SERDP

EXECUTIVE SUMMARY

Multi-Taxa Ecotoxicity of Novel PFAS-Free Firefighting Formulations: Aquatic and Terrestrial Species

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SERDP Project ER20-1531

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ACRONYMS AND ABBREVIATIONS

AFFF	aqueous film-forming foam
CEWL	cutaneous evaporative water loss
COD	chemical oxygen demand
EPA	U.S. Environmental Protection Agency
LC_{50}	Lethal concentration 50%
OECD	Organization for Economic Co-operation and Development
PFAS	per- and polyfluoroalkyl substances
QPL	Qualified Product List

DISCLAIMER

Replacement products studied herein are or were commercially available for purchase but are not currently on the Qualified Product List (QPL) for military performance-based specification (MIL-PRF)-32725(2). Products studied herein comprised similar ingredients to those on the QPL, but are not identical. Products have been stripped of naming to avoid incorrect associations between those studied and those that are currently on the QPL.

1.0 INTRODUCTION

Amid global concern regarding the persistence, bioaccumulation, and ecological impacts of per- and polyfluoroalkyl substances (PFAS), there is an urgent need to develop and validate PFAS-free products across their diverse usage. Historically, aqueous film forming foams (AFFF) used for class B firefighting contained PFAS, with three main types varying in chemical composition. The first type contained perfluorooctanesulfonate and was manufactured in the United States until 2002, followed by fluorotelomer-based AFFF including some long-chain PFAS manufactured until 2016 (Interstate Technology and Regulatory Council 2022). Finally, modern fluoroteolomer AFFF containing predominantly short-chain PFAS was introduced in response to the U.S. Environmental Protection Agency's (EPA) voluntary perfluorooctanoic acid stewardship program.

AFFF have been used widely for fire suppression at firefighting training facilities, airports, oil refineries, and military bases (Ruyle et al. 2023). The properties of PFAS, including their high thermal and physical stability, make them highly efficient in controlling and extinguishing hydrocarbon-based fires (Bourgeois et al. 2015). Due to the concerns regarding the environmental impacts of PFAS, the National Defense Authorization Act of 2020 required a phase out of AFFF use at all military installations (United States Government 2020). Fluorosurfactant-containing foams are required to be phased out by October 2024, with a revised military specification published in January 2023. Consequently, most foam manufacturers are now producing firefighting foams that do not contain PFAS and require validation of their firefighting, toxicological, and biodegradation properties.

At present, few studies have considered the potential environmental fate and toxicity of fluorine-free foams (F3). Taking this into account, this project provided a multi-taxa assessment of F3s and a reference AFFF product incorporating both aquatic and terrestrial species. In addition, the biodegradability of foams and their chemical constituents was assessed. The overarching goal of the project was to provide a synthesis of toxicological and biodegradation data for novel F3s and a reference product.

2.0 OBJECTIVES

The objectives for this project were as follows:

1) Conduct multi-taxa ecotoxicity studies with F3s using both acute and chronic exposure durations.

2) Conduct multi-taxa ecotoxicity studies with a reference product using acute and chronic exposure durations.

3) Determine biodegradation potential of both F3s and a reference product to provide insight on the environmental persistence of the products.

3.0 TECHNOLOGY APPROACH

Overall, a total of six and seven F3s were used for biodegradability and toxicity testing, respectively. Results for F3s were compared to a reference AFFF product, which has been listed on the Department of Defense's Qualified Product Listing since 2004 and has previously been shown to contain various short-chain PFAS (Shojaei et al. 2022). An anonymized list of the products tested is given in Table ES-1.

Table ES-1. List of Products Considered in this Study, Including Full Name, Formulation Type, and the Abbreviation Used.

Product Code	Formulation Type
F3 1	Commercial PFAS-free Formulation*
F3 2	Commercial PFAS-free Formulation
F3 3	Commercial PFAS-free Formulation
F3 4	Commercial PFAS-free Formulation
F3 5	SERDP Developmental Formulation
F3 6	SERDP Developmental Formulation
F3 7	Commercial PFAS-free Formulation
Reference Product	Current Use AFFF

Notes:
* Indicates that this product was considered in aquatic and reptile toxicity studies only.

In terms of toxicity testing, both terrestrial and aquatic species were considered, including birds (bobwhite quail, *Colinus virginianus*), reptiles (brown anole, *Anolis sagrei*), algae (*Raphidocelis subcapitata*), aquatic invertebrates (midge larvae, *Chironomus dilutus*), and fish (*Pimephales promelas*). A summary of all tests conducted, foams assessed, and selected endpoints is given in Table ES-2. Acute and chronic tests were performed for all species excluding algae due to the extremely short life cycle of *R. subcapitata*. Toxicity tests were generally performed according to standardized methods from the EPA or Organization for Economic Co-operation and Development (OECD). For the aquatic tests, survival (all three species), growth (*P. promelas* and *C. dilutus*), and development (*C. dilutus* only) were assessed. For terrestrial studies with *C. virginianus*, survival, reproduction, and offspring development were assessed in acute and chronic (60 day) drinking water studies. Reptile studies focused on acute lethality and effects of chronic exposure via pseudo-gavage (60 day) on growth, condition index, and other sublethal endpoints including bite force and evaporative water loss. Where possible, no and lowest observed effect concentrations were calculated along with effective and lethal concentrations. To validate exposure concentrations and determine the potential for bioaccumulation of product constituents, chemical analysis was performed on dosing solutions used for chronic exposure of aquatic invertebrates, birds, and reptiles. In addition, bioaccumulation of product constituents in reptile and adult quail livers, quail eggs, and quail chick livers was assessed.

Table ES-2. Summary of Acute and Chronic Toxicity Studies with All Taxa Including Foams Assessed, Endpoints Measured, Study Durations, and Guidance Methods.

Organism Group	Study Duration	Products Assessed	Endpoints Measured
Acute Studies			
Algae	96 h	Reference Product, F3s 1 -7	Cell Count
Aquatic Invertebrates	48 h	Reference Product, F3s 1 -7	Survival
Fish	96 h	Reference Product, F3s 1 -7	Survival
Birds	24 h	Reference Product, F3s 2 -7	Survival
Reptiles		Reference Product, F3s 1, 2, 3, 4, and 6	Survival
Chronic Studies			
Aquatic Invertebrates	Up to 60 d	Reference Product, F3s 1 -7	Survival, Growth, Emergence
Fish	7 d	Reference Product, F3s 1 -7	Survival & Growth
Birds	60 d	Reference Product, F3s 2 -7	Survival, Reproduction, Development, Offspring Development
Reptiles	60 d	Reference Product, F3s 1, 2, 3, 4, and 6	Survival Growth, Condition, Water Loss, Bite Force

Notes:
d = day(s)
h = hour(s)

For the biodegradation studies, tests were performed according to a modified Zahn-Wellens test following the OECD 302B (OECD 1992) method. This procedure involves applying three different concentrations of F3s and the reference product to biological reactors to assess the inherent biodegradability of targeted formulation constituents over 28 days. Manometric measurements of oxygen uptake by microorganisms were used to determine the extent of biological degradation of organics, as well as the rate of degradation. Concurrently, soluble chemical oxygen demand (COD) measurements were performed as an additional measure following the EPA's Hach dichromate method. In addition to biodegradability, all formulations were analyzed for total Kjeldahl nitrogen, soluble ammonia, nitrite, and nitrate, as well as total phosphate and volatile suspended solids. Constituents of formulations including surfactants and PFAS for the reference product were analyzed over the 28-day degradation period using liquid chromatography-mass spectrometry.

4.0 RESULTS AND DISCUSSION

4.1 AQUATIC TOXICITY TESTING

For acute toxicity, the majority of tested F3s exhibited greater toxicity compared to the reference product (Figure ES-1). Comparing across species, green algae were the most sensitive of the aquatic organisms tested, with three F3s having lethal concentration 50% (LC_{50}) values < 10 mg/L.

A single F3, F3 2, was designated as very highly toxic to green algae based on the EPA's Alternative Assessment Hazard Criteria, and highly toxic to fish.

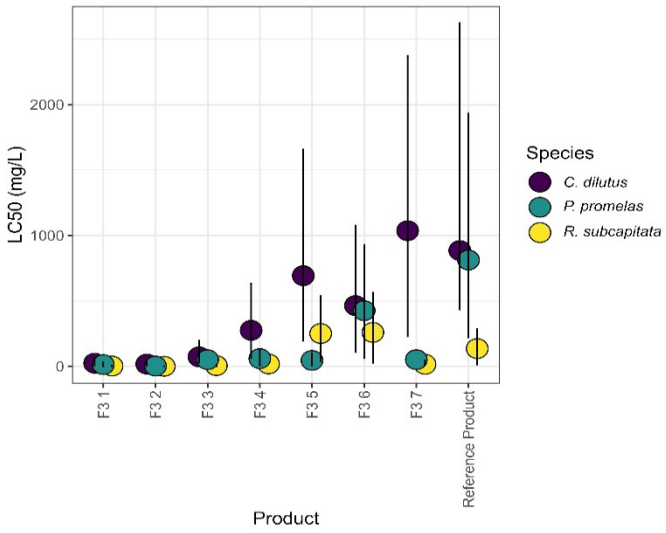

Figure ES-1. Acute LC_{50} Values for Seven F3s and One Reference Product in Aquatic Species.

Similar findings were observed for chronic studies using invertebrates and fish, with the tested F3s exhibiting higher toxicity compared to the reference product.

F3s 1 and 2 were consistently among the most toxic across all aquatic toxicity tests, which was consistent with studies conducted on other aquatic species in related efforts conducted under this Statement of Need (Jones et al. 2022). Based on the EPA's Alternative Assessment Hazard Criteria, F3s 2 and 3 were considered highly toxic based on chronic no observed effect concentrations for *C. dilutus* development.

4.2 TERRESTRIAL TOXICITY TESTING

Results of acute lethality tests with all foams and chronic studies with F3 2 and the reference product are published in Hossain et al. (2022) and Hossain et al. (2023). For acute lethality studies using *C. virginianus*, all the tested formulations had acute lethal dose values at or around the dosing limit of ~1,500 milligrams per kilogram, indicating low or very low toxicity based on the U.S. EPA's Alternatives Assessment Criteria. Results of chronic drinking water studies with *C. virginianus* were complex, with non-monotonic relationships observed for many of the tested formulations. Overall, the most commonly impacted endpoints in bird studies were adult and chick lipid content, chick biometrics, and day of arrested development, which were impacted by 4/7, 4/7, and 3/7 of the tested foams, respectively. Conversely, adult growth rates and the number of eggs produced per hen were only impacted by a single F3. Two F3s caused a significantly greater proportion of arrested embryos relative to controls; however, no significant effects on hatching success or offspring survival were observed. F3 4 had a significant effect on the number of eggs laid per hen; however, effects were only observed at the lowest tested concentration. Exposure to the reference product led to a significantly increased percentage of cracked eggs. Generally, effects were observed at similar nominal concentrations when comparing F3s and the reference AFFF, excluding F3 3 wherein only a single effect was observed at the highest tested concentration.

Bobwhite quail studies incorporated smaller sample sizes relative to previous assessments of chemical of concern effects on bird reproduction, leading to lower statistical power and greater uncertainty in the results.

For quail studies, chemical constituents of foams including surfactants and PFAS compounds were measured in dosing solutions, adult quail livers, eggs, and chick livers. Elevated concentrations of surfactants were generally observed in quail matrices following exposure to F3s, though significant impact was observed in controls likely due to the ubiquity of components such as sodium dodecyl sulfate in bird feed used during studies.

In terms of reptile studies, acute lethality dose values were at the limit of ~1,500 milligrams per kilogram for all foams. For the chronic studies, few overall significant effects were observed, with no significant reductions in growth rate, snout vent length, or condition index following 60 days of exposure to F3s and the reference product. Exposure concentrations used for reptiles were lower than those used for birds, with maximum concentrations of 450 mg/L and 2500 mg/L for reptiles and birds, respectively. Mass of the reptile gastrointestinal tract was impacted in response to two F3s, with significant reductions and increases relative to the controls for F3s 1 and 3, respectively. For the sublethal endpoints, reptile cutaneous evaporative water loss (CEWL) was significantly impacted in response to F3s 1 and 2, which suggested potential impacts on osmoregulation and thermoregulation (Figure ES-2). Finally, reptile bite force was significantly impacted in response to three F3s, though effects varied depending on the timepoint. No significant effects on any measured endpoint were recorded in response to F3 4 and the reference product.

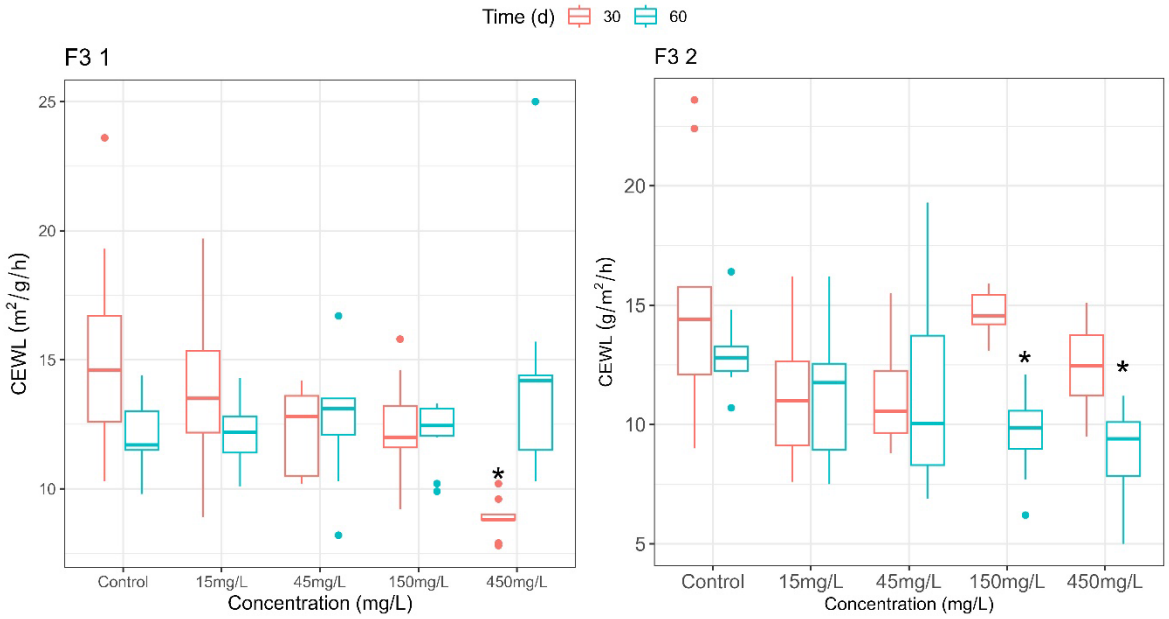

Figure ES-2. CEWL in Brown Anoles Exposed to Two F3s.

* Indicates significant differences to controls at a given time point (30- or 60-day, ANOVA, Post-hoc Dunnett's Test, $p < 0.05$).

Results from the biodegradability studies are summarized in Modiri Gharehveran et al. (2022). Overall, biodegradation testing indicated good biodegradability of all tested F3s over the 28-day period, with F3 6 having the longest overall biodegradation time. Biodegradation was not limited by nutrients or trace minerals; however, all formulations required biomass adaptation to achieve adequate biodegradability. Adaptation was achieved in two days for F3s 2, 3, 4, and 7; three, four, and five days of adaptation were required for the F3 6, F3 5, and the reference product, respectively. Respirometry demonstrated similar oxygen uptake curves for all formulations over the range of concentrations tested, implying that formulations were not significantly toxic to the microbial community. For the reference product, residual COD was observed at the end of the 28-day testing period, which was attributed to the fluorinated fraction. Concentrations of several PFAS, including perfluoro-n-pentanoic acid, perfluorobutanoic acid, and perfluorohexanesulfonate, showed significant increases over the biodegradation testing period as shown in Figure ES-3.

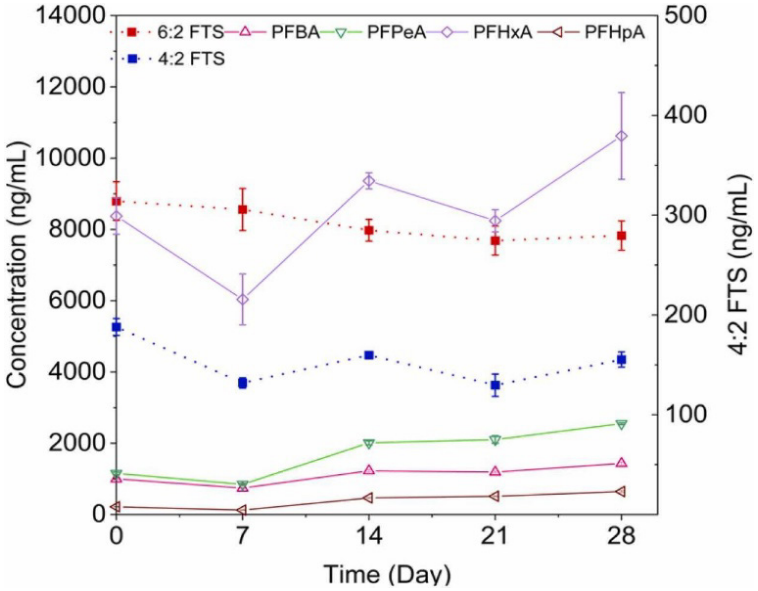

Figure ES-3. Degradation of Tested PFAS in the Reference Product Over the Reaction Period of 28 Days.

The 4:2 FTS concentration is shown on the secondary y-axis.

Chemical constituents of F3s, including several surfactants, were measured over the 28-day biodegradation period. For F3s 2, 3, and 4, all measured constituents had dropped to non-detectable levels by the seventh day of testing. Several foams including F3s 5, 6, and 7, had measurable levels of at least one chemical constituent after 28 days of degradation. Among the F3s, F3 6 had a higher proportion of slowly degradable organics relative to other foams, with some PFAS components of the AFFF showing little degradation over the 28-day period. Consequently, the tested F3s offer a significant advantage over the reference product in terms of overall biodegradation of constituents.

5.0 IMPLICATIONS FOR FUTURE RESEARCH AND BENEFITS

Overall, the present study documented the ecotoxicity and biodegradation potential of novel F3s and a reference product. For the aquatic species, several of the tested F3s were more toxic than the reference product and categorized as highly or very highly toxic based on EPA's Alternatives Assessment Criteria. Terrestrial toxicity studies were more complex, with some effects of both F3s and the reference product on reproductive and lipid parameters in bobwhite quail, and few overall effects of any of the tested products in reptile studies. Biodegradation studies indicated good overall biodegradability of the tested F3s. Taken together, these findings will be used to inform decision making and selection of appropriate PFAS-free firefighting formulations.

6.0 LITERATURE CITED

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