Aquatic systems as a tool for evaluating the toxicity of PFAS-free AFFF

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Perpetual threat of contaminants
Aquatic Systems: Reservoirs For Contaminants
Aquatic Systems: Tools For Toxicology

• Model system for toxicity research
  - Numerous model species
  - Ease of culturing
  - Diverse suite of standardized tests
    • Acute
    • Chronic
  - Large-scale community-level experiments
  - Ease of field sampling
PFAS-free AFFF Alternatives Research

• Per- and polyfluoroalkyl substances (PFAS) are a global concern
  • Contamination, bioaccumulation, and health risks
• Increasing focus on developing PFAS-free foam alternatives to replace these chemicals
• Before the selection and implementation of PFAS-free foam alternatives, research must address their potential environmental impacts
• Contaminants pose a high risk to aquatic systems
  • Limited data for aquatic species
• SERDP-funded studies will assist in the final selection of new PFAS-free foam alternative formulations that minimize potential environmental risks
Aquatic Test Objectives

• Using a suite of ecologically diverse aquatic species, our objectives were to:
  • Determine the acute and chronic toxicity of PFAS-free AFFF alternatives to aquatic species
  • Characterize the relative toxicity of PFAS-free AFFF alternatives compared to PFAS-containing AFFF
  • Assess species-level variation in toxicity of PFAS-free AFFF alternatives
  • Additional objectives
    • Compare toxicity of PFAS-free AFFF alternatives between freshwater and marine species
    • Assess laboratory variation in toxicity results
Tested AFFF Formulations

- Provided by SERDP

<table>
<thead>
<tr>
<th>Formulation name</th>
<th>Formulation type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buckeye Platinum Plus C6 MILSPEC 3%</td>
<td>Reference C6 Formulation</td>
</tr>
<tr>
<td>National Foam AVIO F3 Green KHC 3%</td>
<td>Commercial PFAS-free Formulation</td>
</tr>
<tr>
<td>Bio-Ex ECOPOL A 3% FFF</td>
<td>Commercial PFAS-free Formulation</td>
</tr>
<tr>
<td>Fomtec ENVIRO 2-3% FFF</td>
<td>Commercial PFAS-free Formulation</td>
</tr>
<tr>
<td>Solberg Re-healing Foam RF3 3%</td>
<td>Commercial PFAS-free Formulation</td>
</tr>
<tr>
<td>Angus Fire JetFoam 3%</td>
<td>Commercial PFAS-free Formulation</td>
</tr>
<tr>
<td>National Foam NFD 20-391 Formulation</td>
<td>SERDP Developmental Formulation</td>
</tr>
<tr>
<td>NRL 502W Siloxane-based Formulation</td>
<td>SERDP Developmental Formulation</td>
</tr>
</tbody>
</table>
Acute Toxicity Trials

- Limited data on toxicity of PFAS-free AFFF foams
  - Formulations include alkyl sulfates, alkyl betaines, amphoteric surfactants, non-ionic surfactants, and amines
  - Complex mixtures of ingredients
  - SDS largely report toxicity of specific components rather than the entire foam
- Acute toxicity trials needed to assess basic toxicity and determine range of concentrations for chronic studies
- Pulling efforts across research groups allowed us to develop a more comprehensive assessment of toxicity
# Plants, Invertebrates, And Vertebrates Tested

- **Freshwater and marine (○) species**

## Plant name | Scientific name
--- | ---
Freshwater algae | *Raphidocelis subcapitata*

## Invertebrate name | Scientific name
--- | ---
Water flea | *Ceriodaphnia dubia*
Water flea | *Daphnia magna*
Midge | *Chironomus dilutus*
Amphipod | *Hyalella azteca*
Mud snail | *Tritia obsoleta*

## Amphibian name | Scientific name
--- | ---
Jefferson's salamander | *Ambystoma jeffersonianum*
American toad | *Anaxyrus americanus*
Western chorus frog | *Pseudacris triseriata*
Gray tree frog | *Hyla versicolor*
Northern leopard frog | *Rana pipiens*
Wood frog | *Rana sylvatica*

## Fish name | Scientific name
--- | ---
Fathead minnow | *Pimephales promelas*
Sheepshead minnow | *Cyprinodon variegatus*
Methods Overview

• Sources: Commercial suppliers, lab cultures, or natural populations
• Husbandry: Cultured/reared using standardized protocols
Methods Overview

- Pilot studies used to assess general toxicity of foams prior to main experiments
- Main experiments (EPA or ASTM guidelines):
  - 4 to 9 concentrations (mg/L) tested
  - 2 to 10 replicates per treatment
  - 1 to 20 individuals per replicate
  - 48, 72, or 96-hr tests with mortality checked every 24 hr
- Statistical analyses
  - Calculated LC50 values using the ‘LC_logit’ function in the ecotox package of R
  - Weighted by total individuals tested per treatment
Representative Survival Curves

**Freshwater zooplankton (Daphnia magna)**

- Formulated Product
  - Avio
  - ECOPOL
  - NFD
  - FOMTEC
  - ReHealing
  - 502W
  - Buckeye

**Northern leopard frog (Rana pipiens)**

- Formulated Product
  - Avio
  - ECOPOL A
  - NFD
  - ReHealing
  - FOMTEC
  - 502W
  - Buckeye
• PFAS-free AFFF were generally more acutely toxic than the PFAS-containing AFFF (Buckeye)
• Variation across species in toxicity
• Let’s break this down

<table>
<thead>
<tr>
<th>EPA toxicity category</th>
<th>Acute concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very highly toxic</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Highly toxic</td>
<td>0.1 - 1</td>
</tr>
<tr>
<td>Moderately toxic</td>
<td>&gt;1 - 10</td>
</tr>
<tr>
<td>Slightly toxic</td>
<td>&gt;10 - 100</td>
</tr>
<tr>
<td>Practically nontoxic</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>
Distribution Of LC50 Values Across Invertebrates

- Broadest diversity in phylogeny – crustaceans, insects, snails
- Marine mudsnails were highly sensitive to AFFF formulations (note only marine invert. eval. thus far)
- Buckeye, 502W, and ReHealing were the least toxic
- Similar levels of toxicity among the other formulations
Distribution Of LC50 Values Across Amphibians

- Consistent results across the 6 species
- Buckeye was the least toxic followed by 502W
- Avio was the most toxic
Distribution Of LC50 Values Across Fish

- Buckeye was the least toxic followed by 502W
- Similar levels of toxicity among the other formulations
- Sheepshead minnows tended to be more sensitive (marine species)
- Similar results across our 3 lab groups for fathead minnows
Key Points

- PFAS-free AFFF were generally more acutely toxic than the PFAS-containing AFFF (Buckeye)
  - EPA toxicity category – Slightly toxic
- Consistency among results using the same species and among closely related taxa
- Marine species more sensitive than freshwater species
- Data submitted to *Environmental Science and Technology*
Chronic Toxicity Trials

• Concentrations in nature are expected to be well below the acute toxicity value for most species
• Using the acute toxicity data, we selected a range of concentrations to explore chronic effects on growth, development, and reproduction
• Focal species: water fleas, fathead minnows, and gray tree frogs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Avio Green</th>
<th>Fomtec ENVIRO</th>
<th>Buckeye Platinum</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC50/1000</td>
<td>0.017</td>
<td>0.038</td>
<td>2.96</td>
</tr>
<tr>
<td>LC50/100</td>
<td>0.17</td>
<td>0.38</td>
<td>29.6</td>
</tr>
<tr>
<td>LC50/10</td>
<td>1.73</td>
<td>3.77</td>
<td>295.8</td>
</tr>
<tr>
<td>LC50/5</td>
<td>3.46</td>
<td>7.54</td>
<td>591.5</td>
</tr>
<tr>
<td>LC10</td>
<td>6</td>
<td>3.2</td>
<td>2855.4</td>
</tr>
</tbody>
</table>
Water Flea Experiments

• Goal: Determine the long-term effects of AFFF alternatives on *D. magna* growth, reproduction, and survival

• Approach: Standardized 21-day test
  • Static renewal
  • 5 concentrations per formulation
  • 15 replicates per treatment
  • 1 individual per replicate
• Results: Buckeye Platinum Plus C6
Water Flea Experiments

• Results: National Foam AVIO
Water Flea Experiments

- Results: Bio-Ex ECOPOL
Water Flea Experiments

- Results: Fomtec ENVIRO

[Graphs showing mean clutch size and number of reproductive events for different exposure treatments.]
Fathead Minnow Experiments

- **Goal:** Determine the effects of AFFF alternatives on *P. promelas* growth and survival
- **Approach:** Standardized 7-day test
  - Static renewal
  - 5 concentrations per formulation
  - 4 replicates per treatment
  - 10 individual per replicate
Fathead Minnow Experiments

- Results:

![Graphs showing mean fathead minnow length vs exposure treatments for different AFFF formulations.](image-url)
Upcoming Experiments

Fish reproduction experiments
- Goal: Determine the effects of AFFF alternatives on *P. promelas* reproduction
- Approach: Standardized 28-day test (OECD/EPA)
- Responses:
  - Behavior
  - Fecundity
  - Fertility
  - Endocrine function

Amphibian experiments
- Goal: Determine the effects of AFFF alternatives on *H. versicolor* growth and development
- Approach: Metamorphosis test
- Responses:
  - Mass and SVL at metamorphosis
  - Time to metamorphosis
Incorporating ecology

• Community interactions and ecosystem-level effects
Summary

• Aquatic systems ideally suited for the exploration of PFAS-free AFFF toxicity
• PFAS-free AFFF formulations are generally more acutely toxic than PFAS-containing AFFF
• Substantial variation in chronic toxicity across PFAS-free AFFF formulations
• Additional analyses, experiments, and data synthesis needed to determine the formulations with the lowest environmental risk