBA; C4), perfluoropentanoic acid (PFPeA; C5), perfluorohexanoic acid (PFHxA; C6), and perfluoroheptanoic acid (PFHpA; C7)) are labeled as safer alternatives to legacy PFAS, due to their shorter half-life in animals. Despite the high potential for occupational and environmental dermal exposure, dermal exposure studies are lacking. Using a murine model, the present studies analyzed organ weight, serum chemistries, histology, immune phenotyping, and gene expression to evaluate the systemic toxicity of sub-chronic 28-day dermal PFAS (C4-C8) exposure (1.25-15% or 31.25-375 mg/kg/dose in acetone vehicle). Legacy (PFOA; C8) and alternative (C4-C7) PFAS were absorbed after dermal exposure leading to an increase in detection levels in serum and urine with all PFAS tested. Legacy and alternative PFAS also increased liver weight (% body) and induced histopathology changes in the liver and skin. Gene expression changes were observed with peroxisome proliferator-activated receptors (PPAR) isoforms in the liver and skin along with changes in genes involved in steatosis, fatty acid metabolism, necrosis, skin barrier function, and inflammation. Immune-cell phenotyping identified significant changes in multiple cell sub-populations in the skin, spleen, and skin draining lymph nodes. These findings demonstrate that both legacy and alternative PFAS are absorbed through the skin and can induce systemic changes similar to those reported for oral PFAS exposure. Similar toxicity trends were seen between legacy and alterative PFAS in the liver after dermal exposure, suggesting that chain length may not be the best predictor of toxicity. However, differences in gene expression and certain cell sub-populations were observed, suggesting different mechanisms of toxicity. These findings raise concerns of alternative PFAS being promoted as a safer option and show that further investigation into PFAS dermal exposure is needed to understand the hazards of skin exposure and help promote protective measures.

3760 The Stage-Specific Toxicity of Per- and Polyfluoroalkyl Substances (PFAS) in Nematode *Caenorhabditis elegans*

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Per- and Polyfluoroalkyl Substances (PFAS) are a diverse class of industrial chemicals that have been used for decades in industrial and commercial applications. Due to widespread use and resultant environmental bioaccumulation, PFAS are consistently detectable in the bloodstream of humans. PFAS have been linked to several adverse health outcomes including hepatotoxicity, immunotoxicity, endocrine disruption, tumorigenicity, and neurotoxicity, *specifically for* developmental neurotoxicity (DNT). An increased prevalence of neurodevelopmental disorders in children has been observed and linked to pre- and postnatal exposure to PFAS; however, the mechanisms of adverse neurodevelopmental effects of PFAS are largely unknown. In order to determine PFAS mechanism of action, in-depth toxicological studies are required. Traditional toxicological chemical studies use animal models that are costly, time consuming, and challenging to interpret when testing multiple chemicals at varying concentrations. The nematode *C. elegans* serve as an ideal model organism for neurodevelopmental toxicity studies due to the organism only having 302 neurons, a complete written diagram for its chemical and electrical connections available, and a short lifespan. In this study, 10 PFAS compounds with high occurrence frequency were selected to represent a wide range of typical PFAS structures, including perfluoroalkyl carboxylic acids (PFBA, PFHxA, PFOA), sulfonic acids (PFBS, PFHxS, PFOS), sulfonamides and derivatives (PFOSA, NetFOSAA), fluorotelomers (6:2 FTS), and new substitutes (HFPO-DA, the acid form of GenX). Wild-type worms were exposed to single PFAS at 0, 0.1, 1, 10 ,100 and 200 µM. The toxic effects of PFAS on development, fecundity, and behavior at different larval stages (L1, L2 ,L3 and L4) were investigated using a high-throughput screening (HTS) platform which consisted of a robotic system, COPAS Biosort™. Our results have identified several PFAS indicative of having negative effects on development, fecundity and behavior of *C. elegans*. These results suggest that PFAS could potentially be linked to neurodevelopmental disorders in children with pre- and postnatal exposure. It is important to note that PFAS do not exist independently in the environment; hence, future studies with an environmental relevant mixture of PFAS will be conducted with the defined dose-range obtained from findings of single PFAS.

3761 PFOS Exposure Upregulates CD36 Expression and Induces Translocation of CD36 to the Plasma Membrane of CD4+ T Cells

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Perflurooctane sulfonate (PFOS, an 8-carbon PFAS) is an environmental pollutant that has been detected frequently in the environment. Scientific literature suggests that PFOS exposure can have a negative impact on human health. According to published research, PFOS can promote immunotoxicity in the spleen and alter innate and adaptive immune responses. Although PFOS are regarded as immunological hazards for people, it is still unclear how they cause immunotoxicity. Our preliminary data showed that PFOS exposure increases the expression of the CD36 gene in CD4+ T cells in mice. The scavenger receptor CD36 is an essential metabolic regulator of T cell metabolism in immunological responses. *Therefore, the goal of the current study is to investigate if PFOS-induced alterations in the CD36-lipid metabolism axis contribute to immunotoxicity*. In this study, *in vitro* and *in vivo* settings were used to examine the involvement of a CD36-lipid metabolism axis after PFOS exposure in inducing immunotoxicity. In *in vivo* studies, C57BL/6 mice were exposed to PFOS in drinking water for 7 weeks. The splenic CD4+ T-cells were then isolated from splenic tissue of control and PFOS treated mice and qRT-PCR was used to measure expression of CD36. *In vitro* studies included T cell isolation and PFOS treatment in cell culture. Splenic CD4+ T lymphocytes were stimulated with anti-CD3+/CD28+ activation beads, treated with PFOS, and then analyzed by qRT-PCR and flow cytometry analysis. Flow cytometry is used to quantify changes in the membrane associated CD36 expression due to PFOS exposure. *In vivo* data demonstrate the increase in CD36 mRNA expression in splenic CD4+ T cells from PFOS treated mice as compared to control C57BL/6 mice. We have also demonstrated the increase in CD36 mRNA expression in CD4+ T cells following PFOS exposure *in vitro*. Consistently, flow cytometry analysis suggests that exposure to PFOS leads to an increase in the CD36 level on the cell surface of CD4+ T cells. In summary, our studies demonstrate that PFOS exposure can contribute to increased CD36 expression in splenic CD4+ T cells. These findings also suggest that there is increased abundance of CD36 on the plasma membrane of spleen derived CD4+ T cells. Further studies will determine the functional significance of PFOS-induced CD36 expression in T cell lipid metabolism and immunotoxicity. *Supported in part by NIEHS/NIH grant P42ES007380 and by UK-CARES grant P30ES026529.*

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