Systemic and immunotoxicity induced by topical application of perfluorohexane sulfonic acid (PFHxS) in a murine model_Dataset

Weatherly LM, Shane HL, Jackson LG, Lukomska E, Baur R, Cooper MP, Anderson SE [2024]. Systemic and immunotoxicity induced by topical application of perfluorohexane sulfonic acid (PFHxS) in a murine model. Food Chem Toxicol, 186, 114578. doi.org/10.1016/j.fct.2024.114578

Introductory Information

Per- and polyfluoroalkyl substances (PFAS) are a large group of stable man-made surfactants that are incorporated into numerous products for their water and oil resistance and have been associated with adverse health effects. The present study evaluated the systemic and immunotoxicity of sub-chronic 28- or 10-day dermal exposure of PFHxS (0.625-5% or 15.63-125 mg/kg/dose) in a murine model. Elevated levels of PFHxS were detected in the serum and urine, suggesting that absorption is occurring through the dermal route. Liver weight (% body) significantly increased and spleen weight (% body) significantly deceased with PFHxS exposure supported by histopathological changes. Additionally, PFHxS significantly reduced the humoral immune response and altered immune subsets in the spleen, suggesting immunosuppression. Gene expression changes were observed in the liver, skin, and spleen with genes involved in fatty acid metabolism, necrosis, and inflammation. Immune-cell phenotyping identified significant decreases in B-cells, NK cells, and CD11b+ cells in the spleen along with increases in CD4+ and CD8+ T-cells, NK cells, and neutrophils in the skin. These findings support dermal PFHxS-induced liver damage and immune suppression. These data support PFHxS absorption through the skin and demonstrate immunotoxicity via this exposure route, raising concern about prompting the need for further investigation.

Methods Collection

1. **Animal Exposures**
    - **Female $B_6C_3F_1$ mice (7-8 weeks at start of study)**
    - **Perfluorohexane sulfonic acid (PFHxS) (25 µl/ear; 1.25-5%) or acetone on dorsal surface of both ears for 10 or 28 days**
2. **Tissue Collection**
    - **Thymus were collected, weighed, and then discarded.**

- Kidneys were weighed and then one was discarded and one (right) was collected in 10% formalin for histopathology analysis.
- 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 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. Gene expression
    - Real-time PCR (Applied Biosystems 7500 RT-PCR System).
5. Immune Cell Subsets
    - Flow cytometry using BD LSRII Flow Cytometer and data was analyzed using FlowJo software.
6. Histology
    - Tissue samples were stained via hematoxylin and eosin (H&E) and evaluated by a veterinary pathologist.
7. Spleen IgM response to SRBC
    - The primary IgM response to SRBC was enumerated using a modified hemolytic plaque assay
8. Serum IgM response to SRBC
    - Serum samples were analyzed for anti-SRBC IgM using a commercially available ELISA kit

Publications

Dzubak L, Shane H, Lukomska E, Jackson L, Baur R, Cooper M, Anderson S. Systemic and immunotoxicity induced by topical application of perfluorohexane sulfonic acid (PFHxS) in a murine model. Food Chem Toxicol, 186, 114578. doi.org/10.1016/j.fct.2024.114578

Acknowledgements

This work was supported by NIOSH CAN 9390DU3

Lisa M. Weatherly nux6@cdc.gov

Hillary L. Shane hshane@cdc.gov

Ewa Lukomska uvm3@cdc.gov

Laurel Jackson ghf4@cdc.gov

Rachel Baur oee6@cdc.gov

Madison Cooper ufd8@cdc.gov

Stacey E. Anderson Sanderson4@cdc.gov

Contact

For further information contact:

Allergy and Clinical Immunology Branch (ACIB), Health Effects Laboratory

Division (HELD), National Institute for Occupational Safety and Health (NIOSH),

Morgantown, WV

304.285.6024