

## **Systemic toxicity induced by topical application of perfluoroheptanoic acid (PFHpA), perfluorohexanoic acid (PFHxA), and perfluoropentanoic acid (PFPeA) in a murine model**

### **Introductory Information**

Perfluoroheptanoic acid (PFHpA), perfluorohexanoic acid (PFHxA), and perfluoropentanoic acid (PFPeA) are synthetic chemicals belonging to the per- and polyfluoroalkyl substances (PFAS) group that includes over 5,000 chemicals. PFHpA, PFHxA, and PFPeA are short-chain PFAS (C7, C6, C5, respectively) that have been labeled as a safer alternative to the legacy PFAS perfluorooctanoic acid (PFOA) and perfluorooctane sulfate (PFOS) which have been linked to numerous health effects. This class of chemicals are incorporated into consumer products such as stain resistant carpeting and textiles, nanosprays, medical devices, and fire-fighting foams. There is a high potential for occupational exposure and in the environment, short-chain PFAS have been detected in a variety of water sources leading to concerns for dermal exposure, however, these studies are lacking. In previous studies from our lab, PFOA was demonstrated to be absorbed via the skin and immunomodulatory. Also, the short-chain PFAS, PFBA, was found to have systemic toxic effects on mice with dermal exposure. In the present study, the systemic toxicity of a 28-day dermal PFHpA, PFHxA, and PFPeA (1.25-5% w/v or v/v, or 31.25-125 mg/kg/dose) exposure was evaluated in a murine model.

### **Methods Collection**

#### **1. Animal Exposures**

- Female B<sub>6</sub>C<sub>3</sub>F<sub>1</sub> mice (7-8 weeks at start of study)
- Perfluoroheptanoic acid (PFHpA), perfluorohexanoic acid (PFHxA), and perfluoropentanoic acid (PFPeA) (25 µl/ear; 1.25-5%) or acetone on dorsal surface of both ears for up to 28 days

#### **2. Tissue Collection**

- Kidney and thymus were collected, weighted, and then discarded.
- Liver was weighed, caudate lobe was collected in RNA later and then homogenized on a TissueLyser II in Buffer RLT for RNA extraction. The remainder of the liver was collected in 10% formalin for histopathology analysis.

- Spleen was weighed, 1/2 was collected in 4 mL RPMI and processed into single cell suspensions by mechanical disruption of tissues between frosted microscope slides. The remainder of the spleen was collected in 10% formalin for histopathology analysis.
  - Ear pinnas (1 per mouse; split into ventral and dorsal halves) was collected in RPMI and either and processed into single cell suspensions prepared by incubating with a 0.25 mg/ml Liberase-TL Research grade (Roche) enzymatic digestion for 90 min at 37°C in RMPI with 100 µg/ml DNase I. The second ear pinna (1/2) collected in 10% formalin for histopathology analysis and 1/2 was collected in RNA later and then homogenized on a TissueLyser II in Buffer RLT for RNA extraction.
  - Right and left auricular draining lymph nodes (dLNs) collected in 2 mL sterile phosphate-buffer saline (PBS) (pH 7.4) and single cell suspensions (2 nodes/animal) were prepared by mechanical disruption of tissues between frosted microscope slides
3. Serum chemistries
    - Blood samples were collected via cardiac puncture and separated by centrifugation. Selected serum chemistries were evaluated using Catalyst DX Chemistry Analyzer.
  4. Analytical PFAS detection
    - Serum and urine samples were collected and analyzed for individual PFAS by Vista Analytical Laboratories as described in (Shoemaker et al. 2008).
  5. Gene expression
    - Real-time PCR (Applied Biosystems 7500 RT-PCR System).
  6. Immune Cell Subsets
    - Flow cytometry using BD LSRII Flow Cytometer and data was analyzed using FlowJo software.
  7. Histology
    - Tissue samples were stained via hematoxylin and eosin (H&E) and evaluated by a veterinary pathologist.

## **Publications**

Dzubak L, Shane H, Lukomska E, Baur R, Anderson S [2022]. Systemic toxicity induced by topical application of perfluoroheptanoic acid (PFHpA), perfluorohexanoic acid (PFHxA), and perfluoropentanoic acid (PFPeA) in a murine model. Food Chem Toxicol. 171 (2023) 113515.

## **Acknowledgements**

This work was supported by the National Occupational Research Agenda (NORA)  
CAN 9390HTP

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