

Immunotoxicity of PFCs (perfluoroalkyl compounds) Found in Fire-Fighting Foams

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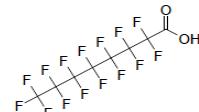
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Perfluorooctanoic Acid (PFOA)

- Perfluorocarbon chain



- Hydrophobic and

lipophobic



- Ubiquitous and persistent in environment

(ATSDR, 2015; NTP, 2016)

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Concentrations of PFOA in Serum



http://www.sitnews.us/PhotoGallery/DaveHull/Training/073003_firefighting.html

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Concentrations of PFOA in Serum

- General population
 - 2 ng/mL
- Ohio River Valley
 - 34 ng/ml
- NORA Sector: Fire Protection
 - Up to 9x general population
- NORA Sector: Chemical Manufacturing
 - Up to 6000x general population

http://www.sitnews.us/PhotoGallery/DaveHull/Training/073003_firefighting.html

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PFOA Immunotoxicity

- “*presumed to be an immune hazard to humans*” by NTP (2016)
- Mechanisms of toxicity are unclear
- Little information on **innate immunity**
 - **‘First responders’** of the immune system
 - Immune cells: **neutrophils**, macrophages

(NTP, 2016; ATSDR, 2015)

Project Goal

Investigate the innate immunotoxicity of PFOA and the molecular pathways involved

- Aim 1: PFOA mortality and sublethal effects
- Aim 2: PFOA effects on innate immunity
- Aim 3: PFOA molecular mechanism of toxicity



Zebrafish embryo (ZFE) model:
High relevance/homology to humans
Ease of use and genetic manipulation

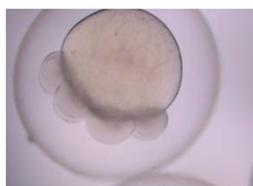
AIM 1

PFOA MORTALITY AND SUBLETHAL EFFECTS

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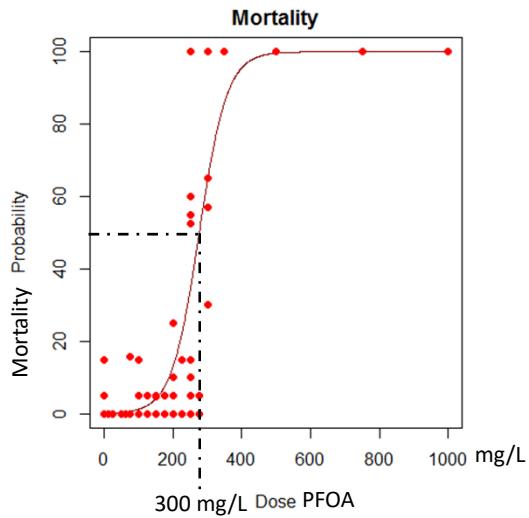
Methods: PFOA Mortality

- Test guidelines: **OECD 236, US EPA 210**
- Analytical chemistry
- 48 hour static exposure at 28°C
- **7 concentrations x 3 replicates x 20 embryos**



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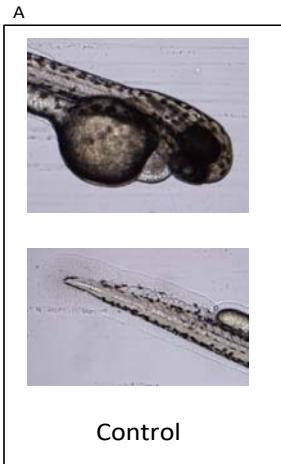
Results: PFOA Mortality



48 hour LC₅₀ = 300 mg/L

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Results: Sublethal developmental malformations



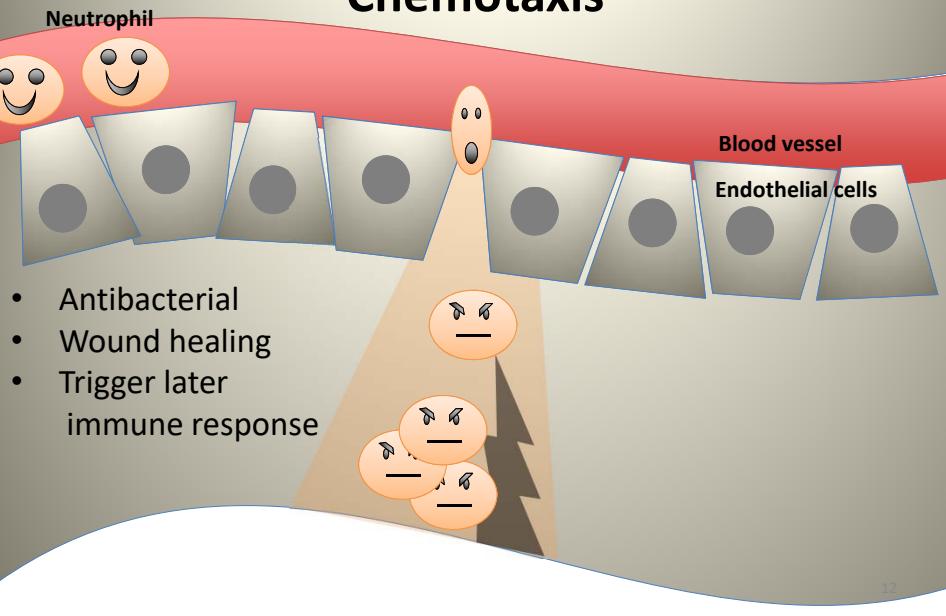
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AIM 2

PFOA EFFECTS ON INNATE IMMUNITY

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Introduction to Neutrophil Chemotaxis

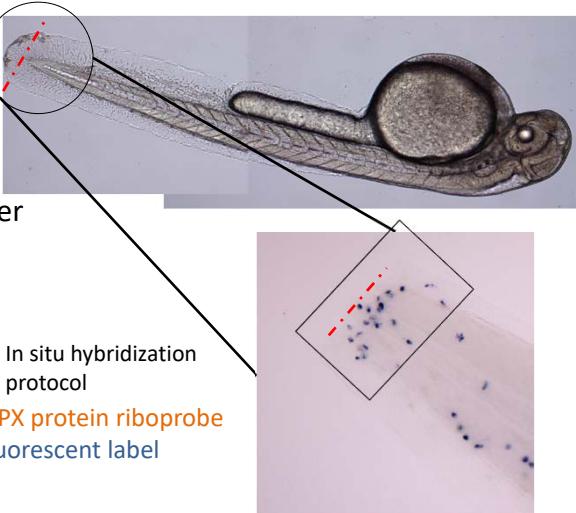


Methods: Assay for measuring neutrophil chemotaxis

- Exposed ZFE 1 hpf to vehicle control, 0.5, or 5.0 mg/L PFOA for 48 hrs
 - Wounded to trigger neutrophil chemotaxis

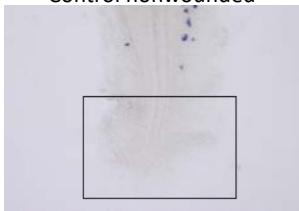
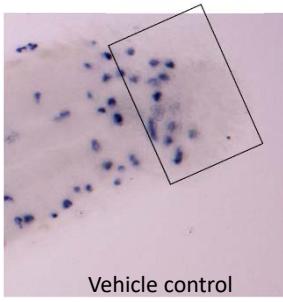
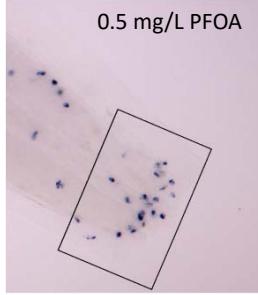
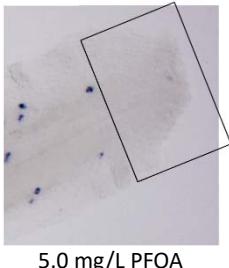
ISH In situ hybridization protocol

- MPX protein riboprobe
- Fluorescent label



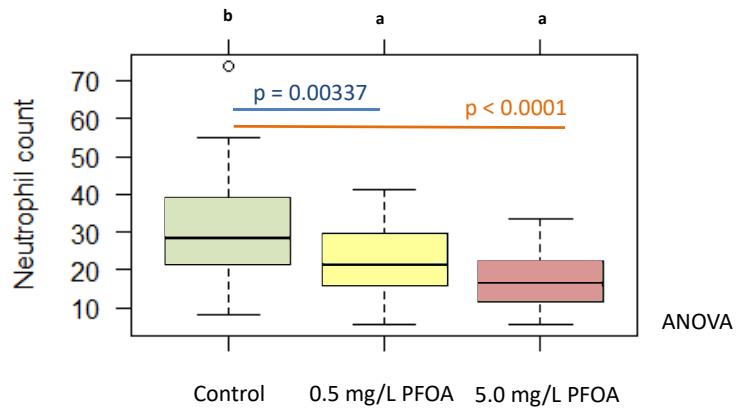
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Histology Results

Control nonwounded		
0.5 mg/L PFOA		
		Vehicle control
		5.0 mg/L PFOA

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Effect on neutrophil count is significant



- **25% reduction** in neutrophils

AIM 3 PFOA MOLECULAR MECHANISM OF TOXICITY

Aim 3 Methods: RNA Sequencing

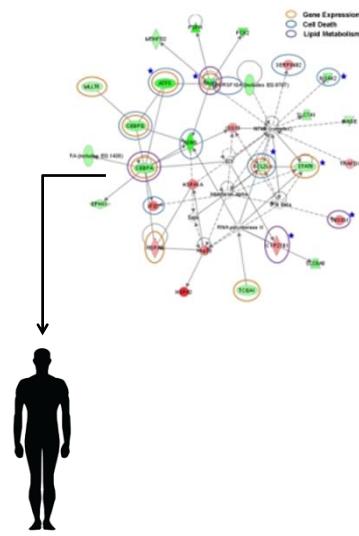
- 48 hour static exposure at 28°C at 0, 0.5, or 5.0 mg/L
 - Wounded for immune stimulus
 - Single embryo sequencing, 10 replicates per treatment
 - LOTS of troubleshooting!

Still awaiting results!

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Critical Questions for Analysis

- What are the molecular mechanisms?
- How does immune stimulation change the molecular profile?
- Is this a recruitment effect (chemotaxis) and/or neutropenia?



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Summary and Conclusions

- PFOA not highly toxic
 - Causes **developmental malformations** in zebrafish embryos
 - Potential window of **susceptibility**?
- PFOA suppresses the innate immune response
 - Reduces **neutrophil recruitment** to the inflammatory site up to **45%**
 - Implications for **reduced wound healing, increased infection**
- **Future Funding:** Preliminary data to be used grant application

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Research-2-Practice



http://www.sitnews.us/PhotoGallery/DaveHull/Training/073003_firefighting.html

- **Occupational cohorts are the most highly exposed**
- **Firefighters increased prevalence for immune disease**
- **Results suggest PFOA is contributing to altered innate immune status and possibly adding to disease burden**
 - Research can inform **mitigation, exposure prevention, biomonitoring**

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Acknowledgements

Mentors:

University of Cincinnati

- Dr. Andy Maier
- Dr. Jagjit Yadav
- Dr. Susan Kasper

Collaborators:

Cincinnati Children's Hospital Medical Center

- Dr. Saulius Sumanas
 - Satish CasieChetty
- Dr. Adam Biales
 - MJ See

Funding:

NIOSH Pilot Research Project Training Program Grant #T42OH008432
Center for Environmental Genetics New Investigator Scholar: NIH/NIEHS
P30 ES006096

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Questions?

- **References**

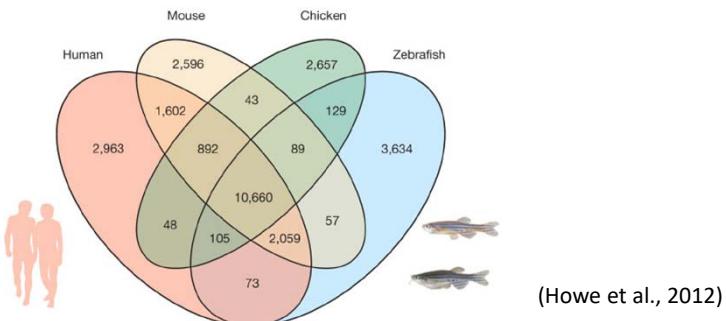
- Agency for Toxic Substances and Disease Registry (ATSDR). (2015). Draft toxicological profile for perfluoroalkyls. US Department of Health and Human Services. August.
- National Toxicology Program (NTP). (2016) Immunotoxicity associated with exposure to perfluorooctanoic acid or perfluorooctane sulfonate. NTP Monographs, US Department of Health and Human Services. September.
- OECD Fish Embryo Toxicity Test Guideline 236
- US EPA Fish Early-life Stage Test Guideline 210

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Zebrafish as an Immune Model for Human Effects

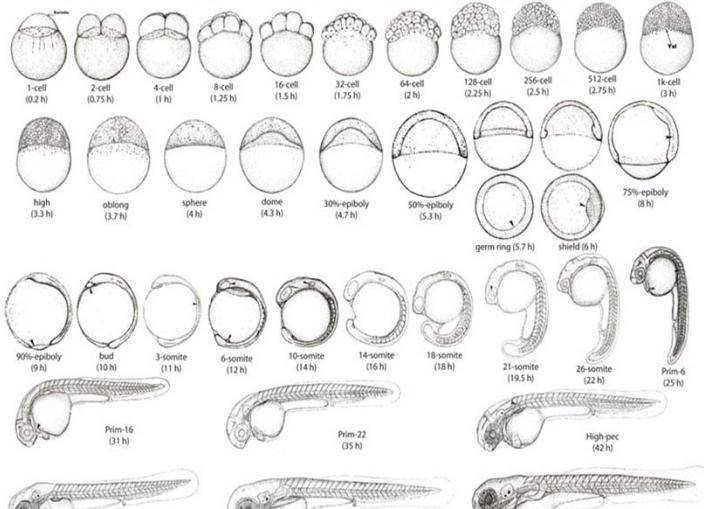


- Innate **immune signaling pathways** conserved
- Similar immune cell types, **neutrophils**

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<http://www.sandeepkhat.info/img/ZebrafishStages.jpg>

STAGES OF EMBRYONIC DEVELOPMENT OF THE ZEBRAFISH



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Systems Biomaging Lab, ECE, UCSB
syb@ece.ucsb.eduSource: <http://www.uoneuro.uoregon.edu/k12/zfk12.html>

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Perfluorooctanoic acid (PFOA)

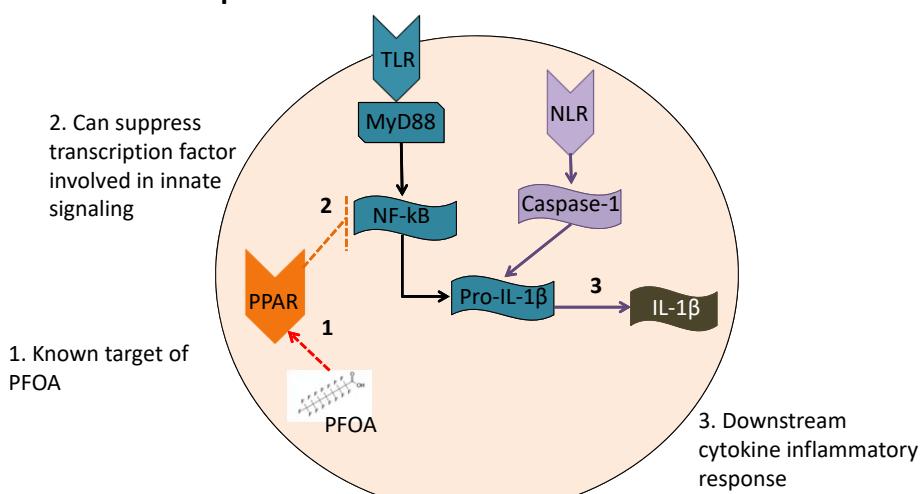
- EPA drinking water health advisories
 - 0.07 µg/L based on developmental effects

State/Gov	RfD (ug/kg-day)	Critical effect
Maine	0.006	liver
Michigan	0.02	developmental
Minnesota	0.02	developmental
New Jersey	0.002	Liver weight
Texas	0.015	developmental
US EPA	0.02	developmental

(EPA, 2016)



Hypothesis: PFCs alter innate immunity pathways during development via dose-dependent activation of PPAR



Zebrafish - Immune Model

- Innate **immune signaling pathways** conserved from fish to mammals
 - contain **similar hematopoietic lineage** and many development processes and genes
 - similar **lymphoid organs** and **immune cell types**
 - close homology to human lymphocytes, neutrophils, monocyte/macrophages, eosinophils, and mast cells



<http://www.sci-news.com/biology/article00415.html>

- **IN VITRO WITH IN VIVO CONTEXT**

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