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

Jamie Suski
Neil Fuller
EA Engineering, Science, and Technology, Inc., PBC

Christopher Salice
Towson University

Todd Anderson
Jennifer Guelfo
Texas Tech University

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Jamie Suski, Christopher J. Salice, Todd Anderson, Neil Fuller

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EA Engineering, Science, and Technology Inc., PBC
225 Schilling Circle, Suite 400
Hunt Valley Maryland 21031

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14. ABSTRACT

Historically, aqueous film-forming foams (AFFF) used in firefighting contained PFAS, with new legislation prompting development and use of PFAS-free firefighting formulations (F3s). To support the implementation of novel PFAS-free F3s, comprehensive testing of their firefighting, biodegradation, and toxicological properties is required. Consequently, this project aimed to elucidate the toxicity of a suite of F3s and a reference AFFF product across multiple taxa including aquatic (algae, invertebrates, and fish) and terrestrial (birds and reptiles) species. In addition, 28-day biodegradation studies were conducted to assess product biodegradability. For the tested aquatic species, the F3s tested were more acute and chronically toxic than the reference product. Studies with bobwhite quail found similar or greater toxicity of several of the F3s relative to the reference AFFF, with effects observed on reproduction, lipid content, and offspring growth, though low sample sizes precluded a robust analysis of effects on reproduction given natural variability. For reptile studies, few overall effects of F3s or the reference AFFF were observed, though one F3 had significant effects on two sublethal endpoints. Overall, biodegradability testing indicated good overall biodegradability of all the tested F3s, with a single F3 having a higher proportion of slowly degrading organics, and the reference AFFF product having residual chemical oxygen demand (COD) and elevated concentrations of perfluorinated chemicals after 28 days. This report contains a synthesis of all toxicity and biodegradation findings of candidate F3s that will facilitate selection of an appropriate replacement F3.

15. SUBJECT TERMS

PFAS-free Firefighting Formulations; Aquatic Toxicity; Reptile Toxicity, Avian Toxicity, Chronic Toxicity; Acute Toxicity; Per- and polyfluoroalkyl Substances (PFAS); Aqueous Film-Forming Foams (AFFF)

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ACRONYMS AND ABBREVIATIONS

°C	Degrees Celsius
µg/mL	Microgram(s) per milliliter
µL	Microliter(s)
µm	Micrometer(s)
4:2 FTS	4:2 Fluorotelomer sulfonic acid
6:2 FTS	6:2 Fluorotelomer sulfonic acid
ACURO	Animal Care and Use Review Office
ADI	Average daily intake
AFFF	Aqueous film-forming foam
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
CEWL	Cutaneous evaporative water loss
COD	Chemical oxygen demand
d3-N-MeFOSAA	N-methyl-d3-perfluoro-1-octanesulfonamidoacetic acid
DGMBE	Diethylene glycol monobutyl ether
DMDA N-O	N, N-Dimethyldecylamine N-oxide
DO	Dissolved oxygen
DoD	Department of Defense
EA	EA Engineering, Science, and Technology, Inc., PBC
EC	Effective concentration
EGMBE	Ethylene Glycol Monobutyl Ether
EPA	U.S. Environmental Protection Agency
ESI	Electrospray ionization
F3	Fluorine-free Foam
g	grams
$g/m^2/h$	grams per square meter per hour
GI	Gastrointestinal
GLM	General linear model
HG	Hexylene glycol
HPLC	High performance liquid chromatograph
HSD	Honestly significant difference
IACUC	Institutional Animal Care and Use Committee
L	Liter(s)
LC	Lethal concentration

LC/MS	Liquid chromatography-mass spectrometry
LC/MS/MS	Liquid chromatography/mass spectrometry/mass spectrometry
LD	Lethal dose
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
LOEC	Lowest observed effect concentration
LOQ	Limit of quantification
mg	Milligram(s)
mg/L	Milligram(s) per liter
mg/kg	Milligram(s) per kilogram
mg/kg/bw-day	Milligram(s) per kilogram of body weight per day
mg/kg/day	Milligram(s) per kilogram per day
Mil-Spec	Military Specification
mL	Milliliter(s)
mm	Millimeter
mM	Millimolar
MPFHxA	Perfluoro-n-[1,2-13C2]-hexanoic acid
N	Newtons
ng/g	Nanogram(s) per gram
ng/mL	Nanogram(s) per milliliter
NOAEL	No observed adverse effect level
NOEC	No observed effect concentration
OECD	Organization for Economic Co-operation and Development
PFAA	Perfluoroalkyl acids
PFAS	Per- and polyfluoroalkyl substances
PFBA	Perfluorobutanoic acid
PFBS	Perfluorobutanesulfonic acid
PFCA	Perfluoroalkyl carboxylic acid
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexanesulfonate
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PFPeA	Perfluoropentanoic acid
PFPeS	Perfluoropentanesulfonic acid
QC	Quality control
rpm	Revolutions per minute
SDS	Sodium dodecyl sulfate

SERDP	Strategic Environmental Research and Development Program
SVL	Snout Vent Length
TCMP	2-chloro-6-(trichloromethyl)pyridine
TNT	2,4,6-trinitrotoluene
tTKN	Total Kjeldahl nitrogen
US	United States
VSS	Volatile suspended solids

KEYWORDS

PFAS-free Firefighting Formulations; Aquatic Toxicity; Reptile Toxicity, Avian Toxicity, Chronic Toxicity; Acute Toxicity; Per- and polyfluoroalkyl Substances (PFAS); Aqueous Film-Forming Foams (AFFF)

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 are comprised of 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.

ABSTRACT

INTRODUCTION AND OBJECTIVES

Due to concerns relating to the persistence, bioaccumulation, and environmental impacts of per- and polyfluoroalkyl substances (PFAS), there is an urgent need to develop PFAS-free products. Aqueous film-forming foams (AFFF) used in firefighting historically contained PFAS, with recent legislation mandating the phase out of PFAS-containing foams. Consequently, novel PFAS-free firefighting formulations (F3s) have been developed and require validation of their performance and environmental impact. This study performed both multi-taxa ecotoxicity assessments and inherent biodegradability tests with a suite of seven F3s and a reference short-chain PFAS-containing AFFF product. The overarching goal of the study was to evaluate the toxicity and biodegradation potential of F3s to facilitate selection of an appropriate replacement foam that minimizes environmental impacts.

TECHNICAL APPROACH

For the toxicity studies, tested taxa included three aquatic species: green algae, *Raphidocelis subcapitata*; midge larvae, *Chironomus dilutus*; and fish, *Pimephales promelas*, and two terrestrial species: bobwhite quail, *Colinus virginianus*; and brown anole, *Anolis sagrei*. Acute and chronic studies were performed to assess the effects of F3s and a reference product (AFFF) on survival, growth, reproduction, and development. For biodegradation studies, a modified Zahn-Wellens test was performed to assess the inherent biodegradability of the foams over 28 days.

RESULTS

For the aquatic species, both acute and chronic toxicity tests indicated higher toxicity of several of the tested F3s relative to the reference product. For bobwhite quail studies, similar effects were observed between several of the tested F3s and the reference product, with effects on endpoints including lipid levels and chick biometrics. For reptiles, several of the tested F3s were more toxic compared to the reference product, with effects recorded on cutaneous water loss and bite force. Finally, biodegradation tests indicated good overall biodegradability of all tested F3s, with one F3 having a higher proportion of slowly degradable organics. The reference product had residual chemical oxygen demand (COD) and elevated concentrations of perfluorinated chemicals after 28 days, indicating persistence of foam constituents.

BENEFITS

Overall, the present study documented the ecotoxicity and biodegradation potential of novel F3s and a reference AFFF product. This report contains a synthesis of toxicity and biodegradation findings of candidate F3s. Results may be used to inform suitable replacements.

EXECUTIVE SUMMARY

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 (PFOS) 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 United States Environmental Protection Agency's voluntary perfluorooctanoic acid (PFOA) 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 (Mil-Spec) 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 F3s. Taking this into account, this project provides 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.

OBJECTIVES

The objectives for this project are 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.

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 as 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 ES1.

Table ES1. 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 ES2. 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 United States Environmental Protection Agency (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 (EC and LC values, respectively). 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 ES2. 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 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.

RESULTS AND DISCUSSION

Aquatic Toxicity Testing

For acute toxicity, the majority of tested F3s exhibited greater toxicity compared to the reference product (Figure ES1). Comparing across species, green algae were the most sensitive of the aquatic organisms tested, with three F3s having 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.

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.

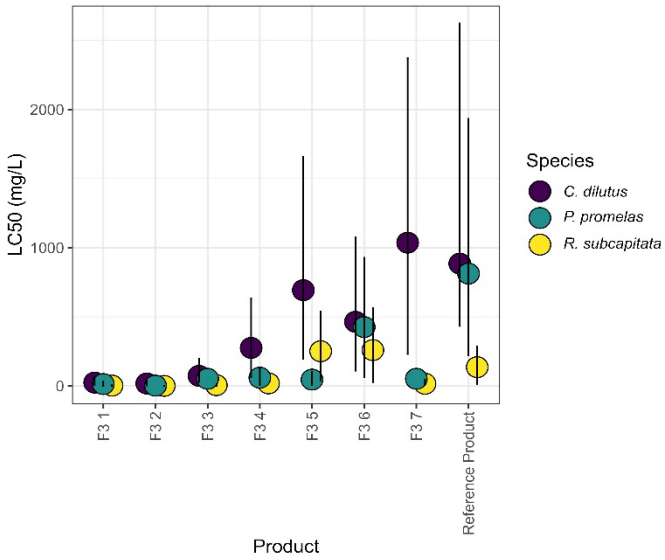

Figure ES1. Acute LC_{50} values for seven F3s and one reference product in aquatic species.

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. (2024). 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 US 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 contaminant 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 contamination 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 suggests potential impacts on osmoregulation and thermoregulation (Figure ES2). Finally, reptile bite force was significantly impacted in response to three F3s, though effects varied dependent on the timepoint. No significant effects on any measured endpoint were recorded in response to F3 4 and the reference product.

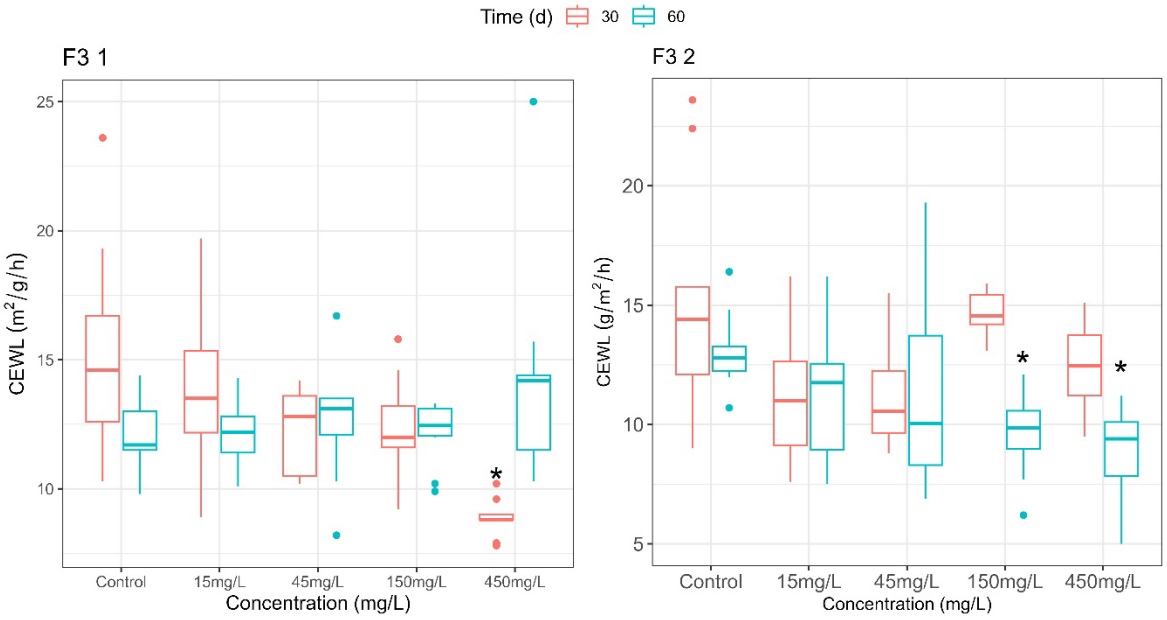

Figure ES2. CEWL in brown anoles exposed to two F3s.

* 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 (PFPeA), perfluorobutanoic acid (PFBA), and perfluorohexanesulfonate (PFHxS) showed significant increases over the biodegradation testing period with the as shown in Figure ES3.

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.

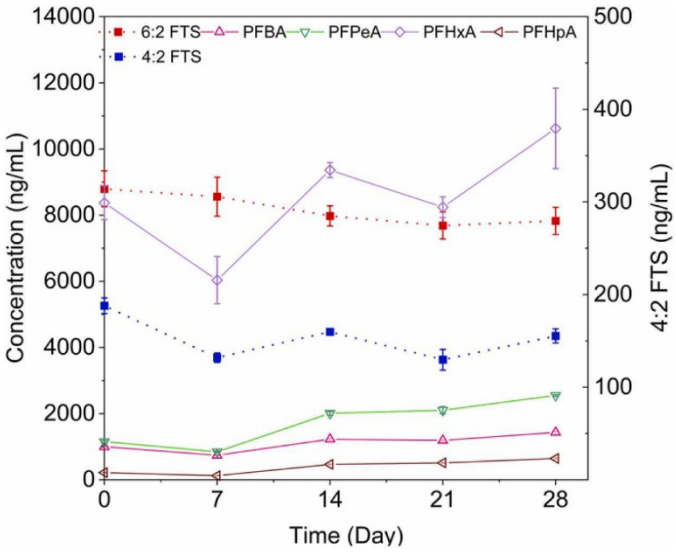

Figure ES3. Degradation of tested PFAS in the reference product over the reaction period of 28 days. The 4:2 FTS concentration is shown on the secondary y-axis.

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.

1.0 OBJECTIVE

The specific technical objectives of this research were developed in response to the Strategic Environmental Research and Development Program's (SERDP), Environmental Restoration Statement Of Need 20-A1, which was focused on quantifying the potential ecotoxicity of Fluorine-Free Surfactant Foam Formulations. In addition, the statement of need identified the need for comparative product toxicity between aqueous film-forming foams (AFFF) containing per- and polyfluoroalkyl substances (PFAS) and novel PFAS-free firefighting formulations (F3s). The technical approach of this project involved ecotoxicity assessment of F3s and AFFF in aquatic and terrestrial taxa, biodegradation experiments, and synthesis of data. The specific objectives 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 AFFF product using acute and chronic exposure durations.
3) Determine biodegradation potential of both F3s and short chain PFAS AFFF to provide insight on the environmental persistence of the products.

2.0 BACKGROUND

Globally, there is growing concern regarding the potential health and environmental effects of PFAS (Baluyot et al. 2021; Kurwadkar et al. 2022; Podder et al. 2021). These compounds have a wide range of uses both commercially and industrially, including in food packaging, nonstick cookware, hydraulic fluids, and AFFF used in firefighting (Glüge et al. 2020). The primary concerns associated with PFAS are related to their persistence in environmental matrices including soil, sediment, and water (Cousins et al. 2020), their bioaccumulation potential and persistence in humans and wildlife (Li et al. 2018; Lupton et al. 2015), and chronic toxicity (Ankley et al. 2021; Fenton et al. 2021). The vast majority of PFAS are designated as very persistent by regulatory agencies (Cousins et al. 2020), do not readily degrade in the environment (Evich et al. 2022), and are toxic (Fenton et al. 2021). Due to these concerns, there is an increasing need to develop alternative products to replace PFAS across their diverse usage.

PFAS-containing AFFF have been widely used 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 fires where flammable volatile liquids constitute the primary fuel source (Bourgeois et al. 2015). Historically, AFFF used in firefighting included three main types: 1) legacy AFFF containing PFOS (manufactured until 2002), 2) legacy fluorotelomer-based AFFF including some long-chain PFAS (manufactured until 2016), and 3) modern fluorotelomer AFFF containing predominantly short-chain PFAS were introduced in response to the United States (US) Environmental Protection Agency's (EPA's) voluntary perfluorooctanoic acid (PFOA) stewardship program (Interstate Technology and Regulatory Council 2022). Herein, the term AFFF will be used to refer to legacy foams containing PFAS, with F3 referring to novel PFAS-free firefighting formulations. The military possesses the largest stockpile of AFFF within the US, with long-term use of AFFF in training, equipment maintenance, and emergency response leading to thousands of PFAS-impacted military sites (Anderson et al. 2021). Given the critical role of AFFF in fire prevention, developing F3s without PFAS at military specification is a priority (Jones et al. 2022). Under the 2020 National Defense Authorization Act, fluorosurfactant-containing foams are required to be phased out by October 2024, with development of a revised military standard fluorine-free foam no later than January 2023 (US Government 2020). Consequently, international effort to develop F3s has yielded some commercially available formulations, with a total of 35 commercially available Class B foams certified as PFAS-free as of February 2023 (Ateia et al. 2023). In January 2023, the Department of Defense (DoD) published a military specification (Mil-Spec) for F3s, which includes criteria for aquatic toxicity and biodegradability (DoD 2023).

The effectiveness of newly developed F3s for fire suppression has been studied for over 20 years (Iglesias et al. 1995) and although they appear to remain inferior in comparison to fluorosurfactant-based AFFF, a satisfactory fire suppression foam with potential reduced environmental impacts is necessary. In fact, RF6, an F3 foam had adequate performance compared to PFOS-based AFFF in meeting the Australian Defence Force Specifications (Schaefer et al. 2007). However, Hinnant et al. (2017) recently found that the RF6 resulted in more rapid degradation during fire suppression when compared to Buckeye 3% (Buckeye Fire Equipment Company, Inc. Bessemer City, North Carolina), a short chain PFAS-containing AFFF, which is currently the most widely used foam by

the US Navy. As F3s are being developed to meet appropriate criteria for firefighting, environmental fate and ecological data are also necessary to understand and minimize, as much as possible, the potential for risks to ecological receptors and systems.

Biodegradation of F3s currently available on the market suggests many have > 60% degradation within the 28-day window of a guideline biodegradation test; likewise, short-chain PFAS AFFF products appear to degrade within the specified testing window (Bourgeois et al. 2015). Although product degradation using standardized methods is important, this method does not provide insight into the degradation of specific product ingredients. It is well known that short-chain PFAS are environmentally persistent (Parsons et al. 2008). With the understanding that fire suppression foams require certain classes of chemicals to be effective, it is also prudent to determine degradation of constituents when replacement chemistry is warranted. In fact, the EPA developed an approach of evaluating such replacement chemistry (Alternatives Assessment). Using EPA's Design for the Environment Program Alternatives Assessment Criteria for Hazard Evaluation (2011) the available product data on F3 foams shows low – high acute toxicity for aquatic organisms. There is no data available on terrestrial organisms and importantly no data available for sub-chronic or chronic exposures.

While additional F3s are being developed and tested, studies determining the environmental fate, behavior, and toxicity of new formulations are needed to inform selection of appropriate products for use in firefighting applications. At present, few studies have considered the potential environmental fate and toxicity of F3s (Hossain et al. 2022; Jones et al. 2022; Yu et al. 2022). Jones et al. (2022) studied the direct lethal effects of seven F3s on a total of 14 aquatic species using a series of acute (48–96 hours) lethal concentration (LC_{50}) tests. Findings were compared to data for an AFFF, with the authors finding that at least one of the seven test foams was more, or as, toxic as the AFFF (Jones et al. 2022). Similarly, Yu et al. (2022) found that several F3s had stronger effects on reproduction compared to AFFF in the model soil organism, *Caenorhabditis elegans*. Finally, a study by Wu et al. (2022) considered the effects of six F3s foams on the terrestrial plant, *Brassica rapa*, finding that the majority of the F3s were more highly toxic than the corresponding short-chain AFFF foam.

Overall, this research project aimed to fill the ecological and environmental concern data gaps associated with new F3 products. Through acute and chronic exposures of relevant test organisms, we intended to synthesize toxicity of F3s (as identified by SERDP) and a reference AFFF containing short-chain PFAS. This research provided a comprehensive toxicological profile of the products to aquatic (algae, invertebrate, and fish) and terrestrial (avian and reptiles) receptors and evaluated environmental degradation.

3.0 MATERIALS AND METHODS

3.1 PRODUCTS TESTED

Overall, a total of seven F3s were used for toxicity testing, and six for biodegradation potential (Table 1). One F3, F3 1, was assessed in reptile and aquatic studies only. The reference AFFF product has been listed on the DoD's Qualified Product Listing since 2004, with previous studies of this formulation identifying various PFAS (Modiri Gharehveran et al. 2022; Shojaei et al. 2022).

Further chemical characterizations of the F3s and reference AFFF product are given in Sections 4.2.1.2 and 4.3.5, respectively, with Table 2 displaying the disclosed ingredients of the F3s.

Table 1. List of products considered in this study including formulation type, and anonymous name given.

Product Name	Formulation Type
F3 2	Commercial PFAS-free Formulation
F3 1	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	Reference C6 Formulation

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

Table 2. List of products considered in this study including full name, formulation type, and the abbreviation used. Constituents of F3s and the reference product are based on Safety Data Sheets.

Chemical Name	Reference Product	F3 3	F3 4	F3 2	F3 7	F3 5	F3 6	F3 1
Hexylene glycol	X					X		
Fluorosurfactants and hydrocarbon surfactants	X							
Diethylene glycol monobutyl ether		X	X	X	X		X	X
Alkyl sulfate		X						
Alkyl betaine		X						
Amphoteric surfactant		X						
Preservative		X						
Sulfuric acid, mono-C12-14-alkyl esters, compds. with triethanolamine			X					
Alcohols, C12-14, ethoxylated, sulfates, sodium salts			X			X		
Amines, C12-14 (even numbered) - alkyldimethyl, N-oxides			X					
Sodium Lauryl Sulfate				X				
Lauramine oxide				X				
Dimethyltetradecylamine oxide				X				
Starch					X			
tris(2-hydroxyethyl)ammonium dodecylsulfate					X			
alpha-sulfo-omega-hydroxy- poly(oxy-1,2-ethanediyl)C9-11 alkyl ethers, sodium salts					X			
1-propanaminium, 3-amino-N- (carboxymethyl)-N,N-dimethyl-, N-coco acyl derivs., hydroxides, inner salts					X			
1-propanaminium, N-(3- aminopropyl)-2-hydroxy-N,N- dimethyl-3-sulfo-, N-coco acyl derivs., hydroxides, inner salts					X			
D-glucopyranose, oligomers, decyl octyl glycosides					X		X	
Sucrose					X			
1-Propanaminium, N-(3-aminopropyl)-2-hydroxy-N,N-dimethyl-3-sulfo-, N-(C8-18(even numbered) acyl) derivs., hydroxides, inner salts						X		
3-(Polyoxyethylene)propylheptamethyltrisiloxane							X	
Diethylene oximide								X

3.2 TOXICITY TESTING

A summary of the exposure durations, endpoints tested, and guidance methods for toxicity tests across all taxa is given in Table 3. The suite of F3s tested was different across taxa, with all eight F3s shown in Table 1 tested for aquatic species, all excluding F3 1 included in bird studies, and F3s 5 and 7 not tested in reptile studies. F3 5 was not tested in reptiles due to product volume limitations. The F3 5 designated for use in reptile studies was needed to complete the bird study that was mid-way through the chronic exposure study. We anticipated receiving additional foam; however, foams were no longer available from the program office or among funded projects. F3 7 was not tested in reptiles as the concentrated foam viscosity increased over time, thus making the foam difficult to uniformly dilute for dosing solutions. Further details on individual tests are provided in the following sections.

Table 3. Summary of acute and chronic toxicity studies with all taxa including foams assessed, endpoints measured, study durations, and guidance methods.

Species	Study Duration	Foams Assessed	Endpoints Measured	Guidance Method
Acute Studies				
Algae (*R. subcapitata*)	96 h	Reference Product, F3s 1-7	Cell Count	EPA 1003.0
Invertebrate (*C. dilutus*)	48 h	Reference Product, F3s 1-7	Survival	OECD 235
Fish (*P. promelas*)	96 h	Reference Product, F3s 1-7	Survival	OECD 203
Bird (*C. virginianus*)	24 h	Reference Product, F3s 2 -7	Survival	OECD 425
Reptile (*A. sagrei*)	24 h	Reference Product, F3s 1, 2, 3, 4, and 6	Survival	OECD 425
Chronic Studies				
Invertebrate (*C. dilutus*)	Up to 60 d	Reference Product, F3s 1-7	Survival, Growth, Emergence	OECD 219
Fish (*P. promelas*)	7 d	Reference Product, F3s 1-7	Survival & Growth	EPA 1000.0
Bird (*C. virginianus*)	60 d	Reference Product, F3s 2 -7	Survival, Reproduction, Development, Offspring Development	OECD 206
Reptiles (*Anolis sagrei*)	60 d	Reference Product, F3s 1, 2, 3, 4, and 6	Survival Growth, Condition, Water Loss, and Bite Force	None

Notes:
d = day(s)
h = hour(s)
OECD = Organization for Economic Co-operation and Development
EPA = United States Environmental Protection Agency

3.2.1 Aquatic Toxicity Testing

3.2.1.1 Exposure Solution Preparation

For all aquatic toxicity tests, F3s and the reference product were received in 1L bottles as concentrates (on a per weight basis) and diluted to the appropriate exposure concentrations using either dechlorinated tap water (*C. dilutus* tests), synthetic moderately hard water (EPA 2002, *P. promelas* tests), or Woods Hole algae media (Stein-Taylor, 1973). The amount of 3% stock solution required was calculated based on the target exposure concentration and added to the appropriate dilution media in either a 300 mL lipless polypropylene beaker (*C. dilutus* tests), 1L polypropylene beaker (*P. promelas* tests), or a 250 mL glass Erlenmeyer flask. All stocks were adequately mixed overnight using a magnetic stir bar prior to dilution.

3.2.1.2 Algae (Raphidocelis subcapitata)

Due to the extremely short life cycle of *R. subcapitata*, a 96-hour exposure was conducted and considered to be reflective of both acute and chronic exposures. Freshwater algae *Raphidocelis subcapitata* were obtained from the University of Waterloo's Canadian Phycological Culture Centre (Waterloo, Ontario, Canada). The algae were cultured in accordance with EPA (2002a) methodology. All algae were 7 days old when used for test initiation. The toxicity testing was conducted following EPA guidance (2002a). The definitive toxicity tests with each product utilized five test concentrations and a control (dechlorinated tap water). The nominal concentrations used for the definitive tests were based on the results of range finding studies with a wider range of test concentrations (Appendix A, Table A1).

All test solutions were prepared from a 3% stock solution on a per weight basis. The 96-hour *R. subcapitata* toxicity tests were conducted in 250-mililiter (mL) Erlenmeyer flasks with loose fitting metal lids. Each replicate chamber contained 100 mL of test solution. Dilution and control water for *R. subcapitata* was Woods Hole algae media, which was prepared fresh within 24 hours of preparation of the test concentrations. The definitive tests had four replicate test chambers per test concentration. At test initiation, the test chambers, including the water quality monitoring chambers, were inoculated with 1 mL of approximately 1,000,000 cells/mL concentration of *R. subcapitata* to give an initial target cell concentration of approximately 10,000 cells/mL. The flasks were placed on a shaker table for the duration of the 96-hour exposure period. The chambers were maintained at a target temperature of 25 ± 1 degrees Celsius (°C) and were exposed to continuous illumination of 400 ± 40-foot candles. Preparation of test solutions and inoculum, and the inoculation of the test chambers were performed following sterile procedures. Temperature and dissolved oxygen (DO) were monitored daily, with conductivity and pH measured at test initiation only. At test termination (96 hours), cell growth was determined visually using a hemacytometer.

3.2.1.3 Invertebrates (Chironomus dilutus)

Acute Testing

The acute testing method for *C. dilutus* was based on the Organization for Economic Co-operation and Development (OECD) 235 Chironomus sp., Acute Immobilisation Test (OECD 2011). All *C. dilutus* individuals were obtained from Aquatic Research Organisms (Hampton, New Hampshire) and acclimated to laboratory water at 20°C. The source of the laboratory water was

the City of Baltimore municipal water system which was passed through a high-capacity activated carbon filtration system to remove contaminants and dechlorinate prior to use. Second instar larvae were used for all toxicity testing.

For each foam, *C. dilutus* were exposed to a total of five test concentrations and a control containing only dechlorinated tap water for 48 hours. Test concentrations were based on the results of preliminary range finding studies using a wide range of concentrations (Appendix A, Table A4). All test solutions were prepared from 3% stock solutions on a per weight basis. Tests were conducted in 1-liter (L) beakers containing 250 mL of test solution on a nominal basis. Each concentration and the control had two replicate beakers containing a total of 10 *C. dilutus* individuals. Target water quality conditions during the acute tests were 20 ± 1°C with a 16-hour light/8-hour dark photoperiod. Water quality measurements and mortality observations were made daily throughout the study and were recorded on the data sheets. Water quality determinations during testing were performed at test initiation and daily for the following parameters: temperature, DO, conductivity, and pH.

Chronic Testing

Toxicity testing for *C. dilutus* was modified from the EPA and OECD guidance to determine the effects of formulations on survival, growth, and emergence with spiked water (EPA 2002, OECD Method 219, OECD 2023). Briefly, *C. dilutus* were received from Aquatic Biosystems (Fort Collins, Colorado) and gradually acclimated to laboratory water at 23°C before 2nd instar individuals were used for testing. A total of eight replicates, each containing 10 *C. dilutus* individuals, were used for each treatment. Each replicate consistent of a 300 mL plastic beaker containing approximately 100 mL of artificial sediment (consisting of 10% peat moss, 20% clay, 70% sand 1% $CaCO_3$, and a moisture level of 45%) and a minimum of 175 mL of overlying water containing the foam solutions at various concentrations and a control containing dechlorinated tap water only. For each formulation, five different concentrations were used along with a control. Target nominal concentrations of formulations were based on the results of the acute testing for *C. dilutus*, with selected concentrations ranging from orders of magnitude below acute toxicity values up to acute (48 hour) LC_{50} concentrations. To validate the nominal exposure concentrations used, a subset of prepared exposure solutions from the chronic *C. dilutus* study were shipped to Texas Tech University for analysis. Methodological details of chemical analysis of selected constituents are given in Section 3.5.1.

Sediment and test solutions were added to the treatment vessels approximately 24 hours prior to introducing *C. dilutus* to allow settlement of any suspended sediment. Throughout the test, vessels were maintained in a water bath at 23°C with a 16-hour light/8-hour dark photoperiod, and individuals were fed 1.5 mL of a 4.0 mg/mL flake food (Tetramin, Blacksburg, Virgina) suspension daily per individual replicate. Overlying water containing formulations was renewed daily using an automated water delivery system. Water quality parameters including temperature, pH, DO, and conductivity were recorded daily on the overlying water in one replicate for each treatment. Following 10 days of exposure, four of the eight replicates were sacrificed for growth and survival analyses. Individuals were retrieved from each test vessel to determine survival, and surviving *C. dilutus* were placed in pre-weighed, ashed crucibles and oven dried for a minimum of 6 hours before reweighing. Next, the crucibles were ashed at 550°C for a minimum of 2 hours, allowed to cool, and reweighed. The mean ash-free dry weight per individual midge was

calculated. The four remaining replicates after day 10 were fitted with emergence traps, and the number of emergent individuals per day recorded until the end of the test. The end of the test was determined as once no emergence had occurred for seven consecutive days. Finally, test vessels were sieved and any individuals remaining in the sediment were recorded.

3.2.1.4 Fish (Pimephales promelas)

Acute Studies

Pimephales promelas (fathead minnow) were obtained from EA Engineering, Science, and Technology, Inc., PBC's (EA's) Culture Facility in Hunt Valley, Maryland. The origin of the brood stock was Aquatic Biosystems (Fort Collins, Colorado). Brood organisms were maintained in recirculating dechlorinated tap water at 25°C in 20-gallon aquaria. The source of the tap water was the City of Baltimore municipal water system. Upon entering the laboratory, the water passed through a high-capacity, activated-carbon filtration system to remove any possible contaminants such as chlorine and trace organic compounds. Eggs produced from the brood system were removed from the brood aquaria and placed into culture water at 25°C until hatched. Hatched larvae were acclimated to the test temperature of 25°C prior to testing. The larvae utilized for testing were less than 14 days old. The *P. promelas* were fed *Artemia* sp. nauplii (< 24 hours old) prior to test initiation and at 48 hours during the testing. Synthetic moderately hard water freshwater (EPA 2002c) was used as the dilution water for the *P. promelas* toxicity tests. Batches of this water were made by passing deionized water through activated carbon, adding reagent grade chemicals, and aerating overnight. The water was stored at 25°C under gentle aeration until needed, up to 14 days.

The toxicity testing was conducted in accordance with OECD guidelines (2019) and EPA guidance (2002c). All fish studies were conducted in accordance with approval from the Institutional Animal Care and Use Committee (IACUC) at EA (Protocol No. 2021.01-1 [Acute], 2021.01-2 [Chronic]) and the Animal Care and Use Review Office (ACURO) of the US Army Medical Research and Development Command. The definitive toxicity tests with each product utilized five test concentrations and a control. Target nominal test concentrations were based on preliminary range finding studies (Appendix A, Table A8). All test solutions were prepared from a 3% stock solution on a per weight basis. The *P. promelas* toxicity tests were conducted in 1 L beakers containing 250 mL of test solution. For the definitive toxicity tests, each concentration and control had two replicates of 10 test organisms. The toxicity tests were maintained at a target temperature of 25 ± 1°C with a 16-hour light/8-hour dark photoperiod. Water quality measurements and mortality observations were made daily throughout the study and were recorded on the data sheets. Water quality determinations during testing were performed on the following schedule: temperature, DO, conductivity, and pH were measured at test initiation, and daily for each exposure concentration and the controls.

Chronic Studies

As highlighted above, methods for chronic *P. promelas* studies were based on the EPA 1000.0 7-day larval growth and survival test (EPA 2002c). Briefly, < 24-hour old *P. promelas* larvae obtained from Aquatic Biosystems were used for testing. Test solutions were prepared using synthetic moderately hard freshwater as dilution and control water (EPA 2002c). A total of five test concentrations and a control were used, with the concentrations based on results of acute and

range finding studies. Tests were conducted in 1,000 mL beakers containing 250 mL of test solution, with a total of four replicates containing ten organisms per treatment. All tests were performed at a target temperature of 25 ± 1°C with a 16-hour light/8-hour dark photoperiod. The test solutions were renewed each day by siphoning approximately 80% of the old test solution from the beaker and replacing it with freshly prepared test solution. Observations of mortality were recorded daily, and dead organisms were removed when observed. Temperature, pH, DO, and conductivity measurements were recorded on one replicate of each concentration daily on the new and old test solutions. The *P. promelas* larvae were fed 0.10 mL of a 0.05 grams/mL suspension of newly hatched brine shrimp nauplii (*Artemia* sp., less than 24 hours old) three times daily throughout the test.

After 7 days of exposure all replicates were analyzed for survival and growth. Test organisms were retrieved from beaker to determine number of surviving organisms. For weight determination, surviving *P. promelas* were placed in a pre-weighed tin weigh boat. Organisms were oven dried for a minimum of 6 hours after which each tin was weighed. The mean dried weight of the *P. promelas* was calculated by subtracting the initial weight of the tin by the oven dry tin with organisms and then dividing by the number of surviving organisms in the replicate.

3.2.2 Terrestrial Toxicity Testing

3.2.2.1 Birds (Colinus virginianus)

Acute Studies

Acute toxicity (as indicated by death or the attainment of a humane endpoint as described below) was evaluated using the Up-and-Down Procedure according to OECD Test 425 (OECD, 2022). The Up-and-Down Procedure was first described by Dixon and Mood (1948). The main advantages of this procedure are that: (1) it ensures the minimum number requirements of animals, (2) testing one sex is generally considered sufficient, (3) the method is easiest to apply to materials that produce death within 1 or 2 days, and (4) a limit test can be used efficiently to identify chemicals that are likely to have low toxicity. Although the Up-and-Down Procedure is conducted over 24–48 hours, it does require moribund animals or animals in pain or showing signs of severe and enduring distress to be humanely euthanized. These animals are considered in the interpretation of the test results in the same way as animals that died during the test (OECD 2022). All studies were conducted in accordance with approval from the IACUC of Texas Tech University (Protocol No. 20073-09) and ACURO of the US Army Medical Research and Development Command.

A total of 37 adult (28 males and 9 females) northern bobwhite quail (*Colinus virginianus*) were obtained from T & T Game Birds and Hunting Preserve, Lubbock, Texas. Birds were housed in a 15-Section Quail Battery Breeding Pen manufactured by GQF (Savannah, Georgia). During a 48-hour acclimation period, birds received Purina Game Bird Breeder Layena® feed and water *ad libitum*. The temperature (22.8 ± 0.46°C) and humidity (16%) of the room was monitored daily, and birds were observed three times per day. Artificial lighting was provided at a 12:12-hour light:dark cycle. Following the acclimation period, birds were weighed and randomly separated into groups of five (four males and one female). The Up-and-Down Procedure began with a "limit test," whereby five animals were dosed at the "limit.". For each F3 and the reference product, a total of 5 bobwhite quail (4 male and 1 female) were exposed for the acute tests. The OECD

guideline indicates the "limit" is generally 2,000 milligrams per kilogram (mg/kg) body weight (sometimes 5,000 mg/kg). Due to the viscosity of the foam products, potential issues were highlighted regarding attempts to force birds to swallow products at 2,000 mg/kg (approximately 420 microliters [µL] of product/bird). A 1,500 mg/kg body weight was determined as the "limit," which was administered neat (i.e., products were not diluted) to minimize the volume of material that the bird needed to swallow. The rationale for choosing 1,500 mg/kg as the limit was based on the following scenario. If a foam product was being sprayed at 3% (the typical application rate for AFFFs and Mil-Spec expectations for new foams) and a puddle of water was created, a quail consuming water directly from that puddle would need to drink 25% of its daily water requirement (Guthery & Koerth 1992) at one time to reach a dose of 1,500 mg/kg body weight.

Birds were dosed using a pseudogavage technique (Weir et al. 2014), whereby the material is administered by pipette to the back of the throat; once the pipette is removed, the bird swallows the material naturally. Each bird received approximately 315 µL of neat product. The birds were closely monitored for 1 hour, and then at 8, 16, and 24 hours post dose. Signs of stress or suffering (such as poor posture, ambulating difficulty, wing drooping) were considered humane endpoints. In such cases, the bird would be euthanized and counted as a mortality. After 24 hours, the surviving birds were euthanized and stored in a freezer (−25°C).

A water avoidance trial was also performed over 5 days using the reference product only on adult male/female pairs of birds at three foam product concentrations (0.5%, 1.5%, and 3% weight in volume) in drinking water. Whereas the primary goal of this short trial was to aid in the selection of exposure concentrations for upcoming chronic toxicity tests, water avoidance also has value as an acute toxicity endpoint. The reference product was the only foam tested for water avoidance to minimize the number of birds used and because the other foam products would not be tested at higher exposure concentrations than the reference product in the upcoming chronic studies; all seven foams would be tested at the same exposure concentrations in the chronic study. Furthermore, in addition to the PFAS components in the reference product, it also contains chemicals (e.g., hexylene glycol [HG], sodium dodecyl sulfate [SDS], diethylene glycol monobutyl ether [DGMBE]) that are common in, or are major components of, the other six foam products. Thus, we would expect similar results for the other foam products in terms of water avoidance. The reference product was added to drinking water on a weight basis, and the concentrations of the reference product were analytical verified using two "marker" PFAS (perfluorobutanoic acid [PFBA] and perfluorohexanoic acid [PFHxA]) present in the formulation. Water consumption was monitored over the 5 days and compared with water consumption by control birds. All birds were housed in a 15-Section Quail Battery Breeding Pen and were observed three times per day. Birds received Purina Game Bird Breeder Layena® feed and water *ad libitum*, and temperature, humidity, and light were the same as described previously. After 5 days, birds were euthanized by CO_2 asphyxiation followed by cervical dislocation following the IACUC protocol and stored in a freezer at −25°C.

Chronic Studies

Adult northern bobwhite quail (*C. virginianus*) were obtained from T & T Game Birds and Hunting Preserve (Lubbock, Texas) for chronic studies. All birds were from disease-free stocks and more than 16 weeks old, approaching sexual maturity. The chronic reproductive toxicity studies were conducted following Protocol 19103-12 approved by the Texas Tech University IACUC

(Approval Number: 2022-1286) and the ACURO of the US Army Medical Research and Development Command. Requirements from Ecological Effects Test Guidelines (Office of Chemical Safety and Pollution Prevention 2012) and OECD Avian Reproduction Test 206 Guidelines (OECD 1984; e.g., bird age, husbandry, environmental conditions, acclimation, health, diet and feeding, maintenance of photoperiod) were followed to ensure study quality and health of the birds. Briefly, the initial weight of the birds was taken, and they were housed randomly as pairs in a 15-section Quail Battery Breeding Pen manufactured by GQF. Initially, the light regime was maintained at a 10.5:13.5-hour light:dark cycle for 7 days as birds acclimated to the laboratory environment. Following acclimation, the light was increased by 20 minutes per day to reach a 16:8-hour light:dark cycle for photostimulation. Purina Game Bird Breeder Layena® feed and deionized water were provided *ad libitum* throughout the study. Cuttlebones were freely supplied to each battery as a calcium supplement. The room was maintained at ambient temperature (19.7 ± 0.2°C) and humidity (47 ± 1.9%).

Once birds reached the reproductive stage (egg production), a post-photostimulation weight was measured. Birds were leg-banded and kept in pairs in the battery, which was labeled according to treatment, with birds for all F3s and the reference product exposed to 100 mg/L, 1,000 mg/L, or 2,500 mg/L. Each battery had a total of 5 rows, with 3 pens in each row, and each pen containing a male/female pair with drop pans beneath each row to catch feces, food, or water. For each exposure, controls were on the top row, low dose (100 mg/L) was on the second row, intermediate dose (1,000 mg/L) was on the third row, and the high dose (2,500 mg/L) on the fourth row. The fifth and bottom row consisted of additional controls and spare pens for when birds needed to be isolated due to potential wounds. This procedure was used to minimize the potential impacts of food, feces, or water falling from a higher dose to a lower dose row. Each pen received light from the front and back, and batteries were moved around daily to ensure all pens received adequate light.

Exposures to products were conducted in blocks, with the products tested together and the number of pairs used per treatment shown in Table 4. For each block, controls were shared between all products tested. For example, the reference product and F3 2 were tested together with three pairs of birds for each exposure treatment (100, 1,500, or 2,500 mg/L in drinking water), and six pairs of birds as a control for both products. For each experimental block, three extra pairs were maintained in case replacement birds were needed as a result of unexpected mortality.

Table 4. Blocks tested for bobwhite quail studies. The number of pairs of birds per individual product and project year of testing is shown in parentheses.

Block	Treatments (mg/L)
F3 2 & Reference Product (Year 1)	Control (6 pairs), 100 (3 pairs), 1,500 (3 pairs), and 2,500 (3 pairs)
F3s 5, and 7. (Year 2)	Control (6 pairs), 100 (3 pairs), 1,500 (3 pairs), and 2,500 (3 pairs)
F3s 3 and 6 (Year 3)	Control (6 pairs), 100 (3 pairs), 1,500 (3 pairs), and 2,500 (3 pairs)

F3 4 (Year 2)	Control (6 pairs), 100 (3 pairs), 1,500 (3 pairs), and 2,500 (3 pairs)

For chronic studies, birds were exposed through drinking water. While wild quail fulfill their water requirements through food, during reproduction quail need access to free water. The exposure portion of the study was 60 days; birds were exposed through drinking water using polypropylene bottles refilled with a known volume of test solution as needed. Exposure solutions were constructed in Milli-Q (> 18MΩ) water by weight; F3 concentrations were verified by analysis of "marker" components (for example, sodium dodecyl sulfate, hexylene glycol, diethylene glycol monobutyl ether) present in a particular foam using liquid chromatography/mass spectrometry (LC-MS, Section 4.3). Exposure solutions were made four times during the course of each block and stored in plastic containers at room temperature. The quantity of water consumed by each pair was recorded at the time water in the bottle was replenished. Birds were monitored at least twice each day. Any wounded birds were treated with an antibiotic ointment. The same humane endpoints as described for the acute test were used. Based on the study protocol, any birds displaying these signs were euthanized and classified as a mortality. After 60 days of exposure, surviving adult birds were euthanized by CO_2 asphyxiation followed by cervical dislocation following the IACUC protocol. Body weight, biometric data (bill-head length, tarsus length, and wing metacarpal lengths), liver weight, and gravimetric lipid content after necropsy were recorded. Carcasses were archived at −25°C.

Eggs collected from each pair were labeled by treatment, replicate, and date. The eggs were set at room temperature, with a portion of eggs incubated and archived every nine days. The numbers of soft and broken eggs were recorded and stored with the unincubated eggs in a freezer for chemical residue analysis. A GQF 1502-Digital Sportsman Incubator (37.7°C and 45%–55% humidity) and a 1550 Digital Hatcher (37°C and 55%–65% humidity) were used for the incubation and hatching of eggs, respectively. Eggs were placed in trays blunt end up in the incubator, where the trays gently tilt or rotate the eggs at regular intervals to simulate the natural process of a bird turning its eggs during the incubation period. Eggs were moved from the incubator to a hatcher on day 21 of incubation. Any unhatched eggs were collected on day 24 and the embryonic stage for those eggs which did not pip or hatch were recorded.

Following hatching, an initial body weight of chicks was recorded, and chicks were wing-banded before placement in a brooder. Feed (Purina Game Bird Breeder Startena®) and clean, filtered, water was provided *ad libitum* during this period. Chicks were reweighed at 7, 14, and 21 days to elucidate chick growth. At 14 days, chicks were moved to a larger brooder followed by euthanasia at day 21 (CO_2 asphyxiation followed by cervical dislocation). Biometric measurements (length of head, tarsus, and metacarpal bone) as well as liver weight were determined at necropsy. Carcasses were archived at −25°C.

3.2.2.2 Reptiles (Anolis sagrei)

All reptilian studies were conducted with subadult, male brown anoles (*Anolis sagrei*). Anoles were selected as a study species because they are invasive in Florida and therefore collections do not have negative impacts on native anole populations. Males were preferentially selected to avoid complexities and variability associated with using females. Previous studies have demonstrated

that gravid females may have up to 20% of their mass in nonmetabolic tissue (internal eggs; Suski et al. 2008) which could influence contaminant accumulation and subsequent effects.

Acute Studies

Experimental methods followed the previously developed "pseudo-gavage" technique that delivers an aqueous dose to the rear of the throat using a calibrated pipette (Suski et al., 2008; Salice et al., 2009; Weir et al., 2014). To assess acute toxicity of products an up-and-down protocol was used in which the starting dose for all products was 1,500 mg/kg mirroring the methods used to assess acute toxicity to bobwhite quail described above. Exposure solutions for acute studies were prepared as 10% dilutions of neat product to enable delivery of an adequate amount of solution (approximately 0.07 mL for a 5 g lizard).

Chronic Studies

Fifty wild-caught, male brown anoles were obtained from a commercial supplier (Miami, Florida). Anoles arrived within a day of shipment. Mass and snout-to-vent length measurements were taken for each specimen upon arrival. Each anole was kept in a 15-quart plastic container that included a substrate mixture of cocochips and cocosoil, one large black hide, two sets of artificial leaves, and a water dish. Containers were kept on shelving units with the front portion situated under a UVB light strip and the back portion placed on top of a strip of heat tape. Anoles acclimated to the laboratory conditions of a 12:12-hour light-dark photoperiod with 55–75% humidity and 27–29ºC temperature for one week prior to the beginning of the study. Containers were misted with deionized water every day, and four crickets covered in multivitamin and calcium powders were fed to the anoles every Monday, Wednesday, and Friday prior to and during the study.

A 60-day chronic exposure study was executed for the F3s and reference AFFF. For each toxicity study, anoles were randomly separated into five groups with the same average total mass. The five groups represented four treatment groups and one control group. Treatment groups consisting of ten anoles were exposed to different concentrations of a formulation mixed with moderately hard water to receive a target dose of the formulation. To prepare dilute solutions, a precalculated volume of neat product was diluted in moderately hard water, with solutions stored in 1L Nalgene polypropylene wide mouth bottles at room temperature on a shaker table to ensure adequate mixing. A single batch of exposure water for each formulation and treatment level was prepared, excluding F3 1 where an additional batch was made on day 18 of the 60-day exposure due to cloudiness in the exposure solution, which has been observed in other previous exposures with this F3 conducted under this project. The target dose was achieved by calculating the dose volume based on the mass of each individual. The target concentrations were 15 mg/L, 45 mg/L, 150 mg/L, and 450 mg/L (mg formulation/L moderately hard water) and the target doses were 0.1 milligrams per kilogram per day (mg/kg/day), 0.3 mg/kg/day, 1 mg/kg/day, and 3 mg/kg/day (mg formulation/kg anole mass/day), respectively. The control group was not exposed to a formulation and as such had a concentration of 0 mg/L and target dose of 0 mg/kg/day.

Anoles were dosed via the pseudo-gavage method every Monday, Wednesday, and Friday (Suski et al., 2008; Salice et al., 2009). This technique involved a researcher firmly holding the anole with one hand and gently rubbing their snout until their mouth opened. The researcher then carefully inserted their finger into the anole's mouth to hold it open, during which time a second researcher

slowly dosed the anole into the back of its throat to allow the anole to swallow voluntarily. A 20-200 µL Fisherbrand™ Elite™ pipette, model No. FBE00200 (US), was used to administer a dose.

Mass and snout-to-vent length were measured and recorded every Wednesday. A Sartorius Lab Instruments GmbH & Co. KG Practum® balance, model No. PRACTUM313-1S (Goettingen, Germany), was used to collect anole mass. A NEIKO Electronic Digital Caliper Measuring Tool, model No. 01407A (China), was utilized to capture snout-to-vent length with a range of accuracy of ± 0.2 millimeters (mm). Condition index was calculated by dividing an individual's snout-to-vent length from its mass. To provide a measure of performance and physiological condition, bite force and cutaneous evaporative water loss, respectively, were collected at the 30- and 60-day points. Anole bite force was measured using a Tekscan, Inc. Economical Load & Force Measurement System with a FlexiForce sensor, model No. B201 (Boston, Massachusetts). The FlexiForce sensor was placed in the mouth of each anole and was subsequently bitten. The Economical Load & Force Measurement System captured and recorded the bite force through time. Cutaneous evaporative water loss was measured using a Delfin Technologies Ltd. Vapometer, model No. SWL4515 (Kuopio, Finland). The Vapometer was positioned on the right, lateral side of the anole trunk and suctioned to the epidermis where evaporative water loss was measured for 20 seconds three times. Average water loss of the three measurements was calculated and reported. At the end of the 60 days, the anoles were euthanized following approved methods and in accordance with Towson University IACUC Protocol #1389 and approved by DoD ACURO. Dissection of the anoles occurred immediately after decapitation, during which the liver, gonads, gastrointestinal tract, and leg muscle were removed and weighed separately, along with a final mass recorded of the remaining carcass. Each were placed in a labeled Whirl-Pak and stored in a freezer at -20ºC.

3.3 BIODEGRADABILITY STUDY

3.3.1 Experimental Design

Prior to biodegradability tests, the formulations were analyzed for total chemical oxygen demand (COD), total Kjeldahl nitrogen (tTKN), soluble ammonia, soluble nitrite, soluble nitrate, total phosphate, and volatile suspended solids (VSS). Further chemical characterization was also performed on the formulations as described in Section 3.3.2.

The tests performed in this experiment were conducted according to a modified Zahn-Wellens test following OECD 302B (OECD 1992) where three different concentrations of each test substance were applied to biological reactors to check the inherent biodegradability of targeted constituents in F3 and AFFF formulations. In this testing, manometric measurement of oxygen uptake by microorganisms was measured as an indicator of how far and how quickly biological degradation of organics were occurring. At the same time, soluble COD measurements were taken periodically as a second measure of how far the biological degradation of organics proceeded and to measure any residual COD that may not be biodegradable. The COD test used was the EPA-approved Hach dichromate method.

Duplicate seed and control reactors were operated. Control reactors included acetate and no seed culture. COD was used as a measure of organic carbon in each reactor. The seed culture was mixed liquor from a local municipal wastewater treatment facility near Fayetteville, Arkansas. Control

seed reactors were prepared in duplicate and did not include AFFF or F3 formulations. All tests were conducted at 25ºC. Nutrients, trace minerals, and buffer were added to ensure that deficiencies (i.e., nutrients and/or trace minerals deficiencies) did not affect the biodegradation. The nitrification inhibitor, 2-chloro-6-(trichloromethyl)pyridine (TCMP), was added to ensure that oxygen uptake measured was due only to organic degradation. Reactors were incubated for 28 days. Oxygen uptake of all reactors was monitored using an aerobic respirometer (RSA PF-8000 respirometer). COD was measured after three hours of incubation and every 7 subsequent days.

In addition to the biodegradability test reactors monitored for COD, seven sacrificial reactors were established (one for each formulation), to allow sampling and analysis of the constituents over the reaction period. The additional reactors were operated on respirometers just as with the biodegradation testing reactors with the same biomass, nutrient, and feed concentrations. The mixed liquors in these additional reactors were sampled on days 0, 7, 13, 21, and 28 and sent to Texas Tech University, Lubbock, Texas for confirmation of targeted analytes including remaining fluorinated compounds where appropriate. For liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) analysis, 1 mL of sample was centrifuged (3,000 revolutions per minute [rpm] for 10 minutes) and then injected (triplicate injections). This method allowed confirmation of the identity of certain chemical components in the non-fluorine foams, allowing targeted analyses of select components in samples from the bioreactors. Samples collected from each sacrificial reactor were analyzed for two to four targeted analytes to further assess biodegradation. For evaluation of potential carryover and system cleaning, pure methanol was injected every five samples. For quality control (QC)/quality assurance purposes and calibration verification, the mid-level PFAS standards were also injected and evaluated every ten samples.

3.3.2 Chemical Characterization

Prior to biodegradability testing, further chemical characterization of the PFAS-free foams was conducted. Target analytes for the F3s were based on material safety data sheets. Neat materials of the identified chemicals from the SDS were purchased and used as standards to support the subsequent targeted analysis. For each foam, 2-4 chemical components that were found to be characteristic of that F3 were used for calibration of the liquid chromatography/mass spectrometry (LC/MS) method. Further details on analytical methods used for analysis and foam constituents tested are given in Section 3.5.

3.3.3 Testing

A series of 32 reactors were set up where 25 were used for the biodegradability testing and the rest of the reactors were used for constituents' analysis. Briefly, biodegradation reactions were initiated by placing test medium (480 mL), VSS, foam solutions, and other feedstocks into the reactors. The seed solution was 3.79 L of mixed liquor from the Fayetteville Wastewater Treatment Plant which was aerated, strained, and centrifuged to concentration solids upon return to the lab. Tap water was then added to the solids to make approximately 20% of the original volume and mixed to re-suspend solids. This sample was analyzed for total suspended solids and VSS. The biomass concentration was diluted to 5,000 mg/L VSS prior to use.

To initiate the biodegradation reaction, TCMP and concentrated seed (20 mL) were added to reactors along with test medium, VSS, foam solutions and other feedstock. Reactors were continuously stirred at 600 rpm while being incubated at 25°C for a total reaction period of 28 days. For compound degradation analysis, 25 mL of mixed liquor from reactors were taken into 50-mL Falcon tubes and were shipped to analytical labs while being kept frozen during shipment. For biodegradability tests, 10 mL of concentrate were taken for pH, COD, NH_3-N, NO_2-N, and NO_3-N measurements on days 0 (i.e., after 3 hours of incubation), 7, 14, 21, and 28 days. pH was adjusted to 7 if lower than 6.5 or higher than 8.0 using concentrated NaOH or HCl, respectively.

3.4 STATISTICAL ANALYSES

3.4.1 Aquatic Toxicity Testing

Statistical analyses used varied depending on the dataset. For acute lethality data, the dose-response modeling R package 'drc' was used to generate LC_{50} values (Ritz et al. 2015). Best fitting models were selected using comparison of log likelihood and Akaike Information Criterion using the 'mselect' function. For chronic tests, statistical analyses varied. For growth data, analysis of variance (ANOVA) with post-hoc Dunnett's test were used. Percentage data such as percent emergence were analyzed using binomial regression. Effective concentrations including EC_{20}, EC_{50}, and EC_{90} were calculated using the function 'ED' in package drc. No observed effect concentrations (NOECs) were defined as the concentration at which no significant effect relative to controls was observed based on the statistical tests mentioned previously. All statistics were conducted in R Studio (2023.06.1). The results of aquatic toxicity tests were compared to the EPA's Alternatives Assessment Criteria for Hazard Evaluation as shown in Table 5.

Table 5. Alternatives Assessment Criteria for Hazard Evaluation from EPA (2011).

Value	Very High	High	Moderate	Low
Acute Aquatic Toxicity (LC or EC_{50}) (mg/L)	< 1.0	1 – 10	> 10 – 100	> 100
Chronic Aquatic Toxicity (NOEC or LOEC) (mg/L)	< 0.1	0.1 – 1	> 1 – 10	> 10

Notes:
EC = effective concentration
LC = lethal concentration
LOEC = Lowest Observed Effect Concentration
mg/L = milligrams per liter
NOEC = No Observed Effect Concentration

3.4.2 Terrestrial Toxicity Testing

For bird studies, data were analyzed using Microsoft Excel and R statistical software (Version 4.1.1). Briefly, ANOVA was used to determine treatment effects, followed by Tukey-Kramer multiple comparisons when appropriate. To avoid potential complications when comparing sex (males vs. females) and repeated measures (e.g., chick growth data), single factor

ANOVAs were run on the data separately rather than combined. When the assumptions of ANOVA including homogeneity of variances and normality of residuals were not met, a Kruskal-Wallis test followed by Dunn's post-hoc test was used.

In terms of the reptile data, changes in body weight, snout-vent length (SVL), and condition index were assessed over the exposure period using a general linear model (GLM) with the week of testing, growth, SVL, condition index, and interaction terms considered. For the organ mass dataset, ANOVA with post-hoc Dunnett's Test was used to determine significant differences in body weight-normalized organ masses relative to the controls. Where the homogeneity of variances and normality of residuals assumptions were violated, Kruskal-Wallis and Dunn's post-hoc tests were used. For the sublethal endpoints, both body weight and SVL have been shown to be significantly correlated with cutaneous evaporative water loss (CEWL) and bite force (Anderson et al. 2008; Weaver et al. 2022). However, preliminary tests indicated no significant relationship between mass or SVL and CEWL (Spearman's rho = 0.04 and -0.09, $p > 0.05$ for mass and SVL, respectively). Consequently, individual ANOVAs on CEWL data collected at days 30 and 60 were performed with no covariate. Conversely, both mass and SVL were positively correlated with bite force (Spearman's rho = 0.157 and 0.285, $p < 0.05$ for mass and SVL, respectively); thus, an analysis of covariance (ANCOVA) using mass and SVL as covariates was conducted on data from days 30 and 60 separately. Post-hoc tests for the ANCOVA were performed using pairwise comparisons of estimated marginal means with a Benjamini-Hochberg false discovery rate correction using the package emmeans_test. All statistics for reptile tests were performed with R (Version 4.3.1). No hazard criteria exist for chronic avian or reptile studies in the EPA's Alternatives Assessment; thus, no comparison to hazard criteria was conducted for these values. For acute avian oral toxicity, < 10 mg/kg, 10 – 50 mg/kg, 51 – 500 mg/kg, 501 – 2,000 mg/kg and > 2,000 mg/kg represent very high, high, moderate, low, and very low toxicity according to the Alternatives Assessment (EPA 2011).

3.5 CHEMICAL CONSTITUENT ANALYSES

Chemical analyses were performed on various matrices from toxicity tests to determine bioaccumulation in organisms and validate levels of constituents in dosing solutions. A summary of which chemical constituents were measured for each foam is given in Table 6. All chemical analyses were performed at Texas Tech University. For the quail studies, chemical constituents were measured in adult livers, chick livers, eggs, dosing solutions, and feed used throughout the study. For chronic reptile studies, livers, and dosing solutions for all seven foams tested were analyzed. Finally, for aquatic studies, dosing solutions of the F3s 2, 5, and 6 from the *C. dilutus* study were analyzed. A summary of analytical methods by matrix is given in this Section.

Table 6. Chemical constituents measured for each F3 and the reference AFFF.

Product	Constituents Measured
F3 2	SDS, DGMBE, DMDA N-O[a]
F3 3	SDS, DGMBE
F3 4	SDS, DGMBE
F3 7	SDS, DGMBE
F3 5	SDS, HG[a]

F3 6	DGMBE
Reference Product	SDS, HG[a], PFBA, PFHxA

Notes:
[a] = Analyzed in dosing solutions only.
DGMBE = Diethylene glycol monobutyl ether
DMDA N-O = N, N-Dimethyldecylamine N-oxide
HG = Hexylene Glycol
PFBA = Perfluorobutanoic acid
PFHxA = Perrfluorohexanoic acid
SDS = Sodium dodecyl sulfate

3.5.1 Dosing Solutions

Exposure solutions from bobwhite quail studies were made four times during the chronic study; the first and final stock solutions were analytically verified. For the reptile studies, initial analysis of exposure solutions from chronic studies indicated a reduction in measured concentrations with holding time of solutions. Thus, exposure solutions (150 mg/L treatment only) were prepared fresh and analyzed immediately. In terms of aquatic studies, exposure solutions for F3s 2, 5 and 6 from the chronic *C. dilutus* studies only were analyzed. All dosing solutions were analyzed by direct injection into an ultra-high-performance liquid chromatograph coupled with mass spectrometry via electrospray ionization (ESI) in the negative mode. For PFAS analyses (reference product only), separation was achieved using an Eclipse Plus C18 column (4.6 × 150 mm × 3.5 micrometers [µm]) at 35°C column oven temperature and 70%:30% 20 millimolar (mM) ammonium acetate in high-performance liquid chromatograph (HPLC)–grade water:methanol eluent, similar to methods reported by Rewerts et al. (2021) and Dennis et al. (2020). HG, DGMBE, and N,N-dimethyldecylamine N-oxide (DMDA N-O) were determined using atmospheric pressure chemical ionization in the positive mode with a Thermo Scientific Hypersil Gold aQ C18 column (100 × 2.1 mm × 1.9 µm) at 40°C and a 0.1% formic acid in HPLC-grade water:0.1% formic acid in acetonitrile mobile phase. Sodium dodecyl sulfate was analyzed by ESI (positive mode) and a Kinetex® C18 100 A° column (50 × 2.1 mm × 2.6 µm) at 35°C with 70%:30% 50 mM formic acid in HPLC-grade water:acetonitrile mobile phase.

3.5.2 Tissue & Feed Analyses

For quail studies, several matrices were analyzed including adult livers, chick liver, eggs, and feed. In terms of reptiles, 8-10 livers from the controls and two highest exposure groups (150 and 450 mg/L) were composited and analyzed. This section describes analyses of tissue from both reptile and quail studies, as well as the analysis of feed used in quail studies. For adult quail, a total of 12 livers (6 male, 6 female) were analyzed from control treatments, with a total of 6 (3 male, 3 female) from the exposure treatments. In addition, 15 quail chick livers were used for analysis per treatment group after euthanasia at day 21 of the experiment, with a total of 10 quail eggs used. For quail feed, a 1 gram sample of both types of food used (Purina Game Bird Breeder Layena® and Purina Game Bird Breeder Stratena®) was extracted. For reptile studies, livers were analyzed from the control and two highest exposure groups. Prior to beginning the extraction procedure, liver samples were homogenized and vortexed for 10-15 minutes, and a subsample of approximately 0.25 grams wet weight was transferred into a conical 50 mL polypropylene centrifuge tube. For eggs, the whole inner content of the egg was transferred into a falcon tube, weighed, and vortexed for 5-10

minutes until homogenized. Extraction and analytical methods differed for PFAS and other constituents (SDS and DGMBE) and are described in the following sections.

In terms of instrumental analysis, all liver, egg, and feed extracts were analyzed by a liquid chromatograph (UltiMate 3000 UHPLC+, Thermo Scientific) coupled with a Thermo Scientific EnduraTM TripleStage Quadrupole Mass Spectrometer for quantification of PFBA, PFHxA, SDS, and DGMBE. First, each analyte, respective surrogates and internal standard was optimized in full scan and selected-reaction-monitoring mode using 10 micrograms per milliliter (µg/mL) of stock solution. The method was developed based on the optimization results by choosing the highest intensities of precursor, target, and confirmation ions. Solvent blanks were run after the standard and after every five samples to reduce the carryover of contamination between samples. One calibration standard was used as a QC check at the very start of the sequence and after every 10 samples run to check the quality and reproducibility of the instrument and data.

Seven livers, eggs, and feed surrogate samples were used to determine the limit of quantification (LOQ). The limit of detection (LOD) was quantified as 10 times lower than the LOQ. A value of 0.5*LOD was substituted for concentrations which were below the LOD and 0.5*LOQ for concentrations higher than the LOD but lower than the LOQ. Specific instrumental details are given for each of the chemical groups below.

3.5.2.1 PFAS Analyses

For PFAS analyses, a 1 mg/L N-methyl-d3-perfluoro-1-octanesulfonamidoacetic acid (d3-N-MeFOSAA, Wellington Laboratories, Ontario, Canada) surrogate mixture prepared in methanol was added to each sample, vortexed for 30 seconds and allowed to settle for 30 minutes. The volume of surrogate mixture added varied depending on the matrix, with 5 µL added for livers and feed, and 20 µL added for egg samples. Next, 2 mL of water and 10 mL of acetonitrile were added to each liver and egg sample, with 4 mL of water and 10 mL acetonitrile added to feed samples. All samples were vortexed for 30 seconds, and salts (4 grams anhydrous magnesium sulfate, 1 gram anhydrous sodium chloride) were added followed by vortex for another 30 seconds. The sample tubes were centrifuged at 3,000 rpm, 0°C for 5 minutes, and the supernatant transferred into a glass tube. The volume of supernatant transferred varied between matrices, with 4 mL transferred for livers, 5.5 mL for feed, and 7.5 mL for eggs. Following transfer, samples were evaporated under nitrogen at 35°C, 5-10 pounds per square inch until the samples were dried. All samples were then reconstituted to a final volume of 1 mL with 975 µL methanol and 25 µL of a 1 mg/L isotopically labeled internal standard perfluoro-n-[1,2-13C2]-hexanoic acid (MPFHxA, Wellington Laboratories) (prepared in methanol). All glass tubes were vortexed for 30 s, filtered through 0.22 µm cellulose filters fitted with a syringe, and transferred into polypropylene LC vials. Water, acetonitrile, and methanol used for extraction method were optima LC/MS grade. All chemical standards PFBA, PFHxA, d3-N-MeFOSAA, and MPFHxA were purchased from Wellington Laboratories (Ontario, Canada) and prepared as a mixture in methanol.

PFAS was analyzed using an Eclipse Plus C18 column (4.6 x 150 mm x 3.5 µm) at negative polarity ESI source, 35°C column oven temperature, 350°C ion transfer tube temperature, and 290°C vaporizer temperature as previously reported by Rewerts et al. (2021) and Dennis et al. (2020). The mobile phases were 60% of 20 mM ammonium acetate in LC-grade water and 40% of LC grade pure methanol. The injection volume was 20 µL and the flow rate was 350 µL/min with a total run time of 20 minutes. The flow gradients of the solvents were 40% of pure methanol

for 0-12.5 minutes, 90% for 12.5-15 minutes, 100% for 15-18 minutes, and 40% for 18-20 minutes. Eluent was diverted to waste at 0-2 minutes and 14-20 minutes to reduce the carryover. The seven-point calibration standards ranging from 1 to 250 nanograms per milliliter (ng/mL) were made from 50 µg/mL standards (Wellington Laboratories). Pure methanol was used as blank, and a 5 ng/mL solution was used for the QC check.

3.5.2.2 SDS and DGMBE

For SDS and DGMBE analyses, 20µL of a 10 mg/L surrogate standard, tris (1-chloro-2-propyl) phosphate (Sigma Aldrich, St Louis, Missouri), in acetonitrile was added to all homogenized liver, feed, and egg samples. Samples were vortexed for 30 seconds and allowed to settle for 30 minutes. The same procedure as for PFAS analyses was followed, with the exception of 5 mL of supernatant transferred and blown down using N_2 evaporation. All samples were reconstituted with 1 mL methanol, filtered through 0.22 µm cellulose filters, and transferred into polypropylene LC vials.

For LC/MS analysis of SDS, samples (20 µL) were injected into an Kinetex® C18 100 A° column (50 x 2.1 mm x 2.6 µm) at 35°C column oven temperature. The sample flow rate was 300 µL/minute at positive polarity using an electrospray ion source. The ion transfer tube and vaporizer temperature were set at 350°C and 290°C, respectively. Mobile phases were 70:30 of 50 mM formic acid in LC-grade water:pure LC-grade acetonitrile. The total run time was 15 minutes with flow gradients of 30% acetonitrile from 0-4 minutes, 70% from 4.100 – 6 minutes, 90% from 6-11 minutes, 100% from 11-12 minutes, and 30% from 12-15 minutes. Eluent diversion to waste was at 0-0.5 minutes and 14-15 minutes. The calibration point samples ranged from 5 to 250 ng/mL and were made from SDS (>97% purity, Tokyo Chemical Industry Co., Ltd.) whereas 5 ng/mL and 10 ng/mL samples were checked as QC.

For DGMBE analysis, samples (20 µL) were injected into a Thermo Scientific Hypersil Gold aQ C18 column (100 x 2.1 mm x 1.9 µm) at 40°C column oven temperature. The sample flow rate was 250 µL/min at positive polarity using an atmospheric pressure chemical ionization source. The ion transfer tube and vaporizer temperature were set at 275°C and 350°C, respectively. Mobile phases were 100:0 of 0.1% formic acid in LC-grade water: 0.1% formic acid in LC-grade pure acetonitrile. The total run time was 15 minutes with flow gradients of 100% of 0.1% formic acid in LC-grade water from 0-9 minutes, 99% of 0.1% formic acid in LC-grade acetonitrile from 9-12 minutes, 100% of 0.1% formic acid in LC grade water from 13-15 minutes. Eluent diversion to waste was at 0-2 minutes and 14-15 minutes. The matrix match calibration standard used ranged from 62.5 to 1250 ng/mL and was made from a DGMBE standard (99% purity, Thermo Fisher). A sample of DGMBE standard at 250 ng/mL was checked for QC purposes.

For both SDS and DGMBE, solvent blanks were run after the standard and after every five samples to reduce the carryover, and one calibration standard was used as QC check at the very first of the sequence followed by after every 10 samples run to check the quality and reproducibility of the instrument and data.

4.0 RESULTS AND DISCUSSION

4.1 AQUATIC TOXICITY TESTING

4.1.1 Acute Studies

4.1.1.1 Algae

Preliminary range finding test results, water quality parameters, and a data summary are given in Appendix A, Tables A1, A2, and A3, respectively. A synthesis of F3 and reference product EC_{50} values are given in Figure 1 and Table 7. Based on the EPA's Alternative Assessment Hazard Criteria, three of the tested F3s (F3s 1, 2, and 3) were characterized as highly or very highly toxic (Table 7).

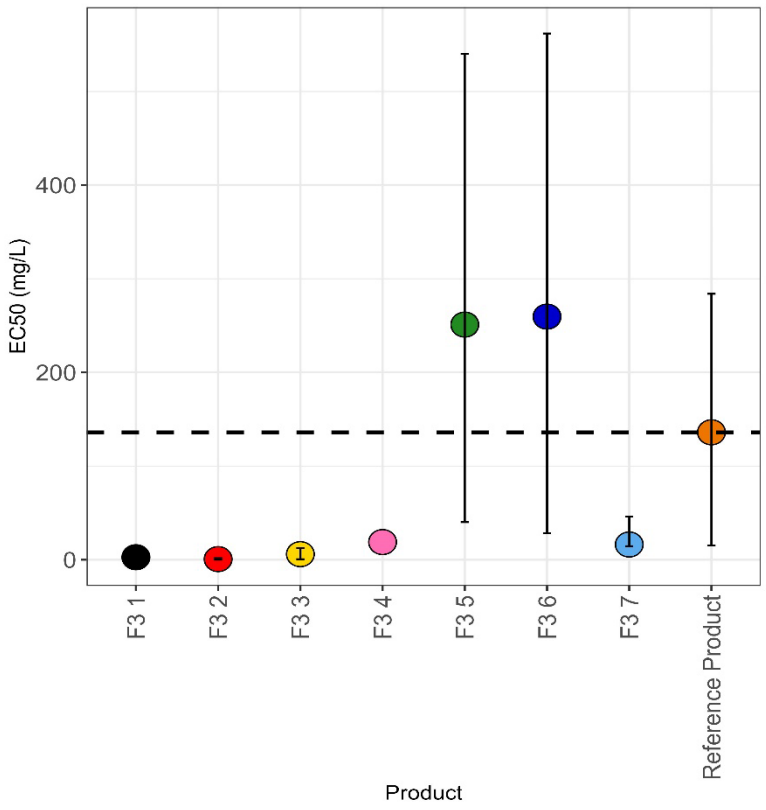

Figure 1. Definitive acute EC_{50} values for *R. subcapitata* exposed to seven F3s and a reference product for 96 hours.
The dashed line represents the value for the reference product.
Error bars are 95 percent confidence intervals.

Table 7. Acute EC_{50} values for *R. subcapitata* exposed to seven F3s and the reference AFFF.

Foam	EC_{50} (mg/L)	Hazard Criteria
F3 1	2.70 (2.10 – 3.90)	High
F3 2	0.69 (0.6 – 0.9)	Very High
F3 3	5.97 (5.40 – 6.60)	High
F3 4	18.9 (NC)	Moderate
F3 5	251(211 – 289)	Low
F3 6	260 (231 – 302)	Low
F3 7	16.3 (1.80 – 29.7)	Moderate
Reference Product	136 (121 – 148)	Low

Notes:
EC = effective concentration
mg/L = milligrams per liter
NC = Not calculated.

4.1.1.2 Invertebrates

Preliminary range finding test results, water quality parameters, and a data summary are given in Appendix A, Tables A5, A6, and A7, respectively. LC_{50} values for the definitive tests are given in Table 8 and Figure 2. A synthesis of F3 and reference product LC_{50} values for *C. dilutus* are given in Figure 2 and Table 8. Three of the tested F3s (F3s 1, 2, and 3) were characterized as moderately toxic to *C. dilutus*, with all other products classified as low toxicity.

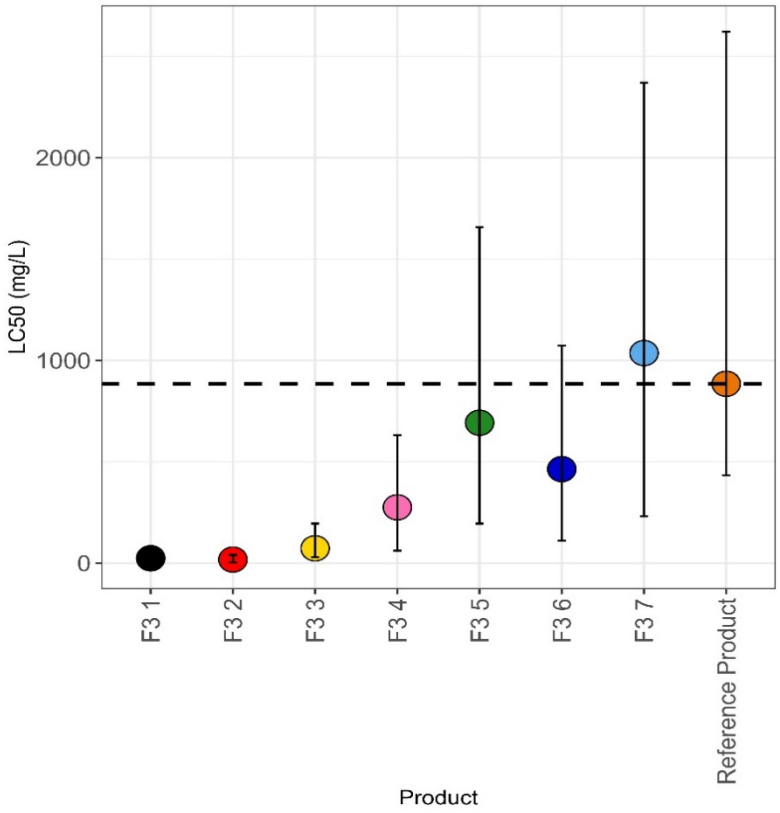

Figure 2. Definitive acute LC_{50} values for *C. dilutus* exposed to seven F3s and one product for 96 hours.
The dashed line represents the value for the reference product.
Error bars represent 95 percent confidence intervals.

Table 8. Acute EC_{50} values for *C. dilutus* exposed to seven F3s and a reference product.

Foam	48 h LC_{50} (mg/L)	Hazard Criteria
F3 1	24.5 (15.6 – 38.1)	Moderate
F3 2	18.0 (14.4 – 22.8)	Moderate
F3 3	52.0 (NC)	Moderate
F3 4	278 (214 – 356)	Low
F3 5	693 (498 – 965)	Low
F3 6	464 (353 – 609)	Low
F3 7	1,040 (806 – 1,330)	Low
Reference Product	885 (451 – 1,740)	Low

Notes:
EC = effective concentration
h = hours
mg/L = milligrams per liter
NC = Not calculated.

4.1.1.3 Fish

Preliminary range finding test results, water quality parameters, and a data summary are given in Appendix A, Tables A8, A9, and A10, respectively. LC_{50} values for the definitive tests are given in Table 8 and Figure 3. A synthesis of acute LC_{50} values for *P. promelas* is given in Figure 3 and Table 8. Based on the alternatives assessment hazard criteria, one F3, F3 2, was classified as highly toxic to *P. promelas*, with four F3s classified as moderately toxic, and all other F3s and the reference product exhibiting low toxicity.

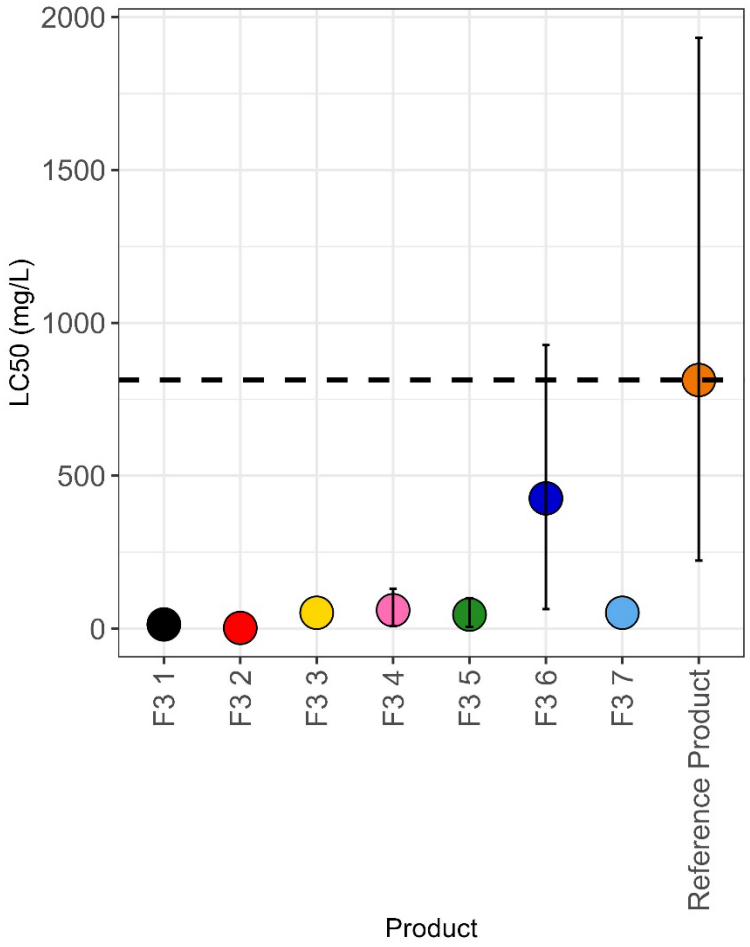

Figure 3. Definitive acute LC50 values for *P. promelas* exposed to seven F3s and one reference product for 96 hours.

The dashed line represents the value for the reference product.
Error bars are 95 percent confidence intervals.

Table 9. Acute EC_{50} values for *P. promelas* exposed to seven F3s and the reference AFFF.

Foam	96 h LC_{50} (mg/L)	Hazard Criteria
F3 1	13.8 (11.4 – 16.5)	Moderate
F3 2	2.31 (NC)	High
F3 3	52.0 (NC)	Moderate
F3 4	60.3 (51.9 – 70.2)	Moderate
F3 5	46.0 (39.3 – 54)	Moderate
F3 6	426 (362 – 501)	Low
F3 7	52.0 (NC)	Moderate
Reference Product	813 (591 – 1,120)	Low

Notes:
h = hours
LC = lethal concentration
mg/L = milligrams per liter
NC = Not calculated.

4.1.1.4 Summary: Acute Tests

A summary of acute LC_{50} (EC_{50} for *R. subcapitata*) values for all species is shown below in Figure 4. Generally, product toxicity was consistent across species. For example, all three species were most sensitive to F3s 1 and 2, with LC/EC_{50} values ranging from 0.7 mg/L (*R. subcapitata*) to 18.0 mg/L (*C. dilutus*). Differences between species were observed for F3 5, which was more toxic to *P. promelas* compared to *C. dilutus* and *R. subcapitata*. Generally, *C. dilutus* appeared the least sensitive of the three tested species when comparing within individual formulations.

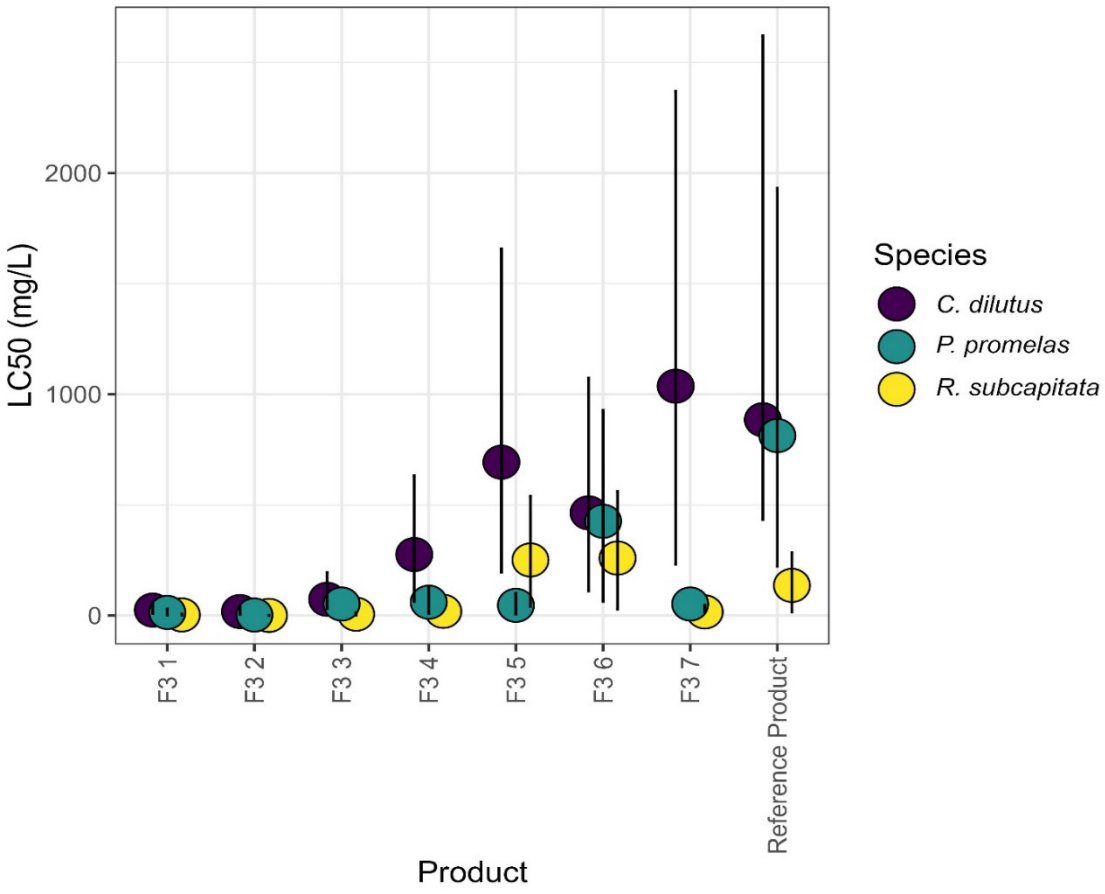

Figure 4. Comparison of acute EC/LC_{50} values for three aquatic species exposed to seven F3s and one reference product.
Error bars are 95 percent confidence intervals.

4.1.2 Chronic Studies

4.1.2.1 Invertebrates

Water quality parameters measured during the invertebrate tests are given in the Appendix A, Table A5. A summary of the chronic invertebrate study is given in Table A7. For the survival endpoint, 7 out of the 8 tested formulations had a significant effect on *C. dilutus*, with F3 3 the only formulation that had no significant effect at nominal concentrations up to 30 mg/L (Binomial GLM, $p > 0.05$).

In terms of the growth endpoint, a total of 6 out of the 8 formulations had significant effects on growth in *C. dilutus* (ANOVA, $p < 0.05$, Table 10, Figure 5), including the reference product and F3s 1, 2, 4, 5, and 7. Exposure to F3s 3 and 6 had no significant effect on *C. dilutus* dry weight (ANOVA, $p > 0.05$). For the F3 1 formulation, a significant reduction in dry weight relative to controls was observed at the 30 mg/L concentration (ANOVA, Post-hoc Dunnett's test, $p < 0.05$), with significant reductions relative to controls at 300 and 900 mg/L and 300 mg/L only in F3s 5 and 7, respectively (ANOVA, Post-hoc Dunnett's test, $p < 0.05$). However, dry weight of *C. dilutus* was significantly increased at 90 mg/L relative to controls in the F3 5 treatment (ANOVA, Post-hoc Dunnett's test, $p < 0.05$). For F3 4, significant reductions in dry weight were observed at 90 mg/L relative to controls (ANOVA, Post-hoc Dunnett's test, $p < 0.05$). For the reference product and F3 2, no significant differences relative to controls were observed; however, a reduction at 900 mg/L relative to 30 and 90 mg/L and a reduction at 30 mg/L relative to 3 and 9 mg/L was observed for the reference product and F3 2, respectively (ANOVA, Post-hoc Tukey's honestly significant difference (HSD), $p < 0.05$). Calculated EC_{50} values were lowest in F3 1 (17.7 mg/L), followed by F3 4 (80.7 mg/L), F3 7 (213 mg/L), and F3 5 (290 mg/L).

For emergence, all seven of the tested F3s and the reference product had a significant effect on percent emergence in *C. dilutus* (Binomial GLM, $p < 0.05$, Table 10). Calculated NOECs, lowest observed effect concentrations (LOECs), and EC values are given in Table 10.

Table 10. Calculated NOEC, LOEC, EC_{20} and LC/EC_{50} values for survival, growth, and emergence endpoints in *C. dilutus* exposed to seven F3s and the reference product. Values in parentheses are 95% confidence intervals.

Foam	Concentration Range (mg/L)	NOEC (mg/L)	LOEC (mg/L)	EC_{20} (mg/L)	LC/EC_{50} (mg/L)	Hazard Criteria
Survival						
F3 1	0.9 – 90	9	30	-	12.2 (0.810 – 23.6)	Moderate
F3 2	0.9 – 90	30	90	-	42.8 (24.5 – 61.2)	Low
Reference Product	30 – 3,000	300	900	-	759 (NC)	Low
F3 3	0.3 – 30	NS	NS	-	-	-
F3 4	9 – 900	9	30	-	36.6 (30.8 – 42.4)	Moderate
F3 5	30 – 3,000	90	300	-	411 (329 – 492)	Low
F3 6	9 – 900	90	300	-	459	Low
F3 7	30 – 3,000	90	300	-	258 (54.0 – 463)	Low
Growth						
F3 1	0.9 – 90	9	30	8.38 (2.18 – 14.6)	17.7 (10.9 – 24.5)	Moderate
F3 2	0.9 – 90	NS	NS	NS	-	-
Reference Product	30 – 3,000	NS	NS	NS	-	-
F3 3	0.3 – 30	9	30	4.69 (NC)	26.3 (NC)	Moderate
F3 4	9 – 900	30	90	44.5 (8.28 – 80.7)	80.7 (49.3 – 112)	Low
F3 5	30 – 3,000	30	90	237 (160 – 634)	279 (132 – 426)	Low
F3 6	9 – 900	NS	NS	NS	-	
F3 7	30 – 3,000	90	300	215 (NC)	253 (NC)	Low
Emergence						
F3 1	0.9 – 90	3	9	4.17 (2.50 – 5.83)	7.02 (5.63 – 8.41)	Moderate
F3 2	0.9 – 90	0.9	3	9.36 (4.76 – 14.0)	15.6 (11.1 – 20.1)	High
Reference Product	30 – 3,000	90	300	59.3 (16.3 – 102)	130 (77.0 – 183)	Low
F3 3	0.3 – 30	0.9	3	0.409 (0.167 – 0.985)	3.97 (0.651 – 7.29)	High
F3 4	9 – 900	9	30	22.7 (18.1 – 27.2)	36.5 (31.7 – 41.4)	Moderate
F3 5	30 – 3,000	30	90	18.4 (11.01 – 47.7)	70.0 (17.1 – 123)	Low
F3 6	9 – 900	30	90	15.6 (0.334 – 30.8)	43.2 (23.3 – 63.0)	Low
F3 7	30 – 3,000	30	90	21.0 (3.86 – 45.9)	64.3 (27.2 – 101)	Low

Notes:
EC = effective concentration
LOEC = Lowest Observed Effect Concentration
mg/L = milligrams per liter
NC = Not Calculated
NOEC = No Observed Effect Concentration
- = Not applicable
NS = Non-significant.

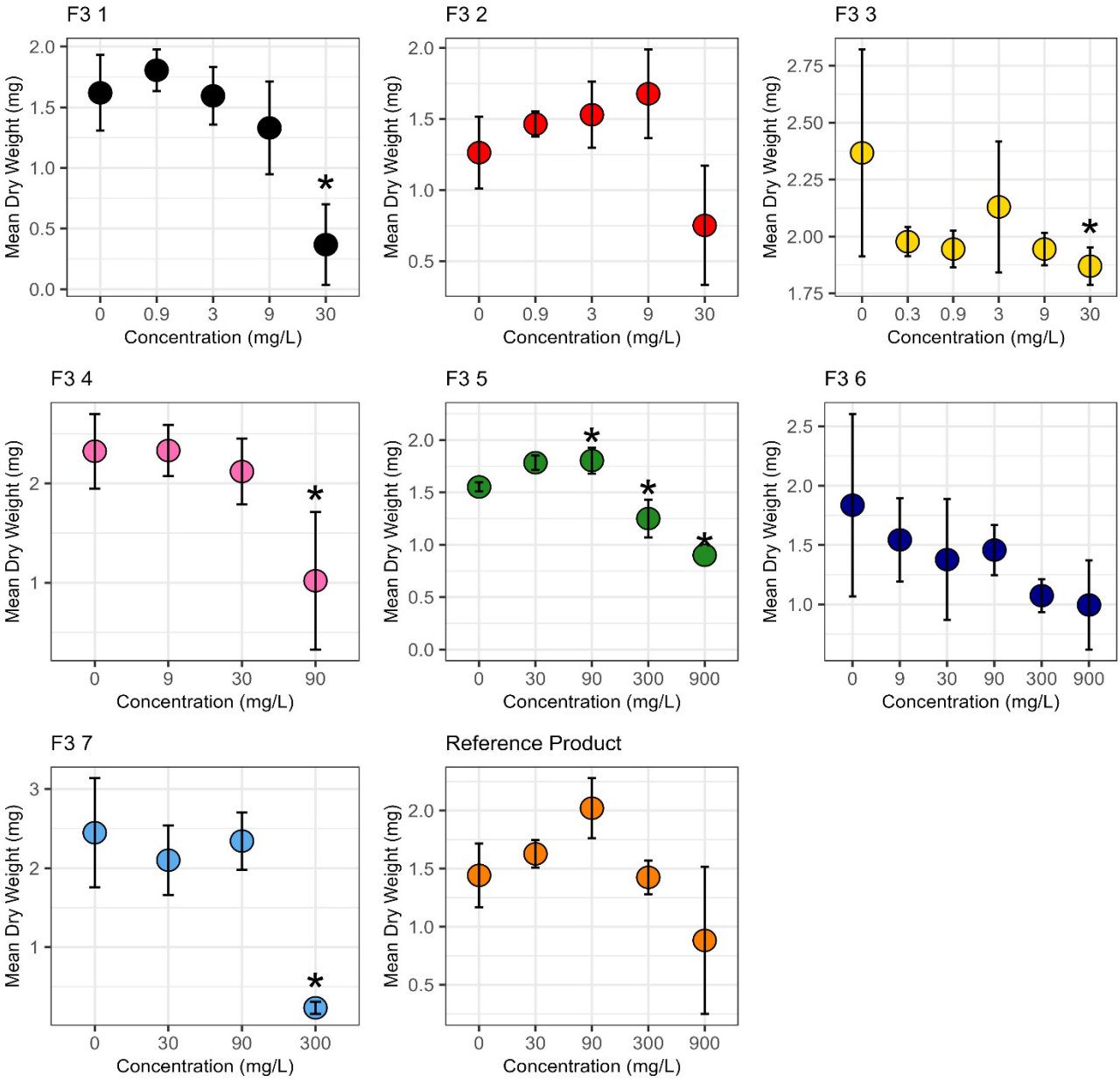

Figure 5. Effects of seven F3s and one reference product on growth (measured as dry weight in milligrams) of *C. dilutus*.

* indicates significant differences from control (Dunnett's Test, $p < 0.05$).
Error bars represent standard deviations.

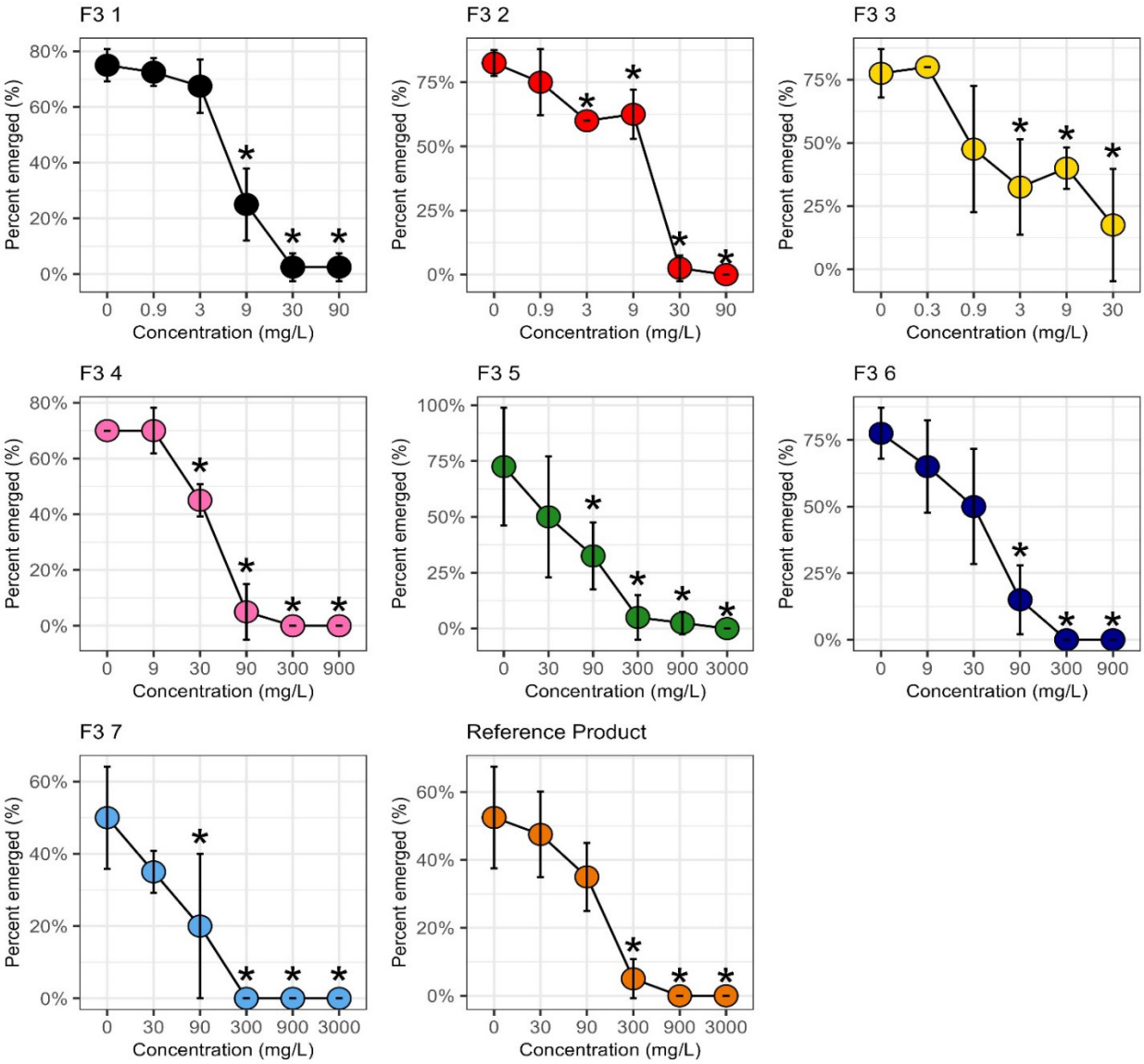

Figure 6. Effects of seven F3s and one reference product on emergence of *C. dilutus*.
* indicates significant differences from control (Dunnett's Test, $p < 0.05$).
Error bars represent standard deviations.

4.1.2.2 Fish

Water quality parameters measured during the chronic fish studies are given in Appendix Table A9. A summary of chronic toxicity data for *P. promelas* is given in Table A11. All seven of the tested F3s and the reference product had a significant effect on survival in *P. promelas* (Binomial GLM, $p < 0.05$). In terms of effects on dry weight, significant differences were observed for 5 of the 8 products (ANOVA, $p < 0.05$, Table 10). However, only a single F3, F3 4, showed significant reductions relative to controls (ANOVA, Post-hoc Dunnett's Test, $p < 0.05$, Table 11, Figure 7). For F3 4, significant reductions in weight were observed at 30 and 90 mg/L relative to controls, with a calculated EC_{20} value of 19.2 mg/L (Table 11). For F3 3, marginally significant differences were observed at 18 and 30 mg/L relative to controls, ANOVA, Post-hoc Dunnett's Test, $p = 0.05$).

Similarly, marginally significant differences were observed at 9 mg/L relative to controls in the F3 2 formulation (ANOVA, Post-hoc Dunnett's Test, $p = 0.07$), with significantly lower *P. promelas* weight at 9 mg/L relative to 1.8 and 3 mg/L for F3 2 (ANOVA, Post-hoc Tukey's HSD, $p < 0.05$). For the F3 5 formulation, significantly increased dry weight was observed at 30 mg/L relative to controls (ANOVA, Post-hoc Dunnett's Test, $p < 0.05$, Figure 7), with significant differences between the 30 and 300 mg/L treatments for F3 6 (ANOVA, Post-hoc Tukey's HSD, $p < 0.05$). Finally, no significant differences in *P. promelas* dry weight were observed for the reference product of F3s 1 and 7 (ANOVA, $p > 0.05$).

Table 11. Calculated NOEC, LOEC, EC_{20} and EC_{50} values (95% confidence intervals) for the growth endpoint in *P. promelas* exposed to seven F3s and a reference product. Values in parentheses are 95% confidence intervals.

Foam	Concentration Range (mg/L)	NOEC (mg/L)	LOEC (mg/L)	EC_{20} (mg/L)	LC/EC_{50} (mg/L)	Hazard Criteria
Survival						
F3 1	0.9 – 90	3	9	-	5.45 (2.35 – 8.55)	Moderate
F3 2	1.8 – 30	3	9	-	8.61 (NC)	Moderate
Reference Product	30 – 3,000	180	300	-	338 (NC)	Low
F3 3	0.3 – 30	30	90	-	41.9 (10.3 – 73.4)	Moderate
F3 4	9 – 180	30	90	-	45.9 (20.9 – 70.9)	Moderate
F3 5	3 – 90	18	30	-	24.5 (18.0 – 30.9)	Moderate
F3 6	9 – 900	300	900	-	379 (164 – 595)	Low
F3 7	6.25 – 100	30	90	-	38.9 (NC)	Moderate
Growth						
F3 1	0.9 – 90	NS	NS	-	-	-
F3 2	1.8 – 30	NS	NS	-	-	-
Reference Product	30 – 3,000	NS	NS	-	-	-
F3 3	0.3 – 30	NS	NS	-	-	-
F3 4	9 – 180	18	30	19.2 (9.82 – 28.6)	40.9 (29.0 – 52.8)	Low
F3 5	3 – 90	Increase	Increase	-	-	-
F3 6	9 – 900	NS	NS	-	-	-
F3 7	6.25 – 100	NS	NS	-	-	-

Notes:
EC = effective concentration
LOEC = Lowest Observed Effect Concentration
mg/L = milligrams per liter
NOEC = No Observed Effect Concentration
NS = Non-significant.
NC = Not Calculated
- = Not applicable

Increase = Significant increase relative to controls.

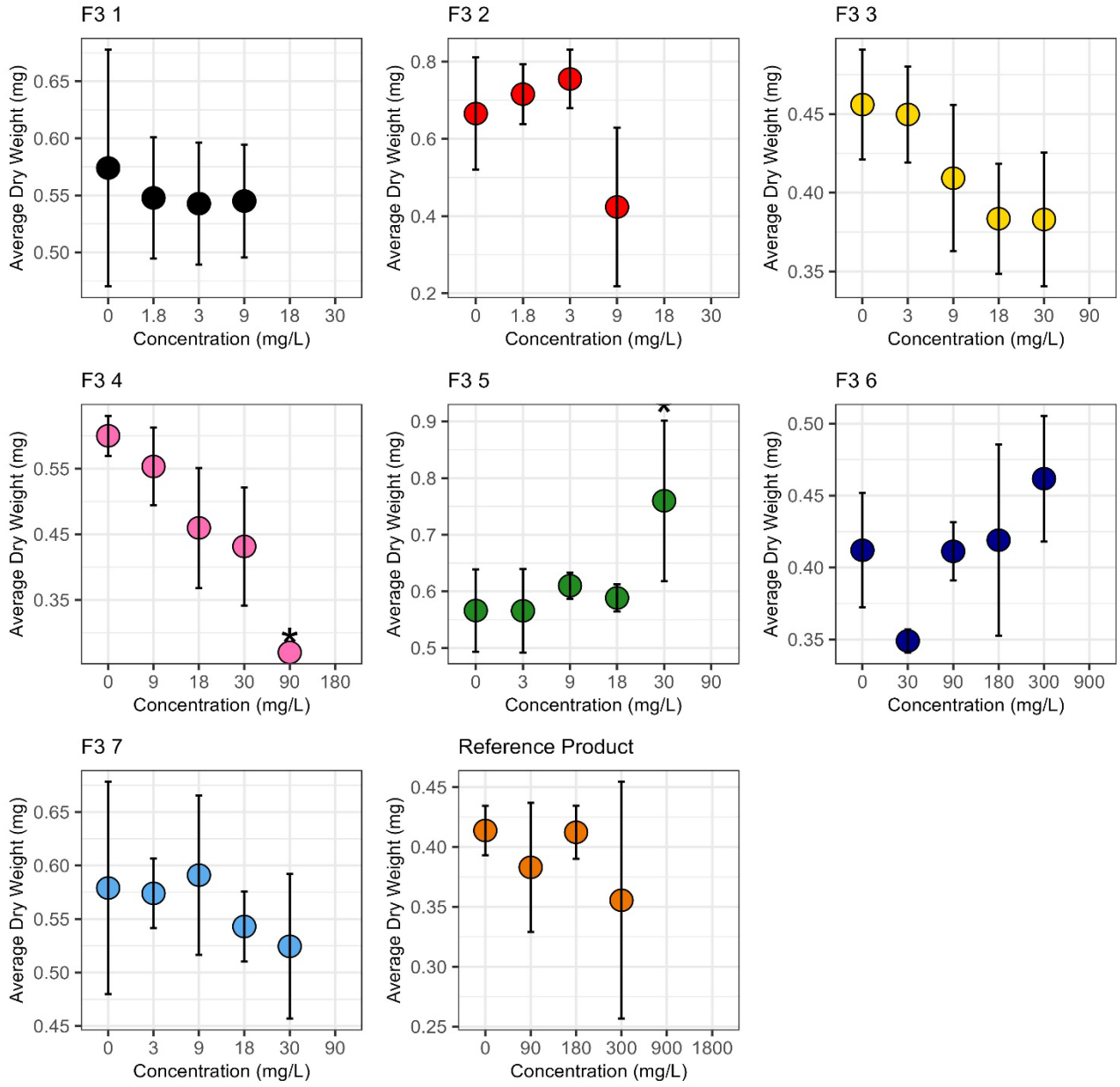

Figure 7. Effects of seven F3s and one reference product on dry weight (in milligrams) of *P. promelas*.

* Indicates significant differences from control (Dunnett's Test, $p < 0.05$).

Error bars are standard deviations.

4.1.2.3 Summary: Chronic Tests

A synthesis of chronic LC_{50} values calculated for *C. dilutus* and *P. promelas* is given in Figure 11. Generally, comparable LC_{50} values were observed between species for the following F3s: F3 1, F3 2, F3 4, and F3 6. Notable differences were observed for F3 5, which had an LC_{50} of 24.5 mg/L in *P. promelas*, whereas the calculated LC_{50} in *C. dilutus* was 411 mg/L.

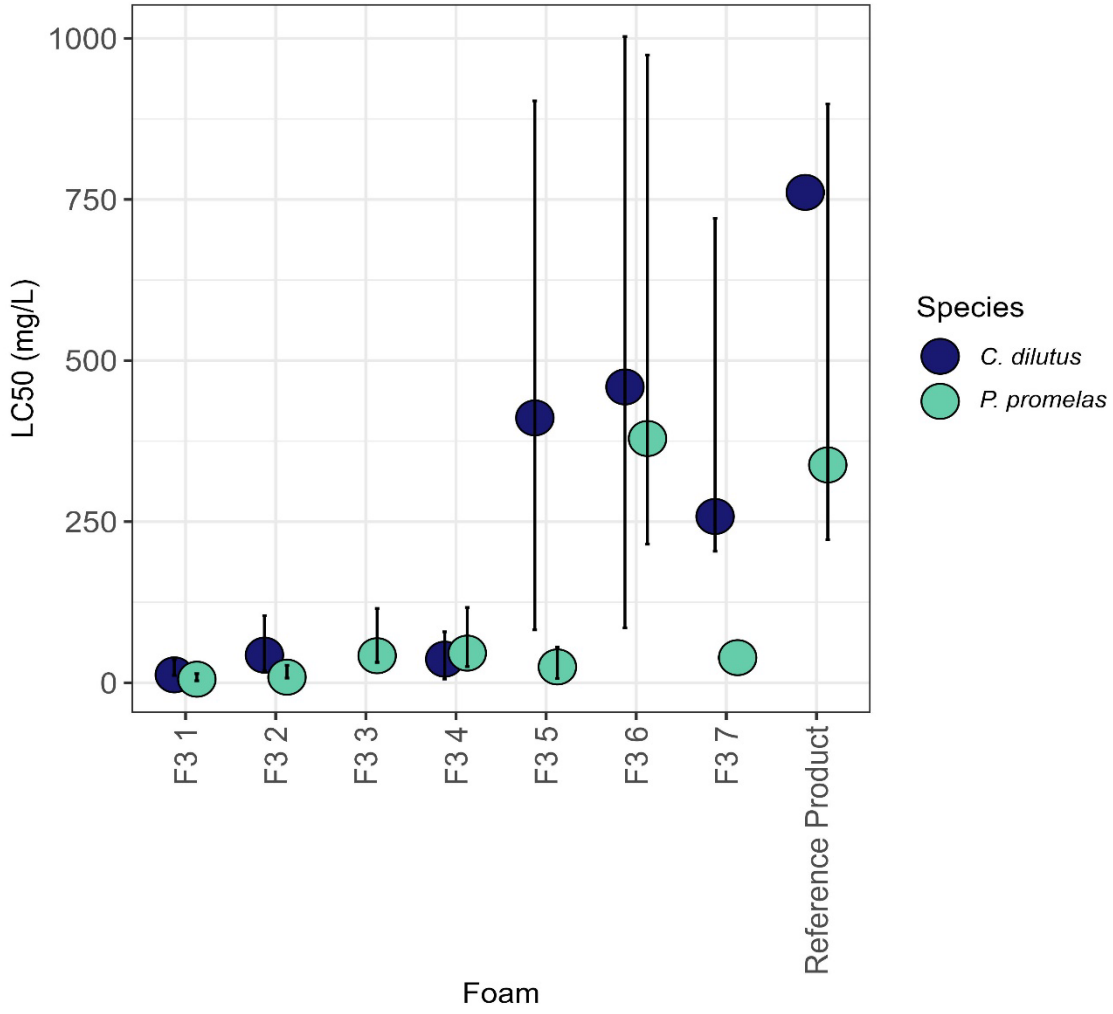

Figure 8. Comparison of chronic LC_{50} values in *C. dilutus* and *P. promelas*.
Error bars represent 95 percent confidence intervals.

For the sublethal endpoints, only a single F3, F3 4, had a significant negative effect on growth in *P. promelas*. For *C. dilutus*, significant sublethal effects on emergence were recorded for all tested F3s and the reference product.

4.1.3 Discussion – Aquatic Toxicity Testing

Acute and chronic toxicity testing using three species and multiple endpoints enabled a synthesis of the toxicity of F3s and a reference product to aquatic species. Comparing the concentrations where significant effects were observed to anticipated environmental exposures is challenging

given that the present study was based on whole foam nominal concentrations and studies of AFFF-impacted areas typically measure concentrations of individual constituents in the environment. Furthermore, detailed site histories including the amounts of AFFF used, frequency of application, and specific chemical compositions of AFFF are rarely available for studies of AFFF-impacted areas. Several studies have reported high concentrations of PFAS associated with historical AFFF in surface water following firefighting activities (Dauchy et al. 2017; Moody et al. 2002; Taniyasu et al. 2015). Moody et al. (2002) measured concentrations of PFAS in surface water samples and fish livers following an accidental release of AFFF at Toronto airport, finding maximum PFAS concentrations (sum of perfluorohexanecsulfonate [PFHxS], PFOS, and PFOA) of 2.27 mg/L two days post-accident. Dauchy et al. (2017) reported maximum concentrations of 1.77 micrograms per liter (equivalent to 0.002 mg/L) 6:2 fluorotelomer sulfonamide alkylbetaine, a major component of the AFFF suspected to have been used, in a river adjacent to the civilian airport (Dauchy et al. 2017). However, these studies have not focused on investigating foam constituents other than PFAS; therefore, there are no data available on environmental presence and/or persistence of other foam ingredients following AFFF release.

Both novel F3s and legacy AFFF are typically applied to fires as 3% concentrates, equivalent to 30,000 mg/L of product. Given that effects were observed at nominal concentrations of 3 mg/L (*C. dilutus* emergence for F3s 2 and 3), equivalent to 10,000 times lower than their recommended usage dilution, it is feasible that environmental exposures following application of foams may exceed effect thresholds measured in these studies. Similar studies assessing the aquatic toxicity of AFFF and fire suppressors found acute and chronic toxicity to aquatic invertebrates at concentrations 100 to 10,000 times lower than their recommended dilutions for application in fire suppression (da Silva et al. 2019; Ueda-De-Carvalho et al. 2019).

4.2 TERRESTRIAL TOXICITY TESTING

4.2.1 Bird Studies

4.2.1.1 Acute Oral Toxicity

Overall, only a single bobwhite quail died during the 24-hour limit tests; a male exposed to the F3 2 formulation. Mortality occurred between 16- and 24-hours following exposure, with the bird showing no visible symptoms of toxicity during the initial monitoring period. All other birds survived the limit test. Furthermore, no humane endpoints (poor posture, wing drooping, ambulating difficulty, distress) that could be indicative of toxicity were reached for the remaining birds. Because fewer than two birds died during the limit test on each product, the full Up-and-Down Procedure was not triggered, and the acute lethal dose (LD_{50}) for each formulated product in adult quail was at or above the limit (~1,500 mg/kg). Table 12 below describes the results of the limit test portion of the Up-and-Down Procedure and the LD_{50} values.

Table 12. Acute LD_{50} values for bobwhite quail exposed to six F3s and the reference product.

Foam	LD_{50} (mg/kg)[1]
F3 2	>1,580
F3 3	>1,608
F3 4	>1,561
F3 5	>1,469
F3 6	>1,629
F3 7	>1,506
Reference Product	>1,597

Notes:
[1]The reported LD_{50} is based on the mass of the formulated product, not the chemical components within each product.
LD = lethal dose
mg/kg = milligrams per kilogram

In terms of the water avoidance trial, birds exposed to 0.5% (5,000 mg/L) of the reference product in water consumed 81% of control bird water. The birds exposed to 1.5% (15,000 mg/L) and 3% (30,000 mg/L) solutions of the reference product consumed 68% and 32%, respectively, of control bird water consumption over the 5-day exposure period. These data were used in determining exposure concentrations in chronic studies to test product toxicity and not toxicity associated with confounding dehydration due to taste aversion.

4.2.1.2 Chronic Studies

Survival

Overall survival in adults during the chronic study was 100% for the reference product and F3s 3, 5, and 7, 94% for F3 2 and F3 6, and 91% for F3 4. All survival rates were above the criteria of 90% for validity of the study (OECD 1984).

Water Consumption and Average Daily Intake

Mean water consumption, body weight, and average daily intake (ADI) for northern bobwhite quail exposed to all foams is given in Table 13. ADI is given for both the formulated product and the measured chemical constituents in each product. Overall, mean water consumption across all formulations and concentrations ranged from 30 ± 1.2 to 41 ± 2.6 mL/bird/day, consistent with previous drinking water exposures of this species to PFAS (Dennis et al. 2020, 2021). Compared to acute studies wherein reductions in water consumption were observed in treatment groups relative to controls, exposed birds consumed similar amounts of water across all exposures. No significant difference in mean water consumption was observed relative to controls (ANOVA, $p > 0.05$).

Table 13. Body weights, water consumption and average daily intake for F3s, the reference AFFF, and individual foam constituents.

Treatment	Mean Body Weight (kg)	Mean Water Consumption (mL/bird/day)	ADI (mg/kg body weight/day)
F3 2			
Control	0.219 ± 0.004	35 ± 0.4	NA
100 mg/L	0.214 ± 0.009	31 ± 4.9	14.5 ± 2.3 (Formulation)
			1.3 ± 0.1 (DGMBE)
			0.37 ± 0.03 (SDS)
			0.81 ± 0.06 (DMDA N-O)
1,000 mg/L	0.225 ± 0.008	35 ± 1.4	156 ± 6.2 (Formulation)
			15.9 ± 0.6 (DGMBE)
			1.83 ± 0.07 (SDS)
			10 ± 0.38 (DMDA N-O)
2,500 mg/L	0.236 ± 0.01	37 ± 3.8	392 ± 40 (Formulation)
			38 ± 2.0 (DGMBE)
			5.71 ± 0.30 (SDS)
			51 ± 2.7 (DMDA N-O)
Reference Product			
Control	0.219 ± 0.004	35 ± 0.4	NA
100 mg/L	0.234 ± 0.01	33 ± 1.3	14.1 ± 0.6 (Formulation)
			$7.6 \times 10^{-5} \pm 3.1 \times 10^{-6}$ (PFHxA)
			< LOD (PFBA)
			1.02 ± 0.04 (HG)
			$1.5 \times 10^{-3} \pm 6.0 \times 10^{-5}$ (SDS)
1,000 mg/L	0.223 ± 0.004	36 ± 1.1	161 ± 4.9 (Formulation)
			$5.9 \times 10^{-4} \pm 1.4 \times 10^{-5}$ (PFHxA)
			$1.2 \times 10^{-4} \pm 2.72 \times 10^{-6}$ (PFBA)
			10.7 ± 0.24 (HG)
			$2.3 \times 10^{-2} \pm 5.3 \times 10^{-4}$ (SDS)
2,500 mg/L	0.215 ± 0.01	35 ± 2.1	407 ± 24 (Formulation)
			$1.3 \times 10^{-3} \pm 7.3 \times 10^{-5}$ (PFHxA)
			$3.4 \times 10^{-4} \pm 1.9 \times 10^{-5}$ (PFBA)
			26 ± 1.5 (HG)

Treatment	Mean Body Weight (kg)	Mean Water Consumption (mL/bird/day)	ADI (mg/kg body weight/day)
			$2.9 \times 10^{-2} \pm 1.6 \times 10^{-3}$ (SDS)
F3 3			
Control	0.224 ± 0.007	36 ± 3.0	NA
100 mg/L	0.209 ± 0.004	30 ± 1.2	14.4 ± 0.51 (Formulation)
			1.4 ± 0.04 (DGMBE)
			0.21 ± 0.007 (SDS)
1,000 mg/L	0.226 ± 0.008	38 ± 1.8	171 ± 5.8 (Formulation)
			13.8 ± 0.47 (DGMBE)
			2.3 ± 0.078 (SDS)
2,500 mg/L	0.225 ± 0.007	38 ± 3.7	421 ± 2.4 (Formulation)
			32 ± 1.9 (DGMBE)
			5.6 ± 0.33 (SDS)
F3 4			
Control	0.244 ± 0.01	34.1 ± 1.2	NA
100 mg/L	0.223 ± 0.01	30.0 ± 2.3	13.5 ± 0.6 (Formulation)
			0.05 ± 0.001 (SDS)
			0.48 ± 0.01 (DGMBE)
1,000 mg/L	0.224 ± 0.01	33.4 ± 1.6	149 ± 6.0 (Formulation)
			0.15 ± 0.004 (SDS)
			6.54 ± 0.18 (DGMBE)
2,500 mg/L	0.257 ± 0.01	37.4 ± 0.2	364 ± 15 (Formulation)
			0.31 ± 0.005 (SDS)
			18.4 ± 0.32 (DGMBE)
F3 5			
Control	0.233 ± 0.0	33.7 ± 1.2	NA
100 mg/L	0.222 ± 0.01	34.4 ± 3.2	15.5 ± 1.4 (Formulation)
			0.09 ± 0.01 (SDS)
			0.2 ± 0.01 (HG)
1,000 mg/L	0.228 ± 0.0	33.2 ± 2.0	146 ± 8.8 (Formulation)
			0.7 ± 0.03 (SDS)
			2.3 ± 0.11 (HG)
2,500 mg/L	0.245 ± 0.01	34.5 ± 1.5	352 ± 15 (Formulation)
			1.3 ± 0.02 (SDS)

Treatment	Mean Body Weight (kg)	Mean Water Consumption (mL/bird/day)	ADI (mg/kg body weight/day)
			4.1 ± 0.08 (HG)
F3 6			
Control	0.224 ± 0.007	36 ± 3.0	NA
100 mg/L	0.230 ± 0.005	37 ± 1.4	16.5 ± 0.69 (Formulation)
			2.6 ± 0.11 (DGMBE)
1,000 mg/L	0.233 ± 0.015	36 ± 6.6	154 ± 2.4 (Formulation)
			27.9 ± 2.83 (DGMBE)
2,500 mg/L	0.235 ± 0.019	41 ± 2.6	445 ± 2.4 (Formulation)
			87 ± 6.1 (DGMBE)
F3 7			
Control	0.233 ± 0.0	33.7 ± 1.2	NA
100 mg/L	0.232± 0.01	37.3 ± 1.5	16 ± 0.6 (Formulation)
			0.24 ± 0.004 (SDS)
			0.74 ± 0.01 (DGMBE)
1,000 mg/L	0.236 ± 0.0	32.0 ± 0.2	136 ± 0.8 (Formulation)
			1.4 ± 0.03 (SDS)
			7.9 ± 0.14 (DGMBE)
2,500 mg/L	0.226 ± 0.0	36.5 ± 1.7	404 ± 19 (Formulation)
			3.5 ± 0.12 (SDS)
			20 ± 0.7 (DGMBE)

Notes:
ADI = Average daily intake
DGMBE = Diethylene glycol monobutyl ether
DMDA N-O = N,N-Dimethyldecylamine N-oxide
HG = Hexylene glycol
kg = kilograms
LOD = Limit of Detection
mg/kg = milligrams per kilogram
mL = milliliter
NA = not applicable
PFHxA = Perfluorohexanoic acid
PFBA = Perfluorobutanoic acid
SDS = Sodium dodecyl sulfate

Growth

A summary of adult growth data is given in Appendix Table A12. Adult weight change over the 60-day study was significantly impacted by exposure to the F3 4 only, with females exposed to the highest concentration of 2,500 mg/L having significantly lower weight gain relative to controls

(ANOVA, $p < 0.05$). No significant effects were observed for males at any concentration of F3 4 (ANOVA, $p > 0.05$), with none of the other tested F3s or the reference product having significant effects on adult growth (ANOVA, $p > 0.05$).

Morphometry

Biometric measurements including bill-head length, left and right tarsus, and metacarpal bone of the left and right wing were determined for both adults and 21-day old chicks. A summary of adult morphometric data is given in Appendix Table A13. In terms of the adults, F3s 2, 3, 4, 6 and the reference product had no significant effect on any biometric endpoints (ANOVA, $p > 0.05$, Table 14). For F3 7, adult males exposed to 100 mg/L and 2,500 mg/L had shorter bill-head lengths relative to controls, with longer left tarsa in males exposed to all concentrations, and longer right tarsa in females exposed to 1,000 mg/L only relative to controls (ANOVA, $p < 0.05$). For F3 5 adult females exposed to the 100 and 2,500 mg/L had longer right tarsa relative to controls, with longer left tarsa observed in the 2,500 mg/L treatment only (ANOVA, $p < 0.05$).

Table 14. Summary of biometric endpoints in chicks following parental exposure to six F3s and the reference AFFF. Arrows indicate the direction of effect relative to controls.

	Nominal Exposure Concentration					
	Males			**Females**		
Foam	**100 mg/L**	**1,000 mg/L**	**2,500 mg/L**	**100 mg/L**	**1,000 mg/L**	**2,500 mg/L**
F3 2	NS	NS	NS	NS	NS	NS
F3 3	NS	NS	NS	NS	NS	NS
F3 4	NS	NS	NS	NS	NS	NS
F3 5	NS	NS	NS	RT↑	NS	RT↑, LT↑
F3 6	NS	NS	NS	NS	NS	NS
F3 7	BH ↓ LT↑	LT↑	BH ↓, LT↑	NS	RT↑	NS
Reference Product	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Notes:
BH = Bill-head length
LW = Left wing metacarpal bone
LT = Left tarsa
mg/L = milligrams per liter
NS = non-significant
RT = Right tarsa
RW = Right wing metacarpal bone

Liver Weights and Percent Lipid

A summary of relative liver weights and percent lipids for all foams is given in Table A14. Across all foams, relative liver weight (absolute liver weight normalized to individual body weight) was significantly different following exposure F3 2 only. Female bobwhite quail exposed to the highest concentration, 2,500 mg/L, had significantly greater liver weights relative to controls (ANOVA, $p < 0.05$). Significant effects on percent liver lipids were observed following exposure to F3s 2, 5,

7, and the reference product. For both F3 2 and the reference product, significantly increased liver lipids were observed for males at the highest concentration of 2,500 mg/L (ANOVA, $p < 0.05$). Conversely, females exposed to 100 and 1,000 mg/L of F3 5 had significantly lower percent lipids, with a significant reduction observed at 100 mg/L only for males (ANOVA, $p < 0.05$). For F3 7, a significant reduction in percent lipid was observed at the lowest exposure concentration of 100 mg/L in females only (ANOVA, $p < 0.05$).

Reproductive Effects

Several reproductive endpoints were assessed in bobwhite quail, including eggs laid per hen, presence of cracked or soft eggs, hatching success, and embryonic development. Table 15 provides a summary of significant effects on all reproductive endpoints. Where possible, reproductive parameters were compared to typical values for bobwhite quail provided in OECD Method 206 (OECD 1984). Means and standard errors for all reproductive endpoints are given in Appendix Table A15. In terms of the number of eggs laid per hen, only one product, F3 4, had a significant effect, with significantly fewer eggs laid per hen in the 100 mg/L treatment (28.3 ± 9.0) relative to controls (59 ± 0.8), but not at 1,000 or 2,500 mg/L. The typical range for the number of eggs laid per hen in bobwhite quail is 28 – 38 (OECD 1984); thus, the significantly reduced egg production following exposure to 100 mg/L F3 4 would still be considered within the typical range. The percentage of cracked eggs was significantly increased following parental exposure to the highest concentration of the reference product, 2,500 mg/L (ANOVA, Post-hoc Dunnett's Test, $p < 0.05$), with no significant effects on this parameter in any other foam relative to controls (ANOVA, $p > 0.05$). However, the average percent cracked eggs in the F3 2 100 and 2500 mg/L treatments, 3.37% ± 1.71% and 3.11 ± 1.71%, exceeded the typical range of 0 – 2% for bobwhite quail (OECD 1984, Table A15).

Table 15. Summary of reproductive effects on bobwhite quail.

Product	Endpoint			
	Number of Eggs per Hen	**Percent Cracked Eggs**	**Percent Arrested Embryos**	**Hatching Success**
F3 2	NS	NS	NS	NS
F3 3	NS	NS	NS	NS
F3 4	↓, 100 mg/L only	NS	↑, 100 mg/L only	NS
F3 5	NS	NS	NS	NS
F3 6	NS	NS	↑, 1000 mg/L only	NS
F3 7	NS	NS	NS	NS
Reference Product	NS	↑, 2500 mg/L only	NS	NS

Notes:
mg/L = milligrams per liter.
NS = non-significant

In terms of effects on embryos, two F3s had a significant effect on the proportion of embryos that were exhibiting arrested development, F3s 4 and 7 (ANOVA, $p < 0.05$). For F3 4, the proportion of arrested embryos was significantly higher at the lowest concentration, 100 mg/L (13.0% ± 5.67%), relative to controls (3.46% ± 4.66%, Post-hoc Dunnett's Test, $p < 0.05$). However, no significant effects were observed at any other tested concentrations for F3 4 (ANOVA, Post-hoc Dunnett's Test, $p > 0.05$). For F3 6, the proportion of arrested embryos was significantly increased at the 1000 mg/L treatment only (17.0% ± 5.33%) relative to controls (2.94% ± 7.40%, Post-hoc Dunnett's Test, $p < 0.05$). The highest percentage of arrested embryos was observed in the F3 2 and reference product exposures, with 19.9% ± 11.5% and 22.0% ± 20.1% percent arrested embryos in the F3 2 1000 mg/L and reference product 1000 mg/L treatments, respectively (Table A15). However, these were not significantly increased relative to controls due to high interindividual variability, low replicate numbers (n of 3 for treatments), and higher percentage of arrested embryos in controls used for the F3 2 and reference product (9.17% ± 1.92%) relative to other foams (control range: 2.93% - 3.46%). In terms of the day of arrested development, significant effects were observed for F3 2, the reference product, and F3 6, with significantly earlier arrested development in embryos at all three concentrations of AF3 2 and the reference product, and at 1,000 mg/L only for F3 6 (ANOVA, $p < 0.05$). Of those eggs that did not experience arrested development, hatching success was not significantly impacted in response to any foam or treatment (ANOVA, $p > 0.05$). Hatching success across all foams and treatments was within the typical range for bobwhite quail of 50% – 90% (OECD 1984), excluding the 1000 mg/L treatment of F3 6, where average hatching success was 48.4% ± 21.4% (Table A15).

Chick Survival, Growth, and Liver Lipid Content

None of the tested foams had a significant effect on chick survival; however, chick growth (measured as weight change) after 21 days was significantly impacted in response to F3s 2, 5, 6, and 7 and the reference product. A summary of chick growth data is given in Appendix Table A16. Significantly greater growth in 21-day old chicks was observed in response to parental exposure to the F3 2, reference product, and F3 6, at concentrations of 2,500 mg/L only, 100 and 1,000 mg/L, and 1,000 mg/L only for F3 2, the reference product, and F3 6, respectively. For parental exposure to F3 7 and F3 5, chicks had significantly reduced growth at 100 mg/L only.

Four of the seven tested foams had a significant effect on chick liver lipid content: F3 2, the reference product, F3 7, and F3 5 (Table 15). For the reference product and F3 7, parental exposure to the highest concentration, 2,500 mg/L, led to significantly reduced liver lipid content in chicks relative to controls. For F3s 2 and 5, parental exposure to 100 and 1,000 mg/L led to significantly reduced liver lipid content in chicks relative to controls, respectively, though no effect was observed at the highest concentration.

Table 16. Summary of significant effects on chick survival, growth, and lipid content.

Product	Endpoint		
	Chick Survival	**Chick Growth**	**Chick Lipid Content**
F3 2	NS	↑, 2500 mg/L only	↓, 100 and 1,000 mg/L
F3 3	NS	NS	NS
F3 4	NS	NS	NS
F3 5	NS	↓, 100 mg/L only	↓, 100 and 1,000 mg/L
F3 6	NS	↑, 1,000 mg/L only	NS
F3 7	NS	↓, 100 mg/L only	↓, 2500 mg/L only
Reference Product	NS	↑, 100 and 1,000 mg/L	↓, 2500 mg/L only

Notes:
mg/L = milligrams per liter
NS = non-significant

Chick Morphometry

A summary of the statistical differences in biometric endpoints for chicks is given in Table 17, with data summarized in Appendix Table A17. Significant effects were observed for F3s 3, 4, 5, 6, and 7. For the F3 4, chicks born following parental exposure to the lowest nominal concentration, 100 mg/L, had significantly smaller right wing metacarpal bones as well as left and right tarsi relative to controls (ANOVA, Post-hoc Tukey-Kramer Test, $p < 0.05$). Additionally, chicks born following parental exposure to 1,000 and 2,500 mg/L had significantly reduced left- and right-wing metacarpal bones, left and right tarsi, and significantly increased liver weights relative to controls (ANOVA, Post-hoc Tukey-Kramer Test, $p < 0.05$). Bill-head length was not significantly impacted at any nominal concentration of F3 4 (ANOVA, $p > 0.05$). In terms of the F3 3 exposure, chicks born following parental exposure to 2,500 mg/L had significantly shorter left wing metacarpal bones. For F3 6, chicks born following parental exposure to 100 mg/L and 2,500 mg/L had larger left metacarpal bones and left tarsus for 100 mg/L and 2,500 mg/L, respectively. For F3 7, all biometric parameters were significantly reduced in chicks born following parental exposure to 100 mg/L, with shorter left and right tarsa only in the 1,000 mg/L treatment, and shorter left wing, left and right tarsa in the 2,500 mg/L treatment. Finally, chicks born following parental exposure to 100 mg/L F3 5 had significantly shorter left- and right-wing metacarpal bones and left and right tarsa, with shorter left- and right-wing metacarpal bones only following parental exposure to 1,000 mg/L, and chicks having larger bill-head lengths and shorter left and right tarsa at 2,500 mg/L.

Table 17. Summary of biometric endpoints in chicks following parental exposure six F3s and a reference product.

	Nominal Exposure Concentration		
Foam	**100 mg/L**	**1,000 mg/L**	**2,500 mg/L**
F3 2	NS	NS	NS
Reference Product	**NS**	**NS**	**NS**
F3 3	NS	NS	LW ↓
F3 4	RW ↓, LT↓, RT ↓	LW ↓, RW ↓, LT ↓, RT ↓	LW ↓, RW ↓, LT ↓, RT ↓
F3 5	LW ↓, RW ↓, LT ↓, RT ↓	LW ↓, LT ↓, RT ↓	LT ↓, RT ↓, BH ↑
F3 6	LW ↑	NS	LT ↑
F3 7	LW ↓, RW ↓, LT ↓, RT ↓, BH ↓	LT ↓, RT ↓	LW ↓, LT ↓, RT ↓

Notes:
Arrows indicate the direction of effect relative to controls.
BH = Bill-head length
LW = Left wing metacarpal bone
LT = Left tarsa
mg/L = milligrams per liter
NS = Non-significant
RT = Right tarsa
RW = Right wing metacarpal bone

Summary

A summary of significant effects induced by each foam during chronic bobwhite quail studies and associated NOECs and LOECs is given in Table 18. Values are given as ADI in milligrams per kilogram of body weight per day (mg/kg/bw-day) and as nominal aqueous exposure concentrations of individual foams. Only endpoints where significant deleterious effects were recorded were included. For example, chick growth was significantly increased following parental exposure to several foams including F3s 2 and 6 and the reference product, which is unlikely to have a significant overall negative impact on organismal health. Conversely, increased lipid weights and percent lipids were retained since such effects may be representative of lipid dysregulation which could lead to concomitant impacts such as fatty liver disease (Zaefarian et al. 2019). Overall, the most commonly impacted endpoints 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 foam, F3 4.

In all foams excluding F3 3, at least one endpoint was significantly impacted at the lowest tested dose of 100 mg/L. Day of arrested development, chick growth, and chick biometrics were the most commonly impacted endpoints at 100 mg/L. However, many of the observed effects were observed only at low concentrations, with no significant effects observed relative to controls at higher concentrations (indicated with * in Table 18). For example, F3 4 exposure induced a significant reduction in the number of eggs per hen at the lowest tested concentration of 100 mg/L; however, no significant effects were observed at 1,000 or 2,500 mg/L.

Table 18. Summary of significant effects and no observed and lowest observed adverse effect levels (NOAELs and LOAELs) in bobwhite quail exposed to F3s and a reference product for 60 days.

Product	Effect	NOAEL (mg/kg/bw-day)	LOAEL (mg/kg/bw-day)
F3 2	Lipid Content (Males)	156 (1,000 mg/L)	**392 (2,500 mg/L)**
	Relative Liver Weight (Females)	156 (1,000 mg/L)	**392 (2,500 mg/L)**
	Arrested Development Day	-	14.5 (100 mg/L)
	Chick Lipid Content	156 (1,000 mg/L)	14.5* (100 mg/L)
Reference Product	Lipid Content (Males)	161 (1,000 mg/L)	**407 (2,500 mg/L)**
	Cracked Eggs	161 (1,000 mg/L)	407 (2,500 mg/L)
	Arrested Development Day	-	14.1 (100 mg/L)
	Chick Lipid Content	161 (1,000 mg/L)	407 (2,500 mg/L)
F3 3	Chick Biometrics (Left Wing Metacarpal)	421 (1,000 mg/L)	171 (2,500 mg/L)
F3 4	Adult Growth (Females)	149 (1,000 mg/L)	364 (2,500 mg/L)
	Number of Eggs per Hen	-	13.5* (100 mg/L)
	Chick Biometrics (Left and Right Tarsa, Right Wing Metacarpal)	-	13.5 (100 mg/L)
	Proportion of Arrested Embryos	-	13.5* (100 mg/L)
F3 5	Lipid Content (Males)	16.1 (100 mg/L)	149* (1,000 mg/L)
	Lipid Content (Females)	-	16.1* (100 mg/L)
	Chick Lipid Content	-	16.1* (100 mg/L)
	Chick Growth	-	16.1* (100 mg/L)
	Chick Biometrics (Left- and Right-Wing Metacarpals, Left and Right Tarsa)	-	16.1 (100 mg/L)
F3 6	Arrested Development Day	16.5 (100 mg/L)	154* (1,000 mg/L)
	Proportion Arrested Embryos	16.5 (100 mg/L)	154* (1,000 mg/L)
F3 7	Lipid Content (Females)	-	16.5* (100 mg/L)
	Chick Lipid Content	137 (1,000 mg/L)	406 (2,500 mg/L)
	Chick Growth	-	16.5* (100 mg/L)

Product	Effect	NOAEL (mg/kg/bw-day)	LOAEL (mg/kg/bw-day)
	Adult Biometrics (Bill-head length)	-	16.5 (100 mg/L)
	Chick Biometrics (Left- and Right-Wing Metacarpals, Left and Right Tarsa)	-	16.5 (100 mg/L)

Notes:
Bold values indicate increases relative to the control.
* Indicates that significant effects were not observed at higher doses.
– indicates where effects were observed at the lowest tested concentration and a NOAEL could not be calculated.
LOAEL = Lowest Observed Adverse Effect Level
mg/kg/bw-day = milligrams per kilogram of bodyweight per day
mg/L = milligrams per liter
NOAEL = No Observed Adverse Effect Level

Discussion

Overall, the acute and chronic studies provided a comprehensive assessment of the effects of F3s and a reference product on northern bobwhite quail. In terms of acute lethality, calculated LD_{50} values were at or above the limit of ~ 1,500 mg/kg, indicating low or very low toxicity based on the Alternatives Assessment Criteria (EPA 2011). However, the results of chronic studies were more complex. A large number of endpoints were considered, encompassing potential effects of F3s and a reference product on survival, growth, reproduction, and offspring fitness. In general, few effects were recorded on adult bobwhite quail, with liver lipid content (measured as percent total lipids) the most commonly impacted endpoint for adults (impacted by exposure to 4/7 tested foams). Liver percent lipids were significantly increased in response to F3 2 and the reference product, but significantly decreased in response to F3s 5 and 7, suggesting disruption of lipid metabolism in response to foam exposure. Lipids play essential roles in egg production, thermogenesis, and growth in avian species; therefore, impacts on lipid content can be considered an adverse effect that may have implications at higher levels of biological organization (Bussière-Côté et al. 2016). Lipid metabolism has been shown to be impacted by a range of contaminants in quail, including metals (Zhu et al. 2023), pharmaceuticals (Bussière-Côté et al. 2016), and pesticides (Han et al. 2023). Given that the majority of foam constituents are not disclosed, it is difficult to attribute impacts on lipid content to a given chemical ingredient. Furthermore, no studies to our knowledge have considered the impacts of the measured chemical constituents, SDS and DGMBE, on avian receptors. However, studies from aquatic species have suggested that SDS can induce lipid peroxidation at high concentrations (Bhattacharya et al. 2023; Freitas et al. 2020), which would be anticipated to have concomitant effects on lipid profiles (Pérez-Rodríguez et al. 2015). Given that foams with higher concentrations of SDS, such as F3 3, had no significant effects on adult or chick lipid content, it is difficult to attribute observed impacts on lipid levels directly to these surfactants.

In terms of effects on reproduction and embryonic development, F3s 2, 4, 6, and the reference product had effects on at least one of the measured endpoints. The percentage of cracked eggs was significantly increased in response to the highest concentration of the reference product, with exposure to 100 and 2500 mg/L of F3 2 leading to a higher percentage of cracked eggs than the

normal range (0% - 2%, OECD 1984) for bobwhite quail. In a previous study, Dennis et al. (2020, 2021) found no significant effect of exposure to PFOS, PFHxS, and PFHxA, or binary PFAS mixtures on the proportion of cracked eggs in bobwhite quail. Conversely, Grote et al. (2006) found a significant increase in the percent of cracked eggs in Japanese quail (*Coturnix coturnix japonica*) exposed to 30 mg/L of the antifungal, fentin hydroxide. In terms of egg production, a single foam, F3 4, caused significantly reduced eggs laid per hen at the lowest tested dose of 100 mg/L; however, effects were not observed at higher exposure levels, indicating a non-monotonic dose response. It is important to consider that the present study utilized three pairs of bobwhite quails per exposure group, which is fewer than similar studies assessing the effects of contaminants on bird reproduction (Bursian et al. 2021; Newsted et al. 2007). For example, Bursian et al. (2021) utilized 16 pairs per dose in a study of the effects of PFOS and AFFF on Japanese quail reproduction, with Newsted et al. (2007) similarly incorporating 16 pairs per treatment group in assessment the effects of dietary exposure to PFOS on bobwhite quail and mallard ducks. Given the significant natural variability in bobwhite quail reproductive parameters (OECD 1984), the small sample sizes and associated lower statistical power used in the present study may have been insufficient to detect effects on reproduction. In addition, EPA guidance for avian reproduction tests recommends a minimum of 12 pairs per treatment group (EPA 1996). Furthermore, the experimental design involved exposing quail in blocks of two products and a single control concurrently, with all experiments conducted over a period of three years. The block design and use of different groups of birds for exposures may have contributed further variability to the data. For example, controls in the F3 2 and reference product exposure had a higher percentage of cracked eggs, 1.64%, compared to controls from all other blocks (0 or 0.17%). As such, significant effects on reproductive endpoints should be interpreted with caution.

The majority of significant effects observed were on chicks born following parental exposure to firefighting foams, with fewer effects observed on adults. Generally, few avian studies include assessment of chicks following parental exposure to contaminants. Newsted et al. (2007) studied the effects of dietary exposure to PFOS on mallard ducks and bobwhite quail, including a 7-week parental exposure followed by assessment of reproduction and 14-day chick survival. Few effects on adult quail were observed, with a LOAEL of 10 mg/kg calculated based on reduced survivorship of 14-day old chicks despite no significant effects on any other reproductive endpoint including number of eggs laid, the percentage of viable embryos, and hatching success (Newsted et al. 2007). Similarly, Gaffard et al. (2022) exposed grey partridge to pesticide mixtures via diet and assessed impacts on parental body condition, reproduction, and chick survival and body condition. Neither parental condition or egg production was significantly impacted by pesticide exposure; however, chick growth and body condition were significantly lower following parental exposure (Gaffard et al. 2022). As mentioned previously, the present assessment used smaller sample sizes than previous assessments of bird reproduction; thus, further study with a larger number of individuals would be useful to further elucidate potential multigenerational effects of F3s and the reference product.

Biometric endpoints including left- and right-wing metacarpal bones, left- and right-tarsa, and bill-head length were also commonly impacted in chicks following parental exposure (impacted in 4/7 foams), but impacted less frequently in adults (impacted in 2/7 foams). Few laboratory studies with avian receptors have considered biometric endpoints, with most studies incorporating these endpoints conducted on field-collected birds. For example, significant correlations between tarsal bone, wing length, and polychlorinated biphenyl concentrations were recorded in field collected

house sparrows, *Passer domesticus*, (Nossen et al. 2016), with another study demonstrating relationships between heavy metal exposure and a suite of biometric parameters in *P. domesticus* (Albayrak and Pekgöz, 2021). Furthermore, the Gaffard et al. (2022) study found that chicks born following parental exposure to pesticides had reduced skeletal growth, which was calculated incorporating measurements of head-bill, tarsus, and wing-length. Given the importance of the tarsa and wings in roosting, foraging, and protection from predation, impacts on these biometrics are anticipated to have concomitant effects at higher levels of organization.

4.2.2 Reptile Studies

4.2.2.1 Acute Studies

Similar to findings for bobwhite quail, acute lethality values for all foams were > 1,500 mg/kg. No available criteria for reptilian toxicity

4.2.2.2 Chronic Studies

Growth, Snout-Vent Length and Condition Index

Body weight, SVL, and condition index were assessed in brown anole after exposure to F3s and a reference AFFF for a period of 10 weeks. A summary of brown anole growth, SVL, and condition index over the exposure period is given in Appendix Table A19. In terms of body weight, all exposed *A. sagrei* gained weight over the exposure period excluding individuals exposed to the highest concentration of F3 4, 450 mg/L, where the average weight change was -2 ± 12% relative to controls after 10 weeks of exposure. However, no significant differences in body weight relative to controls were observed at this concentration due to high interindividual variability and low control growth during this experiment (average weight change of 2 ± 11%). Body weight was only significantly impacted in response to a single F3, F3 2, with significantly increased growth after 10 weeks in individuals exposed to 150 mg/L relative to controls (GLM, $p < 0.05$, Figure 9). No significant effect of F3 2 exposure on SVL change over the course of the experiment was observed (GLM, $p > 0.05$); however, condition index was significantly greater in anoles exposed to the 150 mg/L concentrations after 9 weeks relative to controls (GLM, $p < 0.05$). None of the other tested foams had significant effects on body weight, SVL, or condition index (GLM, $p > 0.05$).

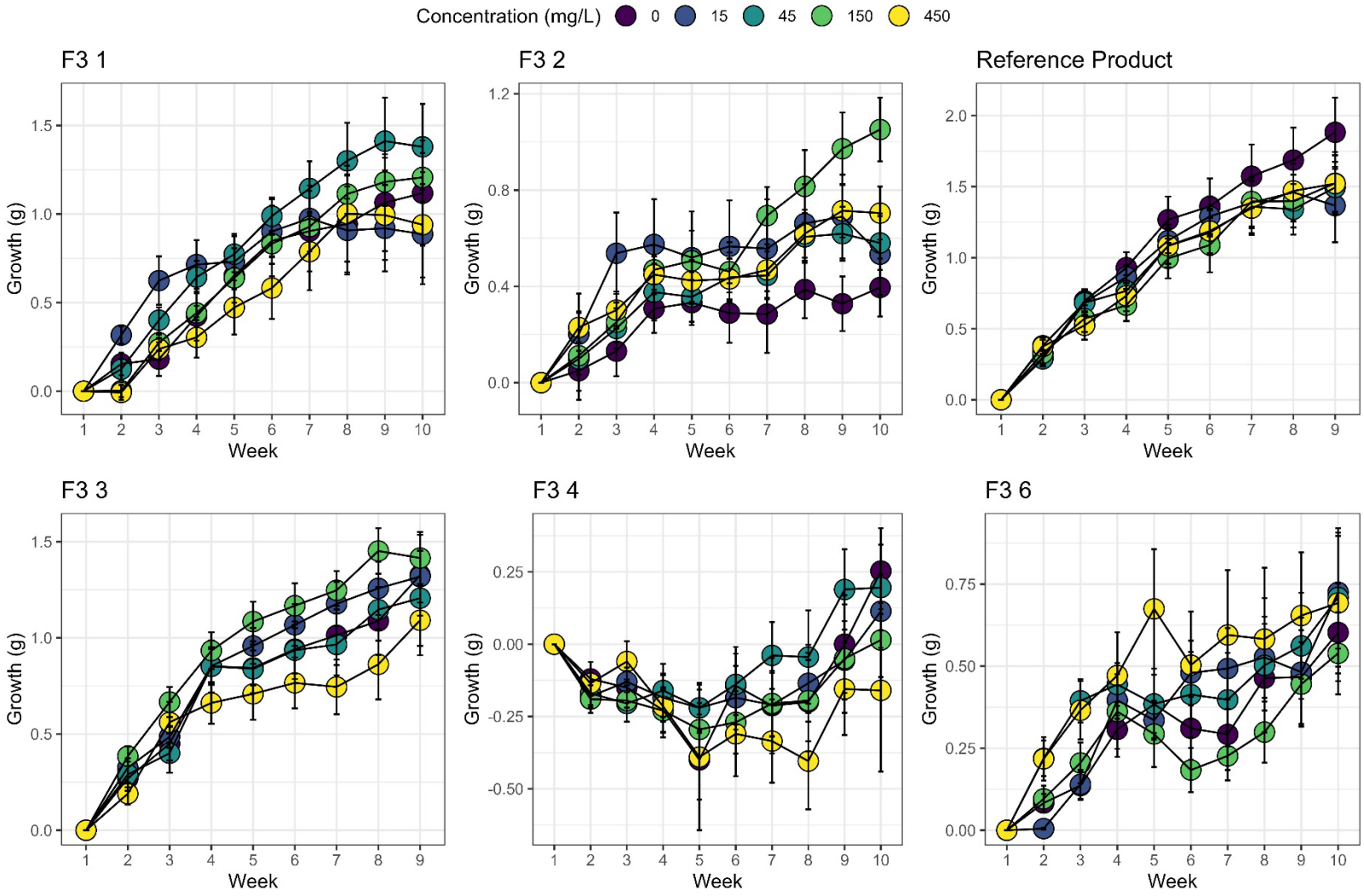

Figure 9. Growth (described as change in mass relative to week 1) in brown anoles exposed to five F3s and one AFFF.
Error bars are ± standard error of the mean.
* Indicates significant difference relative to controls

Organ Masses

Masses of brown anole liver, gonad, gastrointestinal (GI) tract, and muscle were determined following 10-week exposures. A summary of organ masses for all foams is given in Table A20. Values were normalized to the total mass of brown anoles. Overall, exposure to the F3s 2, 4, 6, and the reference product had no significant effect on masses of any of the measured organs (ANOVA, $p > 0.05$). For the F3 3 formulation, individuals exposed to 15 and 450 mg/L had significantly greater mass-normalized GI tract weights relative to controls (Kruskal-Wallis H-test, Post-Hoc Dunn's Test, $p < 0.05$, Figure 10), with significant differences observed between the 15 and 45 mg/L treatments for gonads, but no significant differences relative to controls (Kruskal-Wallis H-test, Post-Hoc Dunn's Test, $p < 0.05$). For F3 1, individuals exposed to 45 mg/L had significantly lower mass-normalized GI tract weights relative to controls (Kruskal-Wallis H-test, Post-Hoc Dunn's Test, $p < 0.05$, Figure 10); though no significant effect at any other concentration was recorded.

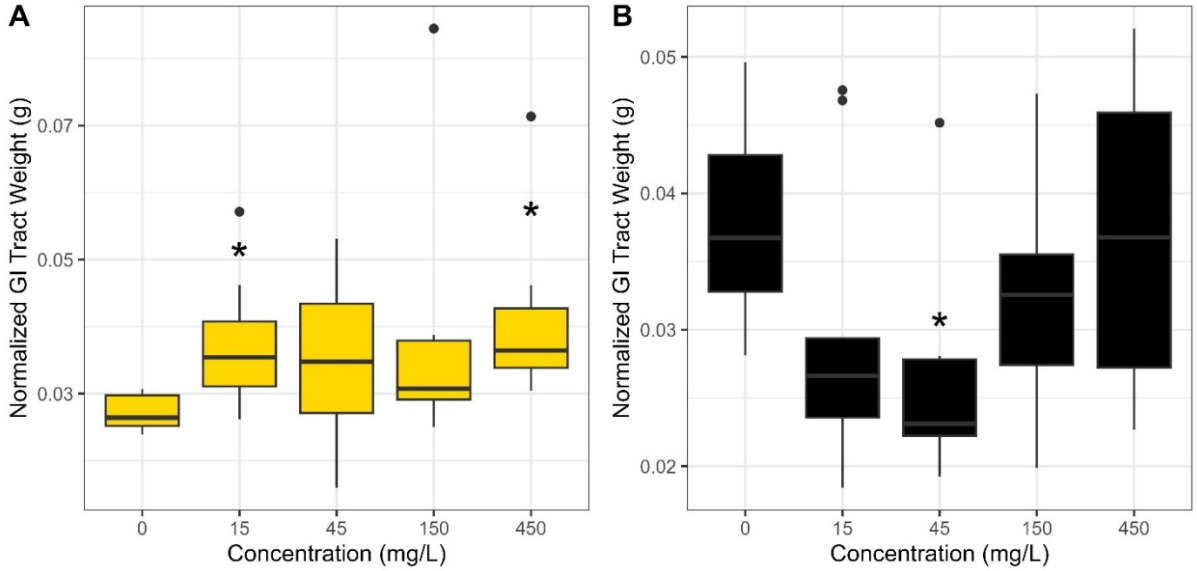

Figure 10. Mass-normalized GI tract weight in brown anoles exposed to F3 3 (A) or F3 1 (B).

* Indicates significant difference relative to controls (Dunn's Test, $p < 0.05$).

Cutaneous Evaporative Water Loss (CEWL)

CEWL was measured in *A. sagrei* at days 30 and 60 of the chronic tests. A summary of all CEWL data is given in Appendix Table A21. Overall, two F3s had a significant effect on CEWL: F3s 1 and 2 (ANOVA, $p < 0.05$, Figure 11). For F3 1, a significant reduction in CEWL was recorded in *A. sagrei* exposed to a nominal concentration of 450 mg/L after 30-day of exposure (ANOVA, Post-hoc Dunnett's Test, $p < 0.05$); however, no significant differences among treatments were observed after 60-day of exposure (ANOVA, Post-hoc Dunnett's Test, $p > 0.05$). For F3 2, no significant effects were recorded after 30 days; however, a significant reduction relative to controls was observed in *A. sagrei* exposed to 150 and 450 mg/L after 60-day (ANOVA, Post-Hoc Dunett's Test, $p < 0.05$)

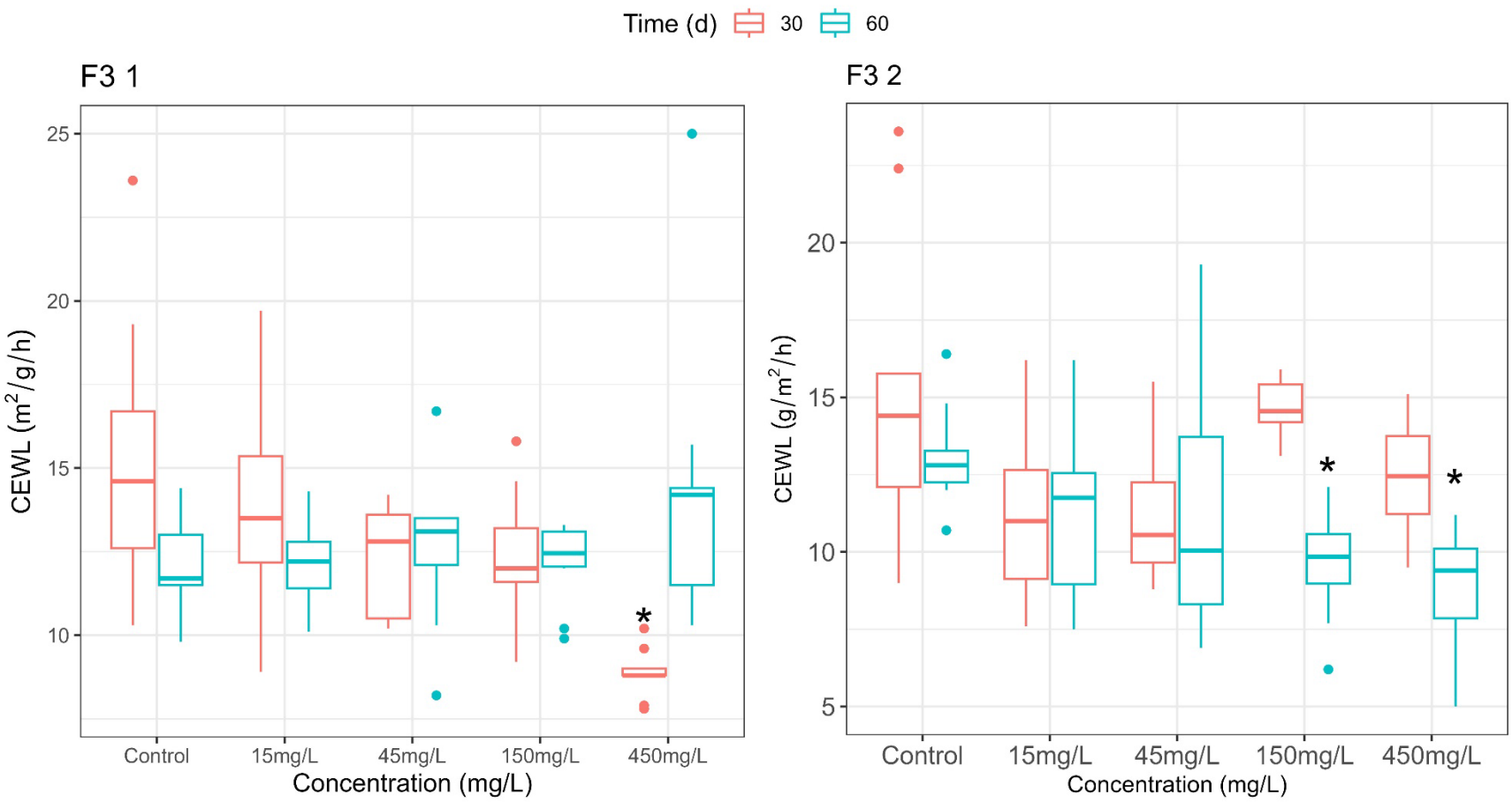

Figure 11. CEWL in brown anoles exposed to two F3s.

* Indicates significant differences to controls at a given time point (30- or 60-day, ANOVA, Post-hoc Dunnett's Test, $p < 0.05$). $g/m^2/h$ = gram per square meter per hour.

Bite Force

Similar to CEWL, bite force was measured at both days 30 and 60 of the test. A summary of all bite force data is given in Appendix Table A22. Overall, three of the six tested foams had a significant effect on bite force at either day 30, day 60, or both: F3s 1, 2, and 6 (Figure 12). For F3 1, no significant effects on bite force were recorded after 30 days(ANCOVA, $p > 0.05$); however, bite force was significantly reduced relative to controls after 60 days (ANCOVA, For F3 2, *A. sagrei* exposed to 450 mg/L had significantly increased bite force relative to controls after 30-day of exposure (ANCOVA, $p < 0.05$), though this was marginally significant after 60-day of exposure (ANCOVA, $p = 0.07$). Furthermore, *A. sagrei* exposed to F3 2 at 150 mg/L had significantly greater bite force relative to controls after 60-day (ANCOVA, $p < 0.05$). For F3 6, bite force was significantly reduced at all concentrations relative to controls following 30 days of exposure (ANCOVA, $p < 0.05$). However, after 60 days of exposure, the inverse was recorded with all concentrations having higher bite force relative to controls, which was significant at 150 mg/L only (ANCOVA, $p < 0.05$)

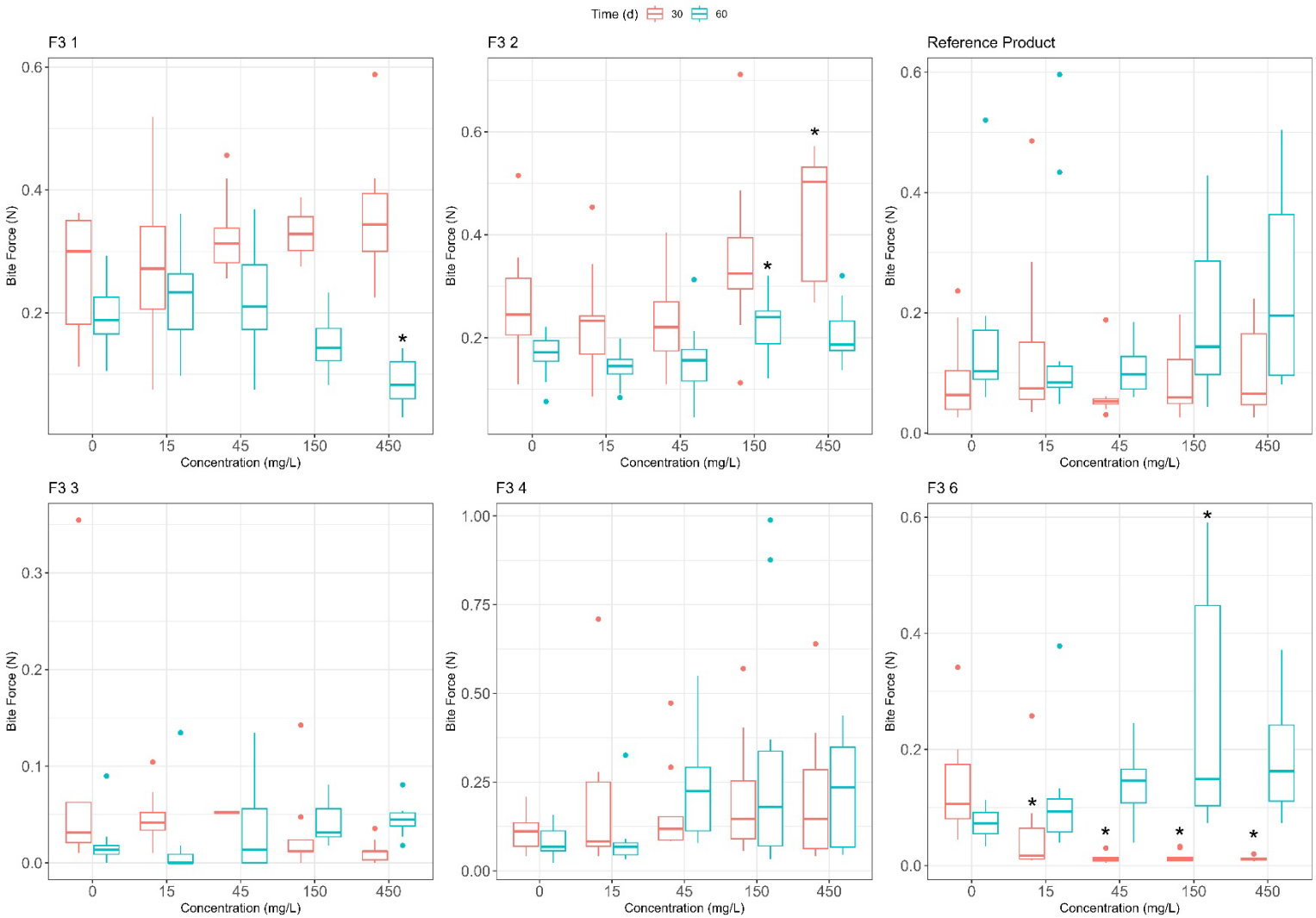

Figure 12. Bite force in *A. sagrei* following exposure to five F3s and one AFFF for 30 or 60 days.

*Indicates significant relative to controls based on a post-hoc test of estimated marginal means using morphometric data as a covariate.

Summary

A summary of all significant deleterious effects for chronic reptile studies is given below in Table 20. Only effects that are anticipated to have adverse effects on overall organismal health are included. For example, individuals exposed to 150 mg/L F3 2 had significantly increased growth, with no concomitant reduction at other exposure concentrations. Similarly, individuals exposed to 450 and 150 mg/L F3 2 had increased bite force at days 30 and 60, respectively, which is unlikely to have an overall negative impact. Increased organ mass was retained as a potential negative effect for data summaries. Overall, F3 1 had the most significant effects on reptiles, with reduced GI mass, CEWL, and bite force. Conversely, F3 4 and the reference product had no significant effect on any of the tested endpoints in reptiles.

Table 19. Summary of effects of five F3s and the reference AFFF on reptiles.

Foam	Effect	NOEC (mg/L)	LOEC (mg/L)
F3 1	Reduced GI Mass	15	45
	Reduced 30 d CEWL	150	450
	Reduced Bite Force	150	450
F3 2	Reduced 60 d CEWL	45	150
Reference Product	**No Significant Effects.**		
F3 3	Increased GI Mass	-	15
F3 4	No Significant Effects.		
F3 6	Reduced 30 d Bite Force	-	15

Notes:
CEWL = cutaneous evaporative water loss
d = day
GI = gastrointestinal
LOEC = Lowest Observed Effect Concentration
mg/L = milligrams per liter
NOEC = No Observed Effect Concentration

Discussion

In the present study, few significant effects of F3 and AFFF exposure on the growth, SVL, and condition index of *A. sagrei* were observed. Chronic exposure to 150 mg/L of F3 2 caused significantly greater growth rates relative to controls, with no significant effects observed at other concentrations or in response to any other F3s or the reference product. Overall, relatively few studies have considered the effects of toxic contaminants on growth in reptiles (Holliday et al. 2009; McFarland et al. 2008, 2009, 2011, 2012; Salice et al. 2009), with this group poorly studied relative to other ecological receptors. Salice et al. (2009) exposed western fence lizards (*Sceloporus occidentalis*) to inorganic lead for sub-chronic (14-day) and chronic (60-day) exposure durations, finding significant weight loss in individuals exposed to 62.5 mg/kg/d lead relative to controls in 14-day studies, but no overall significant dose effect after 60 days. In

contrast, McFarland et al (2008, 2012) assessed the effects of oral exposure to the nitroaromatic explosive, 2,4,6-trinitrotoluene (TNT) on western fence lizards, finding a hormetic response including increased growth at lower concentrations and a concomitant reduction in growth at higher concentrations. Consequently, the significantly increased growth observed in the present study could indicate a hormetic response, with the highest concentrations used not sufficient to cause a subsequent reduction. However, MacFarland et al. (2012) found that hormesis was no longer observed when *ad libitum* feeding was reduced to simulate resource limitation in the natural environment, indicating that this response may be an artifact of laboratory studies where food resources are highly abundant.

In terms of organ masses, significant effects were recorded for F3s 1 and 3, with both having significant effects on GI tract weight when normalized to body weight. For F3 1, significantly reduced GI tract weights were recorded at exposure concentrations of 45 mg/L only, whereas F3 3-exposed individuals had significantly greater GI tract weights at both 15 and 450 mg/L. The aforementioned reptile studies found significant effects of contaminant exposure on organ masses in reptiles, with Salice et al. (2009) reporting significantly increased kidney masses following lead exposure, but reduced testes and body fat masses. Similarly, McFarland et al. (2008) found that TNT exposure significantly increased the mass of liver, kidney, spleen, and brain relative to controls, with a significant reduction in testes relative to controls. Finally, Chang et al. (2016) recorded significantly reduced liver mass in Chinese lizards (*Eremias argus*) following oral exposure to the pyrethroid pesticide lambda-cyhalothrin.

No studies have considered the effects of contaminant exposure on the mass of the GI tract of reptiles to the author's knowledge. This organ includes the mouth, buccal cavity, oropharynx, esophagus, stomach, intestine, and the colon, with important biological functions including food storage, mechanical and enzymatic digestion, and transportation of ingested food (Mitchell and Diaz-Fugueroa 2005). Though mass was not recorded, Cakici and Akat (2012) found significant effects of oral exposure to the carbamate pesticide, carbaryl, on the GI tract of snake eyed lizards (*Ophisops elegans*) with histological defects including large intestine epithelial cell disintegration and effects on the esophageal connective tissue. Furthermore, a study by Chen et al. (2016) found that tissue concentrations of the pyrethroid pesticide, beta-cypermethrin, were highest in the GI tract of *E. argus* following oral gavage relative to other organs. Taken together, these findings may suggest a potential effect of F3 exposure on the digestive system of reptiles, though further study of additional endpoints such as histopathology, cellular biomarkers, and gene expression are necessary to confirm this finding.

In terms of sublethal endpoints, both CEWL and bite force were analyzed at the midpoint and end of the study, with significant effects on CEWL observed for F3s 1 and 2. CEWL refers to evaporation of water across the skin and does not include losses via respiratory, ocular, cloacal or excretory processes (Weaver et al. 2022). CEWL plays a significant role in both osmoregulation and thermoregulation and has been shown to have a high degree of plasticity in response to environmental conditions such as ambient temperature and humidity (Weaver et al. 2022). Importantly, *A. sagrei* exposed to the highest concentrations of the F3 1 showed significantly reduced CEWL after 30 days, but no significant differences after 60 days, which may be indicative of this plasticity and a potential adaptive response. No studies have considered the effects of contaminants on CEWL to the author's knowledge; however, changes to thermoregulatory behavior have been documented in reptiles following pollutant exposure, which could ultimately

be related to changes in water loss (Carpenter et al. 2016; Rohr and Palmer 2005; Yu et al. 2023). Carpenter et al. (2016) and Yu et al. (2023) both documented effects of glyphosate pesticides on the thermoregulatory behavior of reptiles, with changes to heat-seeking behaviors including basking. Given that CEWL is positively related to reptilian body temperature (Weaver et al. 2022), increased time basking in response to contaminant exposure as shown in Carpenter et al. (2016) could be a compensatory mechanism to account for changes in CEWL and concomitant body temperature. Further studies should consider implementing CEWL as a sublethal endpoint in studies of stressor impacts on squamate reptiles to contextualize these findings.

Exposure to three F3s led to significant effects on bite force measured either at 60 days only (F3 1), or both 30 and 60 days (F3s 2 and 6). Importantly, the direction of effects appeared to differ over time for the F3 6 foam, with significant reductions relative to controls at all concentrations in bite force recorded after 30 days, but an increase at all concentrations after 60 days which was significant at 150 mg/L. Similar to the findings for CEWL highlighted previously, this may suggest an adaptive response or recovery over chronic exposure durations.

Bite force is an important determinant of diet and prey handling and has been shown to influence antagonistic interactions among conspecifics in lizards (Boronow and Landkilde 2009; Huyghe et al. 2005). As such, bite force is postulated to be related to overall fitness given that a greater bite force is likely to increase proficiency in capturing and swallowing prey as well as determining the outcome of competitive interactions (Anderson et al. 2008). However, few studies have considered the impacts of stressors on bite force of reptiles, with most of the research focused on relating bite force to morphometric endpoints including head and body size (Anderson et al. 2008; Deeming 2022; Isip et al. 2022). A previous study assessing the sublethal effects of fire ant venom (*Solenopsis invicta*) on the eastern fence lizard (*Scloporus undulatus*), found no significant effects of fire ant venom on bite force. To the author's knowledge, no other studies have considered the effects of contaminants or other stressors on bite force. Several studies have related bite force to body and ambient temperatures (Anderson et al. 2008; Vicenzi et al. 2020) or behavioral state (Anderson et al. 2008). For example, Anderson et al. (2008) found significantly higher bite force in Tokay geckos (*Gekko gecko*) at elevated body temperatures, with Vicenzi et al. (2020) finding an inverse relationship for leopard iguanas (*Diplolaemus leopardinus*). CEWL, which is closely related to temperature regulation of lizards, was significantly impacted by exposure to F3s 1 and 2 as well as bite force. Thus, it is possible that observed effects on bite force could be related to changes in thermogenesis mediated by changes to CEWL following foam exposure. Given the limited implementation of bite force as a sublethal endpoint in contaminant studies, contextualizing the findings of the current study is challenging.

4.3 CHEMICAL ANALYSES

4.3.1 Aquatic Studies

For the aquatic studies, three dosing solutions from the chronic *C. dilutus* exposures were analyzed for chemical constituents (Table 21). For F3 2, measured concentrations ranged from 40% – 183% of nominal, with average concentrations of 0.9 mg/L ethylene glycol monobutyl ether (EGMBE) measured in control exposure solutions. For F3 6, measured concentrations ranged from 38% - 73% of nominal concentrations, with average concentrations of 0.6 mg/L measured in control exposure solutions. Finally, SDS measurements in F3 5 exposure solutions ranged from 105 – 229% of nominal, with 19.5 mg/L measured in controls.

Table 20. Measured concentrations of chemical constituents in three F3s used for aquatic toxicity testing.

Nominal Concentration (mg/L)	Measured Concentration (mg/L)	Percent of Nominal (%)
F3 2 (EGMBE)		
0	0.9 ± 0	NA
0.9	1.65 ± 0	183%
3	2.7 ± 0	90%
9	3.6 ± 0.3	40%
30	16.5 ± 0.6	55%
90	48.3 ± 1.5	54%
F3 6 (EGMBE)		
0	0.6 ± 0.3	NA
9	5.7 ± 0	63%
30	11.4 ± 0.3	38%
90	66 ± 6.3	73%
300	142 ± 4.50	47%
900	415 ± 6.3	46%
F3 5 (SDS)		
0	19.5 ± 0	NA
30	68.7 ± 0.9	229%
90	115 ± 3	128%
300	315 ± 40.2	105%
900	1326 ± 74.4	147%
1500	2291 ± 60.3	153%

4.3.2 Bobwhite Quail Studies

4.3.2.1 Chemical Analysis of Dosing Solutions and Feed

Chemical characterization of the dosing solutions for the F3s and reference product are given in Tables 22 and 23, respectively. Chemical analysis for the feed provided to adult and juvenile quail throughout the studies, Layena and Stratena, respectively, are given in Table 24.

Table 21. Measured chemical constituents in dosing solutions used for chronic bobwhite quail tests with six F3s.

Foam	Nominal Concentration (mg/L)	SDS (mg/L)	DGMBE (mg/L)	DMDA N-O (mg/L)	HG (mg/L)
F3 2	100	2.5	8.99	5.58	na
	1,000	11.8	102	65	na
	2,500	36.7	245	331	na
F3 3	100	1.4	10	na	na
	1,000	14	81	na	na
	2,500	34	192	na	na
F3 4	100	0.342	3.52	na	na
	1,000	0.945	42	na	na
	2,500	2.02	118	na	na
F3 5	100	0.6	na	na	1.2
	1,000	4.7	na	na	16
	2,500	9.3	na	na	29
F3 6	100	na	16	na	na
	1,000	na	182	na	na
	2,500	na	492	na	na
F3 7	100	1.5	4.6	na	na
	1,000	11	58	na	na
	2,500	22	121	na	na

Notes:
DGMBE = Diethylene glycol monobutyl ether
DMDA N-O = N,N-Dimethyldecylamine N-oxide
HG = Hexylene glycol
mg/L = milligrams per liter
na = Not analyzed
SDS = Sodium dodecyl sulfate

Table 22. Measured chemical constituents in the reference AFFF product for chronic bobwhite quail tests.

Product	Nominal Concentration (mg/L)	SDS (mg/L)	HG (mg/L)	PFBA (mg/L)	PFHxA (mg/L)
Reference Product	100	0.02	7.12	<LOD	0.0005
	1,000	0.15	67	0.0007	0.0037
	2,500	0.18	162	0.002	0.008

Notes:
HG = Hexylene glycol
LOD = limit of detection
mg/L = milligrams per liter
PFBA = Perfluorobutanoic acid
PFHxA = Perfluohexanoic acid
SDS = Sodium dodecyl sulfate

Table 23. Measured chemical constituents in feed used for chronic bobwhite quail tests. Layena and Stratena were given to adult and juvenile bobwhite quail, respectively.

Experimental Exposures	Feed Type	SDS (ng/g)	DGMBE (ng/g)	PFBA (ng/g)	PFHxA (ng/g)
F3 2 and Reference Product	Layena	36	74	nd	nd
	Stratena	40	77	nd	nd
F3s 5 and 7	Layena	2.8	102	nd	nd
	Stratena	2.8	80	nd	nd
F3s 3 and 6	Layena	30.4	94	nd	nd
	Stratena	24.7	90	nd	nd
F3 4	Layena	2.8	102	nd	nd
	Stratena	2.8	80	nd	nd

Notes:
DGMBE = Diethylene glycol monobutyl ether
DMDA N-O = N,N-Dimethyldecylamine N-oxide
HG = Hexylene glycol
nd = not detected
ng/g = nanograms per gram
PFBA = Perfluorobutanoic acid
PFHxA = Perfluohexanoic acid
SDS = Sodium dodecyl sulfate

4.3.2.2 Analysis of Eggs, Juvenile and Adult Bobwhite Quail Livers

Results of SDS and DGMBE analysis in F3s 2, 3, 4, and 7 are given in Figure 13. A summary of chemical residue data is given in Appendix Table A18. For F3s 5 and 6, only SDS and DGMBE were analyzed, respectively, with results shown in Figure 14. Finally, the results of the PFAS and SDS analyses in the reference product are shown in Figure 15. Generally, chemical constituent results were complex, with non-monotonic tissue concentrations for many foams and constituents. Furthermore, elevated levels of constituents in control tissue were observed for most of the tested foams. In addition, results appeared to be matrix-dependent, with data for chemical constituents measured in eggs generally showing an increase at higher exposure concentrations, which was not typically the case for adult and chick livers. Results are discussed by individual foam below.

For the F3 2 foam, the highest concentrations of SDS were observed in eggs following parental exposure to 2,500 mg/L (36.7 mg/L SDS measured), with concentrations of 1,486 ±223 nanograms per gram (ng/g) wet weight observed, significantly greater than eggs in all other treatments (ANOVA, Post-hoc Dunnett's Test, $p < 0.05$). Comparatively, maximum levels of SDS in adult livers following exposure to F3 2 were 367 ± 74.6 ng/g wet weight in the 2,500 mg/L treatment. In terms of DGMBE concentrations following F3 2 exposure, the highest concentrations were observed in chick livers following parental exposure to 1,000 mg/L (102 mg/L DGMBE measured), with concentrations of 569 ± 30.2 ng/g wet weight observed, significantly higher than all other treatments (ANOVA, Post-Hoc Dunnett's Test, $p < 0.05$). Maximum concentrations of 224 ± 24.7 ng/g wet weight were observed in eggs following parental exposure to 2,500 mg/L (245 mg/L DGMBE measured), significantly greater than controls (18.9 ± 4.8 ng/g wet weight, ANOVA, Post-Hoc Dunnett's Test, $p < 0.05$).

For F3 3, maximum concentrations of SDS were observed in juvenile livers from the control treatment (198 ± 69.6 ng/g wet weight); however, no significant differences among treatments were observed (ANOVA, Post-hoc Dunnett's Test, $p > 0.05$). For adult livers, highest levels were observed in the 2,500 mg/L treatment (34 mg/L measured); however, no significant differences between treatments were observed (ANOVA, Post-hoc Dunnett's Test, $p > 0.05$). Conversely, a dose-response relationship was observed for eggs, with maximum concentrations of 119 ± 16.3 ng/g following parental exposure to 2,500 mg/L, significantly greater than all other treatments (ANOVA, Post-hoc Dunnett's Test, $p < 0.05$). For DGMBE, maximum concentrations were in adult livers exposed to 100 mg/L, with average concentrations of 619 ± 87 ng/g wet weight; however, no significant differences among treatments were observed (ANOVA, Post-hoc Dunnett's Test, $p > 0.05$). Comparable findings were observed for DGMBE in juvenile livers and eggs, with higher concentrations at 100, 1,000, and 2,500 mg/L relative to controls, but not significantly so (ANOVA, Post-hoc Dunnett's Test, $p > 0.05$).

In terms of F3 4, maximum concentrations of SDS were observed in adult livers exposed to the highest concentration, 2,500 mg/L (2.02 mg/L SDS measured), with average liver concentrations of 450 ± 98 ng/g wet weight, significantly higher than all other groups (ANOVA, Post-hoc Dunnett's Test, $p < 0.05$). For DGMBE, elevated levels in the control were observed for liver samples, with maximum concentrations of 1,587 ± 97 ng/g wet weight and 1,372 ± 94 ng/g observed in adult and juvenile livers, respectively, significantly greater than the 100 and 1,000 mg/L treatments (ANOVA, Post-hoc Dunnett's Test, $p < 0.05$). While elevated levels of DGMBE were also observed in eggs from the control treatment (570 ± 16 ng/g wet weight), higher concentrations were observed in all other treatments, with maximum concentrations of 1,163 ± 84 ng/g in eggs following parental exposure to the 2,500 mg/L treatment (118 mg/L DGMBE measured), significantly higher than all other treatments (ANOVA, Post-Hoc Dunnett's Test, $p < 0.05$). Similar findings were observed for F3 7, with highest adult and juvenile liver concentrations of SDS and DGMBE occurring in the control treatment, and significantly lower concentrations in all other treatments (ANOVA, Post-Hoc Dunnett's Test, $p < 0.05$). Conversely, egg concentrations for both SDS and DGMBE increased with increasing parental exposure, with significantly higher concentrations in the 2,500 mg/L treatment (121 mg/L measured), 176 ± 18.4 ng/g, relative to controls (17.6 ± 1.88 ng/g, ANOVA, Post-Hoc Dunnett's Test, $p < 0.05$).

For F3 5, non-monotonic relationships were observed, with the highest concentrations of SDS observed in adult livers from the 100 mg/L treatment (0.6 mg/L SDS measured), 397 ± 217 ng/g

wet weight, but lower concentrations at higher exposure levels, and no significant differences between treatments (ANOVA, Post-hoc Dunnett's Test, $p > 0.05$). For juvenile livers, comparable SDS concentrations were observed at all treatments including the control, with no statistically significant differences (ANOVA, $p > 0.05$). Finally, maximum concentrations of SDS were observed in eggs following parental exposure to the 2,500 mg/L treatment (9.3 mg/L SDS measured), with concentrations of 30.7 ± 4.22 ng/g observed, significantly higher than the 1,000 mg/L treatment (4.7 mg/L SDS measured, 5.82 ± 1.42 ng/g), but not the control treatment (ANOVA, Post-hoc Dunnett's Test, $p > 0.05$). Similar findings were observed for DGMBE following exposure to F3 6 with highest concentrations observed in adult livers following exposure to the lowest concentration, 100 mg/L (16 mg/L DGMBE measured), with average levels of 516 ± 73 ng/g, and lower levels at higher exposure levels; though no overall statistically significant differences were observed (ANOVA, $p > 0.05$). For juvenile livers, comparable concentrations were observed in the 100 mg/L (16 mg/L DGMBE measured) and 1,000 mg/L treatments (182 mg/L DGMBE measured), with average concentrations of 348 ± 66 and 350 ± 61 ng/g, respectively, and significantly lower concentrations in the control treatment (492 mg/L DGMBE measured, 96 ± 18 ng/g, ANOVA, Post-hoc Dunnett's Test, $p < 0.05$).

Results for accumulation of measured PFAS and SDS in quail following exposure to the reference product are given in Figure 15. Concentrations of PFBA and PFHxA were below the limit of detection in control treatments for all adult liver samples. In terms of SDS in adult livers following exposure to the reference product, higher levels were observed in all treatment groups relative to controls, though this was not statistically significant. Maximum SDS concentrations were observed in the 1,000 mg/L treatment (0.15 mg/L SDS measured) for adult livers, with average concentrations of 225 ± 72 ng/g, significantly higher than controls (31.6 ± 4 ng/g, ANOVA, Post-hoc Dunnett's Test, $p < 0.05$). For chick livers, highest levels were observed in the 100 mg/L treatment (0.02 mg/L SDS measured), with lower concentrations in other treatments, and no significant differences among treatments (ANOVA, $p > 0.05$). Similarly, no significant differences among treatments were observed for egg concentrations, with maximum average concentrations of 239 ± 87 ng/g in the 1,000 mg/L treatment, but levels comparable or lower than controls at 100 and 2,500 mg/L (ANOVA, $p > 0.05$).

Overall, results for chemical residues analyses were complex and showed non-monotonic relationships or elevated levels in controls relative to treatment groups. The elevated presence of these surfactants in controls can be attributed to their widespread nature in products such as feed used throughout chronic studies. Furthermore, the finding of reduced chemical constituents at higher foam exposure levels in adults and chicks likely reflects the capacity to metabolize and eliminate these compounds, which may be upregulated at higher exposure concentrations. For eggs, increasing constituent concentrations were generally observed at higher concentrations, which is likely due to the reduced contaminant metabolic capacity of developing embryos relative to adults (Liu et al. 2019).

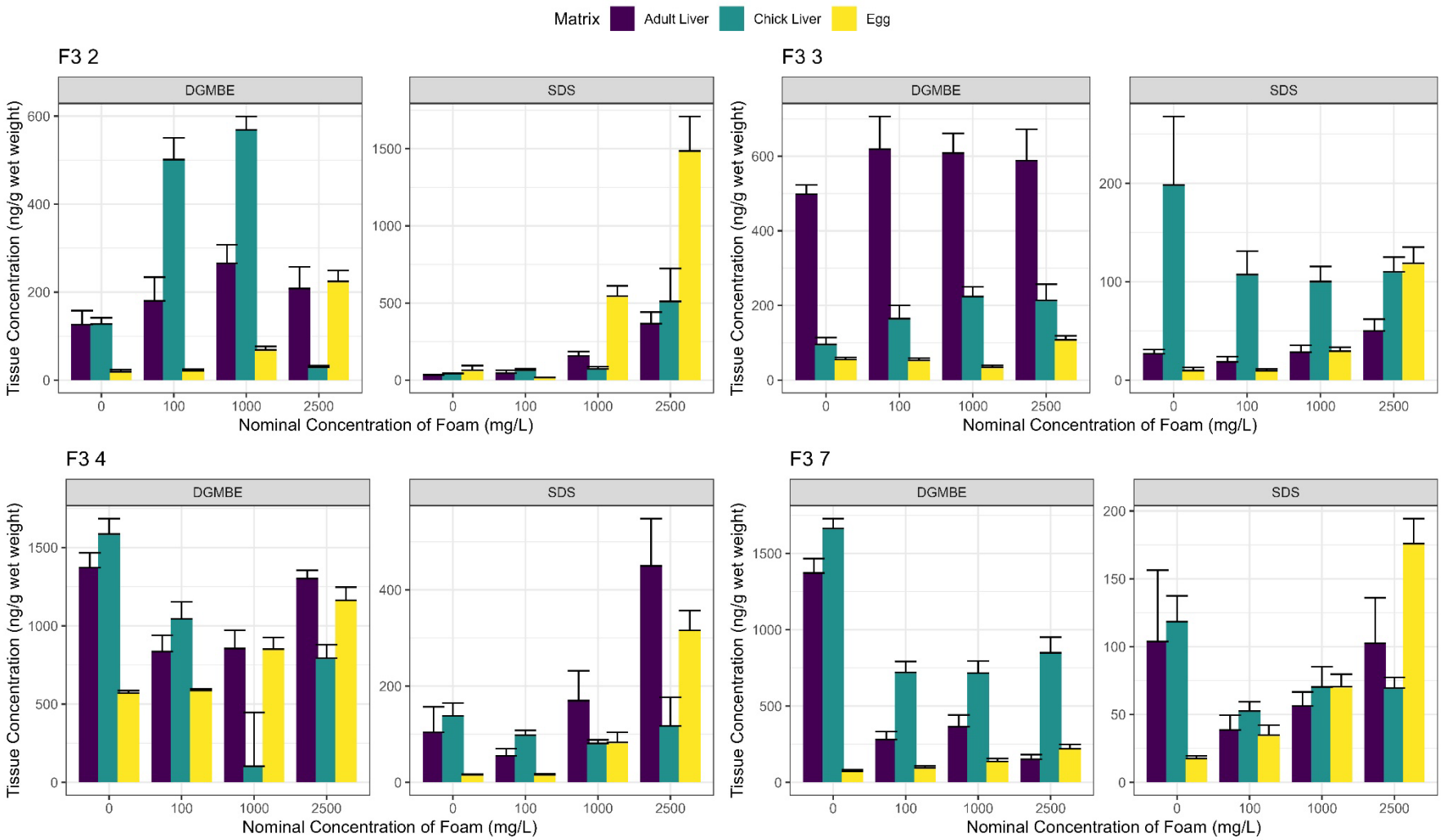

Figure 13. Tissue concentrations of chemical constituents in adult bobwhite quail liver, chick bobwhite quail liver, and eggs following chronic parental exposure to four F3s for 60 days.

SDS = sodium dodecyl sulfate, DGMBE = Diethylene Glycol Monobutyl Ether.
Error bars are standard error of the mean.

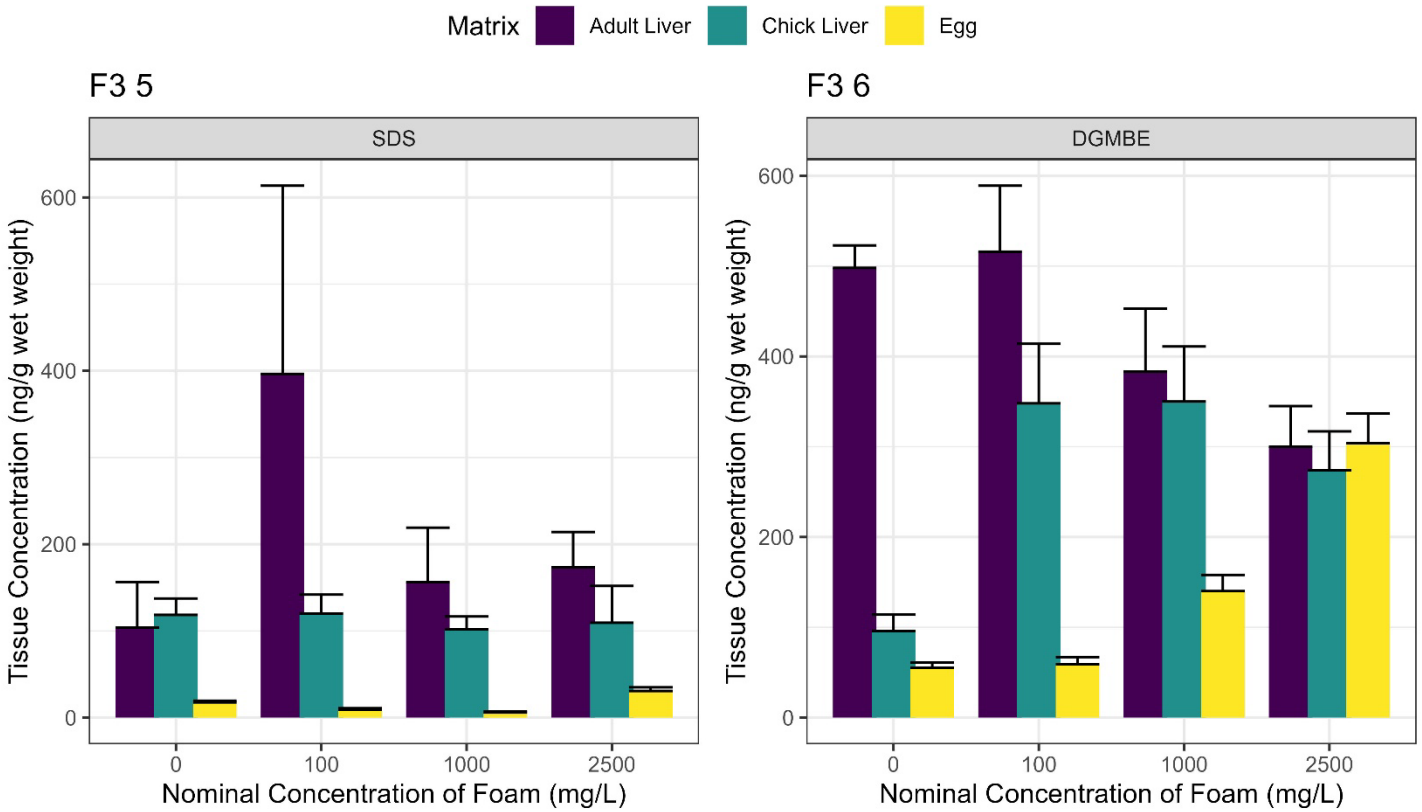

Figure 14. Tissue concentrations of chemical constituents in adult bobwhite quail liver, chick bobwhite quail liver, and eggs following chronic parental exposure to two F3s for 60 days.
SDS = sodium dodecyl sulfate, DGMBE = Diethylene Glycol Monobutyl Ether.
Error bars are standard error of the mean.

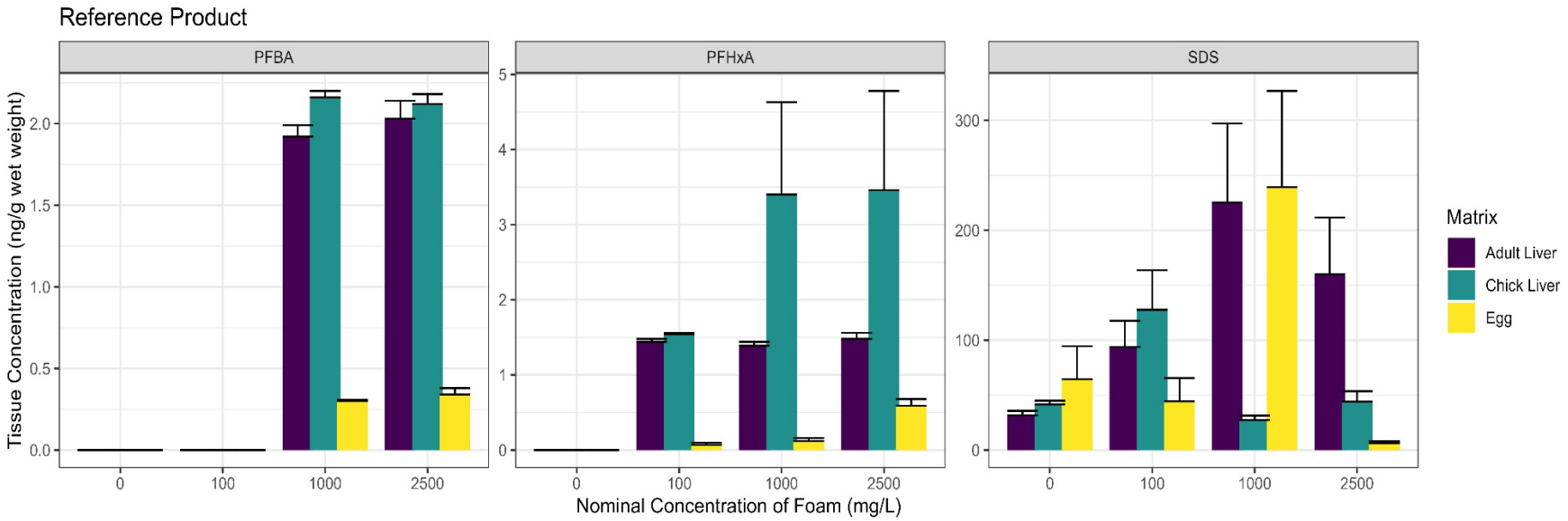

Figure 15. Tissue concentrations of chemical constituents in adult bobwhite quail liver, chick bobwhite quail liver, and eggs following chronic parental exposure to an AFFF for 60 days.
PFBA = Perfluorobutanoic acid. PFHxA = Perfluorohexanoic Acid. SDS = sodium dodecyl sulfate.
Error bars are standard error of the mean.

4.3.3 Brown Anole Studies

4.3.3.1 Dosing Solutions

During analysis of dosing solutions used for reptile studies, issues associated with the holding time between preparation of solutions and subsequent chemical analysis were noted. For example, solutions of F3 1 analyzed six weeks after preparation had measured concentrations of DGMBE ranging from 147–175% the expected levels based on nominal concentrations. F3 1 solutions prepared and analyzed within three weeks had marginally improved measured DGMBE concentrations of 47–107% relative to nominal. Consequently, two batches of reptile exposure solutions (150 mg/L only) prepared on different days in January 2024 were shipped and analyzed as measures of the accuracy of prepared nominal exposure solutions. All samples were analyzed within 14 days of receipt. Results are shown for four F3s and the reference product in Table 24. Overall, measured concentrations based on DGMBE ranged from 71–121% of nominal, indicating good overall accuracy in preparation of dosing solutions.

Table 24. Measured concentrations of DGMBE in reptile dosing solutions.

Foam	Date Prepared	Nominal Concentration (mg/L)	Measured DGMBE Concentration (mg/L)	Percent Relative to Nominal
F3 1	1/10/2024	150	159 ± 9	106%
	1/15/2024	150	152 ± 6	101%
F3 2	1/10/2024	150	134 ± 4	89%
	1/15/2024	150	106 ± 5	71%
F3 3	1/10/2024	150	153 ± 8	102%
	1/15/2024	150	172 ± 11	115%
F3 4	1/10/2024	150	147 ± 6	98%
	1/15/2024	150	145 ± 4	97%
Reference Product	1/10/2024	150	170 ± 3	113%
	1/15/2024	150	181 ± 5	121%

Notes:
DGMBE = Diethylene glycol monobutyl ether
mg/L = milligrams per liter

4.3.3.2 Reptile Livers

Two constituents, DGMBE and SDS, were measured in three replicates of three composite reptile livers from each F3 in controls, 150 mg/L, and 450 mg/L exposed groups. A summary of all chemical residue data is given in Appendix Table A23. DGMBE was not detected in any of the F3 1 samples (Figure 16). In terms of SDS, higher concentrations were typically observed in controls

relative to the treatment groups, with the exception of F3 4 (Figure 17). For F3 4 the highest SDS concentrations were observed in livers of the 150 mg/L group (32.1 ± 11.3 ng/g, average ± standard error), followed by the controls (26.1 ± 25.6 ng/g), and 450 mg/L treatment (13.1 ± 13.1 ng/g). For DGMBE, residues in livers increased with treatment concentrations for F3 2 and F3 3, with maximum concentrations in livers of 110 ± 58.9 ng/g and 69.0 ± 69.0 ng/g in the 450 mg/L treatment for F3 2 and F3 3, respectively. Conversely, DGMBE levels decreased with increasing F3 concentration for F3s 4 and 6, with maximum concentrations in control livers of 77.3 ± 77.3 ng/g and 261 ± 107 ng/g for F3 4 and F3 6, respectively.

Similar to the results for bobwhite quail livers highlighted above, the elevated presence of chemical constituents in reptiles from control treatments likely reflects the widespread nature of these surfactants in various matrices. Furthermore, the lower liver residues of surfactants at higher treatment concentration of F3s may be reflective of elevated metabolism at higher exposure concentrations.

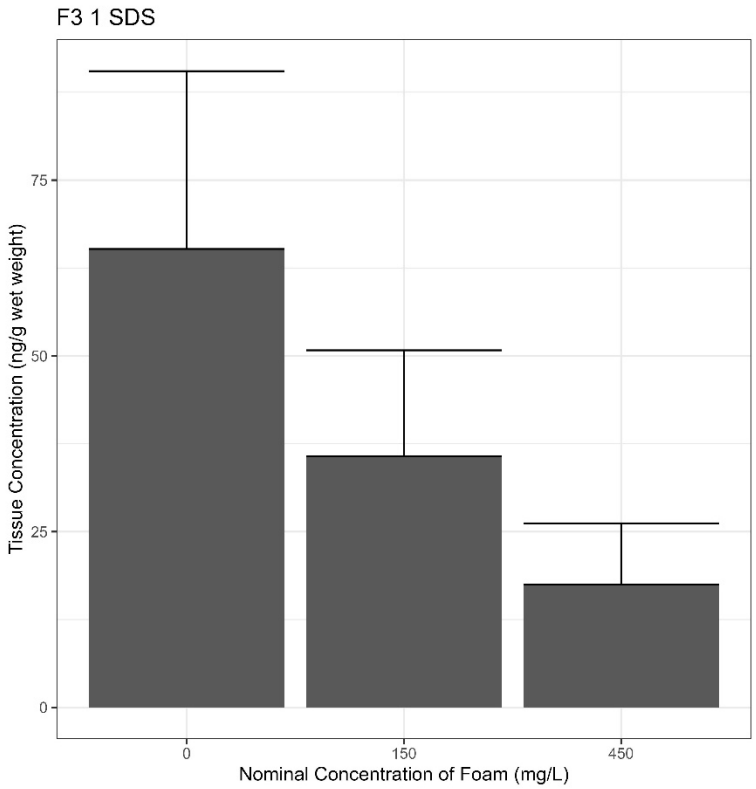

Figure 16. Concentrations of SDS in reptile livers following 60-day exposure to F3 1.

Error bars are standard error of the mean.
SDS = Sodium dodecyl sulfate.

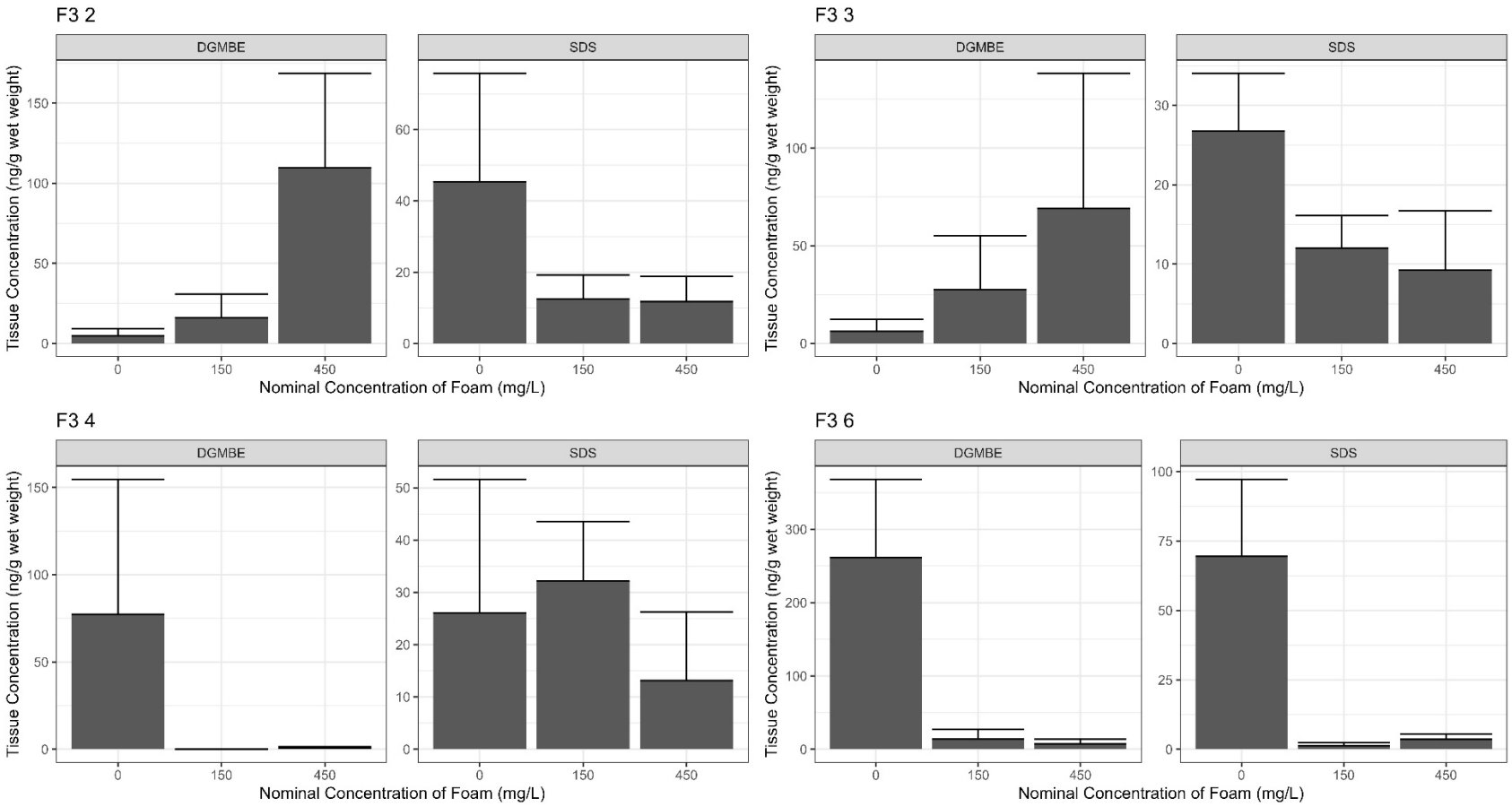

Figure 17. Concentrations of SDS and DGMBE in reptile livers following 60-day exposure to four F3s.

Error bars are standard error of the mean.
SDS = Sodium dodecyl sulphate.
DGMBE = Diethylene Glycol Monobutyl Ether.

4.3.4 Comparison of Chemical Constituents in Bobwhite Quail and Brown Anole Livers

A comparison of chemical constituents in adult bobwhite quail liver and brown anole liver is given in Table 25. In terms of SDS, broadly similar concentrations were observed between adult bobwhite quail exposed to 100 mg/L and brown anoles exposed at 150 mg/L. For DGMBE however, differences of over an order of magnitude were observed in liver concentrations between bobwhite quail and brown anoles following exposure to F3s 2, 3, and 6.

Due to the presence of chemical constituents in controls and the lack of dose-dependent increases in bioaccumulation, relating the measured concentrations of F3 constituents in quail and anole livers to measured amounts in exposure solutions is challenging. For bobwhite quail, measured concentrations of SDS in the F3s ranged from 0.342 – 2.50 mg/L in the 100 mg/L exposure group, with measured concentrations of 18.6 – 397 ng/g SDS in adult bobwhite livers exposed to this treatment, indicating low bioaccumulation of this constituent which has been observed previously (Comber et al. 2003). In addition, concentrations of SDS in bobwhite quail livers were not proportional with levels in dosing solutions. For example, the highest concentrations of SDS were observed in the F3 2 dosing solution for quail, with measured concentrations of 36.7 mg/L in the 2,500 mg/L treatment. However, the highest concentrations of SDS were observed in birds exposed to F3 4 (maximum of 450 ng/g, Table 25), despite lower concentrations of SDS in exposure solutions. Similar findings were observed for DGMBE, with the highest measured concentrations observed in dosing solutions for F3 6, but higher concentrations observed in livers of quail and anoles exposed to other F3s. Bioaccumulation of DGMBE was similarly low across both species and all treatments, which is in line with model estimates for this compound from EPA's EpiSuite software.

Table 25. Measured concentrations of DGMBE and SDS in adult bobwhite quail and brown anole livers.

Foam	Matrix	Nominal Concentration (mg/L)	SDS (ng/g)	DGMBE (ng/g)
F3 2	Adult Bobwhite Quail Liver	0	31.6 ± 4.0	126 ± 31.8
		100	45.2 ± 18.7	180 ± 53.7
		1,000	157 ± 18.7	266 ± 42.1
		2,500	367 ± 74.7	208 ± 49.3
	Brown Anole Liver	0	65.2 ± 25.3	4.63 ± 4.63
		150	35.7 ± 15.1	16.2 ± 14.7
		450	17.5 ± 8.70	110 ± 58.9
F3 3	Adult Bobwhite Quail Liver	0	27.0 ± 4.20	498 ± 25.0
		100	18.6 ± 5.40	619 ± 97.0
		1,000	28.5 ± 7.10	608 ± 53.0
		2,500	50.1 ± 11.9	588 ± 84.0
	Brown Anole Liver	0	26.8 ± 7.20	6.18 ± 6.18
		150	12.0 ± 4.10	27.6 ± 27.6
		450	9.20 ± 7.50	69.0 ± 69.0
F3 4		0	104 ± 53.0	1370 ± 94.0

Foam	Matrix	Nominal Concentration (mg/L)	SDS (ng/g)	DGMBE (ng/g)
	Adult Bobwhite Quail Liver	100	55.0 ± 15.0	836 ± 104
		1,000	170 ± 62.0	855 ± 117
		2,500	450 ± 98.0	1300 ± 53.0
	Brown Anole Liver	0	26.1 ± 25.6	77.3 ± 77.3
		150	32.2 ± 11.3	nd
		450	13.1 ± 13.1	0.700 ± 0.700
F3 6	Adult Bobwhite Quail Liver	0	na	498 ± 25.0
		100		516 ± 73.0
		1,000		383 ± 70.0
		2,500		300 ± 45.0
	Brown Anole Liver	0	69.5 ± 27.7	261 ± 107
		150	1.30 ± 1.20	13.8 ± 13.0
		450	3.60 ± 1.80	7.30 ± 6.40

na = not analyzed
nd = not detected.

4.4 BIODEGRADABILITY TESTING

4.4.1 Chemical Oxygen Demand, Nitrogen, and Phosphate

COD was tested in all F3 and the reference product. Higher COD levels indicate a greater amount of oxidizable organic material in the sample, which will ultimately reduce DO levels in the aquatic environment. Formulations with higher COD values are more likely to have a greater environmental impact with equivalent release volumes and formulation concentrations A summary table of COD for all F3s and the reference product as well as other characteristics is given below in Table 26.

Results indicated that F3 2 had the highest COD of 783,200 mg/L, followed by F3 6 (576,320 mg/L), F3 7 (426,400 mg/L), the reference product (400,160 mg/L), F3 3 (394,960 mg/L), F3 4 (233,040 mg/L), and F3 5 (159,960 mg/L). Overall, COD values were low compared to other published values for firefighting foams (i.e., Zhang et al. 2017). This was likely due to volatilization of alcohols and organics during the drying process leading to a false low solid result in the elemental analysis and total and volatile solids tests.

Nitrogen concentrations were also highly variable between foams. In terms of tTKN, highest levels were observed for F3 4 (18,000 mg/L), with the lowest amount in the F3 6 formulation (398 mg/L). tTKN reflects the levels of organic nitrogen plus ammonia (NH_3) and ammonium (NH_{4+}), but does not include other forms of inorganic nitrogen. Concomitantly, soluble ammonia was highest in the F3 4 formulation, and below detection limits in all other formulations excluding F3 3 (230 mg/L). For soluble nitrite and nitrate, levels were highest in the reference product (9,920 mg/L) and F3 2 (136 mg/L), respectively. Finally, total phosphate was highest in the F3 2 formulation (876 mg/L)

over an order of magnitude higher than any other formulation (73 and 38 mg/L in F3 6 and the reference product, respectively, below detection limit in all other foams).

Table 26. Summary of chemical formulations and total COD, tTKN, soluble ammonia, soluble nitrite, soluble nitrate, and total phosphate values for six F3s and one AFFF.

Formulation	Chemical Formula	Total COD (mg/L)	tTKN (mg/L)	sNH_3 (mg/L)	sNO_3 (mg/L)	sNO_2 (mg/L)	tPO_4 (mg/L)
F3 2	$C_{1.5}H_{3.1}ON_{0.08}$	783,200	5,560	BDL	BDL	136	876
F3 3	$C_{1.6}H_{3.7}ON_{0.31}$	394,960	4,280	230	369	BDL	BDL
F3 4	$C_{1.5}H_{3.2}ON_{0.04}$	233,040	18,800	330	448	BDL	BDL
F3 5	$C_{2.1}H_{4.6}ON_{0.13}$	158,960	2,172	BDL	5360	BDL	BDL
F3 6	$C_{1.9}H_{3.8}O$	576,320	398	BDL	540	BDL	38
F3 7	$C_{3.4}H_{7.2}ON_{0.13}$	426,400	1,976	BDL	556	BDL	BDL
Reference Product	$C_{1.3}H_{2.5}ON_{0.021}$	400,160	2,176	BDL	9920	BDL	73

Notes:
BDL = Below detection limit
COD = chemical oxygen demand
mg/L = milligrams per liter
sNH_3 = soluble ammonia
SNO_2 = soluble nitrate
sNO_3 = soluble nitrite
tPO_4 = total phosphate
tTKN = total Kjeldahl nitrogen

4.4.2 Oxygen Uptake as Percent Chemical Oxygen Demand

Most of the formulations showed good biodegradation characteristics with 28-day oxygen uptake equal to approximately 100% of the initial COD added to the reactors (Figure 18). A summary of oxygen uptake rates relative to initial COD is given in Appendix Table A24. For F3s 4 and 5 and the reference product, oxygen uptake as a percentage of COD was similar for all three concentrations tested. Oxygen uptakes exceeding 100% of the initial applied COD implied the actual COD of the formulation was higher than was measured in the standard COD test. For F3 6, oxygen uptake reactions were variable in the different reactors, which was attributed to challenges associated with dosing viscous material. In particular, the curves for 120 and 240 mg/L COD in F3 6 formulation were similar, suggesting the 180 mg/L COD curve as a potential outlier.

4.4.3 Cumulative Oxygen Uptake

The cumulative oxygen uptake rates of most of the formulations showed two portions for organic material degradation with different times of adaptation. For F3 2, a major portion of organic material degraded in the first 24 h of incubation as indicated by the first large oxygen uptake rate peak, while the second portion of organic material required approximately 2 days of adaptation before it began to be degraded as indicated by the second oxygen uptake rate peak (Figure 19). For F3 3, oxygen uptake rates showed initial biodegradation of a portion of organic material followed by a similar second portion that required approximately 2 days of adaptation before it began to be

degraded. For F3 4, oxygen uptake rates showed a very rapid initial biodegradation of a portion of organic material with a high rate that was comparable to the control. Similar to the other formulations, the second portion of organic material required approximately 2 days of adaptation before degradation. For the F3 7 formulation, oxygen uptake rates showed rapid initial biodegradation of a portion of organic material. A second portion of organic material required approximately 2 days of adaptation before it began to be degraded. For F3 6, oxygen uptake rates showed slow initial biological degradation through day 3 suggesting that the formulation was undergoing biological hydrolysis. For F3 5, oxygen uptake rates showed rapid initial biodegradation of a portion of organic material with the second portion requiring approximately four days of adaptation before degradation. For the reference product, oxygen uptake rates showed initial biodegradation of a portion of organic material with the second portion needing five days of adaptation before it began to be degraded.

For F3s 2, 3, 4, 5, 7 and the reference product, measurement of soluble COD concentration for all doses approached zero after 28 days of incubation, translating to complete biodegradability as previously defined by Tang et al. (2019) (Figure 20). The similarity of the adaptation curves at all concentrations indicates that these formulations were not toxic to the microbial community at any concentration tested. For F3 6, soluble COD concentrations over time for the 180 and 240 mg/L were higher than in all other formulations. Subsequent CODs after 28 days of incubation were 15 mg/L and 17 mg/L, respectively. While these values only represent 8% and 8.5% of the initial feed COD, they indicate that part of the organic material in F3 7 degraded more slowly than in other formulations. Overall, the formulation showed good biodegradability at the end of 28 days.

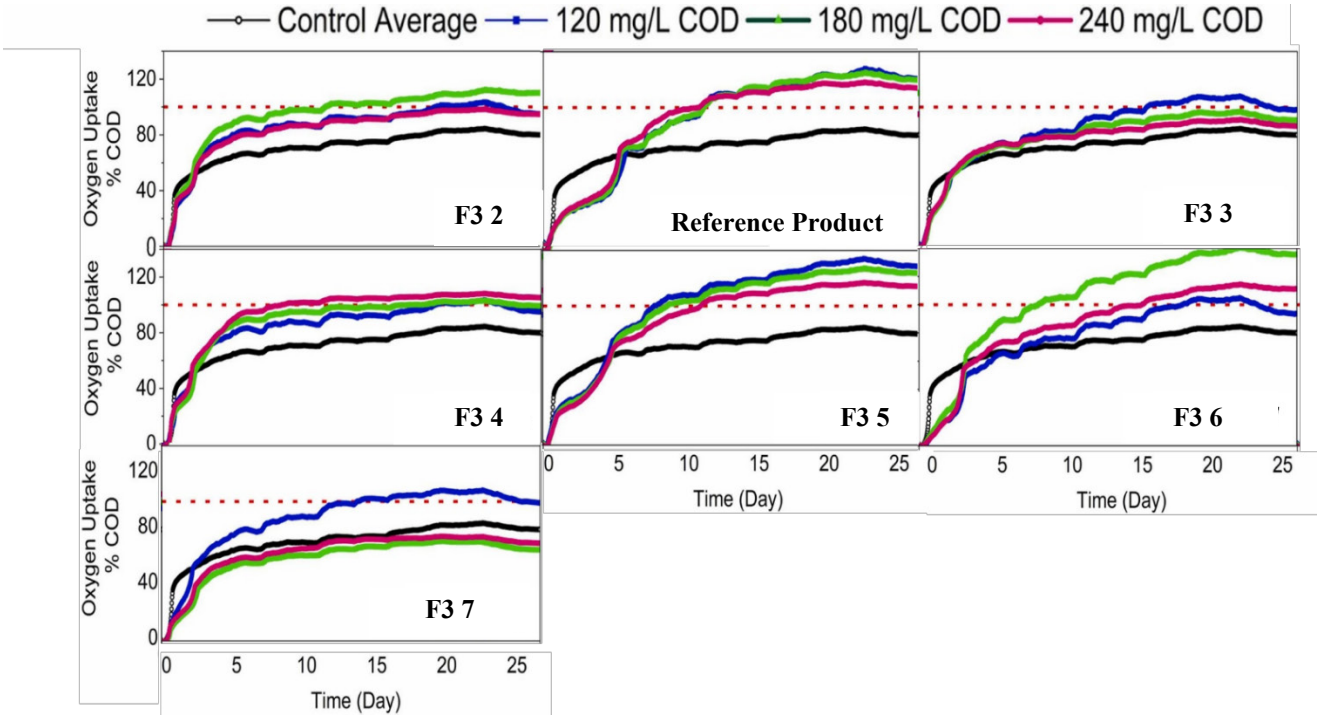

Figure 18. Oxygen uptake rates as percent COD in six F3s and a reference product over the 28 day biodegradation period.

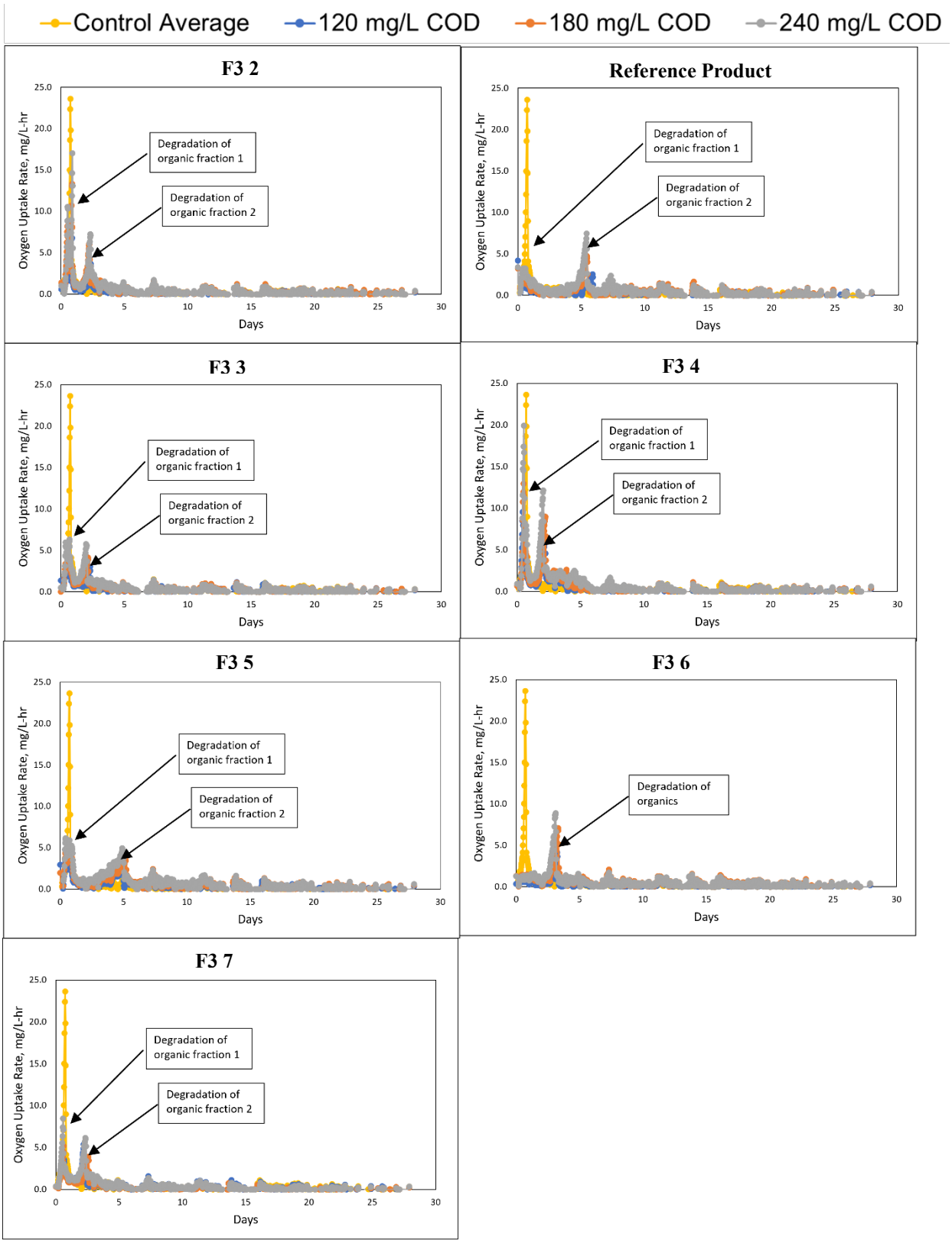

Figure 19. Cumulative oxygen uptake rates at different concentrations of 120, 180, and 240 mg/L COD for six F3s and one AFFF.

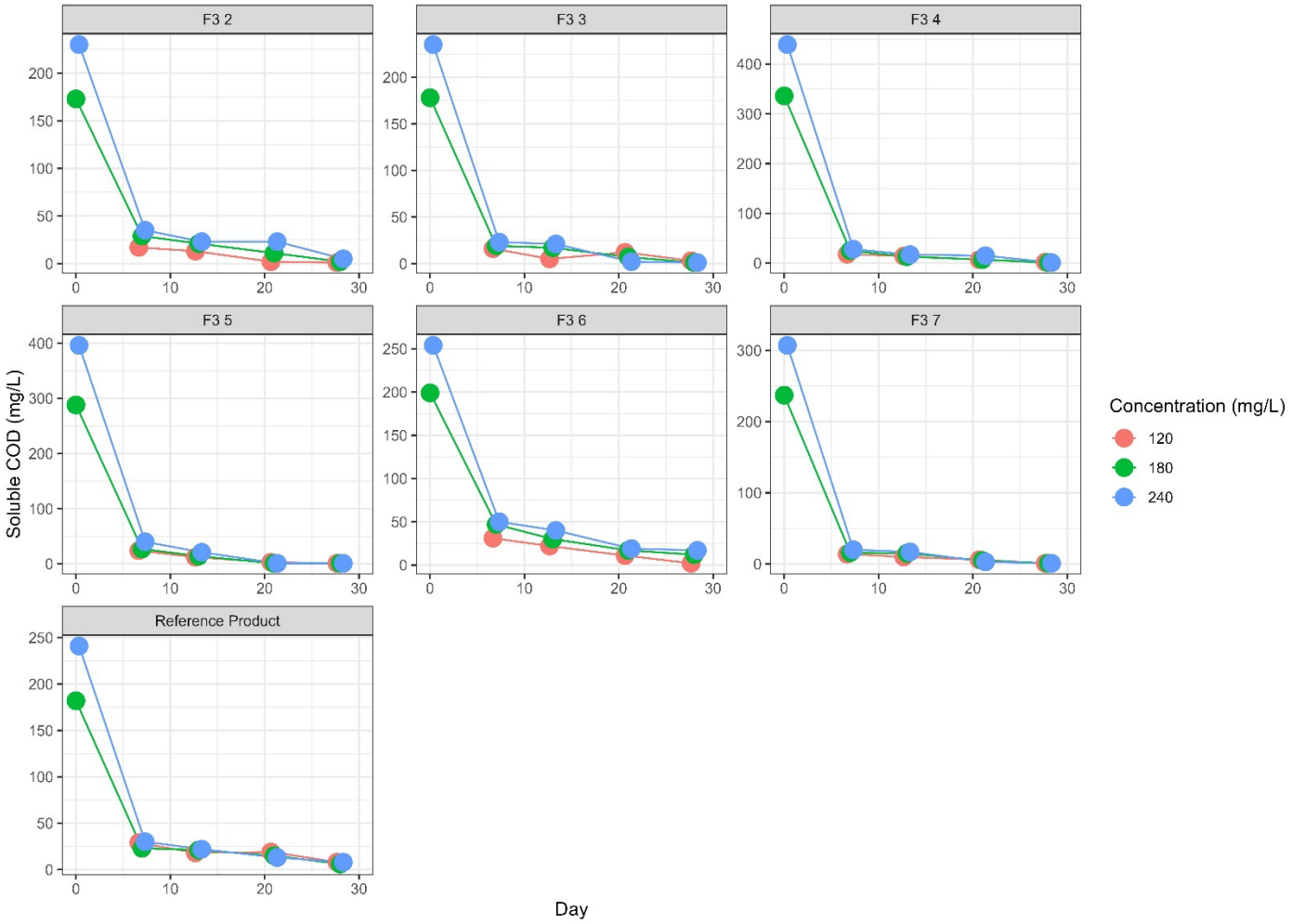

Figure 20. Soluble COD in six F3s and a reference product over the 28-day period.

4.4.4 Biodegradation Of Constituents

Effluents from the biodegradability testing of the formulations were analyzed by LC/MS/MS for the following constituents: DGMBE, SDS, DMDA N-O, and HG. Analysis demonstrated that all constituents had dropped to non-detectable levels by the seventh day of testing for F3s 2, 3, and 4 (Figure 21). For F3 6, DGMBE, and DMDA N-O had dropped to non-detectable levels by the seventh day of testing while the SDS concentration dropped during the first seven days of testing but remained at approximately 15% of the initial concentration through the end of the testing (Figure 21) For F3 5, SDS and HG dropped to non-detectable levels by the seventh day of testing whereas concentrations of DGMBE dropped during the first seven days of biodegradability testing but remained detectable at approximately 20% of initial levels through the end of the test (Figure 22). For F3 7, DGMBE dropped to non-detectable levels by day 14 of testing, with a small amount of SDS present at approximately 0.2% of initial levels by the end of testing (Figure 22).

For the reference product, SDS and HG dropped to non-detectable levels by the seventh day of testing and DMDA N-O by the twenty-first day of testing (Figure 23). While DGMBE decreased during the first seven days of biodegradability testing, its concentration remained at approximately

30% of the original concentration through the end of the test. Similarly, measured PFAS constituents including PFBA and PFHxA showed an increase in concentration through day 21 of testing, declining to 99.6 and 85.3% of initial levels by day 28 for PFBA and PFHxA, respectively (Figure 23).

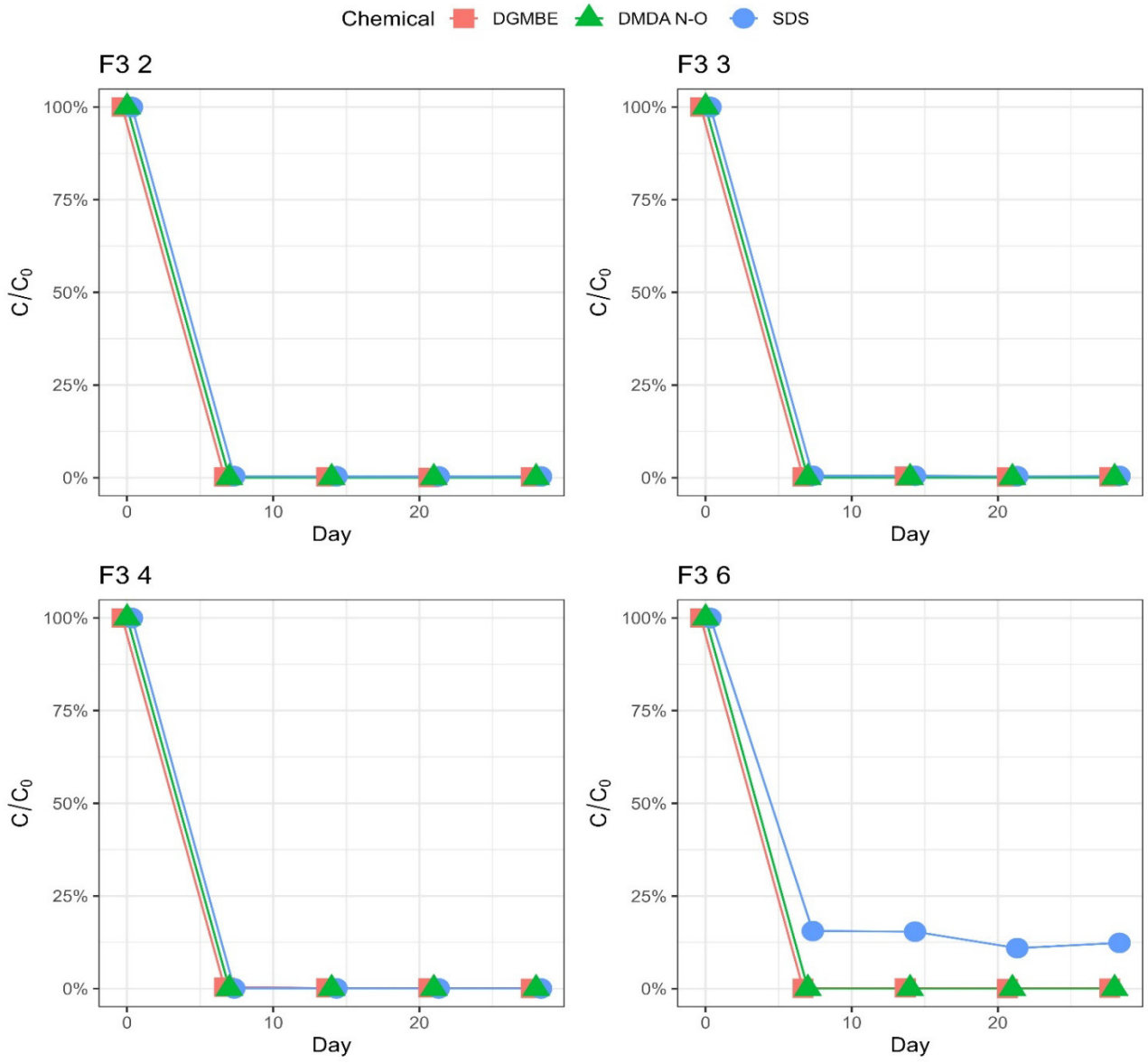

Figure 21. Biodegradation of chemical constituents DGMBE, DMDA N-O, and SDS in four F3s. Values are shown as concentration at a given time point (C) relative to initial concentration (C0).

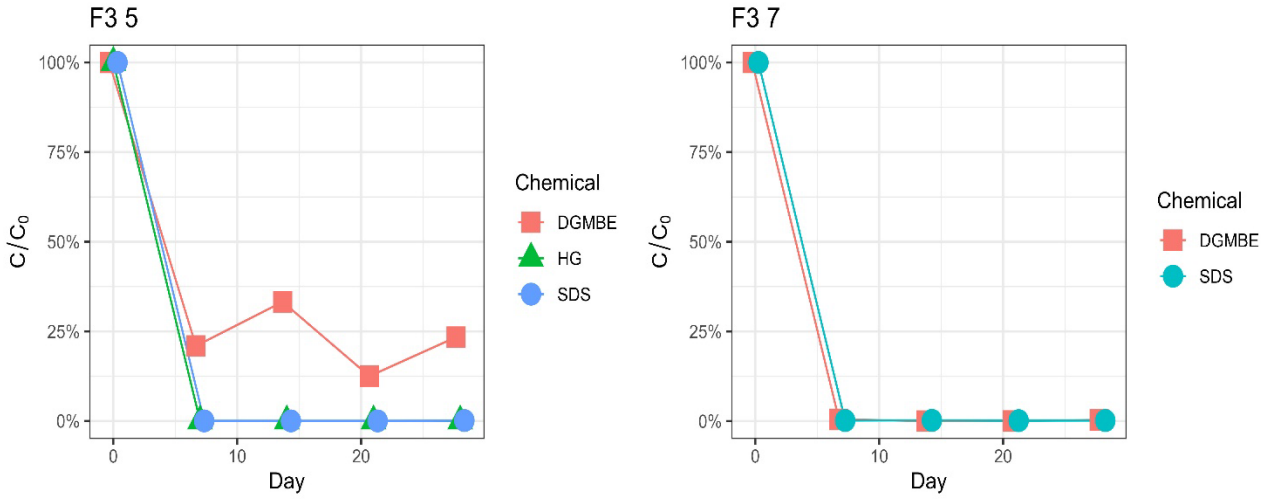

Figure 22. Biodegradation of chemical constituents DGMBE, HG (F3 5 only), and SDS in two F3s. Values are shown as concentration at a given time point (C) relative to initial concentration (C0).

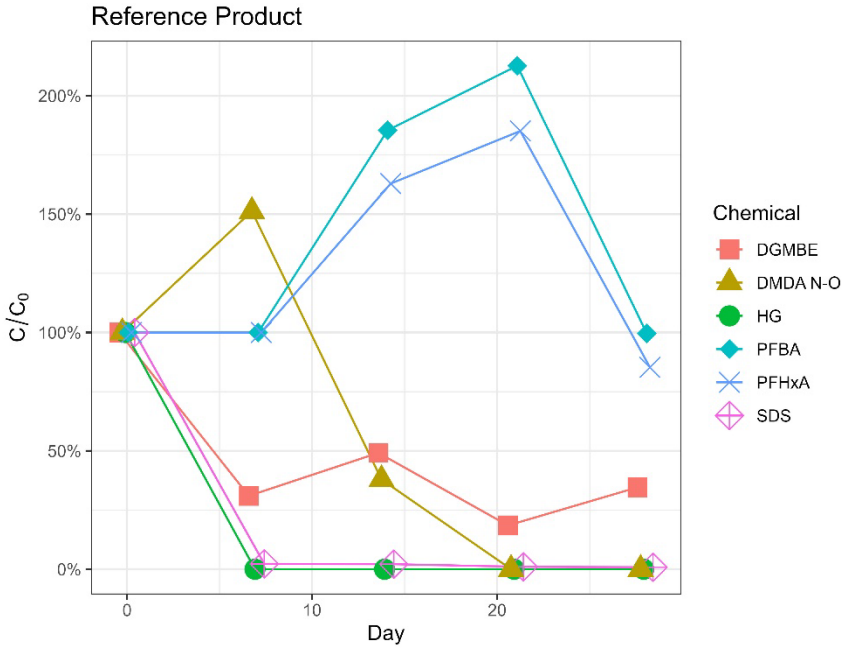

Figure 23.
Biodegradation of chemical constituents DGMBE, DMDA N-O, HG, SDS, PFBA, and PFHxA in the reference product. Values are shown as concentration at a given time point (C) relative to initial concentration (C_0).

4.4.5 PFAS Characterization in the Reference Product

The safety data sheet for the reference product indicates that the fluorine fraction of the formulation is < 5%. The residual COD from the biodegradability testing may be associated with the fluorine fraction of the formulation since fluorinated organics have been shown to be recalcitrant to both

aerobic and anaerobic biological degradation. A suite of PFAS were measured at various concentrations in the reference product as shown in Table 27.

Table 27. Characterization of PFAS measured in the reference product.

Component Name	Concentration (ng/mL)
4:2 Fluorotelomer sulfonic acid (4:2 FTS)	420
6:2 Fluorotelomer sulfonic acid (6:2 FTS)	9,126
Perfluorobutanesulfonic acid (PFBS)	26
Perfluorobutanoic acid (PFBA)	1,239
Perfluoroheptanoic acid (PFHpA)	43
Perfluorohexanoic acid (PFHxA)	2,870
Perfluorohexanesulfonic acid (PFHxS)	8
Perfluorononanoic acid (PFNA)	< 0.0033
Perfluoropentanesulfonic acid (PFPeS)	58
Perfluorooctanoic acid (PFOA)	< 0.0007
Perfluoropentanoic acid (PFPeA)	342

Notes:
ng/mL = nanograms per milliliter

In terms of degradation of the PFAS constituents, results indicated that 4:2 fluorotelomer sulfonic acid (4:2 FTS) and 6:2 fluorotelomer sulfonic acid (6:2 FTS) concentrations decreased while perfluoropentanoic acid (PFPeA), PFBA, and PFHxA concentrations increased within the samples at days 14, 21, and 28 (Figure 24). Both 4:2 FTS and 6:2 FTS appeared to experience degradation on these same sample days and may be transforming to terminal perfluoroalkyl acids (PFAA). Statistical analysis was also performed to evaluate if the values at each time point were significantly different from the initial timepoint. For 6:2 FTS, significant differences were observed between the 21-day and day 0 time points only (t-test, $p < 0.05$), with values at 14 and 28-day approaching significance (p values of 0.08 and 0.07, respectively). For 4:2 FTS, significant differences were observed at all timepoints relative to d0 (t-test, $p < 0.05$).

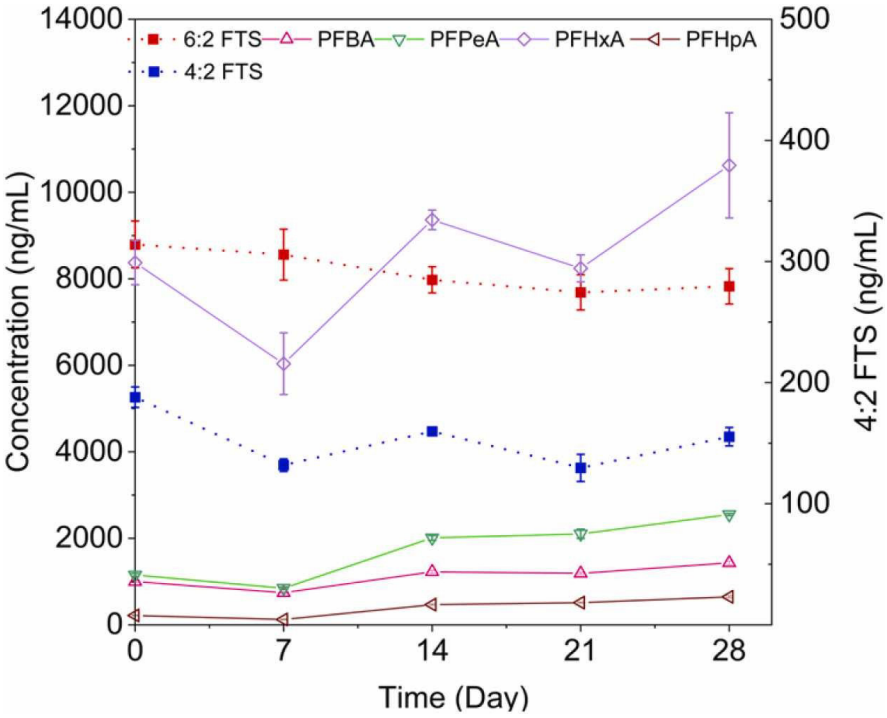

Figure 24. Degradation of tested PFAS in the reference product AFFF formulation over the reaction period of 28 days.
4:2 FTS concentration is shown on the secondary y-axis.

4.4.6 Discussion

Respirometry coupled with analysis of constituents confirmed good biodegradability of all tested F3s with F3 6 having the longest biodegradation time. Biodegradability results indicated that the nitrification inhibitor was effective, and that biodegradation was not nutrient or trace metal limited. 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 F3s 6, 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.

The results for constituents from biodegradability tests of all formulations indicated that the concentrations of DGMBE, SDS, DMDA N-O, and HG were rapidly reduced with degradation proceeding to completion within 7 days of biologically active treatments with some exceptions. By the end of the 28-day test period, DGMBE in reference product and F3 5 approached approximately 30% and 20% of starting values, respectively. For F3 6, SDS reached approximately 15% of starting values, and DMDA N-O in the reference product had an increase on day 7 followed by a rapid decrease and then reaching complete degradation by day 21. The rapid degradation limited the determination of biodegradation rate constants for individual compounds; however, the level of compound degradation for most F3 formulations tested was comparable.

Interestingly for the reference product, residual COD was observed at the end of the 28-day test period which was attributed to the fluorine fraction of the formulation. Shorter chain PFAA detected high concentrations in the reference product, with PFHxA at approximately 2,870 ng/mL and the precursors fluorotelomer sulfonate 6:2 FTS at approximately 9,126 ng/mL and 4:2 FTS at 420 ng/mL. Results indicated that both 6:2 FTS and 4:2 FTS were degraded in the reference product through the 28-day test period while some of the PFAAs including perfluoroheptanoic acid (PFHpA), PFHxA, PFPeA, and PFBA were being generated and simultaneously being degraded. This implies perfluoroalkyl carboxylic acids (PFCAs) generation from FTSs and/or other precursors present in the reference product. This is consistent with previous biodegradation studies where fluorotelomer precursors have been shown to degrade to PFCAs forming C8-C6 from 8:2 FTS transformation and C6-C4 from a 6:2 FTS (Carrillo-Abad et al. 2018; Shaw et al. 2019; Weber et al. 2017) suggesting a similar pathway for 4:2 FTS to PFBA.

From a previous study on the same reference product used in this work (Shojaei et al. 2022), there are other 6:2 fluorotelomer precursors present including 6:2 fluorotelomer sulfonamide propyl methyl amine (6:2 FTSA-Pr-MeAn), 6:2 Fluorotelomer Sulfon Amido Propyl Dimethyl Ammoniohydroxy Propyl Sulfonate (6:2 FTSAPr-AmHOPrS), and 6:2 Fluorotelomer Sulfonamide Propyl methyl Ammoniohydroxy Propyl Sulfonate (6:2 FTSAPr-MeAOHPrS) which possibly yield to 6:2 FTS and PFAAs generation. In other words, 6:2 FTS is very likely to undergo simultaneous generation and biodegradation. Additionally, Shojaei et al. (2022) performed the total oxidizable precursor assay on the reference product, finding significant increases in concentrations of PFPeA, PFBA, and PFHxA. Similarly, a study by Houtz and Sedlak (2012) found generation of PFCAs in 6:2 FT-based PFAS. Overall, Shojaei et al. (2022) reported that the total oxidizable precursor assay data implies > 99% of PFAS was not identified during targeted PFAS analysis in the reference product.

Given the lack of information on emerging F3s, comparable data to contextualize the biodegradability findings of the present work is scarce. However, another study by McDonald et al. (2022) performed an evaluation of the chemical and physical properties, including biodegradability, of a suite of F3s including F3s 2, 3, 4, and 7 that were included in the present study. Methods used to assess biodegradability in the McDonald et al. (2022) study were different to those used in the present work; thus, a direct comparison to our findings was not possible. For example, McDonald et al. (2022) calculated a biodegradability index as the ratio between biological oxygen after 20 days and COD. However, some commonalities were observed with the present work. For example, F3 2 was found to have the highest COD of all tested foams in the McDonald study (893,000 mg/L), with the authors suggesting that this formulation may cause a reduction in DO in the aquatic environment. Similar to the present study, all tested formulations showed adequate biodegradability in the environment as defined by US military specifications of a biodegradability index > 0.65.

5.0 CONCLUSIONS AND IMPLICATIONS

5.1 AQUATIC TOXICITY

To synthesize the aquatic toxicity data, the lowest effect concentrations used to derive Alternatives Assessment Hazard Criteria were compiled across all tested species and study durations (Table 29). As shown in Table 28, one of the tested F3s, F3 2, was very highly toxic to aquatic organisms, with two F3s, F3s 1 and 3, highly toxic to aquatic organisms. Three F3s were considered moderately toxic, including F3s 4, 5, and 7, with F3 6 exhibiting low toxicity.

Table 28. Lowest effect concentrations and alternatives assessment criteria for all aquatic studies.

Foam	Value Type	Effect Concentration (mg/L)	Test	EPA Alternatives Assessment Hazard Criteria
F3 1	EC_{50}	2.70	*R. subcapitata* Cell Count	Highly Toxic
F3 2	EC_{50}	0.69	*R. subcapitata* Cell Count	Very Highly Toxic
F3 3	NOEC	0.9	*C. dilutus* Emergence	Highly Toxic
F3 4	EC_{50}	18.9	*R. subcapitata* Cell Count	Moderately Toxic
F3 5	LC_{50}	46.0	*P. promelas* Survival	Moderately Toxic
F3 6	NOEC	43.2	*C. dilutus* Emergence	Low Toxicity
F3 7	EC_{50}	16.3	*R. subcapitata* Cell Count	Moderately Toxic
Reference Product	NOEC	90	*C. dilutus* Emergence	Low Toxicity

Notes:
EC = effective concentration
LC = lethal concentration
mg/L = milligrams per liter

5.2 TERRESTRIAL TOXICITY

5.2.1 Birds

A summary of all significant effects on birds is given in Table 29 below. Effects were most commonly recorded on lipid levels, with significant responses in 4/7 tested products, and F3s 2 and 5 having significant effects on three endpoints relating to lipids. Comparatively, growth was rarely impacted, with significant effects of 2/7 of the tested products, and only a reduction in adult growth rate at a single concentration of a tested F3 (F3, female growth rate, Section 4.2.2.1). Effects on a number of reproductive parameters including the percentage of cracked eggs, number of arrested embryos, and number of eggs per hen were observed for 4 of the 7 tested products. However, given the low sample sizes and potential effects of the block experimental design, effects on reproductive parameters and offspring fitness should be interpreted with caution.

In terms of the Alternatives Assessment Criteria used to contextualize the aquatic findings, acute oral toxicity of 501 -2,000 mg/kg is considered to be low toxicity, with > 2,000 mg/kg considered very low toxicity. Though an exact value for acute avian toxicity could not be calculated using the

Up and Down approach, values > 1,500 mg/kg for all tested products were recorded, indicating either low or very low acute avian toxicity for all F3s and the reference product based on the Alternatives Assessment Criteria (EPA 2011).

Table 29. Summary of F3 and reference product toxicity to bobwhite quail based on chronic studies.

Foam	Number of Reproductive Effects	Number of Growth Effects	Number of Offspring Effects	Number of Lipid Effects
F3 2	1 (Percent Cracked Eggs)	0	0	3 (Adult Male and Chick Lipid Content, Relative Liver Weight)
F3 3	0	0	1 (Chick Biometrics)	0
F3 4	2 (Arrested Embryos, Number of Eggs per Hen)	1 (Adult Growth Rate)	1 (Chick Biometrics)	0
F3 5	0	0	2 (Chick Growth, Chick Biometrics)	3 (Adult Male and Female Lipid Content, Chick Lipid Content)
F3 6	2 (Percent Arrested Embryos, Hatching Success)	0	0	0
F3 7	0	1 (Adult Biometrics)	2 (Chick Growth, Chick Biometrics)	2 (Adult Female and Chick Lipid Content)
Reference Product	1 (Percent Cracked Eggs)	0	0	2 (Adult and Chick Lipid Content)

5.2.2 Reptiles

In terms of the reptile studies, relatively few significant effects were observed overall, with no significant negative effects on growth and condition index over chronic exposure durations. A synthesis of the number of significant effects for each F3, and the minimum LOEC is given in Table 30. F3 1 had significant effects on three different endpoints, including organ mass (GI tract), bite force, and CEWL, with F3 4 and the reference products having no significant effect on any endpoint tested. Effects observed for F3 3 increased organ mass at two concentrations, 15 and 450 mg/L relative to controls, with no significant effects at other concentrations or for other organs. Compared with F3 2, where clear dose-dependent reductions in CEWL were recorded after 60 days of exposure, the implications of increased GI mass for overall organismal health are less clear. Similarly, though dose-dependent reductions in bite force were recorded for F3 6 after 30 days of exposure, a complete recovery in bite force was recorded after 60 days, with all treatments having higher levels relative to controls; thus, the overall impact of F3 6 on this endpoint is unclear.

Table 30. Summary of F3 and reference product toxicity based on chronic reptile studies.

Foam	Number of Significant Effects	Minimum LOEC (mg/L)
F3 1	3	45 (Reduced GI Mass)
F3 2	1	150 (Reduced CEWL)
F3 3	1	15 (Increased Organ Mass)
F3 4	No Significant Effects.	
F3 6	1	15 (Reduced Bite Force)
Reference Product	No Significant Effects.	

Notes:
GI = gastrointestinal
mg/L = milligrams per liter

5.3 BIODEGRADABILITY

As discussed in Section 4.3, all tested F3s showed good biodegradability over the 28-day period. In terms of soluble COD concentrations over time, F3 6 had higher concentrations after 28 days of incubation relative to all other tested foams, suggesting slower degradation. For chemical constituents during the biodegradation period, PFAS and other chemical constituents were persistent in the reference product; thus, this foam was considered less biodegradable compared to F3s.

5.4 CONCLUSIONS

Overall, the present study aimed to provide an assessment of multi-taxa toxicity and biodegradation potential for a suite of candidate F3s and a reference product. The objectives were met through aquatic and chronic toxicity tests with a total of five species including algae, aquatic invertebrates, fish, birds, and reptiles. For aquatic tests, several of the tested F3s were more toxic than the reference products. Results from quail studies were more complex, with significant effects of both F3s and the reference product on select endpoints including lipid levels, reproductive endpoints, and offspring growth, though small sample sizes precluded a robust analysis. Few effects were observed in reptile studies, though two of the tested F3s had significant effects on CEWL which could impact thermoregulation. Concurrent biodegradability tests with F3s and the reference AFFF were performed, enabling an understanding of the inherent biodegradation potential of all products. These findings will be implemented in the selection and introduction of novel F3s that minimize impacts on ecological receptors and the wider environment.

6.0 LITERATURE CITED

Albayrak, T., & Pekgöz, A. K. (2021). Heavy metal effects on bird morphometry: A case study on the house sparrow Passer domesticus. *Chemosphere*, *276*, 130056.

Anderson, R. A., McBrayer, L. D., & Herrel, A. (2008). Bite force in vertebrates: opportunities and caveats for use of a nonpareil whole-animal performance measure. *Biological Journal of the Linnean Society*, *93*(4), 709-720.

Anderson, R. H., Thompson, T., Stroo, H. F., & Leeson, A. (2021). US Department of Defense–funded fate and transport research on per-and polyfluoroalkyl substances at aqueous film–forming foam–impacted sites. *Environmental Toxicology and Chemistry*, *40*(1), 37.

Ankley, G. T., Cureton, P., Hoke, R. A., Houde, M., Kumar, A., Kurias, J., ... & Valsecchi, S. (2021). Assessing the ecological risks of per-and polyfluoroalkyl substances: Current state-of-the science and a proposed path forward. *Environmental toxicology and chemistry*, *40*(3), 564-605.

Ateia, M., Buren, J. V., Barrett, W., Martin, T., & Back, G. G. (2023). Sunrise of PFAS Replacements: A Perspective on Fluorine-Free Foams. *ACS Sustainable Chemistry & Engineering*. *11,* 21, 7986–7996/

Baluyot, J. C., Reyes, E. M., & Velarde, M. C. (2021). Per-and polyfluoroalkyl substances (PFAS) as contaminants of emerging concern in Asia's freshwater resources. *Environmental Research*, *197*, 111122.

Bhattacharya, R., Chatterjee, A., Chatterjee, S., & Saha, N. C. (2022). Commonly used surfactants sodium dodecyl sulphate, cetylpyridinium chloride and sodium laureth sulphate and their effects on antioxidant defence system and oxidative stress indices in *Cyprinus carpio* L.: An integrated *in silico* and *in vivo* approach. *Environmental Science and Pollution Research*, *29*(20), 30622-30637.

Boronow, K. E., & Langkilde, T. (2010). Sublethal effects of invasive fire ant venom on a native lizard. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *313*(1), 17-23.

Bourgeois, A., Bergendahl, J., & Rangwala, A. (2015). Biodegradability of fluorinated fire-fighting foams in water. *Chemosphere*, *131*, 104-109.

Bursian, S. J., Link, J. E., McCarty, M., & Simcik, M. F. (2021). The subacute toxicity of perfluorooctane sulfonate and/or perfluorooctanoic acid and legacy aqueous film-forming foams to Japanese quail (*Coturnix japonica*) chicks. *Environmental Toxicology and Chemistry*, *40*(3), 695-710.

Bussière-Côté, S., Omlin, T., de Càssia Pinheiro, E., & Weber, J. M. (2016). Gemfibrozil disrupts the metabolism of circulating lipids in bobwhite quails. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *179*, 137-143.

Cakici, O., & Akat, E. (2012). Histopathological effects of carbaryl on digestive system of snake-eyed lizard, *Ophisops elegans*. *Bulletin of environmental contamination and toxicology*, *88*, 685-690.

Carpenter, J. K., Monks, J. M., & Nelson, N. (2016). The effect of two glyphosate formulations on a small, diurnal lizard (*Oligosoma polychroma*). *Ecotoxicology*, *25*, 548-554.

Carrillo-Abad, J., Pérez-Herranz, V., & Urtiaga, A. (2018). Electrochemical oxidation of 6: 2 fluorotelomer sulfonic acid (6: 2 FTSA) on BDD: electrode characterization and mechanistic investigation. *Journal of Applied Electrochemistry*, *48*, 589-596.

Chang, J., Li, J., Wang, H., Wang, Y., Guo, B., Yin, J., ... & Xu, P. (2016). Tissue distribution, metabolism and hepatic tissue injury in Chinese lizards (*Eremias argus*) after a single oral administration of lambda-cyhalothrin. *Environmental pollution*, *218*, 965-972.

Chen, L., Xu, P., Diao, J., Di, S., Li, R., & Zhou, Z. (2016). Distribution, metabolism and toxic effects of beta-cypermethrin in lizards (*Eremias argus*) following oral administration. *Journal of hazardous materials*, *306*, 87-94.

Comber, M. H. I., De Wolf, W., Cavalli, L., Van Egmond, R., Steber, J., Tattersfield, L., & Priston, R. A. (2003). Assessment of bioconcentration and secondary poisoning of surfactants. *Chemosphere*, *52*(1), 23-32.

Cousins, I. T., DeWitt, J. C., Glüge, J., Goldenman, G., Herzke, D., Lohmann, R., ... & Wang, Z. (2020). Strategies for grouping per-and polyfluoroalkyl substances (PFAS) to protect human and environmental health. *Environmental Science: Processes & Impacts*, *22*(7), 1444-1460.

da Silva, S. C., Pusceddu, F. H., dos Santos Barbosa Ortega, A., de Souza Abessa, D. M., Pereira, C. D. S., & Maranho, L. A. (2019). Aqueous Film-Forming Foams (AFFFs) are very toxic to aquatic microcrustaceans. *Water, Air, & Soil Pollution*, *230*, 1-8.

Dauchy, X., Boiteux, V., Bach, C., Rosin, C., & Munoz, J. F. (2017). Per-and polyfluoroalkyl substances in firefighting foam concentrates and water samples collected near sites impacted by the use of these foams. *Chemosphere*, *183*, 53-61.

Deeming, D. C. (2022). Inter-relationships among body mass, body dimensions, jaw musculature and bite force in reptiles. *Journal of Zoology*, *318*(1), 23-33.

Dennis, N. M., Karnjanapiboonwong, A., Subbiah, S., Rewerts, J. N., Field, J. A., McCarthy, C., ... & Anderson, T. A. (2020). Chronic reproductive toxicity of perfluorooctane sulfonic acid and a simple mixture of perfluorooctane sulfonic acid and perfluorohexane sulfonic

acid to northern bobwhite quail (*Colinus virginianus*). *Environmental Toxicology and Chemistry*, *39*(5), 1101-1111.

Dennis, N. M., Hossain, F., Subbiah, S., Karnjanapiboonwong, A., Dennis, M. L., McCarthy, C., ... & Anderson, T. A. (2021). Chronic reproductive toxicity thresholds for northern bobwhite quail (*Colinus virginianus*) exposed to perfluorohexanoic acid (PFHxA) and a mixture of perfluorooctane sulfonic acid (PFOS) and PFHxA. *Environmental Toxicology and Chemistry*, *40*(9), 2601-2614.

Depew, D. C., Basu, N., Burgess, N. M., Campbell, L. M., Evers, D. C., Grasman, K. A., & Scheuhammer, A. M. (2012). Derivation of screening benchmarks for dietary methylmercury exposure for the common loon (*Gavia immer*): rationale for use in ecological risk assessment. *Environmental Toxicology and Chemistry*, *31*(10), 2399-2407.

Dixon, W. J., & Mood, A. M. (1948). A method for obtaining and analyzing sensitivity data. Journal of the American Statistical Association, 43(241), 109-126.

Evich, M. G., Davis, M. J., McCord, J. P., Acrey, B., Awkerman, J. A., Knappe, D. R., ... & Washington, J. W. (2022). Per-and polyfluoroalkyl substances in the environment. *Science*, *375*(6580), eabg9065.

Fenton, S. E., Ducatman, A., Boobis, A., DeWitt, J. C., Lau, C., Ng, C., ... & Roberts, S. M. (2021). Per-and polyfluoroalkyl substance toxicity and human health review: Current state of knowledge and strategies for informing future research. *Environmental toxicology and chemistry*, *40*(3), 606-630.

Freitas, R., Silvestro, S., Coppola, F., Costa, S., Meucci, V., Battaglia, F., ... & Faggio, C. (2020). Toxic impacts induced by Sodium lauryl sulfate in *Mytilus galloprovincialis*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *242*, 110656.

Gaffard, A., Pays, O., Monceau, K., Teixeira, M., Bretagnolle, V., & Moreau, J. (2022). Feeding on grains containing pesticide residues is detrimental to offspring development through parental effects in grey partridge. *Environmental Pollution*, *312*, 120005.

Glüge, J., Scheringer, M., Cousins, I. T., DeWitt, J. C., Goldenman, G., Herzke, D., ... & Wang, Z. (2020). An overview of the uses of per-and polyfluoroalkyl substances (PFAS). *Environmental Science: Processes & Impacts*, *22*(12), 2345-2373.

Grote, K., Niemann, L., Gericke, C., Selzsam, B., & Chahoud, I. (2006). Effects of fentin hydroxide on reproduction of the Japanese quail (*Coturnix coturnix japonica*). *Environmental research*, *101*(1), 81-88.

Guthery, F. S., & Koerth, N. E. (1992). Substandard water intake and inhibition of bobwhite reproduction during drought. *The Journal of wildlife management*, 760-768.

Han, B., Li, J., Li, S., Liu, Y., & Zhang, Z. (2023). Effects of thiacloprid exposure on microbiota–gut–liver axis: Multiomics mechanistic analysis in Japanese quails. *Journal of Hazardous Materials*, *442*, 130082.

Hinnant, K. M., Conroy, M. W., & Ananth, R. (2017). Influence of fuel on foam degradation for fluorinated and fluorine-free foams. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *522*, 1-17.

Holliday, D. K., Elskus, A. A., & Roosenburg, W. M. (2009). Impacts of multiple stressors on growth and metabolic rate of *Malaclemys terrapin*. *Environmental Toxicology and Chemistry: An International Journal*, *28*(2), 338-345.

Hossain, F., Dennis, N. M., Subbiah, S., Karnjanapiboonwong, A., Guelfo, J. L., Suski, J., & Anderson, T. A. (2022). Acute Oral Toxicity of Nonfluorinated Fire-Fighting Foams to Northern Bobwhite Quail (*Colinus virginianus*). *Environmental Toxicology and Chemistry*, *41*(8), 2003-2007.

Hossain, F., Dennis, N. M., Karnjanapiboonwong, A., Subbiah, S., Longwell, A. S., Suski, J. G., ... & Anderson, T. A. (2023). Evaluation of the Chronic Reproductive Toxicity of a Fluorine-free Firefighting Foam and a Short-Chain Fluorinated Foam to Northern Bobwhite Quail (*Colinus virginianus*). *Environmental Toxicology and Chemistry*, *43*(1), 211-221.

Houtz, E. F., & Sedlak, D. L. (2012). Oxidative conversion as a means of detecting precursors to perfluoroalkyl acids in urban runoff. *Environmental science & technology*, *46*(17), 9342-9349.

Huyghe, K., Vanhooydonck, B., Scheers, H., Molina-Borja, M., & Van Damme, R. (2005). Morphology, performance and fighting capacity in male lizards, *Gallotia galloti*. *Functional Ecology*, 800-807.

Iglesias, E., Anderez, J., Forgiarini, A., & Salager, J. L. (1995). A new method to estimate the stability of short-life foams. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *98*(1-2), 167-174.

Interstate Technology and Regulatory Council. (2022). Aqueous Film-Forming Foam (AFFF). Fact Sheet. Updated September 2023.

Isip, J. E., Jones, M. E., & Cooper, N. (2022). Clade-wide variation in bite-force performance is determined primarily by size, not ecology. *Proceedings of the Royal Society B*, *289*(1969), 20212493.

Jones, D. K., Quinlin, K. A., Wigren, M. A., Choi, Y. J., Sepúlveda, M. S., Lee, L. S., ... & Hoverman, J. T. (2022). Acute toxicity of eight aqueous film-forming foams to 14 aquatic species. *Environmental Science & Technology*, *56*(10), 6078-6090.

Kurwadkar, S., Dane, J., Kanel, S. R., Nadagouda, M. N., Cawdrey, R. W., Ambade, B., ... & Wilkin, R. (2022). Per-and polyfluoroalkyl substances in water and wastewater: A critical review of their global occurrence and distribution. *Science of The Total Environment*, *809*, 151003.

Lagarde, F., Beausoleil, C., Belcher, S. M., Belzunces, L. P., Emond, C., Guerbet, M., & Rousselle, C. (2015). Non-monotonic dose-response relationships and endocrine disruptors: a qualitative method of assessment. *Environmental Health*, *14*, 1-15.

Li, Y., Fletcher, T., Mucs, D., Scott, K., Lindh, C. H., Tallving, P., & Jakobsson, K. (2018). Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. *Occupational and environmental medicine*, *75*(1), 46-51.

Liu, X., Liu, C., Wang, P., Liang, Y., Zhan, J., Zhou, Z., & Liu, D. (2019). Distribution, metabolism and metabolic disturbances of alpha-cypermethrin in embryo development, chick growth and adult hens. *Environmental pollution*, *249*, 390-397.

Lupton, S. J., Dearfield, K. L., Johnston, J. J., Wagner, S., & Huwe, J. K. (2015). Perfluorooctane sulfonate plasma half-life determination and long-term tissue distribution in beef cattle (*Bos taurus*). *Journal of Agricultural and Food Chemistry*, *63*(51), 10988-10994.

McDonald, R., Lumley, A., Johnson, G., Broxson, S., Luckarift, H., & Bridgett, A. (2022). Evaluation of the Chemical and Physical Properties of Commercial Fluorine Free Foams. *Arctos Technology Solutions*.

McFarland, C. A., Quinn Jr, M. J., Bazar, M. A., Remick, A. K., Talent, L. G., & Johnson, M. S. (2008). Toxicity of oral exposure to 2, 4, 6-trinitrotoluene in the western fence lizard (*Sceloporus occidentalis*). *Environmental Toxicology and Chemistry: An International Journal*, *27*(5), 1102-1111.

McFarland, C. A., Quinn Jr, M. J., Bazar, M. A., Talent, L. G., & Johnson, M. S. (2009). Toxic effects of oral hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine in the western fence lizard (*Sceloporus occidentalis*). *Environmental Toxicology and Chemistry: An International Journal*, *28*(5), 1043-1050.

McFarland, C. A., Quinn Jr, M. J., Boyce, J., LaFiandra, E. M., Bazar, M. A., Talent, L. G., & Johnson, M. S. (2011). Toxic effects of oral 2-amino-4, 6-dinitrotoluene in the western fence lizard (*Sceloporus occidentalis*). *Environmental Pollution*, *159*(2), 466-473.

McFarland, C. A., Talent, L. G., Quinn, M. J., Bazar, M. A., Wilbanks, M. S., Nisanian, M., ... & Gust, K. A. (2012). Multiple environmental stressors elicit complex interactive effects in the western fence lizard (*Sceloporus occidentalis*). *Ecotoxicology*, *21*, 2372-2390.

Mitchell, M. A., & Diaz-Figueroa, O. (2005). Clinical reptile gastroenterology. *Veterinary Clinics: Exotic Animal Practice*, *8*(2), 277-298.

Modiri Gharehveran, M., Walus, A. M., Anderson, T. A., Subbiah, S., Guelfo, J., Frigon, M., ... & Suski, J. G. (2022). Per-and polyfluoroalkyl substances (PFAS)-free aqueous film forming foam formulations: Chemical composition and biodegradation in an aerobic environment. *Journal of Environmental Chemical Engineering*, *10*(6), 108953.

Moody, C. A., Martin, J. W., Kwan, W. C., Muir, D. C., & Mabury, S. A. (2002). Monitoring perfluorinated surfactants in biota and surface water samples following an accidental release of fire-fighting foam into Etobicoke Creek. *Environmental science & technology*, *36*(4), 545-551.

Newsted, J. L., Coady, K. K., Beach, S. A., Butenhoff, J. L., Gallagher, S., & Giesy, J. P. (2007). Effects of perfluorooctane sulfonate on mallard and northern bobwhite quail exposed chronically via the diet. *Environmental Toxicology and Pharmacology*, *23*(1), 1-9.

Nossen, I., Ciesielski, T. M., Dimmen, M. V., Jensen, H., Ringsby, T. H., Polder, A., ... & Styrishave, B. (2016). Steroids in house sparrows (*Passer domesticus*): Effects of POPs and male quality signalling. *Science of the total environment*, *547*, 295-304.

Organization for Economic Co-operation and Development (OECD). (1984). Test Guideline No. 206. Avian Reproduction Test. Organisation for Economic Co-operation and Development, Paris.

OECD. (1992). Test Guideline No. 302B. Zahn-Wellens/EMPA Test. Organisation for Economic Co-operation and Development, Paris.

OECD. (2011). Test Guideline No. 235. Chironomus sp., Acute Immobilisation Test. Organisation for Economic Co-operation and Development, Paris.

OECD. (2019). Test Guideline No. 203. Fish, Acute Toxicity Test. Organisation for Economic Co-operation and Development, Paris.

OECD. (2022). Test Guideline No. 425: Acute Oral Toxicity: Up-and-Down Procedure. Organisation for Economic Co-operation and Development, Paris

OECD. (2023). Test Guideline No. 219. Sediment-Water Chironomid Toxicity Using Spiked Water. Organisation for Economic Co-operation and Development, Paris.

Parsons, J. R., Sáez, M., Dolfing, J., & De Voogt, P. (2008). Biodegradation of perfluorinated compounds. *Reviews of Environmental Contamination and Toxicology Vol 196*, 53-71.

Pérez-Rodríguez, L., Romero-Haro, A. A., Sternalski, A., Muriel, J., Mougeot, F., Gil, D., & Alonso-Alvarez, C. (2015). Measuring oxidative stress: the confounding effect of lipid concentration in measures of lipid peroxidation. *Physiological and Biochemical Zoology*, *88*(3), 345-351.

Podder, A., Sadmani, A. A., Reinhart, D., Chang, N. B., & Goel, R. (2021). Per and poly-fluoroalkyl substances (PFAS) as a contaminant of emerging concern in surface water: A transboundary review of their occurrences and toxicity effects. *Journal of hazardous materials*, *419*, 126361.

Rewerts, J. N., Christie, E. C., Robel, A. E., Anderson, T. A., McCarthy, C., Salice, C. J., & Field, J. A. (2021). Key considerations for accurate exposures in ecotoxicological assessments of perfluorinated carboxylates and sulfonates. *Environmental Toxicology and Chemistry*, *40*(3), 677-688.

Ritz, C., Baty, F., Streibig, J. C., & Gerhard, D. (2015). Dose-response analysis using R. *PloS one*, *10*(12), e0146021.

Rohr, J. R., & Palmer, B. D. (2005). Aquatic herbicide exposure increases salamander desiccation risk eight months later in a terrestrial environment. *Environmental Toxicology and Chemistry: An International Journal*, *24*(5), 1253-1258.

Ruyle, B. J., Thackray, C. P., Butt, C. M., LeBlanc, D. R., Tokranov, A. K., Vecitis, C. D., & Sunderland, E. M. (2023). Centurial Persistence of Forever Chemicals at Military Fire Training Sites. *Environmental Science & Technology*, 57(21), 8096-8106.

Salice, C. J., Suski, J. G., Bazar, M. A., & Talent, L. G. (2009). Effects of inorganic lead on Western fence lizards (*Sceloporus occidentalis). Environmental Pollution*, *157*(12), 3457-3464.

Sample, B. E., Beyer, W. N., & Wentsel, R. (2019). Revisiting the avian Eco-SSL for lead: Recommendations for revision. *Integrated Environmental Assessment and Management*, *15*(5), 739-749.

Schaefer, T. H., Dlugogorski, B. Z., & Kennedy, E. M. (2007). Vapour suppression of n-heptane with fire fighting foams using laboratory flux chamber. In *Proceedings of the 7th Asia-Oceania Symposium. Fire Science Technology*.

Shaw, D. M., Munoz, G., Bottos, E. M., Duy, S. V., Sauvé, S., Liu, J., & Van Hamme, J. D. (2019). Degradation and defluorination of 6: 2 fluorotelomer sulfonamidoalkyl betaine and 6: 2 fluorotelomer sulfonate by Gordonia sp. strain NB4-1Y under sulfur-limiting conditions. *Science of the Total Environment*, *647*, 690-698.

Shojaei, M., Joyce, A. S., Ferguson, P. L., & Guelfo, J. L. (2022). Novel per-and polyfluoroalkyl substances in an active-use C6-based aqueous film forming foam. *Journal of Hazardous Materials Letters*, *3*, 100061.

Stein-Taylor, J. R. (1973). *Handbook of phycological methods: culture methods and growth measurements, edited by JR Stein* (Vol. 1). Cambridge University Press.

Suski, J. G., Salice, C., Houpt, J. T., Bazar, M. A., & Talent, L. G. (2008). Dose-related effects following oral exposure of 2, 4-dinitrotoluene on the western fence lizard, *Sceloporus occidentalis*. *Environmental Toxicology and Chemistry: An International Journal*, *27*(2), 352-359.

Taniyasu, S., Yamashita, N., Yamazaki, E., Rostkowski, P., Yeung, L. W. Y., Kurunthachalam, S. K., ... & Loganathan, B. G. (2015). Contamination profiles of perfluorinated chemicals in the inland and coastal waters of Japan following the use of fire-fighting foams. In *Water challenges and solutions on a global scale* (pp. 221-244). American Chemical Society.

Ueda-De-Carvalho, M., Buruaem-Moreira, L., Alves-Maranho, L., & Moledo-de-Souza-Abessa, D. (2019). Chronic effects of fire suppressors on the reproduction of the copepod *Nitocra* sp. *Revista de Ciencias Agrícolas*, *36*(SPE), 82-94.

United States Department of Defense (DoD). (2023). Performance Specification. Fire Extinguishing Agent, Fluorine-Free Foam (F3) Liquid Concentrate, for Land-Based, Fresh Water Applications. MIL-PRF-32725. 6 January 2023.

United States Environmental Protection Agency (EPA). (1996). Ecological Effects Test Guidelines. OPPTS 850.2300. Avian Reproduction Test. Office of Pollution prevention & Toxics.

United States Environmental Protection Agency. (2002a). Method 1003.0: Green Alga, *Selanastrum capricornutum,* Growth Test; Chronic Toxicity. Fourth Edition. October 2002. EPA-821-R-02-013.

United States Environmental Protection Agency. (2002b). Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Fourth Edition. October 2002. EPA-821-R-02-013.

United States Environmental Protection Agency. (2002c). Method 1000.0: Fathead Minnow, *Pimephales promelas,* Larval Survival and Growth; Chronic Toxicity. Fourth Edition. October 2002. EPA-821-R-02-013.

United States Environmental Protection Agency (EPA). (2003). Guidance for Developing Ecological Soil Screening Levels. November 2003. Office of Solid Waste and Emergency Response.

United States Environmental Protection Agency (EPA). (2011). Design for the Environment Program Alternatives Assessment Criteria for Hazard Evaluation. Version 2, August 2011. Office of Pollution prevention & Toxics.

United States Government. (2020). National Defense Authorization Act for Fiscal Year 2020. Public Law No. 116-92 (12/20/2019).

Vicenzi, N., Laspiur, A., Sassi, P. L., Massarelli, R., Krenz, J., & Ibargüengoytía, N. R. (2020). Impact of temperature on bite force and bite endurance in the leopard iguana (Diplolaemus leopardinus) in the Andes Mountains. *Journal of Experimental Biology*, *223*(12), jeb221382.

Weaver, S. J., Edwards, H., McIntyre, T., Temple, S. M., Alexander, Q., Behrens, M. C., ... & Taylor, E. N. (2022). Cutaneous evaporative water loss in lizards is variable across body regions and plastic in response to humidity. *Herpetologica*, *78*(3), 169-183.

Weber, A. K., Barber, L. B., LeBlanc, D. R., Sunderland, E. M., & Vecitis, C. D. (2017). Geochemical and hydrologic factors controlling subsurface transport of poly-and perfluoroalkyl substances, Cape Cod, Massachusetts. *Environmental science & technology*, *51*(8), 4269-4279.

Weir, S. M., Talent, L. G., Anderson, T. A., & Salice, C. J. (2014). Unraveling the relative importance of oral and dermal contaminant exposure in reptiles: insights from studies using the western fence lizard (*Sceloporus occidentalis*). PloS one, 9(6), e99666.

Wu, X., Nguyen, H., Kim, D., & Peng, H. (2022). Chronic toxicity of PFAS-free AFFF alternatives in terrestrial plant *Brassica rapa*. *Science of The Total Environment*, *850*, 158100.

Yu, X., Ceja-Navarro, J. A., & Wu, X. (2022). Sublethal toxicity of fluorine-free firefighting foams in soil invertebrate *Caenorhabditis elegans*. *Environmental Science & Technology Letters*, *9*(6), 561-566.

Yu, X., Yu, X., Lin, Y., Li, H., Li, G., & Zong, R. (2023). Comparative Study on Interfacial Properties, Foam Stability, and Firefighting Performance of C6 Fluorocarbon Surfactants with Different Hydrophilic Groups. *Langmuir*, *39*(46), 16336-16348.

Yu, S., Nie, Y., Wang, Z., Zhang, L., Liu, R., Liu, Y., ... & Diao, J. (2023). Glyphosate-based herbicide (GBH) challenged thermoregulation in lizards (*Eremias argus*), compensatory warming could mitigate this effect. *Science of The Total Environment*, *897*, 165287.

Zhang, X. Z., Bao, Z. M., Hu, C., Li-Shuai, J., & Chen, Y. (2017). Organic pollutant loading and biodegradability of firefighting foam. *IOP Conference Series: Earth and Environmental Science*, 94(1), 012137.

Zaefarian, F., Abdollahi, M. R., Cowieson, A., & Ravindran, V. (2019). Avian liver: the forgotten organ. *Animals*, *9*(2), 63.

Zhu, X., He, Y., Zhang, Q., Ma, D., & Zhao, H. (2023). Lead induced disorders of lipid metabolism and glycometabolism in the liver of developmental Japanese quails (*Coturnix japonica*) via inhibiting PI3K/Akt signaling pathway. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *263*, 109489.

APPENDIX A SUPPORTING DATA

Table A1. Results of Range Finding Tests with *R. subcapitata*.

Foam	Concentration Range (mg/L)	EC_{50} (mg/L)
F3 1	0 – 30,000	3.9 (-0.03 – 7.78)
F3 2	0 – 30,000	0.7 (0.6 – 0.8)
Reference Product	0 – 30,000	95.9 (75.3 – 116.4)
F3 3	0 – 30,000	7 (6.1 – 7.8)
F3 4	0 – 30,000	13.5 (9.8 – 17.2)
F3 5	0 – 30,000	193 (150 – 234)
F3 6	0 – 30,000	147 (44.4 – 250)
F3 7	0 – 30,000	14.2 (5.9 – 22.6)

Notes:
Values in parentheses are 95 percent confidence intervals.
EC = Effective Concentration
mg/L = milligrams per liter

Table A2. Water quality measured parameters during *R. subcapitata* testing.

Foam	Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Conductivity (µS/cm)
F3 1	24.0 – 24.0	7.7 – 8.4	7.0 – 9.1	300 – 411
F3 2	24.0 – 24.8	7.6 – 8.1	5.4 – 8.8	303 – 328
Reference Product	24.0 – 24.1	7.7 – 8.4	5.5 – 9.4	288 – 378
F3 3	24.0 – 25.1	7.6 – 8.6	6.0 – 9.1	308 – 371
F3 4	24.0 – 24.0	7.7 – 8.6	7.1– 9.0	305 – 374
F3 5	24.0 – 24.4	7.7 – 8.6	7.1 – 8.9	289 – 355
F3 6	24.0 – 24.2	7.8 – 8.4	6.9 – 8.7	294 – 331
F3 7	24.0 – 24.6	8.0– 8.7	6.1 – 9.1	303 – 390

Notes:

°C = degrees Celsius
µS/cm = microsiemens per centimeter
mg/L = milligrams per liter

Table A3. Data summary from *R. subcapitata* testing.

Foam	Foam Concentration (mg/L)	Cell Density (cells/mL)
F3 1	0	1,258,750
	0.3	1,286,250
	0.9	931,875
	3	585,000
	9	226,875
	30	53,125
F3 2	0	1,006,250
	0.3	943,750
	0.9	284,375
	3	53,125
	9	15,000
	30	1,250
Reference Product	0	1,478,125
	9	1,278,125
	30	1,263,750
	90	886,250
	300	215,625
	900	7,500
F3 3	0	1,142,500
	0.3	1,126,250
	0.9	1,137,500
	3	764,375
	9	373,125
	30	201,875
F3 4	0	1,165,625
	0.3	1,124,375
	0.9	1,143,750
	3	860,625
	9	695,625
	30	456,250
F3 5	0	1,084,375
	30	1,051,875
	90	1,002,500
	300	402,500
	900	6,875
	3000	3,125
F3 6	0	1,146,250
	30	1,186,875
	90	1,245,625
	300	454,375
	900	3,125
	3000	1,250
F3 7	0	1,410,000
	0.3	1,337,500
	0.9	1,139,375
	3	893,125
	9	758,125
	30	605,000

Notes:
mg/L = milligrams per liter
cells\mL = cells per milliliter

Table A4. Results of Range Finding Tests with *C. dilutus*.

Foam	Concentration Range (mg/L)	48 hour LC_{50} (mg/L)
F3 1	0 – 30,000	24.5 (6.6 – 50.3)
F3 2	0 – 30,000	30 (27.8 – 32.2)
Reference Product	0 – 30,000	311 (180 – 443)
F3 3	0 – 30,000	94.5 (NC)
F3 4	0 – 30,000	39 (NC)
F3 5	0 – 30,000	10.3 (NC)
F3 6	0 – 30,000	300 (NC)
F3 7	0 – 30,000	533 (NC)

Notes:
Values in parentheses are 95 percent confidence intervals.
LC = lethal concentration
mg/L = milligrams per liter
NC = not calculated

Table A5. Water quality parameters measured during acute and chronic tests with *C. dilutus*.

Foam	Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Conductivity (µS/cm)
Acute Studies				
F3 1	19.0-20.0	7.7-8.0	7.9-9.3	426-448
F3 2	20.7-21.0	6.9-7.9	7.6-8.9	355-385
Reference Product	19.0-20.2	7.5-8.0	6.9-9.3	411-585
F3 3	19.0-20.5	7.7-8.0	7.6-9.3	426-454
F3 4	19.0-20.2	8.0-8.1	6.9-9.2	437-468
F3 5	19.0-20.1	7.7-7.9	7.8-9.2	410-506
F3 6	19.0 – 19.6	7.1 – 8.1	7.7 – 9.3	445–467
F3 7	19.0-20.0	7.8-8.1	5.2-9.4	438-499
Chronic Studies				
F3 1	22.0-24.0	6.9-8.3	0.7-8.6	306-630
F3 2	22.0-24.0	7.2-8.5	4.3-8.5	291-434
Reference Product	22.0-24.0	6.8-8.6	3.7-9.0	312-634
F3 3	22.0-24.0	7.2-8.8	4.0-9.4	343-529
F3 4	22.0-24.0	7.0-8.3	0.2-8.9	301-575
F3 5	22.0-24.0	7.1-8.4	4.3-9.0	330-521
F3 6	22.0-24.0	6.7-8.7	4.6-9.1	300-486
F3 7	22.0-24.0	6.5-8.9	2.2-9.1	310-678

Notes:
°C = degrees Celsius
µS/cm = microsiemens per centimeter
mg/L = milligrams per liter

Table A6. Data summary for acute *C. dilutus* testing.

Foam	Concentration (mg/L)	48-hour Survival (%)
F3 2	0	95
	3	85
	9	65
	30	60
	90	0
	300	0
F3 1	0	90
	3	95
	9	85
	30	15
	90	0
	300	0
Reference Product	0	100
	30	80
	90	65
	300	85
	900	65
	3,000	0
F3 3	0	95
	9	80
	30	70
	90	50
	300	0
	900	0
F3 4	0	100
	9	100
	30	100
	90	100
	300	45
	900	0
F3 5	0	100
	30	95
	90	100
	300	85
	900	40
	3,000	0
F3 6	0	100
	30	100
	90	100
	300	80
	900	10
	3,000	0
F3 7	0	100
	30	100
	90	100
	300	100
	900	60
	3,000	0

Notes: mg/L = milligrams per liter

Table A7. Summary of data for chronic tests with *C. dilutus*.

Foam	Concentration (mg/L)	Average Survival	Survival Standard Deviation	Average Emergence	Emergence Standard Deviation	Average Dry Weight (mg)	Dry Weight Standard Deviation
F3 1	0	83%	5%	75%	6%	1.619	0.312
F3 1	0.9	90%	0%	73%	5%	1.805	0.172
F3 1	3	83%	5%	68%	10%	1.594	0.237
F3 1	9	58%	5%	25%	13%	1.330	0.383
F3 1	30	10%	0%	3%	5%	0.368	0.333
F3 1	90	0%	0%	3%	5%	0.000	0.000
F3 2	0	80%	16%	83%	5%	1.264	0.254
F3 2	0.9	83%	21%	75%	13%	1.465	0.088
F3 2	3	88%	19%	60%	0%	1.531	0.232
F3 2	9	78%	5%	63%	10%	1.678	0.312
F3 2	30	63%	17%	3%	5%	0.752	0.420
F3 2	90	0%	0%	0%	0%	0.000	0.000
Reference Product	0	90%	0%	53%	15%	1.443	0.274
Reference Product	30	90%	0%	48%	13%	1.628	0.120
Reference Product	90	90%	0%	35%	10%	2.020	0.257
Reference Product	300	90%	0%	5%	6%	1.425	0.146
Reference Product	900	20%	8%	0%	0%	0.883	0.633
Reference Product	3,000	0%	0%	0%	0%	NA	NA
F3 3	0	98%	5%	78%	10%	2.368	0.454
F3 3	0.3	93%	5%	80%	0%	1.978	0.064
F3 3	0.9	80%	0%	48%	25%	1.945	0.081
F3 3	3	80%	0%	33%	19%	2.130	0.287
F3 3	9	80%	0%	40%	8%	1.945	0.071
F3 3	30	80%	0%	18%	22%	1.870	0.083
F3 4	0	85%	6%	70%	0%	2.323	0.376
F3 4	9	80%	8%	70%	8%	2.331	0.257
F3 4	30	53%	15%	45%	6%	2.119	0.330
F3 4	90	8%	5%	5%	10%	0.765	0.761
F3 4	300	0%	0%	0%	0%	0.000	0.000
F3 4	900	0%	0%	0%	0%	0.000	0.000

Table A7. Summary of data for chronic tests with *C. dilutus*.

Foam	Concentration (mg/L)	Average Survival	Survival Standard Deviation	Average Emergence	Emergence Standard Deviation	Average Dry Weight (mg)	Dry Weight Standard Deviation
F3 5	0	95%	6%	73%	26%	1.552	0.044
F3 5	30	95%	6%	50%	27%	1.783	0.071
F3 5	90	88%	15%	33%	15%	1.805	0.124
F3 5	300	70%	14%	5%	10%	1.250	0.181
F3 5	900	5%	10%	3%	5%	0.225	0.450
F3 5	3,000	0%	0%	0%	0%	0.000	0.000
F3 7	0	90%	0%	50%	14%	2.448	0.688
F3 7	30	90%	0%	35%	6%	2.100	0.439
F3 7	90	93%	5%	20%	20%	2.343	0.363
F3 7	300	18%	10%	0%	0%	0.233	0.077
F3 7	900	0%	0%	0%	0%	0.000	0.000
F3 7	3,000	0%	0%	0%	0%	0.000	0.000
F3 6	0	95%	6%	78%	10%	1.836	0.769
F3 6	9	93%	10%	65%	17%	1.544	0.351
F3 6	30	90%	0%	50%	22%	1.378	0.509
F3 6	90	88%	15%	15%	13%	1.458	0.211
F3 6	300	75%	6%	0%	0%	1.074	0.139
F3 6	900	8%	10%	0%	0%	0.498	0.614

Notes:
mg = milligrams
mg/L = milligrams per liter

Table A8. Results of Range Finding Tests with *P. promelas*.

Foam	Concentration Range (mg/L)	96 h LC_{50} (mg/L)
F3 3	0 – 30,000	120 (NC)
F3 1	0 – 30,000	26.3 (6.63 – 46.1)
F3 4	0 – 30,000	48.5 (NC)
F3 7	0 – 30,000	60.0 (NC)
Reference Product	0 – 30,000	944.6 (NC)
F3 2	0 – 30,000	3.6 (NC)
F3 6	0 – 30,000	46.1 (NC)
F3 5	0 – 30,000	37.9 (NC)

Notes:
Values in parentheses are 95 percent confidence intervals.
LC = Lethal Concentration
mg/L = milligrams per liter

Table A9. Water quality parameters measured during chronic tests with *P. promelas*

Foam	Temperature (°C)	pH	Dissolved Oxygen (mg/L)	Conductivity (µS/cm)
Acute Studies				
F3 1	25.1 – 26.0	7.8 – 8.3	6.8 – 8.5	313 – 330
F3 2	24.0 – 25.0	7.7 – 8.5	5.4 – 8.7	325 – 341
Reference Product	24.0 – 24.8	7.4 – 8.2	3.7 – 8.3	325 – 425
F3 3	24.4 – 26.0	7.7 – 8.2	6.7 – 8.4	310 – 325
F3 4	24.0 – 24.7	7.2 – 8.3	2.0 – 8.5	323 – 363
F3 5	24.0 – 25.9	7.6 – 8.6	7.1 – 8.5	303 – 325
F3 6	24.0 – 26.0	7.6 – 8.5	6.6 – 8.4	314 – 330
F3 7	24.0 – 24.8	7.5 – 8.5	4.5 – 8.5	326 – 356
Chronic				
F3 1	24.0 – 24.0	7.7 – 8.4	7.0 – 9.1	300 – 411
F3 2	24.0 – 24.8	7.6 – 8.1	5.4 – 8.8	303 – 328
Reference Product	24.0 – 24.1	7.7 – 8.4	5.5 – 9.4	288 – 378
F3 3	24.0 – 25.1	7.6 – 8.6	6.0 – 9.1	308 – 371
F3 4	24.0 – 24.0	7.7 – 8.6	7.1– 9.0	305 – 374
F3 5	24.0 – 24.4	7.7 – 8.6	7.1 – 8.9	289 – 355
F3 6	24.0 – 24.2	7.8 – 8.4	6.9 – 8.7	294 – 331
F3 7	24.0 – 24.6	8.0– 8.7	6.1 – 9.1	303 – 390

Notes:
°C = degrees Celsius
µS/cm = microsiemens per centimeter
mg/L = milligrams per liter

Table A10. Summary of acute toxicity data for *P. promelas*.

Foam	Concentration (mg/L)	96-hour Survival (%)
F3 1	0	100
	3	100
	9	85
	30	0
	90	0
	300	0
F3 2	0	100
	1.8	100
	3	0
	9	0
	18	0
	30	0
Reference Product	0	95
	9	100
	18	95
	300	90
	900	70
	1,800	0
F3 3	0	100
	18	100
	30	100
	90	0
	180	0
	300	0
F3 4	0	90
	30	90
	90	15
	180	0
	300	0
	900	0
F3 5	0	95
	9	95
	30	85
	90	0
	180	0
	300	0
F3 6	0	100
	30	100
	90	95
	180	100
	300	80
	900	0
F3 7	0	95
	30	100
	90	0
	180	0
	300	0
	900	0

Notes: mg/L = milligrams per liter

Table A11. Summary of chronic toxicity studies with *P. promelas*.

Foam	Concentration (mg/L)	Average Survival (%)	Survival Standard Deviation	Average Dry Weight (mg)	Standard Deviation Dry Weight (mg)
F3 1	0	100%	0%	0.574	0.104
F3 1	1.8	100%	0%	0.548	0.053
F3 1	3	95%	6%	0.543	0.053
F3 1	9	8%	10%	0.545	0.049
F3 1	18	0%	0%	na	na
F3 1	30	0%	0%	na	na
F3 2	0	95%	6%	0.666	0.146
F3 2	1.8	98%	5%	0.716	0.078
F3 2	3	98%	5%	0.755	0.076
F3 2	9	33%	13%	0.424	0.205
F3 2	18	0%	0%	na	na
F3 2	30	0%	0%	na	na
Reference Product	0	100%	0%	0.414	0.021
Reference Product	90	95%	6%	0.383	0.054
Reference Product	180	98%	5%	0.412	0.022
Reference Product	300	58%	25%	0.356	0.099
Reference Product	900	0%	0%	na	na
Reference Product	1800	0%	0%	na	na
F3 3	0	90%	8%	0.456	0.035
F3 3	3	100%	0%	0.450	0.031
F3 3	9	83%	15%	0.409	0.046
F3 3	18	85%	17%	0.384	0.035
F3 3	30	73%	15%	0.383	0.042
F3 3	90	0%	0%	na	na
F3 4	0	98%	5%	0.600	0.031
F3 4	9	98%	5%	0.553	0.059
F3 4	18	98%	5%	0.460	0.091
F3 4	30	93%	5%	0.432	0.090
F3 4	90	3%	5%	0.270	na
F3 4	180	0%	0%	na	na
F3 5	0	100%	0%	0.566	0.073
F3 5	3	100%	0%	0.566	0.074
F3 5	9	100%	0%	0.610	0.023
F3 5	18	95%	10%	0.589	0.024
F3 5	30	13%	13%	0.760	0.142
F3 5	90	0%	0%	na	na
F3 6	0	100%	0%	0.412	0.040
F3 6	30	95%	10%	0.349	0.008
F3 6	90	88%	15%	0.411	0.020
F3 6	180	90%	12%	0.419	0.066
F3 6	300	78%	5%	0.462	0.044
F3 6	900	0%	0%	na	na
F3 7	0	100%	0%	0.579	0.099

Foam	Concentration (mg/L)	Average Survival (%)	Survival Standard Deviation	Average Dry Weight (mg)	Standard Deviation Dry Weight (mg)
F3 7	3	100%	0%	0.574	0.032
F3 7	9	100%	0%	0.591	0.075
F3 7	18	100%	0%	0.543	0.033
F3 7	30	98%	5%	0.525	0.068
F3 7	90	0%	0%	na	na

Notes: mg = milligrams
mg/L = milligrams per liter
na = not applicable

Table A12. Summary of adult bobwhite growth rates over the 60 day exposure.

Foam	Treatment	Adult Weight Change (g)	Adult Growth Rate (g/d)
F3 2	Control	9.8 ± 3.1	0.16 ± 0.05
F3 2	100 mg/L	1.2 ± 7.2	0.02 ± 0.12
F3 2	1,000 mg/L	4.0 ± 7.3	0.07 ± 0.12
F3 2	2,500 mg/L	16 ± 6.4	0.27 ± 0.11
Reference Product	Control	9.8 ± 3.1	0.16 ± 0.05
Reference Product	100 mg/L	9.7 ± 3.2	0.16 ± 0.05
Reference Product	1,000 mg/L	8.4 ± 4.2	0.14 ± 0.07
Reference Product	2,500 mg/L	-2.2 ± 3.7	-0.04 ± 0.06
F3 3	Control	0.73 ± 6.3	0.01 ± 0.11
F3 3	100 mg/L	-14.2 ± 13.9	-0.24 ± 0.23
F3 3	1,000 mg/L	9.6 ± 7.8	0.16 ± 0.13
F3 3	2,500 mg/L	11.2 ± 4.3	0.19 ± 0.07
F3 6	Control	0.73 ± 6.3	0.01 ± 0.11
F3 6	100 mg/L	9.1 ± 8.0	0.15 ± 0.13
F3 6	1,000 mg/L	16 ± 4.7	0.27 ± 0.08
F3 6	2,500 mg/L	5.6 ± 7.4	0.09 ± 0.12
F3 4	Control	22 ± 6.2	0.37 ± 0.10
F3 4	100 mg/L	8.0 ± 7.4	0.13 ± 0.12
F3 4	1,000 mg/L	14.9 ± 4.8	0.25 ± 0.08
F3 4	2,500 mg/L	33.9 ± 7.2	0.57 ± 0.12
F3 5	Control	22.6 ± 6.2	0.38 ± 0.10
F3 5	100 mg/L	18.0 ± 4.3	0.30 ± 0.07
F3 5	1,000 mg/L	8.5 ± 5.4	0.14 ± 0.09
F3 5	2,500 mg/L	24.4 ± 3.2	0.41 ± 0.05
F3 7	Control	22.6 ± 6.2	0.38 ± 0.10
F3 7	100 mg/L	7.8 ± 4.5	0.13 ± 0.07

F3 7	1,000 mg/L	27.8 ± 2.5	0.46 ± 0.04
F3 7	2,500 mg/L	6.9 ± 10.5	0.11 ± 0.17

Notes:
mg/L = milligrams per liter
g = grams
g/d = grams per day

Tabe A13. Summary of adult bobwhite quail biometrics following 60 days of exposure.

Foam	Treatment	Male Left Tarsus (mm)	Male Right Tarsus (mm)	Male Left Wing (mm)	Male Right Wing (mm)	Male Bill-Head Length (mm)	Female Left Tarsus (mm)	Female Right Tarsus (mm)	Female Left Wing (mm)	Female Right Wing (mm)	Female Bill-Head Length (mm)
F3 2	Control	41 ± 0.8	41 ± 0.8	105 ± 4.9	108 ± 2.5	39 ± 0.4	40 ± 0.4	40 ± 0.3	107 ± 2.0	109 ± 1.7	39 ± 0.4
F3 2	100 mg/L	41 ± 0.7	41 ± 0.7	108 ± 3.1	111 ± 1.3	40 ± 0.5	41 ± 0.5	40 ± 0.2	107 ± 4.8	112 ± 2.4	39 ± 0.2
F3 2	1,000 mg/L	42 ± 0.7	41 ± 0.7	112 ± 2.5	115 ± 1.5	40 ± 0.6	39 ± 0.8	39 ± 1.1	105 ± 3.2	110 ± 2.5	38 ± 0.4
F3 2	2,500 mg/L	41 ± 0.9	41 ± 0.8	111 ± 0.6	108 ± 2.3	40 ± 0.5	41 ± 0.6	41 ± 0.5	104 ± 5.1	109 ± 3.5	40 ± 1
Reference Product	Control	41 ± 0.8	41 ± 0.8	105 ± 4.9	108 ± 2.5	39 ± 0.4	40 ± 0.4	40 ± 0.3	107 ± 2.0	109 ± 1.7	39 ± 0.4
Reference Product	100 mg/L	41 ± 1.6	41 ± 1.1	107 ± 1.3	108 ± 1.5	40 ± 1.0	41 ± 0.2	42 ± 0.3	109 ± 3.8	110 ± 3.9	39 ± 0.6
Reference Product	1,000 mg/L	40 ± 0.3	41 ± 0.5	100 ± 5.1	106 ± 3.6	39 ± 1.8	41 ± 0.2	41 ± 0.3	106 ± 2.7	106 ± 2.1	40 ± 0.1
Reference Product	2,500 mg/L	40 ± 0.6	40 ± 0.4	110 ± 1.4	111 ± 0.8	38 ± 0.6	40 ± 0.2	40 ± 0.3	110 ± 2.6	112 ± 0.5	38 ± 0.8
F3 3	Control	40 ± 0.7	40 ± 0.6	103 ± 2.5	106 ± 2	39 ± 0.3	39 ± 0.7	39 ± 0.4	101 ± 4.9	86 ± 15.5	39 ± 0.2
F3 3	100 mg/L	39 ± 0.1	40 ± 0.1	102 ± 3.5	105 ± 3.8	37 ± 0.9	39 ± 0.3	39 ± 0.7	105 ± 3.9	107 ± 3.0	39 ± 0.9
F3 3	1,000 mg/L	41 ± 0.8	40 ± 1.0	110 ± 1.3	109 ± 2.2	40 ± 0.8	40 ± 0.6	39 ± 0.7	103 ± 6.3	108 ± 3.9	38 ± 0.6
F3 3	2,500 mg/L	40 ± 0.4	40 ± 0.3	108 ± 2.8	109 ± 1.2	39 ± 0.9	39 ± 0.5	39 ± 0.7	104 ± 0.5	97 ± 10.5	38 ± 0.3
F3 6	Control	40 ± 0.7	40 ± 0.6	103 ± 2.5	106 ± 2	39 ± 0.3	39 ± 0.7	39 ± 0.4	101 ± 4.9	86 ± 15.5	39 ± 0.2
F3 6	100 mg/L	42 ± 0.5	41 ± 0.4	109 ± 2.3	111 ± 2.1	40 ± 0.5	40 ± 0.1	40 ± 0.9	112 ± 0.9	109 ± 1.5	37 ± 0.6
F3 6	1,000 mg/L	39 ± 1.5	40 ± 0.9	106 ± 2.1	109 ± 6.1	39 ± 0.5	39 ± 1.3	37 ± 1.4	105 ± 5.2	109 ± 1.7	38 ± 0.9
F3 6	2,500 mg/L	42 ± 0.7	42 ± 1.0	100 ± 2.5	106 ± 2.1	39 ± 0.7	39 ± 0.2	39 ± 0.1	109 ± 1.9	110 ± 2.4	38 ± 0.5
F3 4	Control	38.0 ± 1.0	38 ± 1.5	107 ± 3.2	97 ± 4.6	42 ± 0.40	38 ± 0.8	36 ± 0.7	107 ± 3.2	103 ± 3.2	39 ± 0.60
F3 4	100 mg/L	39 ± 1.3	36 ± 1.6	104 ± 4.4	105 ± 5.2	40 ± 3.0	39 ± 0.9	37 ± 0.2	91 ± 8.1	84 ± 5.2	40 ± 0.70
F3 4	1,000 mg/L	40 ± 0.97	39 ± 0.33	100 ± 3.0	103 ± 0.95	41 ± 0.54	38 ± 0.89	37 ± 0.70	102 ± 2.1	97 ± 6.2	39 ± 0.69
F3 4	2,500 mg/L	41 ± 0.30	40 ± 0.37	95 ± 5.5	105 ± 1.3	43 ± 1.2	40 ± 1.6	40 ± 0.6	104 ± 3.3	102 ± 6.9	40 ± 0.11
F3 5	Control	38 ± 1	39 ± 2	107 ± 3.2	97 ± 4.6	42 ± 0.4	38 ± 0.8	36 ± 0.7	107 ± 3.2	103 ± 3.2	39 ± 0.6

Tabe A13. Summary of adult bobwhite quail biometrics following 60 days of exposure.

Foam	Treatment	Male Left Tarsus (mm)	Male Right Tarsus (mm)	Male Left Wing (mm)	Male Right Wing (mm)	Male Bill-Head Length (mm)	Female Left Tarsus (mm)	Female Right Tarsus (mm)	Female Left Wing (mm)	Female Right Wing (mm)	Female Bill-Head Length (mm)
F3 5	100 mg/L	38 ± 0.7	38 ± 0.4	98 ± 2.6	99 ± 4.4	41 ± 0.7	41 ± 0.4	40 ± 0.9	103 ± 4	102 ± 5.9	40 ± 0.7
F3 5	1,000 mg/L	39 ± 1.1	39 ± 0.6	105 ± 8.2	102 ± 5.3	41 ± 1.8	37 ± 1.2	39 ± 1.1	108 ± 2.9	108 ± 4.3	39 ± 0.7
F3 5	2,500 mg/L	39 ± 0.4	40 ± 1.4	105 ± 3.8	110 ± 1	40 ± 1.1	42 ± 0.4	40 ± 0.5	101 ± 2.2	103 ± 3.7	40 ± 0.9
F3 7	Control	38 ± 1	39 ± 2	107 ± 3.2	97 ± 4.6	42 ± 0.4	38 ± 0.8	36 ± 0.7	107 ± 3.2	103 ± 3.2	39 ± 0.6
F3 7	100 mg/L	42 ± 0.4	41 ± 0.3	105 ± 3.4	109 ± 0.5	39 ± 1.1	38 ± 1.4	39 ± 1	103 ± 3.1	104 ± 1.3	39 ± 1
F3 7	1,000 mg/L	42 ± 0.4	41 ± 0.3	110 ± 1.4	110 ± 0.8	40 ± 0.5	39 ± 0.1	40 ± 0.8	111 ± 1.3	111 ± 1.7	38 ± 0.3
F3 7	2,500 mg/L	41 ± 0.3	40 ± 0.3	109 ± 1.8	104 ± 0.6	39 ± 0.1	40 ± 0.8	39 ± 0.6	105 ± 5.1	107 ± 3.5	38 ± 1

Notes:
mg/L = milligrams per liter
mm = millimeters
All values are mean ± standard error of the mean.

Table A14. Summary of lipid content and liver weights in adult and juvenile bobwhite quail.

Foam	Treatment	Male Liver Lipid Content	Female Liver Lipid Content	Female Relative Liver Weight	Male Relative Liver Weight	Chick Liver Lipid Content
F3 2	Control	2.89% ± 0.57%	5.33% ± 1.26%	0.034 ± 0.002	na	4.94% ± 0.37%
F3 2	100 mg/L	5.98% ± 0.61%	8.76% ± 1.78%	0.030 ± 0.001	na	2.97% ± 0.23%
F3 2	1,000 mg/L	6.56% ± 1.21%	4.25% ± 0.49%	0.033 ± 0.003	na	3.51% ± 0.18%
F3 2	2,500 mg/L	8.20% ± 1.14%	6.68% ± 1.45%	0.042 ± 0.001	na	4.53% ± 0.30%
Reference Product	Control	2.89% ± 0.57%	5.33% ± 1.26%	0.034 ± 0.002	na	4.94% ± 0.37%
Reference Product	100 mg/L	5.89% ± 0.50%	6.82% ± 1.14%	0.036 ± 0.003	na	3.96% ± 0.43%
Reference Product	1,000 mg/L	6.40% ± 1.67%	8.49% ± 0.54%	0.034 ± 0.001	na	3.81% ± 0.36%
Reference Product	2,500 mg/L	6.87% ± 0.34%	8.85% ± 1.69%	0.037 ± 0.0003	na	3.40% ± 0.40%
F3 3	Control	2.71% ± 0.83%	3.17% ± 0.41%	0.040 ± 0.0009	0.029 ± 0.007	4.18% ± 0.27%
F3 3	100 mg/L	3.23% ± 1.62%	2.59% ± 0.11%	0.045 ± 0.005	0.026 ± 0.003	3.42% ± 0.23%
F3 3	1,000 mg/L	0.78% ± 0.27%	2.79% ± 1.83%	0.041 ± 0.004	0.031 ± 0.005	3.38% ± 0.18%
F3 3	2,500 mg/L	2.93% ± 0.79%	4.42% ± 0.24%	0.039 ± 0.002	0.025 ± 0.001	3.65% ± 0.23%
F3 6	Control	2.71% ± 0.83%	3.17% ± 0.41%	0.040 ± 0.0009	0.029 ± 0.007	4.18% ± 0.27%
F3 6	100 mg/L	2.28% ± 0.59%	4.82% ± 0.86%	0.036 ± 0.002	0.025 ± 0.0006	4.04% ± 0.30%
F3 6	1,000 mg/L	5.46% ± 1.27%	4.26% ± 0.52%	0.032 ± 0.006	0.038 ± 0.004	4.67% ± 0.31%
F3 6	2,500 mg/L	3.47% ± 0.58%	4.77% ± 0.82%	0.042 ± 0.002	0.026 ± 0.001	4.62% ± 0.29%
F3 4	Control			0.040 ± 0.003	0.021 ± 0.001	1.05 ± 0.08
F3 4	100 mg/L			0.031 ± 0.003	0.027 ± 0.006	1.05 ± 0.09
F3 4	1,000 mg/L			0.038 ± 0.004	0.022 ± 0.002	1.24 ± 0.08
F3 4	2,500 mg/L			0.041 ± 0.004	0.023 ± 0.002	1.17 ± 0.04
F3 5	Control	6.53% ± 0.59%	7.45% ± 0.77%	0.040 ± 0.003	0.021 ± 0.001	4.19% ± 0.33%
F3 5	100 mg/L	4.21% ± 0.49%	3.87% ± 0.42%	0.036 ± 0.003	0.023 ± 0.002	2.46% ± 0.48%
F3 5	1,000 mg/L	3.55% ± 0.66%	3.88% ± 1.02%	0.054 ± 0.012	0.025 ± 0.003	2.49% ± 0.42%
F3 5	2,500 mg/L	5.37% ± 0.60%	4.34% ± 0.17%	0.034 ± 0.002	0.033 ± 0.008	2.76% ± 0.50%
F3 7	Control	6.53% ± 0.59%	7.45% ± 0.77%	0.040 ± 0.003	0.021 ± 0.001	4.19% ± 0.33%
F3 7	100 mg/L	4.99% ± 0.93%	4.51% ± 0.55%	0.036 ± 0.003	0.023 ± 0.0003	3.45% ± 0.37%
F3 7	1,000 mg/L	4.14% ± 0.35%	4.74% ± 0.1%	0.039 ± 0.001	0.027 ± 0.003	3.31% ± 0.36%
F3 7	2,500 mg/L	4.53% ± 1.14%	4.62% ± 0.51%	0.037 ± 0.0006	0.029 ± 0.006	2.75% ± 0.39%

Notes:
mg/L = milligrams per liter
na = not analyzed
All values are mean ± standard error of the mean.

Table A15. Summary of reproductive parameters in adult bobwhite quail.

Endpoint	Treatment	Mean ± SEM
F3 2		
Eggs laid per hen	Control	53.0 ± 1.83
Number of Cracked/Soft Eggs		0.833 ± 0.40
Cracked Eggs (%)		1.64% ± 0.787%
Hatching Success (%)		75.5% ± 0.787%
Percent Arrested Embryos (%)		9.17% ± 1.92%
Day of Arrested Development		18.8 ± 1.24
Chick Survival (%)		96. 1% ± 0.839%
Eggs laid per hen	100 mg/L	34.7 ±14.6
Number of Cracked/Soft Eggs		1.67 ± 0.882
Cracked Eggs (%)		3.37% ± 1.71%
Hatching Success (%)		73.9% ± 12.2%
Percent Arrested Embryos (%)		16.0% ± 8.73%
Day of Arrested Development		5.78 ± 2.45
Chick Survival (%)		100% ± 0%
Eggs laid per hen	1,000 mg/L	49.3 ± 2.19
Number of Cracked/Soft Eggs		1.00 ± 1.00
Cracked Eggs (%)		1.92% ± 1.92%
Hatching Success (%)		57.1% ± 19.4%
Percent Arrested Embryos (%)		19.9% ± 11.5%
Day of Arrested Development		13.6 ± 1.24
Chick Survival (%)		87.8% ± 10.6%
Eggs laid per hen	2,500 mg/L	50.7 ± 4.33
Number of Cracked/Soft Eggs		1.67 ± 0.882
Cracked Eggs (%)		3.11% ± 1.70%
Hatching Success (%)		66.3% ± 12.6%
Percent Arrested Embryos (%)		16.7% ± 6.65%
Day of Arrested Development		5.96 ± 1.13
Chick Survival (%)		90.9% ± 2.45%
Reference Product		
Eggs laid per hen	Control	53.0 ± 1.83
Number of Cracked/Soft Eggs		0.833 ± 0.40
Cracked Eggs (%)		1.64% ± 0.787%
Hatching Success (%)		75.5% ± 0.787%
Percent Arrested Embryos (%)		9.17% ± 1.92%
Day of Arrested Development		18.8 ± 1.24
Chick Survival (%)		96. 1% ± 0.839%
Eggs laid per hen		45.7 ± 7.31

Table A15. Summary of reproductive parameters in adult bobwhite quail.

Endpoint	Treatment	Mean ± SEM
Number of Cracked/Soft Eggs	100 mg/L	1.33 ± 0.882
Cracked Eggs (%)		2.45% ± 1.53%
Hatching Success (%)		70.3% ± 22.7%
Percent Arrested Embryos (%)		19.7% ± 16.7%
Day of Arrested Development		12.4 ± 1.44
Chick Survival (%)		94.4% ± 5.56%
Eggs laid per hen	1,000 mg/L	52.3 ± 0.333
Number of Cracked/Soft Eggs		1.67 ± 1.20
Cracked Eggs (%)		3.20% ± 2.31%
Hatching Success (%)		61.9% ± 31.2%
Percent Arrested Embryos (%)		22.0% ± 20.1%
Day of Arrested Development		2.14 ± 0.794
Chick Survival (%)		98.0 %± 2.00%
Eggs laid per hen	2,500 mg/L	44.3 ± 3.18
Number of Cracked/Soft Eggs		5.67 ± 1.33
Cracked Eggs (%)		12.5% ± 2.28%
Hatching Success (%)		53.3% ± 27.3
Percent Arrested Embryos (%)		21.9% ± 17.2%
Day of Arrested Development		2.67 ± 0.881
Chick Survival (%)		100% ± 0%
F3 4		
Eggs laid per hen	Control	59 ± 0.8
Number of Cracked/Soft Eggs		0 ± 0
Cracked Eggs (%)		0% ± 0%
Hatching Success (%)		80% ± 4.50%
Percent Arrested Embryos (%)		3.46% ± 4.66%
Day of Arrested Development		15.7 ± 1.9
Chick Survival (%)		89.0% ± 4.20%
Eggs laid per hen	100 mg/L	28.3 ± 9.02
Number of Cracked/Soft Eggs		0 ± 0
Cracked Eggs (%)		0% ± 0%
Hatching Success (%)		76.6% ± 10.0%
Percent Arrested Embryos (%)		13.0% ± 5.67%
Day of Arrested Development		15.1 ± 1.84
Chick Survival (%)		82.5% ± 11.8%
Eggs laid per hen	1,000 mg/L	51.3 ± 4.26
Number of Cracked/Soft Eggs		0.333 ± 0.333
Cracked Eggs (%)		0.585% ± 0.585%
Hatching Success (%)		82.6% ± 4.60%

Table A15. Summary of reproductive parameters in adult bobwhite quail.

Endpoint	Treatment	Mean ± SEM
Percent Arrested Embryos (%)		5.22% ± 2.19%
Day of Arrested Development		16.0 ± 3.01
Chick Survival (%)		90.9% ± 3.80%
Eggs laid per hen		56.3 ± 1.76
Number of Cracked/Soft Eggs		0 ± 0
Cracked Eggs (%)		0% ± 0%
Hatching Success (%)	2,500 mg/L	77.5% ± 16.2%
Percent Arrested Embryos (%)		4.21% ± 2.22%
Day of Arrested Development		18 ± 1.85
Chick Survival (%)		86.7% ± 9.46%
F3 5		
Eggs laid per hen		58.2 ± 0.792
Number of Cracked/Soft Eggs		0.167 ± 0.167
Cracked Eggs (%)		0.287% ± 0.287%
Hatching Success (%)	Control	82.3% ± 4.70%
Percent Arrested Embryos (%)		3.46% ± 4.66%
Day of Arrested Development		14.7 ± 2.25
Chick Survival (%)		87.2% ± 5.47%
Eggs laid per hen		48.7 ± 5.78
Number of Cracked/Soft Eggs		0 ± 0
Cracked Eggs (%)		0% ± 0%
Hatching Success (%)	100 mg/L	83% ± 4.00%
Percent Arrested Embryos (%)		7.74% ± 2.80%
Day of Arrested Development		16.3 ± 2.7
Chick Survival (%)		72% ± 12.0%
Eggs laid per hen		53.3 ± 7.97
Number of Cracked/Soft Eggs		0 ± 0
Cracked Eggs (%)		0% ± 0%
Hatching Success (%)	1,000 mg/L	79% ± 9%
Percent Arrested Embryos (%)		8.22% ± 4.13%
Day of Arrested Development		13.3 ± 2.3
Chick Survival (%)		77% ± 5%
Eggs laid per hen		55.0 ± 2.31
Number of Cracked/Soft Eggs		0 ± 0
Cracked Eggs (%)		0% ± 0%
Hatching Success (%)	2,500 mg/L	80% ± 16%
Percent Arrested Embryos (%)		8.65% ± 6.79%
Day of Arrested Development		19.0 ± 1.2
Chick Survival (%)		84% ± 6%
F3 7		

Table A15. Summary of reproductive parameters in adult bobwhite quail.

Endpoint	Treatment	Mean ± SEM
Eggs laid per hen	Control	58.2 ± 0.792
Number of Cracked/Soft Eggs		0.167 ± 0.167
Cracked Eggs (%)		0.287% ± 0.287%
Hatching Success (%)		82.3% ± 4.70%
Percent Arrested Embryos (%)		3.46% ± 4.66%
Day of Arrested Development		14.7 ± 2.25
Chick Survival (%)		87.2% ± 5.47%
Eggs laid per hen	100 mg/L	53.3 ± 0.667
Number of Cracked/Soft Eggs		0 ± 0
Cracked Eggs (%)		0% ± 0%
Hatching Success (%)		90% ± 4%
Percent Arrested Embryos (%)		4.44% ± 1.72%
Day of Arrested Development		16.3 ± 2.2
Chick Survival (%)		82% ± 5%
Eggs laid per hen	1,000 mg/L	57.7 ± 0.882
Number of Cracked/Soft Eggs		0.667 ± 0.667
Cracked Eggs (%)		1.19% ± 1.19%
Hatching Success (%)		90% ± 3%
Percent Arrested Embryos (%)		4.07% ± 1.19%
Day of Arrested Development		19.7 ± 1.9
Chick Survival (%)		85% ± 6%
Eggs laid per hen	2,500 mg/L	56.3 ± 3.67
Number of Cracked/Soft Eggs		0 ± 0
Cracked Eggs (%)		0% ± 0%
Hatching Success (%)		93% ± 4%
Percent Arrested Embryos (%)		3.03% ± 1.54%
Day of Arrested Development		21.4 ± 0.6
Chick Survival (%)		74 ± 8%
F3 3		
Eggs laid per hen	Control	50.8 ± 5.77
Number of Cracked/Soft Eggs		0 ± 0
Cracked Eggs (%)		0 ± 0%
Hatching Success (%)		81.9% ± 5.45%
Percent Arrested Embryos (%)		2.94% ± 0.740%
Day of Arrested Development		14.5 ± 2.10
Chick Survival (%)		92.0 ± 4.11%
Eggs laid per hen	100 mg/L	37.3 ± 7.72
Number of Cracked/Soft Eggs		0 ± 0
Cracked Eggs (%)		0 ± 0%
Hatching Success (%)		82.4 ± 3.81%

Table A15. Summary of reproductive parameters in adult bobwhite quail.

Endpoint	Treatment	Mean ± SEM
Percent Arrested Embryos (%)		7.84% ± 3.12%
Day of Arrested Development		12.6 ± 2.79
Chick Survival (%)		85.0 ± 6.34%
Eggs laid per hen		40.0 ± 7.90
Number of Cracked/Soft Eggs		0.200 ± 0.183
Cracked Eggs (%)		0.423 ± 0.389%
Hatching Success (%)	1,000 mg/L	57.4 ± 14.6%
Percent Arrested Embryos (%)		10.0% ± 6.82%
Day of Arrested Development		17.2 ± 0.877
Chick Survival (%)		69.3 ± 14.9%
Eggs laid per hen		41.7 ± 5.54
Number of Cracked/Soft Eggs		0 ± 0
Cracked Eggs (%)		0 ± 0%
Hatching Success (%)	2,500 mg/L	88.1 ± 5.53%
Percent Arrested Embryos (%)		5.79% ± 4.83%
Day of Arrested Development		16.6 ± 2.18
Chick Survival (%)		93.3 ± 4.71%
F3 6		
Eggs laid per hen		50.8 ± 5.77
Number of Cracked/Soft Eggs		0 ± 0
Cracked Eggs (%)		0 ± 0%
Hatching Success (%)	Control	81.9 ± 5.45%
Percent Arrested Embryos (%)		2.94% ± 0.740%
Day of Arrested Development		14.5 ± 2.10
Chick Survival (%)		92.0 ± 4.11%
Eggs laid per hen		52.7 ± 3.0
Number of Cracked/Soft Eggs		0 ± 0
Cracked Eggs (%)		0 ± 0%
Hatching Success (%)	100 mg/L	85.1 ± 10.4%
Percent Arrested Embryos (%)		4.50% ± 2.68%
Day of Arrested Development		16.7 ± 1.46
Chick Survival (%)		93.3 ± 6.67%
Eggs laid per hen		53.3 ± 3.3
Number of Cracked/Soft Eggs		0 ± 0
Cracked Eggs (%)		0 ± 0%
Hatching Success (%)	1,000 mg/L	48.4 ± 24.1%
Percent Arrested Embryos (%)		17.0% ± 5.33%
Day of Arrested Development		2.82 ± 0.901
Chick Survival (%)		82.0 ± 0%
Eggs laid per hen		54.0 ± 2.0

Table A15. Summary of reproductive parameters in adult bobwhite quail.

Endpoint	Treatment	Mean ± SEM
Number of Cracked/Soft Eggs	2,500 mg/L	0 ± 0
Cracked Eggs (%)		0 ± 0%
Hatching Success (%)		53.2 ± 14.3%
Percent Arrested Embryos (%)		15.6% ± 6.45%
Day of Arrested Development		9.00 ± 1.61
Chick Survival (%)		90.7 ± 1.76%

Notes:
mg/L = milligrams per liter
SEM = standard error of the mean.
All values are mean ± standard error of the mean.

Table A16. Summary of chick growth after 21 days.

Foam	Treatment	Chick 21 Day Weight (g)	Chick 21 Day Growth Rate (g/d)
F3 2	Control	54.3 ± 0.6	2.27 ± 0.03
F3 2	100 mg/L	56.7 ± 1.13	2.39 ± 0.05
F3 2	1,000 mg/L	57.1 ± 0.8	2.40 ± 0.04
F3 2	2,500 mg/L	58.7 ± 0.8	2.44 ± 0.03
Reference Product	Control	54.3 ± 0.6	2.27 ± 0.03
Reference Product	100 mg/L	57.7 ± 0.6	2.41 ± 0.03
Reference Product	1,000 mg/L	58.3 ± 0.9	2.45 ± 0.04
Reference Product	2,500 mg/L	51.9 ± 0.7	2.15 ± 0.03
F3 3	Control	53.1 ± 1.1	2.21 ± 0.05
F3 3	100 mg/L	51.0 ± 1.0	2.1 ± 0.05
F3 3	1,000 mg/L	52.7 ± 0.9	2.2 ± 0.04
F3 3	2,500 mg/L	53.1 ± 0.7	2.2 ± 0.03
F3 6	Control	53.1 ± 1.1	2.21 ± 0.05
F3 6	100 mg/L	55.3 ± 0.7	2.3 ± 0.03
F3 6	1,000 mg/L	59 ± 1.4	2.5 ± 0.06
F3 6	2,500 mg/L	52.5 ± 1.3	2.2 ± 0.06
F3 4	Control	44.6 ± 0.8	2.12 ± 0.04
F3 4	100 mg/L	43.3 ± 1.0	2.07 ± 0.05
F3 4	1,000 mg/L	43.3 ± 1.0	2.07 ± 0.05
F3 4	2,500 mg/L	45.1 ± 1.1	2.15 ± 0.05
F3 5	Control	51.4 ± 0.8	2.12 ± 0.04
F3 5	100 mg/L	45.5 ± 1.4	1.85 ± 0.06
F3 5	1,000 mg/L	49.3 ± 0.8	2.04 ± 0.04
F3 5	2,500 mg/L	53.2 ± 1.0	2.22 ± 0.04
F3 7	Control	51.4 ± 0.8	2.12 ± 0.04
F3 7	100 mg/L	45.1 ± 1.0	1.84 ± 0.05
F3 7	1,000 mg/L	51.9 ± 0.8	2.15 ± 0.04
F3 7	2,500 mg/L	52.2 ± 1.0	2.17 ± 0.04

Notes:
mg/L = milligrams per liter
g = grams
g/d = grams per day.
All values are mean ± standard error of the mean.

Table A17. Summary of chick biometrics after 21 days.

Foam	Treatment	Chick Left Tarsus (mm)	Chick Right Tarsus (mm)	Chick Left Wing (mm)	Chick Right Wing (mm)	Chick Bill-Head Length (mm)
F3 2	Control	30 ± 0.2	30 ± 0.2	76 ± 0.4	78 ± 0.4	29 ± 0.1
F3 2	100 mg/L	31 ± 0.2	31 ± 0.2	78 ± 0.5	81 ± 0.5	29 ± 0.2
F3 2	1,000 mg/L	30 ± 0.2	30 ± 0.2	78 ± 0.6	79 ± 0.5	30 ± 0.2
F3 2	2,500 mg/L	30 ± 0.3	31 ± 0.2	79 ± 0.5	81 ± 0.5	30 ± 0.2
Reference Product	Control	30 ± 0.2	30 ± 0.2	76 ± 0.4	78 ± 0.4	29 ± 0.1
Reference Product	100 mg/L	31 ± 0.2	31 ± 0.2	78 ± 0.4	80 ± 0.4	30 ± 0.2
Reference Product	1,000 mg/L	32 ± 0.2	32 ± 0.2	77 ± 0.7	79 ± 0.6	30 ± 0.2
Reference Product	2,500 mg/L	29 ± 0.2	29 ± 0.2	78 ± 0.5	79 ± 0.4	28 ± 0.2
F3 3	Control	28 ± 0.3	28 ± 0.3	73 ± 0.6	75 ± 0.6	28 ± 0.2
F3 3	100 mg/L	28 ± 0.3	27 ± 0.3	72 ± 0.7	74 ± 0.6	27 ± 0.2
F3 3	1,000 mg/L	28 ± 0.3	28 ± 0.3	73 ± 0.7	75 ± 0.6	27 ± 0.2
F3 3	2,500 mg/L	28 ± 0.2	27 ± 0.2	71 ± 0.7	73 ± 0.5	28 ± 0.2
F3 6	Control	28 ± 0.3	28 ± 0.3	73 ± 0.6	75 ± 0.6	28 ± 0.2
F3 6	100 mg/L	29 ± 0.2	29 ± 1.0	75 ± 0.5	77 ± 0.4	28 ± 0.2
F3 6	1,000 mg/L	29 ± 0.3	29 ± 0.3	74 ± 0.8	74 ± 2.0	28 ± 0.2
F3 6	2,500 mg/L	28 ± 0.3	28 ± 0.4	73 ± 0.7	75 ± 0.6	27 ± 0.2
F3 4	Control	29 ± 0.8	29 ± 0.8	71 ± 0.6	73 ± 0.5	29 ± 0.2
F3 4	100 mg/L	25 ± 0.3	25 ± 0.3	70 ± 0.8	70 ± 0.6	29 ± 0.4
F3 4	1,000 mg/L	25 ± 0.3	24 ± 0.2	67 ± 0.9	69 ± 0.8	29 ± 0.4
F3 4	2,500 mg/L	25 ± 0.2	24 ± 0.3	67 ± 1.2	69 ± 0.6	29 ± 0.3
F3 5	Control	29 ± 0.8	29 ± 0.8	72 ± 0.5	73 ± 0.5	29 ± 0.8
F3 5	100 mg/L	24 ± 0.3	22 ± 0.3	64 ± 1.2	67 ± 1.1	29 ± 0.2
F3 5	1,000 mg/L	24 ± 0.1	22 ± 0.2	67 ± 1.6	73 ± 0.6	29 ± 0.2
F3 5	2,500 mg/L	26 ± 0.4	25 ± 0.4	69 ± 0.6	72 ± 0.6	30 ± 0.2
F3 7	Control	29 ± 0.8	29 ± 0.8	72 ± 0.5	73 ± 0.5	29 ± 0.8
F3 7	100 mg/L	25 ± 0.2	24 ± 0.3	67 ± 0.9	69 ± 0.9	28 ± 0.2
F3 7	1,000 mg/L	25 ± 0.1	24 ± 0.2	69 ± 0.5	72 ± 0.4	29 ± 0.3
F3 7	2,500 mg/L	25 ± 0.2	24 ± 0.2	67 ± 1.5	72 ± 0.5	29 ± 0.2

Notes:
mg/L = milligrams per liter
mm = millimeters
All values are mean ± standard error of the mean.

Table A18. Summary of chemical constituents measured in adult bobwhite quail livers, chick livers, and eggs.

Foam	Compound	Matrix	Treatment (mg/L)	Average Concentration (ng/g wet weight)	SEM Concentration (ng/g wet weight)
F3 2	SDS	Adult Liver	0	31.6	4.0
F3 2	SDS	Chick Liver	0	41.6	3.4
F3 2	SDS	Egg	0	64.5	30.0
F3 2	SDS	Adult Liver	100	45.2	18.7
F3 2	SDS	Chick Liver	100	64.1	11.0
F3 2	SDS	Egg	100	17.1	1.8
F3 2	SDS	Adult Liver	1000	156.9	29.1
F3 2	SDS	Chick Liver	1000	72.6	15.4
F3 2	SDS	Egg	1000	545.7	66.0
F3 2	SDS	Adult Liver	2500	366.6	74.7
F3 2	SDS	Chick Liver	2500	510.7	213.0
F3 2	SDS	Egg	2500	1485.5	223.0
F3 2	DGMBE	Adult Liver	0	126.2	31.8
F3 2	DGMBE	Chick Liver	0	127.6	14.3
F3 2	DGMBE	Egg	0	18.9	4.8
F3 2	DGMBE	Adult Liver	100	180.3	53.7
F3 2	DGMBE	Chick Liver	100	501.3	49.4
F3 2	DGMBE	Egg	100	21.4	3.5
F3 2	DGMBE	Adult Liver	1000	265.7	42.1
F3 2	DGMBE	Chick Liver	1000	568.8	30.2
F3 2	DGMBE	Egg	1000	68.8	8.0
F3 2	DGMBE	Adult Liver	2500	208.3	49.3
F3 2	DGMBE	Chick Liver	2500	29.4	3.6
F3 2	DGMBE	Egg	2500	224.5	24.7
Reference Product	SDS	Adult Liver	0	31.6	4.0
Reference Product	SDS	Chick Liver	0	41.6	3.4
Reference Product	SDS	Egg	0	64.5	30.0
Reference Product	SDS	Adult Liver	100	93.8	23.7
Reference Product	SDS	Chick Liver	100	127.7	35.9
Reference Product	SDS	Egg	100	44.5	21.0
Reference Product	SDS	Adult Liver	1000	225.1	72.0
Reference Product	SDS	Chick Liver	1000	27.4	4.0
Reference Product	SDS	Egg	1000	239.2	87.5
Reference Product	SDS	Adult Liver	2500	160.2	51.4

Foam	Compound	Matrix	Treatment (mg/L)	Average Concentration (ng/g wet weight)	SEM Concentration (ng/g wet weight)
Reference Product	SDS	Chick Liver	2500	44.1	9.5
Reference Product	SDS	Egg	2500	6.4	1.8
Reference Product	PFBA	Adult Liver	0	0.0	0.0
Reference Product	PFBA	Chick Liver	0	0.0	0.0
Reference Product	PFBA	Egg	0	0.0	0.0
Reference Product	PFBA	Adult Liver	100	0.0	0.0
Reference Product	PFBA	Chick Liver	100	0.0	0.0
Reference Product	PFBA	Egg	100	0.0	0.0
Reference Product	PFBA	Adult Liver	1000	1.9	0.1
Reference Product	PFBA	Chick Liver	1000	2.2	0.0
Reference Product	PFBA	Egg	1000	0.3	0.0
Reference Product	PFBA	Adult Liver	2500	2.0	0.1
Reference Product	PFBA	Chick Liver	2500	2.1	0.1
Reference Product	PFBA	Egg	2500	0.3	0.0
Reference Product	PFHxA	Adult Liver	0	0.0	0.0
Reference Product	PFHxA	Chick Liver	0	0.0	0.0
Reference Product	PFHxA	Egg	0	0.0	0.0
Reference Product	PFHxA	Adult Liver	100	1.4	0.0
Reference Product	PFHxA	Chick Liver	100	1.5	0.0
Reference Product	PFHxA	Egg	100	0.1	0.0
Reference Product	PFHxA	Adult Liver	1000	1.4	0.1
Reference Product	PFHxA	Chick Liver	1000	3.4	1.2
Reference Product	PFHxA	Egg	1000	0.1	0.0
Reference Product	PFHxA	Adult Liver	2500	1.5	0.1
Reference Product	PFHxA	Chick Liver	2500	3.5	1.3
Reference Product	PFHxA	Egg	2500	0.6	0.1
F3 3	SDS	Adult Liver	0	27.0	4.2
F3 3	SDS	Chick Liver	0	198.2	69.6
F3 3	SDS	Egg	0	9.8	3.3
F3 3	SDS	Adult Liver	100	18.6	5.4
F3 3	SDS	Chick Liver	100	107.4	23.8
F3 3	SDS	Egg	100	9.7	1.9
F3 3	SDS	Adult Liver	1000	28.5	7.1
F3 3	SDS	Chick Liver	1000	100.2	15.3
F3 3	SDS	Egg	1000	29.4	4.1
F3 3	SDS	Adult Liver	2500	50.1	11.9

Foam	Compound	Matrix	Treatment (mg/L)	Average Concentration (ng/g wet weight)	SEM Concentration (ng/g wet weight)
F3 3	SDS	Chick Liver	2500	110.1	14.8
F3 3	SDS	Egg	2500	118.8	16.3
F3 3	DGMBE	Adult Liver	0	498.0	25.0
F3 3	DGMBE	Chick Liver	0	96.0	18.0
F3 3	DGMBE	Egg	0	55.0	6.0
F3 3	DGMBE	Adult Liver	100	619.0	87.0
F3 3	DGMBE	Chick Liver	100	165.0	35.0
F3 3	DGMBE	Egg	100	53.0	6.0
F3 3	DGMBE	Adult Liver	1000	608.0	53.0
F3 3	DGMBE	Chick Liver	1000	224.0	26.0
F3 3	DGMBE	Egg	1000	35.0	5.0
F3 3	DGMBE	Adult Liver	2500	588.0	84.0
F3 3	DGMBE	Chick Liver	2500	214.0	43.0
F3 3	DGMBE	Egg	2500	108.0	11.0
F3 4	SDS	Adult Liver	0	104.0	53.0
F3 4	SDS	Chick Liver	0	138.0	27.0
F3 4	SDS	Egg	0	16.0	1.1
F3 4	SDS	Adult Liver	100	55.0	15.0
F3 4	SDS	Chick Liver	100	98.0	10.0
F3 4	SDS	Egg	100	16.0	1.5
F3 4	SDS	Adult Liver	1000	170.0	62.0
F3 4	SDS	Chick Liver	1000	81.0	7.4
F3 4	SDS	Egg	1000	83.0	21.0
F3 4	SDS	Adult Liver	2500	450.0	98.0
F3 4	SDS	Chick Liver	2500	117.0	60.0
F3 4	SDS	Egg	2500	316.0	41.0
F3 4	DGMBE	Adult Liver	0	1372.0	94.0
F3 4	DGMBE	Chick Liver	0	1587.0	97.0
F3 4	DGMBE	Egg	0	570.0	16.0
F3 4	DGMBE	Adult Liver	100	836.0	104.0
F3 4	DGMBE	Chick Liver	100	1045.0	108.0
F3 4	DGMBE	Egg	100	587.0	11.0
F3 4	DGMBE	Adult Liver	1000	855.0	117.0
F3 4	DGMBE	Chick Liver	1000	103.0	343.0
F3 4	DGMBE	Egg	1000	851.0	75.0
F3 4	DGMBE	Adult Liver	2500	1302.0	53.0

Foam	Compound	Matrix	Treatment (mg/L)	Average Concentration (ng/g wet weight)	SEM Concentration (ng/g wet weight)
F3 4	DGMBE	Chick Liver	2500	794.0	85.0
F3 4	DGMBE	Egg	2500	1163.0	84.0
F3 5	SDS	Adult Liver	0	103.8	52.6
F3 5	SDS	Chick Liver	0	118.5	19.0
F3 5	SDS	Egg	0	17.6	1.9
F3 5	SDS	Adult Liver	100	396.5	217.0
F3 5	SDS	Chick Liver	100	120.0	21.7
F3 5	SDS	Egg	100	9.4	1.3
F3 5	SDS	Adult Liver	1000	156.2	62.9
F3 5	SDS	Chick Liver	1000	101.8	14.8
F3 5	SDS	Egg	1000	5.8	1.4
F3 5	SDS	Adult Liver	2500	173.4	40.7
F3 5	SDS	Chick Liver	2500	109.4	42.4
F3 5	SDS	Egg	2500	30.7	4.2
F3 6	DGMBE	Adult Liver	0	498.0	25.0
F3 6	DGMBE	Chick Liver	0	96.0	18.0
F3 6	DGMBE	Egg	0	55.0	6.0
F3 6	DGMBE	Adult Liver	100	516.0	73.0
F3 6	DGMBE	Chick Liver	100	348.0	66.0
F3 6	DGMBE	Egg	100	59.0	8.0
F3 6	DGMBE	Adult Liver	1000	383.0	70.0
F3 6	DGMBE	Chick Liver	1000	350.0	61.0
F3 6	DGMBE	Egg	1000	140.0	18.0
F3 6	DGMBE	Adult Liver	2500	300.0	45.0
F3 6	DGMBE	Chick Liver	2500	274.0	43.0
F3 6	DGMBE	Egg	2500	304.0	33.0
F3 7	SDS	Adult Liver	0	103.8	52.6
F3 7	SDS	Chick Liver	0	118.5	19.0
F3 7	SDS	Egg	0	17.6	1.9
F3 7	SDS	Adult Liver	100	38.7	10.8
F3 7	SDS	Chick Liver	100	52.6	6.8
F3 7	SDS	Egg	100	34.9	7.2
F3 7	SDS	Adult Liver	1000	56.3	10.3
F3 7	SDS	Chick Liver	1000	70.3	14.9
F3 7	SDS	Egg	1000	70.6	9.1
F3 7	SDS	Adult Liver	2500	102.5	33.5

Foam	Compound	Matrix	Treatment (mg/L)	Average Concentration (ng/g wet weight)	SEM Concentration (ng/g wet weight)
F3 7	SDS	Chick Liver	2500	69.6	7.8
F3 7	SDS	Egg	2500	175.9	18.4
F3 7	DGMBE	Adult Liver	0	1372.3	94.3
F3 7	DGMBE	Chick Liver	0	1664.5	63.5
F3 7	DGMBE	Egg	0	71.6	10.8
F3 7	DGMBE	Adult Liver	100	280.4	52.7
F3 7	DGMBE	Chick Liver	100	720.5	71.9
F3 7	DGMBE	Egg	100	94.1	13.0
F3 7	DGMBE	Adult Liver	1000	364.3	77.6
F3 7	DGMBE	Chick Liver	1000	717.1	78.4
F3 7	DGMBE	Egg	1000	136.3	20.3
F3 7	DGMBE	Adult Liver	2500	152.1	29.6
F3 7	DGMBE	Chick Liver	2500	849.0	102.8
F3 7	DGMBE	Egg	2500	219.8	28.7

Notes:
mg/L = milligrams per liter
ng/g = nanograms per gram
SEM = standard error of the mean
SDS = sodium dodecyl sulfate
DGMBE = Diethylene glycol monobutyl ether
PFBA = Perfluorobutanoic acid
PFHxA = Perfluorohexanoic acid

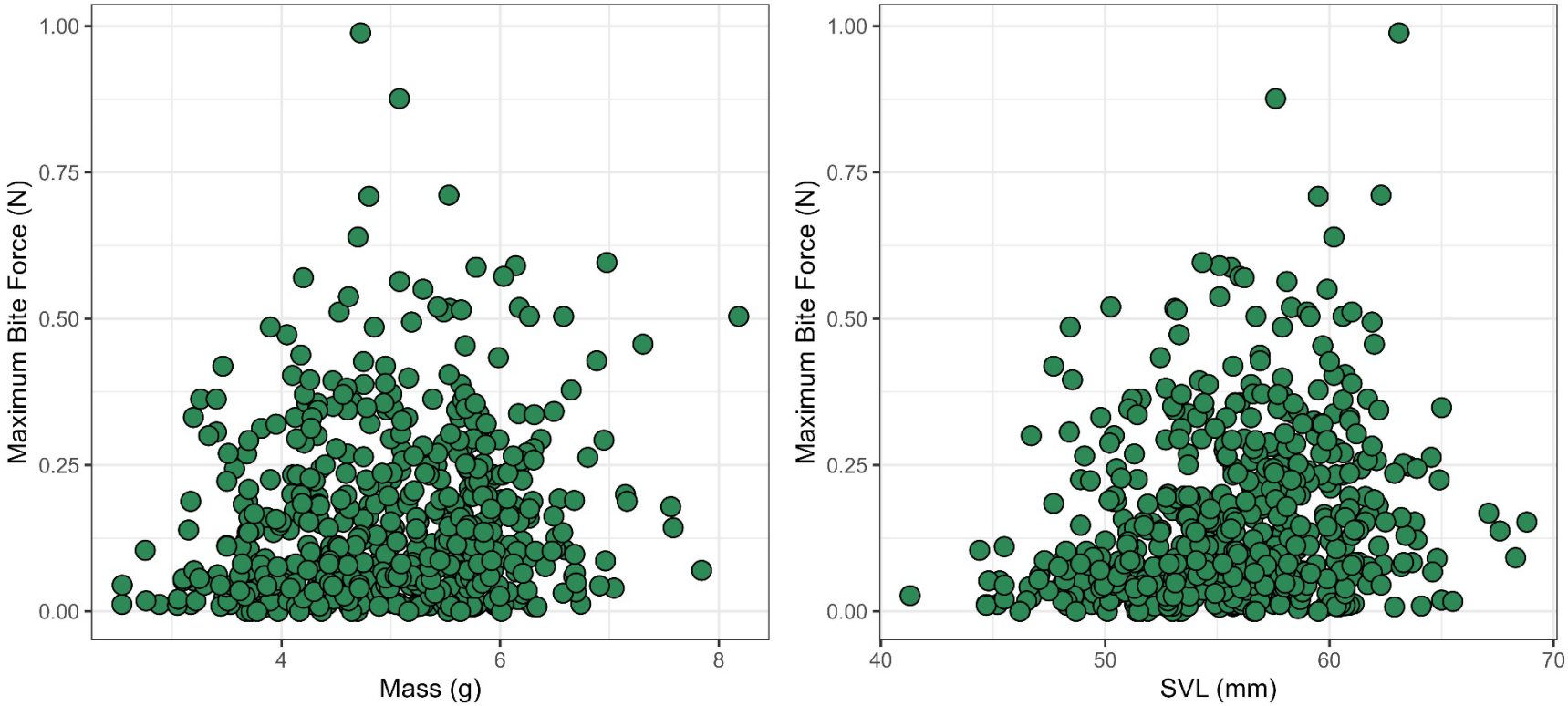

Figure A1. Relationship between mass, snout-vent length, and maximum bite force in brown anoles.

Table A19. Summary of brown anole weight, snout vent length, and condition index over the exposure period.

Foam	Week	Group	Average Weight (g)	SEM (g)	Average SVL (mm)	SEM (mm)	Average CI (g/mm)	SEM (g/mm)
F3 1	1	Control	4.13	0.359	52.8	1.707	0.077	0.004
F3 1	2	Control	4.28	0.399	52.0	1.197	0.082	0.006
F3 1	3	Control	4.31	0.381	54.3	1.416	0.078	0.005
F3 1	4	Control	4.41	0.409	55.2	1.190	0.079	0.006
F3 1	5	Control	4.64	0.392	55.3	1.208	0.083	0.005
F3 1	6	Control	4.83	0.399	55.1	1.319	0.087	0.006
F3 1	7	Control	4.89	0.376	56.1	1.046	0.087	0.005
F3 1	8	Control	4.93	0.416	56.4	0.532	0.087	0.007
F3 1	9	Control	5.05	0.365	56.3	1.108	0.089	0.005
F3 1	10	Control	5.10	0.391	54.5	1.236	0.093	0.006
F3 1	1	15mg	4.65	0.292	55.9	1.346	0.083	0.004
F3 1	2	15mg	4.97	0.317	56.4	1.393	0.088	0.004
F3 1	3	15mg	5.28	0.319	57.1	1.005	0.092	0.004
F3 1	4	15mg	5.37	0.267	57.8	1.053	0.093	0.004
F3 1	5	15mg	5.39	0.227	57.5	0.961	0.093	0.003
F3 1	6	15mg	5.56	0.223	59.3	0.880	0.094	0.003
F3 1	7	15mg	5.63	0.196	58.0	1.223	0.097	0.003
F3 1	8	15mg	5.58	0.227	58.3	1.203	0.096	0.003
F3 1	9	15mg	5.59	0.252	59.0	1.255	0.095	0.003
F3 1	10	15mg	5.63	0.255	59.7	1.214	0.094	0.003
F3 1	1	45mg	4.29	0.333	52.6	1.775	0.081	0.004
F3 1	2	45mg	4.41	0.349	53.2	1.389	0.082	0.005
F3 1	3	45mg	4.80	0.383	55.4	1.296	0.086	0.005
F3 1	4	45mg	5.05	0.405	55.8	1.770	0.089	0.005
F3 1	5	45mg	5.18	0.387	57.0	1.243	0.090	0.005
F3 1	6	45mg	5.39	0.305	57.3	1.529	0.094	0.003
F3 1	7	45mg	5.55	0.283	57.5	1.516	0.096	0.003
F3 1	8	45mg	5.70	0.269	58.1	1.116	0.098	0.003
F3 1	9	45mg	5.81	0.250	58.2	0.960	0.100	0.003
F3 1	10	45mg	5.78	0.261	58.8	1.135	0.098	0.003
F3 1	1	150mg	3.95	0.256	51.6	1.491	0.076	0.003
F3 1	2	150mg	3.95	0.250	52.4	1.409	0.075	0.004
F3 1	3	150mg	4.23	0.261	53.3	1.457	0.079	0.003
F3 1	4	150mg	4.39	0.278	53.1	1.417	0.082	0.004
F3 1	5	150mg	4.59	0.280	54.3	1.406	0.084	0.004
F3 1	6	150mg	4.78	0.268	53.5	1.419	0.089	0.003
F3 1	7	150mg	4.88	0.262	53.2	1.555	0.091	0.003
F3 1	8	150mg	5.06	0.256	56.0	1.355	0.090	0.003
F3 1	9	150mg	5.13	0.263	54.9	0.880	0.093	0.004
F3 1	10	150mg	5.16	0.269	55.1	1.465	0.093	0.003
F3 1	1	450mg	4.00	0.318	52.0	1.574	0.076	0.004
F3 1	2	450mg	3.99	0.292	51.6	1.419	0.077	0.004
F3 1	3	450mg	4.24	0.343	51.2	1.415	0.082	0.005
F3 1	4	450mg	4.31	0.320	51.3	1.193	0.083	0.005

Foam	Week	Group	Average Weight (g)	SEM (g)	Average SVL (mm)	SEM (mm)	Average CI (g/mm)	SEM (g/mm)
F3 1	5	450mg	4.45	0.317	51.7	1.094	0.086	0.005
F3 1	6	450mg	4.56	0.311	53.7	1.165	0.085	0.005
F3 1	7	450mg	4.76	0.298	54.3	1.059	0.087	0.004
F3 1	8	450mg	4.98	0.233	54.6	1.070	0.091	0.003
F3 1	9	450mg	4.97	0.271	52.6	1.371	0.094	0.004
F3 1	10	450mg	4.92	0.273	55.1	0.995	0.089	0.004
Reference Product	1	Control	4.05	0.237	50.9	0.888	0.079	0.004
Reference Product	2	Control	4.33	0.232	50.3	0.868	0.086	0.004
Reference Product	3	Control	4.74	0.200	50.2	0.799	0.094	0.003
Reference Product	4	Control	4.98	0.193	53.4	0.825	0.093	0.003
Reference Product	5	Control	5.31	0.174	53.7	0.685	0.099	0.003
Reference Product	6	Control	5.41	0.144	54.5	0.756	0.099	0.003
Reference Product	7	Control	5.60	0.176	53.1	0.849	0.106	0.003
Reference Product	8	Control	5.72	0.185	52.7	0.840	0.108	0.003
Reference Product	9	Control	5.93	0.175	51.9	0.780	0.114	0.003
Reference Product	1	15mg	3.98	0.408	50.2	1.639	0.078	0.006
Reference Product	2	15mg	4.36	0.447	50.4	2.086	0.085	0.006
Reference Product	3	15mg	4.66	0.437	51.3	1.378	0.089	0.006
Reference Product	4	15mg	4.84	0.415	52.4	1.584	0.091	0.006
Reference Product	5	15mg	5.10	0.361	52.7	1.212	0.096	0.005
Reference Product	6	15mg	5.27	0.340	53.2	1.146	0.098	0.005
Reference Product	7	15mg	5.36	0.353	53.4	0.643	0.100	0.006
Reference Product	8	15mg	5.44	0.312	52.0	1.181	0.104	0.005
Reference Product	9	15mg	5.23	0.365	51.5	0.784	0.101	0.006
Reference Product	1	45mg	4.23	0.292	50.2	1.245	0.084	0.004
Reference Product	2	45mg	4.52	0.291	52.4	1.407	0.086	0.004
Reference Product	3	45mg	4.80	0.294	52.3	1.134	0.091	0.004
Reference Product	4	45mg	4.90	0.301	53.3	1.605	0.091	0.003
Reference Product	5	45mg	5.23	0.307	53.8	0.757	0.097	0.005
Reference Product	6	45mg	5.32	0.331	53.8	1.123	0.098	0.005
Reference Product	7	45mg	5.63	0.310	53.0	1.243	0.106	0.004
Reference Product	8	45mg	5.63	0.312	53.2	1.103	0.105	0.004
Reference Product	9	45mg	5.76	0.322	52.8	1.342	0.109	0.005
Reference Product	1	150mg	4.37	0.254	51.6	0.985	0.084	0.004
Reference Product	2	150mg	4.66	0.262	52.2	0.808	0.089	0.004
Reference Product	3	150mg	5.05	0.283	53.3	0.774	0.095	0.005
Reference Product	4	150mg	5.14	0.258	53.3	0.854	0.096	0.004
Reference Product	5	150mg	5.45	0.287	53.4	0.722	0.102	0.005
Reference Product	6	150mg	5.54	0.283	53.6	0.745	0.103	0.005
Reference Product	7	150mg	5.73	0.319	53.2	0.635	0.107	0.005
Reference Product	8	150mg	5.70	0.289	52.1	0.894	0.109	0.004
Reference Product	9	150mg	5.87	0.240	53.0	0.986	0.111	0.004
Reference Product	1	450mg	4.08	0.429	49.9	1.727	0.080	0.006
Reference Product	2	450mg	4.45	0.413	50.1	1.788	0.086	0.006
Reference Product	3	450mg	4.55	0.417	52.7	1.759	0.085	0.005
Reference Product	4	450mg	4.75	0.432	51.9	1.263	0.091	0.007

Foam	Week	Group	Average Weight (g)	SEM (g)	Average SVL (mm)	SEM (mm)	Average CI (g/mm)	SEM (g/mm)
Reference Product	5	450mg	5.16	0.398	52.3	1.010	0.098	0.006
Reference Product	6	450mg	5.27	0.387	52.5	1.205	0.100	0.005
Reference Product	7	450mg	5.43	0.360	52.8	1.241	0.102	0.005
Reference Product	8	450mg	5.55	0.370	52.8	1.354	0.104	0.005
Reference Product	9	450mg	5.60	0.392	53.8	1.320	0.103	0.005
F3 2	1	Control	4.49	0.183	52.0	1.323	0.086	0.003
F3 2	2	Control	4.54	0.220	54.0	0.707	0.084	0.003
F3 2	3	Control	4.62	0.243	54.5	1.062	0.085	0.003
F3 2	4	Control	4.80	0.229	54.4	0.870	0.088	0.003
F3 2	5	Control	4.82	0.209	54.9	1.533	0.088	0.004
F3 2	6	Control	4.78	0.255	57.8	0.987	0.083	0.004
F3 2	7	Control	4.78	0.265	57.7	0.922	0.082	0.004
F3 2	8	Control	4.88	0.228	58.5	0.604	0.083	0.003
F3 2	9	Control	4.82	0.221	57.4	1.074	0.084	0.004
F3 2	10	Control	4.89	0.222	NA	NA	NA	NA
F3 2	1	15mg	4.70	0.176	53.9	0.967	0.087	0.002
F3 2	2	15mg	4.90	0.182	53.4	0.809	0.092	0.003
F3 2	3	15mg	5.24	0.177	54.9	0.696	0.095	0.003
F3 2	4	15mg	5.28	0.203	55.2	0.827	0.095	0.003
F3 2	5	15mg	5.22	0.213	58.1	0.975	0.090	0.003
F3 2	6	15mg	5.27	0.216	57.2	0.547	0.092	0.003
F3 2	7	15mg	5.26	0.227	57.7	1.046	0.091	0.003
F3 2	8	15mg	5.36	0.209	58.7	0.790	0.091	0.003
F3 2	9	15mg	5.39	0.210	64.3	1.109	0.084	0.003
F3 2	10	15mg	5.24	0.225	NA	NA	NA	NA
F3 2	1	45mg	4.33	0.237	52.1	1.269	0.083	0.003
F3 2	2	45mg	4.43	0.191	51.8	1.053	0.086	0.004
F3 2	3	45mg	4.56	0.225	53.4	0.828	0.085	0.004
F3 2	4	45mg	4.71	0.274	54.0	0.843	0.087	0.004
F3 2	5	45mg	4.69	0.264	57.0	0.939	0.082	0.004
F3 2	6	45mg	4.77	0.270	56.6	0.942	0.084	0.004
F3 2	7	45mg	4.78	0.272	57.7	0.963	0.082	0.004
F3 2	8	45mg	4.94	0.271	56.7	1.023	0.087	0.003
F3 2	9	45mg	4.95	0.281	58.2	0.985	0.085	0.004
F3 2	10	45mg	4.91	0.272	NA	NA	NA	NA
F3 2	1	150mg	4.59	0.241	54.2	0.991	0.084	0.004
F3 2	2	150mg	4.70	0.197	56.3	0.748	0.083	0.003
F3 2	3	150mg	4.84	0.203	56.0	0.978	0.086	0.003
F3 2	4	150mg	5.06	0.187	55.1	1.026	0.092	0.003
F3 2	5	150mg	5.09	0.167	56.9	0.888	0.090	0.003
F3 2	6	150mg	5.05	0.249	57.1	0.967	0.088	0.004
F3 2	7	150mg	5.28	0.203	60.0	1.217	0.088	0.003
F3 2	8	150mg	5.40	0.192	59.1	1.293	0.092	0.003
F3 2	9	150mg	5.56	0.160	60.5	0.918	0.092	0.002
F3 2	10	150mg	5.64	0.177	NA	NA	NA	NA
F3 2	1	450mg	4.61	0.180	54.4	0.771	0.085	0.003

Foam	Week	Group	Average Weight (g)	SEM (g)	Average SVL (mm)	SEM (mm)	Average CI (g/mm)	SEM (g/mm)
F3 2	2	450mg	4.84	0.170	55.2	0.852	0.088	0.003
F3 2	3	450mg	4.91	0.198	54.9	0.773	0.089	0.003
F3 2	4	450mg	5.06	0.170	55.9	0.736	0.090	0.003
F3 2	5	450mg	5.03	0.168	57.5	0.832	0.088	0.003
F3 2	6	450mg	5.04	0.158	56.8	0.642	0.089	0.003
F3 2	7	450mg	5.08	0.150	58.4	0.795	0.087	0.002
F3 2	8	450mg	5.23	0.143	59.2	0.851	0.088	0.002
F3 2	9	450mg	5.33	0.154	59.4	0.721	0.090	0.002
F3 2	10	450mg	5.32	0.133	NA	NA	NA	NA
F3 3	1	Control	3.49	0.325	47.4	1.429	0.073	0.005
F3 3	2	Control	3.76	0.315	48.5	1.421	0.077	0.005
F3 3	3	Control	4.06	0.340	48.8	1.434	0.082	0.005
F3 3	4	Control	4.46	0.348	49.7	1.387	0.089	0.005
F3 3	5	Control	4.33	0.325	50.5	1.243	0.088	0.005
F3 3	6	Control	4.43	0.337	50.4	0.961	0.087	0.005
F3 3	7	Control	4.50	0.311	52.1	1.191	0.086	0.004
F3 3	8	Control	4.58	0.310	52.1	1.381	0.087	0.004
F3 3	9	Control	4.80	0.359	53.5	1.017	0.089	0.005
F3 3	1	15mg	3.36	0.386	47.4	1.869	0.069	0.005
F3 3	2	15mg	3.69	0.350	47.8	1.388	0.076	0.005
F3 3	3	15mg	3.85	0.368	48.6	1.699	0.078	0.005
F3 3	4	15mg	4.21	0.377	49.0	1.528	0.085	0.005
F3 3	5	15mg	4.32	0.376	49.9	1.495	0.085	0.005
F3 3	6	15mg	4.43	0.362	50.8	1.325	0.086	0.005
F3 3	7	15mg	4.54	0.383	50.4	1.310	0.089	0.006
F3 3	8	15mg	4.62	0.372	51.3	1.665	0.089	0.005
F3 3	9	15mg	4.76	0.401	52.6	1.385	0.089	0.005
F3 3	1	45mg	3.23	0.290	44.6	1.156	0.072	0.005
F3 3	2	45mg	3.52	0.282	47.6	1.082	0.073	0.004
F3 3	3	45mg	3.63	0.246	47.7	1.151	0.075	0.004
F3 3	4	45mg	4.08	0.254	49.3	1.228	0.082	0.004
F3 3	5	45mg	4.07	0.269	50.2	1.174	0.081	0.004
F3 3	6	45mg	4.17	0.251	50.7	0.943	0.082	0.004
F3 3	7	45mg	4.20	0.231	51.0	0.999	0.082	0.003
F3 3	8	45mg	4.38	0.237	51.6	1.281	0.085	0.003
F3 3	9	45mg	4.64	0.244	52.4	0.787	0.087	0.003
F3 3	1	150mg	3.17	0.256	44.5	1.173	0.071	0.004
F3 3	2	150mg	3.56	0.223	47.0	1.351	0.075	0.003
F3 3	3	150mg	3.84	0.228	47.8	1.423	0.080	0.003
F3 3	4	150mg	4.10	0.253	50.6	1.406	0.081	0.003
F3 3	5	150mg	4.25	0.208	51.1	1.194	0.083	0.002
F3 3	6	150mg	4.34	0.232	51.1	1.025	0.084	0.003
F3 3	7	150mg	4.42	0.215	51.0	1.003	0.086	0.003
F3 3	8	150mg	4.62	0.206	51.5	0.711	0.089	0.003
F3 3	9	150mg	4.66	0.230	51.5	1.514	0.090	0.003
F3 3	1	450mg	3.31	0.283	48.3	0.930	0.068	0.005

Foam	Week	Group	Average Weight (g)	SEM (g)	Average SVL (mm)	SEM (mm)	Average CI (g/mm)	SEM (g/mm)
F3 3	2	450mg	3.50	0.290	47.8	1.255	0.072	0.004
F3 3	3	450mg	3.87	0.369	48.9	1.084	0.078	0.006
F3 3	4	450mg	3.97	0.325	50.3	1.255	0.078	0.005
F3 3	5	450mg	4.01	0.310	51.4	0.928	0.078	0.005
F3 3	6	450mg	4.07	0.292	50.9	0.868	0.079	0.004
F3 3	7	450mg	4.05	0.280	51.4	1.358	0.078	0.003
F3 3	8	450mg	4.17	0.303	51.8	0.922	0.080	0.005
F3 3	9	450mg	4.40	0.313	52.1	1.068	0.084	0.005
F3 4	1	Control	4.36	0.273	NA	NA	NA	NA
F3 4	2	Control	4.24	0.262	56.4	0.718	0.075	0.004
F3 4	3	Control	4.21	0.257	56.3	0.704	0.075	0.004
F3 4	4	Control	4.14	0.274	56.6	0.598	0.073	0.004
F3 4	5	Control	4.00	0.274	57.9	0.885	0.069	0.004
F3 4	6	Control	4.26	0.262	57.9	0.631	0.073	0.004
F3 4	7	Control	4.19	0.227	56.9	0.484	0.073	0.004
F3 4	8	Control	4.20	0.228	56.4	0.559	0.075	0.004
F3 4	9	Control	4.40	0.237	57.5	0.879	0.076	0.004
F3 4	10	Control	4.50	0.253	56.9	0.519	0.079	0.004
F3 4	1	15mg	4.42	0.296	NA	NA	NA	NA
F3 4	2	15mg	4.24	0.273	55.5	1.066	0.076	0.004
F3 4	3	15mg	4.29	0.273	55.2	0.984	0.077	0.004
F3 4	4	15mg	4.23	0.243	55.8	0.932	0.076	0.004
F3 4	5	15mg	4.20	0.238	56.0	0.926	0.075	0.004
F3 4	6	15mg	4.23	0.219	57.2	0.739	0.074	0.003
F3 4	7	15mg	4.21	0.219	56.0	0.980	0.075	0.003
F3 4	8	15mg	4.28	0.208	56.9	0.846	0.075	0.003
F3 4	9	15mg	4.42	0.220	56.7	1.258	0.078	0.003
F3 4	10	15mg	4.59	0.239	57.2	1.083	0.080	0.003
F3 4	1	45mg	4.81	0.208	NA	NA	NA	NA
F3 4	2	45mg	4.64	0.178	58.0	1.058	0.080	0.002
F3 4	3	45mg	4.60	0.175	58.0	0.913	0.079	0.002
F3 4	4	45mg	4.65	0.152	58.2	1.028	0.080	0.002
F3 4	5	45mg	4.59	0.180	58.2	0.959	0.079	0.003
F3 4	6	45mg	4.70	0.186	59.0	0.690	0.080	0.003
F3 4	7	45mg	4.80	0.184	58.8	0.807	0.081	0.002
F3 4	8	45mg	4.79	0.178	59.1	1.191	0.081	0.002
F3 4	9	45mg	5.03	0.195	59.1	1.024	0.085	0.003
F3 4	10	45mg	5.03	0.196	59.5	0.866	0.084	0.002
F3 4	1	150mg	4.46	0.252	NA	NA	NA	NA
F3 4	2	150mg	4.27	0.230	56.8	1.190	0.075	0.003
F3 4	3	150mg	4.27	0.221	56.8	0.891	0.075	0.003
F3 4	4	150mg	4.23	0.212	57.4	0.734	0.074	0.003
F3 4	5	150mg	4.17	0.197	56.7	0.991	0.073	0.003
F3 4	6	150mg	4.19	0.211	58.7	1.016	0.071	0.003
F3 4	7	150mg	4.26	0.172	57.9	0.828	0.073	0.002
F3 4	8	150mg	4.27	0.172	57.9	0.586	0.074	0.003

Foam	Week	Group	Average Weight (g)	SEM (g)	Average SVL (mm)	SEM (mm)	Average CI (g/mm)	SEM (g/mm)
F3 4	9	150mg	4.41	0.141	57.8	0.767	0.076	0.002
F3 4	10	150mg	4.47	0.216	58.2	1.206	0.077	0.003
F3 4	1	450mg	4.66	0.305	NA	NA	NA	NA
F3 4	2	450mg	4.52	0.293	57.0	1.408	0.079	0.003
F3 4	3	450mg	4.60	0.298	58.3	1.181	0.078	0.004
F3 4	4	450mg	4.44	0.266	56.9	1.725	0.078	0.003
F3 4	5	450mg	4.27	0.244	58.1	1.359	0.073	0.003
F3 4	6	450mg	4.35	0.261	58.4	1.187	0.074	0.004
F3 4	7	450mg	4.32	0.250	58.1	1.355	0.074	0.003
F3 4	8	450mg	4.25	0.289	56.1	1.186	0.075	0.004
F3 4	9	450mg	4.50	0.276	57.2	1.123	0.079	0.005
F3 4	10	450mg	4.58	0.342	59.2	1.649	0.077	0.004
F3 6	1	Control	5.32	0.200	56.9	0.885	0.093	0.003
F3 6	2	Control	5.31	0.199	57.9	0.897	0.092	0.003
F3 6	3	Control	5.40	0.204	56.4	0.754	0.096	0.003
F3 6	4	Control	5.60	0.222	57.2	0.651	0.098	0.003
F3 6	5	Control	5.65	0.254	54.6	0.554	0.103	0.004
F3 6	6	Control	5.60	0.206	55.1	0.702	0.102	0.003
F3 6	7	Control	5.53	0.240	55.1	0.543	0.100	0.004
F3 6	8	Control	5.67	0.299	60.2	0.669	0.094	0.005
F3 6	9	Control	5.62	0.320	58.6	0.530	0.096	0.005
F3 6	10	Control	5.75	0.364	NA	NA	NA	NA
F3 6	1	15mg	5.28	0.142	58.1	1.134	0.093	0.003
F3 6	2	15mg	5.20	0.141	57.5	0.704	0.090	0.002
F3 6	3	15mg	5.42	0.146	57.2	0.632	0.095	0.002
F3 6	4	15mg	5.68	0.140	56.1	0.652	0.101	0.002
F3 6	5	15mg	5.62	0.162	62.1	0.938	0.091	0.003
F3 6	6	15mg	5.75	0.134	55.9	0.689	0.103	0.002
F3 6	7	15mg	5.75	0.159	55.6	0.266	0.103	0.003
F3 6	8	15mg	5.80	0.139	60.2	0.694	0.096	0.002
F3 6	9	15mg	5.75	0.193	57.6	0.561	0.100	0.003
F3 6	10	15mg	6.01	0.154	NA	NA	NA	NA
F3 6	1	45mg	4.93	0.230	57.2	0.896	0.086	0.004
F3 6	2	45mg	5.15	0.248	56.6	0.893	0.091	0.004
F3 6	3	45mg	5.33	0.227	56.8	0.722	0.094	0.004
F3 6	4	45mg	5.38	0.231	55.6	0.732	0.097	0.004
F3 6	5	45mg	5.30	0.240	58.2	0.911	0.091	0.004
F3 6	6	45mg	5.32	0.270	57.3	1.241	0.093	0.004
F3 6	7	45mg	5.30	0.270	56.8	0.685	0.093	0.005
F3 6	8	45mg	5.34	0.312	57.2	0.991	0.093	0.005
F3 6	9	45mg	5.38	0.262	56.3	1.077	0.095	0.004
F3 6	10	45mg	5.54	0.292	NA	NA	NA	NA
F3 6	1	150mg	5.30	0.252	57.3	1.039	0.092	0.004
F3 6	2	150mg	5.31	0.244	57.9	1.078	0.092	0.004
F3 6	3	150mg	5.45	0.245	56.4	0.700	0.097	0.005
F3 6	4	150mg	5.63	0.236	57.9	1.094	0.097	0.004

Foam	Week	Group	Average Weight (g)	SEM (g)	Average SVL (mm)	SEM (mm)	Average CI (g/mm)	SEM (g/mm)
F3 6	5	150mg	5.53	0.252	58.2	0.957	0.095	0.004
F3 6	6	150mg	5.41	0.259	56.3	0.891	0.096	0.005
F3 6	7	150mg	5.44	0.243	56.9	1.080	0.096	0.004
F3 6	8	150mg	5.53	0.242	59.2	1.012	0.093	0.004
F3 6	9	150mg	5.70	0.212	56.1	0.744	0.102	0.004
F3 6	10	150mg	5.81	0.202	NA	NA	NA	NA
F3 6	1	450mg	4.96	0.216	57.2	1.113	0.087	0.003
F3 6	2	450mg	5.08	0.302	58.1	0.813	0.087	0.004
F3 6	3	450mg	5.32	0.248	56.3	0.737	0.094	0.004
F3 6	4	450mg	5.39	0.274	53.9	0.944	0.100	0.005
F3 6	5	450mg	5.60	0.266	56.2	0.621	0.100	0.005
F3 6	6	450mg	5.32	0.274	58.0	0.849	0.092	0.005
F3 6	7	450mg	5.39	0.281	58.1	1.065	0.093	0.005
F3 6	8	450mg	5.38	0.280	59.7	1.008	0.090	0.004
F3 6	9	450mg	5.53	0.266	57.5	0.951	0.096	0.004
F3 6	10	450mg	5.61	0.265	NA	NA	NA	NA

Notes:
mg/L = milligrams per liter
g = grams
mm = millimeters
SEM = standard error of the mean
SVL = snout-vent length
CI = condition index
g/mm = grams per millimeter

Table A20. Summary of brown anole organ masses normalized to body weight.

Chemical	Treatment	Normalized Average Liver Weight (g)	Liver SEM (g)	Normalized Average Muscle Weight (g)	Muscle SEM (g)	Normalized Average Gonad Weigh (g)	Gonad SEM (g)	Normalized Average GI Tract Weight (g)	GI Tract SEM (g)
F3 1	Control	0.0394	0.0152	0.0206	0.0023	0.0632	0.0321	0.0380	0.00239
F3 1	15 mg/L	0.0202	0.0016	0.0263	0.0132	0.0151	0.0042	0.0297	0.00347
F3 1	45 mg/L	0.0193	0.0029	0.0209	0.0066	0.0228	0.0107	0.0262	0.00257
F3 1	150 mg/L	0.0204	0.0021	0.0162	0.0016	0.0164	0.0021	0.0326	0.00260
F3 1	450 mg/L	0.0160	0.0018	0.0164	0.0021	0.0307	0.0160	0.0360	0.00352
F3 2	Control	0.0413	0.0143	0.0129	0.0008	0.0224	0.0020	0.0397	0.00351
F3 2	15 mg/L	0.0275	0.0020	0.0171	0.0011	0.0215	0.0012	0.0398	0.00237
F3 2	45 mg/L	0.0228	0.0016	0.0152	0.0025	0.0209	0.0013	0.0587	0.01740
F3 2	150 mg/L	0.0217	0.0014	0.0171	0.0015	0.0225	0.0017	0.0438	0.00226
F3 2	450 mg/L	0.0254	0.0016	0.0159	0.0013	0.0232	0.0013	0.0417	0.00300
Reference Product	Control	0.0194	0.0014	0.0197	0.0028	0.0222	0.0007	0.0369	0.00252
Reference Product	15 mg/L	0.0213	0.0023	0.0204	0.0022	0.0255	0.0027	0.0364	0.00356
Reference Product	45 mg/L	0.0222	0.0014	0.0207	0.0021	0.0193	0.0014	0.0379	0.00316
Reference Product	150 mg/L	0.0221	0.0012	0.0211	0.0012	0.0193	0.0018	0.0383	0.00340
Reference Product	450 mg/L	0.0242	0.0014	0.0209	0.0015	0.0216	0.0013	0.0386	0.00282
F3 3	Control	0.0303	0.0027	0.0098	0.0031	0.0353	0.0165	0.0271	0.00088
F3 3	15 mg/L	0.0423	0.0144	0.0101	0.0012	0.0385	0.0152	0.0374	0.00292
F3 3	45 mg/L	0.0246	0.0030	0.0164	0.0030	0.0183	0.0008	0.0352	0.00365
F3 3	150 mg/L	0.0240	0.0025	0.0192	0.0051	0.0218	0.0018	0.0373	0.00609
F3 3	450 mg/L	0.0286	0.0019	0.0153	0.0019	0.0223	0.0022	0.0402	0.00383
F3 4	Control	0.0285	0.0126	0.0023	0.0005	0.0150	0.0014	0.0416	0.01009
F3 4	15 mg/L	0.0127	0.0020	0.0048	0.0009	0.0141	0.0015	0.0223	0.00279
F3 4	45 mg/L	0.0167	0.0016	0.0054	0.0015	0.0165	0.0019	0.0206	0.00206

Table A20. Summary of brown anole organ masses normalized to body weight.

Chemical	Treatment	Normalized Average Liver Weight (g)	Liver SEM (g)	Normalized Average Muscle Weight (g)	Muscle SEM (g)	Normalized Average Gonad Weigh (g)	Gonad SEM (g)	Normalized Average GI Tract Weight (g)	GI Tract SEM (g)
F3 4	150 mg/L	0.0176	0.0012	0.0055	0.0012	0.0164	0.0029	0.0363	0.00466
F3 4	450 mg/L	0.0242	0.0039	0.0051	0.0012	0.0187	0.0036	0.0269	0.00473
F3 6	Control	0.0224	0.0020	0.0152	0.0009	0.0188	0.0014	0.0466	0.00443
F3 6	15 mg/L	0.0212	0.0011	0.0189	0.0022	0.0182	0.0013	0.0464	0.00244
F3 6	45 mg/L	0.0201	0.0012	0.0163	0.0020	0.0176	0.0011	0.0486	0.00199
F3 6	150 mg/L	0.0199	0.0007	0.0158	0.0016	0.0163	0.0010	0.0457	0.00283
F3 6	450 mg/L	0.0200	0.0008	0.0153	0.0011	0.0323	0.0137	0.0444	0.00471

Notes:
mg/L = milligrams per liter
g = grams
GI = gastrointestinal
SEM = standard error of the mean

Table A21. Summary of brown anole water loss at days 30 and 60.

Foam	Group	Average day 30 CEWL ($g/m^2/h$)	SEM day 30 ($g/m^2/h$)	Average day 60 CEWL ($g/m^2/h$)	SEM day 60 ($g/m^2/h$)
F3 1	Control	15.1	1.41	12.0	0.489
F3 1	15mg/L	13.9	0.924	12.3	0.432
F3 1	45mg/L	12.2	0.562	12.6	0.784
F3 1	150mg/L	12.4	0.587	12.2	0.384
F3 1	450mg/L	8.87	0.248	14.4	1.45
F3 2	Control	15.0	1.53	13.1	0.497
F3 2	15mg/L	11.1	0.835	11.3	0.849
F3 2	45mg/L	11.2	0.655	11.2	1.21
F3 2	150mg/L	14.7	0.281	9.60	0.545
F3 2	450mg/L	12.4	0.579	8.91	0.578
Reference Product	Control	13.7	0.680	12.1	0.470
Reference Product	15mg/L	11.8	0.266	12.8	0.386
Reference Product	45mg/L	13.1	0.885	11.9	0.973
Reference Product	150mg/L	12.0	0.424	12.2	0.691
Reference Product	450mg/L	12.7	0.737	11.1	0.466
F3 3	Control	10.3	1.29	9.93	0.849
F3 3	15mg/L	10.8	0.970	9.73	1.40
F3 3	45mg/L	10.3	0.648	10.73	1.79
F3 3	150mg/L	10.6	1.23	8.50	0.805
F3 3	450mg/L	10.5	0.706	9.27	0.464
F3 4	Control	13.1	1.87	13.9	1.21
F3 4	15mg/L	17.2	3.19	11.5	1.02
F3 4	45mg/L	13.1	1.59	16.9	5.79
F3 4	150mg/L	13.9	1.99	11.6	1.05
F3 4	450mg/L	12.2	1.15	16.1	3.63
F3 6	Control	10.1	0.948	11.6	1.10
F3 6	15mg/L	10.2	0.451	11.9	1.07
F3 6	45mg/L	9.50	0.808	11.5	0.652
F3 6	150mg/L	10.5	0.604	11.3	0.318
F3 6	450mg/L	13.4	1.78	13.5	4.19

Notes:
mg/L = milligrams per liter
CEWL = Cutaneous Evaporative Water Loss
$g/m^2/h$ = grams per square meter per hour
SEM = standard error of the mean.

Table A22. Summary of brown anole bite force at days 30 and 60.

Foam	Treatment	Time (days)	Average Bite Force (N)	SEM Bite Force (N)
F3 1	Control	30	0.268	0.032
F3 1	Control	60	0.196	0.018
F3 1	15 mg/L	30	0.297	0.035
F3 1	15 mg/L	60	0.223	0.025
F3 1	45 mg/L	30	0.328	0.023
F3 1	45 mg/L	60	0.224	0.032
F3 1	150 mg/L	30	0.329	0.012
F3 1	150 mg/L	60	0.149	0.014
F3 1	450 mg/L	30	0.360	0.036
F3 1	450 mg/L	60	0.090	0.013
F3 2	Control	30	0.265	0.036
F3 2	Control	60	0.166	0.014
F3 2	15 mg/L	30	0.231	0.034
F3 2	15 mg/L	60	0.140	0.011
F3 2	45 mg/L	30	0.232	0.028
F3 2	45 mg/L	60	0.156	0.024
F3 2	150 mg/L	30	0.356	0.051
F3 2	150 mg/L	60	0.225	0.021
F3 2	450 mg/L	30	0.440	0.039
F3 2	450 mg/L	60	0.208	0.018
Reference Product	Control	30	0.088	0.023
Reference Product	Control	60	0.155	0.043
Reference Product	15 mg/L	30	0.137	0.046
Reference Product	15 mg/L	60	0.167	0.059
Reference Product	45 mg/L	30	0.063	0.014
Reference Product	45 mg/L	60	0.107	0.014
Reference Product	150 mg/L	30	0.087	0.018
Reference Product	150 mg/L	60	0.191	0.041
Reference Product	450 mg/L	30	0.098	0.024
Reference Product	450 mg/L	60	0.244	0.053
F3 3	Control	30	0.073	0.036
F3 3	Control	60	0.020	0.009
F3 3	15 mg/L	30	0.047	0.008
F3 3	15 mg/L	60	0.017	0.013
F3 3	45 mg/L	30	0.0522	
F3 3	45 mg/L	60	0.037	0.018
F3 3	150 mg/L	30	0.032	0.015
F3 3	150 mg/L	60	0.042	0.008
F3 3	450 mg/L	30	0.012	0.004
F3 3	450 mg/L	60	0.045	0.005

Table A22. Summary of brown anole bite force at days 30 and 60.

Foam	Treatment	Time (days)	Average Bite Force (N)	SEM Bite Force (N)
F3 4	Control	30	0.113	0.019
F3 4	Control	60	0.084	0.017
F3 4	15 mg/L	30	0.187	0.071
F3 4	15 mg/L	60	0.0898	0.030
F3 4	45 mg/L	30	0.167	0.044
F3 4	45 mg/L	60	0.243	0.053
F3 4	150 mg/L	30	0.206	0.053
F3 4	150 mg/L	60	0.308	0.109
F3 4	450 mg/L	30	0.195	0.075
F3 4	450 mg/L	60	0.232	0.056
F3 6	Control	30	0.133	0.028
F3 6	Control	60	0.074	0.009
F3 6	15 mg/L	30	0.055	0.027
F3 6	15 mg/L	60	0.113	0.031
F3 6	150 mg/L	30	0.015	0.003
F3 6	150 mg/L	60	0.255	0.065
F3 6	45 mg/L	30	0.0121	0.002
F3 6	45 mg/L	60	0.139	0.018
F3 6	450 mg/L	30	0.0123	0.001
F3 6	450 mg/L	60	0.190	0.035

Notes:
mg/L = milligrams per liter
N = Newtons
SEM = standard error of the mean

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Table A23. Summary of brown anole liver chemical constituent concentrations

Foam	Treatment	Compound	Average Measured Concentration (ng/g wet weight)	SEM (ng/g wet weight)
F3 1	Control	DGMBE	nd	nd
F3 1	150 mg/L	DGMBE	nd	nd
F3 1	450 mg/L	DGMBE	nd	nd
F3 1	Control	SDS	65.2	25.3
F3 1	150 mg/L	SDS	35.7	15.1
F3 1	450 mg/L	SDS	17.5	8.7
F3 2	Control	DGMBE	4.63	4.63
F3 2	150 mg/L	DGMBE	16.2	14.7
F3 2	450 mg/L	DGMBE	109.6	58.9
F3 2	Control	SDS	45.4	30.3
F3 2	150 mg/L	SDS	12.5	6.69
F3 2	450 mg/L	SDS	11.7	7.15
F3 3	Control	DGMBE	6.18	6.18
F3 3	150 mg/L	DGMBE	27.6	27.6
F3 3	450 mg/L	DGMBE	69.0	69.0
F3 3	Control	SDS	26.8	7.2
F3 3	150 mg/L	SDS	12.0	4.1
F3 3	450 mg/L	SDS	9.2	7.5
F3 4	Control	DGMBE	77.3	77.3
F3 4	150 mg/L	DGMBE	nd	nd
F3 4	450 mg/L	DGMBE	0.7	0.7
F3 4	Control	SDS	26.1	25.6
F3 4	150 mg/L	SDS	32.2	11.3
F3 4	450 mg/L	SDS	13.1	13.1
F3 6	Control	DGMBE	261.2	107.1
F3 6	150 mg/L	DGMBE	13.8	13.0
F3 6	450 mg/L	DGMBE	7.3	6.4

Table A23. Summary of brown anole liver chemical constituent concentrations

Foam	Treatment	Compound	Average Measured Concentration (ng/g wet weight)	SEM (ng/g wet weight)
F3 6	Control	DGMBE	69.5	27.7
F3 6	150 mg/L	DGMBE	1.3	1.2
F3 6	450 mg/L	DGMBE	3.6	1.8

Notes;
mg/L = milligrams per liter
ng/g = nanograms per gram
SDS = sodium dodecyl sulfate
DGMBE = Diethylene glycol monobutyl ether

Table A24. Oxygen Uptake as Percent Chemical Oxygen Demand for 3 Foams over the 28 day biodegradation test.

Time (h)	Control	F3 2 120 mg/L COD	F3 2 180 mg/L COD	F3 2 240 mg/L COD	Reference Product 120 mg/L COD	Reference Product 180 mg/L COD	Reference Product 240 mg/L COD	F3 3 120 mg/L COD	F3 3 180 mg/L COD	F3 3 240 mg/L COD
24	26.4%	19.9%	23.0%	20.5%	13.2%	13.4%	14%	17.5%	16.9%	17.6%
	2.2%	1.7%	1.9%	1.7%	0.9%	0.9%	1%	1.3%	1.3%	1.4%
48	53.4%	55.6%	62.6%	54.8%	28.0%	28.5%	30%	49.8%	50.0%	52.1%
	0.3%	1.2%	1.2%	1.1%	0.2%	0.2%	0%	0.9%	0.9%	0.9%
72	60.9%	74.2%	82.8%	71.9%	34.3%	35.4%	38%	63.6%	64.5%	66.6%
	0.2%	0.3%	0.3%	0.3%	0.3%	0.3%	0%	0.3%	0.3%	0.3%
96	65.6%	81.5%	89.9%	78.9%	54.1%	59.7%	63%	72.3%	71.1%	72.5%
	0.1%	0.2%	0.2%	0.2%	1.3%	1.4%	1%	0.2%	0.2%	0.2%
120	66.0%	81.6%	90.7%	80.2%	70.9%	71.5%	77%	72.8%	71.5%	73.3%
	0.0%	0.1%	0.1%	0.0%	0.1%	0.1%	0%	0.1%	0.1%	0.0%
144	68.5%	85.1%	93.9%	83.3%	79.5%	79.0%	87%	77.9%	74.9%	76.1%
	0.1%	0.2%	0.2%	0.2%	0.4%	0.3%	0%	0.3%	0.2%	0.1%
168	70.0%	87.0%	96.1%	85.6%	86.1%	85.5%	94%	81.0%	77.2%	77.7%
	0.1%	0.1%	0.1%	0.1%	0.4%	0.3%	0%	0.2%	0.1%	0.1%
192	70.7%	87.5%	97.3%	86.6%	90.7%	90.9%	98%	82.5%	78.6%	78.6%
	0.0%	0.0%	0.0%	0.0%	0.2%	0.2%	0%	0.0%	0.0%	0.0%
216	70.4%	86.4%	96.9%	86.6%	93.2%	94.1%	99%	82.3%	78.7%	78.4%
	0.0%	0.1%	0.0%	0.0%	0.1%	0.1%	0%	0.0%	0.0%	0.0%
240	73.0%	90.2%	100.0%	89.0%	103.1%	102.3%	104%	88.3%	83.2%	81.1%
	0.2%	0.3%	0.2%	0.2%	0.6%	0.5%	0%	0.4%	0.3%	0.2%
264	74.7%	92.7%	101.9%	90.3%	109.6%	107.9%	108%	92.7%	86.7%	82.7%
	0.0%	0.0%	0.0%	0.0%	0.1%	0.1%	0%	0.1%	0.0%	0.0%
288	74.3%	91.8%	101.4%	90.3%	109.7%	109.1%	108%	93.3%	86.9%	82.6%
	0.0%	0.1%	0.0%	0.1%	0.1%	0.2%	0%	0.2%	0.1%	0.0%
312	75.0%	92.4%	102.2%	91.3%	113.1%	113.6%	111%	97.0%	89.0%	83.9%
	0.0%	0.0%	0.0%	0.0%	0.1%	0.1%	0%	0.1%	0.1%	0.0%

Table A24. Oxygen Uptake as Percent Chemical Oxygen Demand for 3 Foams over the 28 day biodegradation test.

Time (h)	Control	F3 2 120 mg/L COD	F3 2 180 mg/L COD	F3 2 240 mg/L COD	Reference Product 120 mg/L COD	Reference Product 180 mg/L COD	Reference Product 240 mg/L COD	F3 3 120 mg/L COD	F3 3 180 mg/L COD	F3 3 240 mg/L COD
336	75.0%	91.9%	101.9%	91.3%	112.9%	113.9%	111%	97.1%	89.0%	83.9%
	0.0%	0.1%	0.0%	0.0%	0.1%	0.0%	0%	0.0%	0.0%	0.0%
360	77.6%	94.1%	104.0%	93.7%	115.9%	116.8%	113%	101.6%	91.1%	86.2%
	0.1%	0.1%	0.1%	0.1%	0.2%	0.2%	0%	0.2%	0.1%	0.1%
384	79.1%	95.8%	105.2%	94.4%	117.7%	118.2%	114%	102.8%	92.2%	87.3%
	0.1%	0.1%	0.0%	0.0%	0.1%	0.0%	0%	0.0%	0.0%	0.0%
408	80.3%	97.0%	105.9%	95.3%	118.7%	118.9%	115%	103.6%	92.8%	88.0%
	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0%	0.1%	0.1%	0.1%
432	82.6%	100.5%	108.3%	97.2%	122.7%	121.9%	116.8%	106.6%	95.1%	89.7%
	0.081%	0.130%	0.086%	0.058%	0.149%	0.120%	0.084%	0.113%	0.087%	0.058%
456	83.0%	101.0%	108.6%	97.5%	123.0%	122.2%	116.9%	106.7%	95.2%	89.8%
	0.012%	0.033%	0.025%	0.008%	0.061%	0.043%	0.034%	0.047%	0.035%	0.021%
480	83.3%	101.4%	109.0%	97.7%	123.2%	122.2%	116.6%	106.3%	94.8%	90.0%
	0.037%	0.068%	0.070%	0.025%	0.128%	0.063%	0.035%	0.043%	0.038%	0.034%
504	84.2%	103.0%	111.0%	98.2%	126.6%	123.9%	117.6%	107.3%	95.8%	90.7%
	0.029%	0.056%	0.088%	0.018%	0.132%	0.079%	0.049%	0.039%	0.039%	0.026%
528	83.4%	101.5%	111.3%	97.3%	126.4%	123.5%	117.3%	105.1%	94.6%	90.0%
	0.052%	0.095%	0.025%	0.052%	0.064%	0.054%	0.039%	0.128%	0.074%	0.051%
552	81.8%	98.5%	110.2%	96.0%	123.8%	121.6%	115.9%	101.5%	92.4%	88.4%
	0.061%	0.120%	0.035%	0.051%	0.096%	0.071%	0.056%	0.138%	0.086%	0.063%
576	80.5%	96.1%	109.4%	95.0%	121.4%	120.0%	114.6%	98.8%	90.7%	87.0%
	0.035%	0.069%	0.025%	0.018%	0.079%	0.059%	0.043%	0.071%	0.047%	0.042%
600	80.1%	95.3%	109.6%	94.8%	120.7%	119.4%	114.1%	98.1%	90.2%	86.5%
	0.015%	0.030%	0.021%	0.009%	0.038%	0.026%	0.019%	0.024%	0.019%	0.023%
624	79.9%	94.9%	109.6%	94.7%	120.2%	119.0%	113.9%	97.8%	90.0%	86.2%
	0.015%	0.028%	0.019%	0.014%	0.034%	0.022%	0.017%	0.032%	0.021%	0.016%

Notes:
h = hour
mg/L = milligrams per liter
COD = chemical oxygen demand

Table A25. Oxygen Uptake as Percent Chemical Oxygen Demand for four Foams over the 28 day biodegradation

Time (h)	F3 4 120 mg/L COD	F3 4 180 mg/L COD	F3 4 240 mg/L COD	F3 5 120 mg/L COD	F3 5 180 mg/L COD	F3 5 240 mg/L COD	F3 6 120 mg/L COD	F3 6 180 mg/L COD	F3 6 240 mg/L COD	F3 7 120 mg/L COD	F3 7 180 mg/L COD	F3 7 240 mg/L COD
24	21.4%	17.7%	20.1%	16.4%	14.6%	13.2%	6.0%	8.9%	6.0%	14.3%	9.8%	11.6%
	1.6%	1.4%	1.6%	1.3%	1.2%	1.1%	0.7%	0.8%	0.6%	1.3%	0.8%	0.9%
48	61.6%	53.0%	59.5%	34.6%	32.4%	30.3%	23.4%	30.9%	26.4%	51.5%	32.4%	37.2%
	1.5%	1.3%	1.1%	0.3%	0.3%	0.3%	0.8%	0.9%	1.2%	1.1%	1.0%	1.0%
72	88.4%	78.1%	79.3%	50.2%	47.8%	46.6%	52.5%	70.0%	60.0%	69.1%	48.3%	53.4%
	0.5%	0.5%	0.5%	0.9%	0.8%	0.8%	0.3%	0.6%	0.4%	0.3%	0.3%	0.2%
96	97.6%	87.5%	92.3%	78.1%	74.9%	71.6%	62.2%	84.5%	70.8%	77.7%	54.3%	58.7%
	0.2%	0.2%	0.3%	0.6%	0.8%	0.5%	0.4%	0.5%	0.3%	0.3%	0.2%	0.1%
120	98.6%	88.7%	95.2%	86.3%	84.2%	77.8%	64.0%	88.7%	73.8%	79.0%	55.2%	59.5%
	0.1%	0.0%	0.0%	0.2%	0.2%	0.2%	0.1%	0.0%	0.0%	0.1%	0.0%	0.0%
144	102.5%	91.8%	98.2%	96.6%	92.2%	84.5%	70.4%	95.7%	78.7%	84.0%	58.0%	62.3%
	0.2%	0.2%	0.1%	0.5%	0.4%	0.3%	0.3%	0.3%	0.2%	0.3%	0.1%	0.1%
168	105.1%	93.6%	100.4%	104.0%	98.6%	90.3%	74.2%	100.9%	82.2%	87.2%	59.8%	64.3%
	0.1%	0.1%	0.1%	0.3%	0.3%	0.3%	0.2%	0.3%	0.2%	0.2%	0.1%	0.1%
192	106.1%	94.4%	101.5%	107.2%	102.1%	94.3%	76.0%	103.8%	84.2%	88.8%	60.7%	65.8%
	0.0%	0.0%	0.0%	0.1%	0.1%	0.1%	0.0%	0.1%	0.1%	0.0%	0.0%	0.1%
216	105.5%	94.1%	101.5%	107.8%	103.3%	97.2%	76.1%	104.7%	85.1%	88.9%	60.8%	66.5%
	0.1%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
240	108.6%	96.5%	103.5%	112.7%	107.9%	102.7%	82.1%	111.4%	90.0%	94.3%	63.4%	69.5%
	0.2%	0.2%	0.1%	0.3%	0.3%	0.3%	0.4%	0.4%	0.3%	0.4%	0.2%	0.2%

Table A25. Oxygen Uptake as Percent Chemical Oxygen Demand for four Foams over the 28 day biodegradation

Time (h)	F3 4 120 mg/L COD	F3 4 180 mg/L COD	F3 4 240 mg/L COD	F3 5 120 mg/L COD	F3 5 180 mg/L COD	F3 5 240 mg/L COD	F3 6 120 mg/L COD	F3 6 180 mg/L COD	F3 6 240 mg/L COD	F3 7 120 mg/L COD	F3 7 180 mg/L COD	F3 7 240 mg/L COD
264	110.5%	97.8%	104.7%	115.8%	111.3%	106.0%	85.7%	116.4%	93.7%	98.3%	65.3%	71.7%
	0.0%	0.0%	0.0%	0.0%	0.1%	0.1%	0.0%	0.1%	0.1%	0.1%	0.0%	0.0%
288	109.7%	97.4%	104.4%	116.2%	112.3%	106.6%	85.8%	116.9%	95.2%	98.8%	65.6%	71.8%
	0.0%	0.0%	0.0%	0.1%	0.2%	0.1%	0.1%	0.1%	0.2%	0.1%	0.1%	0.0%
312	110.2%	98.0%	104.8%	118.8%	115.6%	108.7%	89.5%	120.6%	98.7%	101.8%	67.1%	72.9%
	0.0%	0.0%	0.0%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.0%	0.0%
336	109.8%	97.7%	104.6%	118.9%	115.9%	108.9%	89.7%	121.0%	99.6%	101.7%	67.1%	72.7%
	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.1%	0.1%	0.0%	0.0%
360	110.8%	98.8%	105.3%	122.9%	118.6%	110.8%	95.6%	125.7%	104.0%	103.5%	69.0%	73.4%
	0.1%	0.1%	0.0%	0.1%	0.1%	0.1%	0.2%	0.2%	0.1%	0.1%	0.1%	0.0%
384	111.5%	99.5%	105.7%	124.6%	120.0%	112.1%	98.0%	128.6%	105.8%	104.4%	69.4%	73.7%
	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%
408	111.8%	99.9%	105.9%	126.1%	121.0%	112.9%	99.6%	130.8%	107.5%	104.8%	69.7%	73.8%
	0.1%	0.1%	0.0%	0.1%	0.1%	0.1%	0.1%	0.2%	0.1%	0.1%	0.0%	0.0%
432	113.7%	101.5%	107.1%	129.7%	123.5%	114.8%	103.1%	135.2%	110.6%	107.2%	70.7%	74.7%
	0.065%	0.057%	0.044%	0.123%	0.088%	0.069%	0.134%	0.152%	0.104%	0.094%	0.040%	0.039%
456	113.5%	101.6%	107.2%	130.5%	123.9%	115.1%	103.4%	136.0%	111.6%	107.3%	70.7%	74.6%
	0.045%	0.024%	0.016%	0.030%	0.021%	0.017%	0.046%	0.036%	0.032%	0.051%	0.022%	0.026%
480	113.5%	101.5%	107.3%	131.6%	124.5%	115.4%	103.2%	136.9%	112.7%	106.7%	70.5%	74.3%
	0.045%	0.029%	0.024%	0.076%	0.059%	0.039%	0.047%	0.094%	0.055%	0.040%	0.012%	0.014%
504	114.6%	102.3%	107.9%	133.3%	126.0%	116.4%	104.2%	139.4%	114.0%	107.6%	70.6%	74.5%
	0.039%	0.030%	0.023%	0.061%	0.059%	0.042%	0.050%	0.100%	0.043%	0.037%	0.016%	0.013%
528	113.5%	101.7%	107.5%	132.6%	125.7%	116.2%	101.9%	139.1%	113.4%	105.9%	69.2%	73.6%
	0.074%	0.044%	0.033%	0.062%	0.037%	0.024%	0.141%	0.052%	0.044%	0.105%	0.076%	0.057%
552	111.3%	100.3%	106.5%	130.7%	124.5%	115.3%	97.9%	137.3%	112.2%	102.8%	67.1%	72.0%
	0.080%	0.054%	0.036%	0.071%	0.044%	0.036%	0.155%	0.073%	0.043%	0.120%	0.080%	0.062%
576	109.6%	99.1%	105.6%	129.1%	123.5%	114.5%	94.8%	135.6%	111.3%	100.3%	65.5%	70.7%

Table A25. Oxygen Uptake as Percent Chemical Oxygen Demand for four Foams over the 28 day biodegradation

Time (h)	F3 4 120 mg/L COD	F3 4 180 mg/L COD	F3 4 240 mg/L COD	F3 5 120 mg/L COD	F3 5 180 mg/L COD	F3 5 240 mg/L COD	F3 6 120 mg/L COD	F3 6 180 mg/L COD	F3 6 240 mg/L COD	F3 7 120 mg/L COD	F3 7 180 mg/L COD	F3 7 240 mg/L COD
	0.045%	0.033%	0.024%	0.040%	0.034%	0.024%	0.077%	0.050%	0.021%	0.079%	0.042%	0.036%
600	109.2%	98.8%	105.3%	128.7%	123.3%	114.3%	93.8%	135.2%	111.3%	99.3%	64.9%	70.2%
	0.020%	0.014%	0.013%	0.019%	0.015%	0.013%	0.034%	0.024%	0.010%	0.039%	0.017%	0.017%
624	108.9%	98.6%	105.2%	128.5%	123.1%	114.1%	93.3%	134.9%	111.1%	98.8%	64.7%	70.0%
	0.022%	0.015%	0.011%	0.024%	0.016%	0.012%	0.036%	0.024%	0.018%	0.026%	0.018%	0.013%

Notes:
h = hour
mg/L = milligrams per liter
COD = chemical oxygen demand

Table A26. Soluble chemical oxygen demand over time for all tested foams.

Day	Foam	Concentration (mg/L)	Soluble COD (mg/L)
0	F3 2	120	125
7	F3 2	120	17
13	F3 2	120	13
21	F3 2	120	2
28	F3 2	120	1
0	F3 2	180	173
7	F3 2	180	29
13	F3 2	180	21
21	F3 2	180	11
28	F3 2	180	2
0	F3 2	240	230
7	F3 2	240	35
13	F3 2	240	23
21	F3 2	240	23
28	F3 2	240	5
0	Reference Product	120	122
7	Reference Product	120	29
13	Reference Product	120	18
21	Reference Product	120	19
28	Reference Product	120	8
0	Reference Product	180	182
7	Reference Product	180	23
13	Reference Product	180	21
21	Reference Product	180	15
28	Reference Product	180	6
0	Reference Product	240	241
7	Reference Product	240	30
13	Reference Product	240	22
21	Reference Product	240	13

Table A26. Soluble chemical oxygen demand over time for all tested foams.

Day	Foam	Concentration (mg/L)	Soluble COD (mg/L)
28	Reference Product	240	8
0	F3 3	120	131
7	F3 3	120	16
13	F3 3	120	5
21	F3 3	120	12
28	F3 3	120	3
0	F3 3	180	178
7	F3 3	180	19
13	F3 3	180	17
21	F3 3	180	7
28	F3 3	180	1
0	F3 3	240	235
7	F3 3	240	23
13	F3 3	240	21
21	F3 3	240	2
28	F3 3	240	1
0	F3 4	120	242
7	F3 4	120	18
13	F3 4	120	14
21	F3 4	120	7
28	F3 4	120	2
0	F3 4	180	336
7	F3 4	180	25
13	F3 4	180	13
21	F3 4	180	7
28	F3 4	180	1
0	F3 4	240	439
7	F3 4	240	28
13	F3 4	240	17
21	F3 4	240	15
28	F3 4	240	1
0	F3 5	120	200
7	F3 5	120	24
13	F3 5	120	12
21	F3 5	120	3
28	F3 5	120	1
0	F3 5	180	288

Table A26. Soluble chemical oxygen demand over time for all tested foams.

Day	Foam	Concentration (mg/L)	Soluble COD (mg/L)
7	F3 5	180	27
13	F3 5	180	14
21	F3 5	180	1
28	F3 5	180	1
0	F3 5	240	396
7	F3 5	240	40
13	F3 5	240	21
21	F3 5	240	1
28	F3 5	240	1
0	F3 6	120	138
7	F3 6	120	31
13	F3 6	120	22
21	F3 6	120	11
28	F3 6	120	2
0	F3 6	180	199
7	F3 6	180	47
13	F3 6	180	30
21	F3 6	180	17
28	F3 6	180	12
0	F3 6	240	254
7	F3 6	240	50
13	F3 6	240	40
21	F3 6	240	19
28	F3 6	240	17
0	F3 7	120	156
7	F3 7	120	14
13	F3 7	120	10
21	F3 7	120	6
28	F3 7	120	1
0	F3 7	180	237
7	F3 7	180	16
13	F3 7	180	15
21	F3 7	180	5
28	F3 7	180	1
0	F3 7	240	307
7	F3 7	240	20
13	F3 7	240	17

Table A26. Soluble chemical oxygen demand over time for all tested foams.

Day	Foam	Concentration (mg/L)	Soluble COD (mg/L)
21	F3 7	240	3
28	F3 7	240	1

Notes:

mg/L = milligrams per liter
COD = chemical oxygen demand

Table A27. Degradation of chemical constituents over the 28 day biodegradation study.

Foam	Day	C/C_0	Chemical
F3 2	0	100%	DGMBE
F3 2	7	0.18%	DGMBE
F3 2	14	0.28%	DGMBE
F3 2	21	0.11%	DGMBE
F3 2	28	0.20%	DGMBE
F3 2	0	100%	SDS
F3 2	7	0.41%	SDS
F3 2	14	0.38%	SDS
F3 2	21	0.35%	SDS
F3 2	28	0.33%	SDS
F3 2	0	100%	DMDA N-O
F3 2	7	0%	DMDA N-O
F3 2	14	0%	DMDA N-O
F3 2	21	0%	DMDA N-O
F3 2	28	0%	DMDA N-O
Reference Product	0	100%	DGMBE
Reference Product	7	31%	DGMBE
Reference Product	14	49%	DGMBE
Reference Product	21	19%	DGMBE
Reference Product	28	35%	DGMBE
Reference Product	0	100%	HG
Reference Product	7	0.00%	HG
Reference Product	14	0.00%	HG
Reference Product	21	0.00%	HG
Reference Product	28	0.00%	HG
Reference Product	0	100%	SDS
Reference Product	7	2%	SDS
Reference Product	14	2%	SDS
Reference Product	21	1%	SDS
Reference Product	28	1%	SDS

Table A27. Degradation of chemical constituents over the 28 day biodegradation study.

Foam	Day	C/C_0	Chemical
Reference Product	0	100%	DMDA N-O
Reference Product	7	151%	DMDA N-O
Reference Product	14	38%	DMDA N-O
Reference Product	21	0%	DMDA N-O
Reference Product	28	0%	DMDA N-O
Reference Product	0	100%	PFBA
Reference Product	7	100%	PFBA
Reference Product	14	185%	PFBA
Reference Product	21	213%	PFBA
Reference Product	28	100%	PFBA
Reference Product	0	100%	PFHxA
Reference Product	7	100%	PFHxA
Reference Product	14	163%	PFHxA
Reference Product	21	185%	PFHxA
Reference Product	28	85%	PFHxA
F3 3	0	100%	DGMBE
F3 3	7	0%	DGMBE
F3 3	14	0%	DGMBE
F3 3	21	0%	DGMBE
F3 3	28	0%	DGMBE
F3 3	0	100%	SDS
F3 3	7	1%	SDS
F3 3	14	1%	SDS
F3 3	21	0%	SDS
F3 3	28	0%	SDS
F3 3	0	100%	DMDA N-O
F3 3	7	0%	DMDA N-O
F3 3	14	0%	DMDA N-O
F3 3	21	0%	DMDA N-O
F3 3	28	0%	DMDA N-O
F3 4	0	100%	DGMBE
F3 4	7	0%	DGMBE
F3 4	14	0%	DGMBE
F3 4	21	0%	DGMBE
F3 4	28	0%	DGMBE
F3 4	0	100%	SDS
F3 4	7	0%	SDS
F3 4	14	0%	SDS
F3 4	21	0%	SDS
F3 4	28	0%	SDS

Table A27. Degradation of chemical constituents over the 28 day biodegradation study.

Foam	Day	C/C_0	Chemical
F3 4	0	100%	DMDA N-O
F3 4	7	0%	DMDA N-O
F3 4	14	0%	DMDA N-O
F3 4	21	0%	DMDA N-O
F3 4	28	0%	DMDA N-O
F3 5	0	100%	DGMBE
F3 5	7	21%	DGMBE
F3 5	14	33%	DGMBE
F3 5	21	13%	DGMBE
F3 5	28	23%	DGMBE
F3 5	0	100%	HG
F3 5	7	0%	HG
F3 5	14	0%	HG
F3 5	21	0%	HG
F3 5	28	0%	HG
F3 5	0	100%	SDS
F3 5	7	0%	SDS
F3 5	14	0%	SDS
F3 5	21	0%	SDS
F3 5	28	0%	SDS
F3 6	0	100%	DGMBE
F3 6	7	0%	DGMBE
F3 6	14	0%	DGMBE
F3 6	21	0%	DGMBE
F3 6	28	0%	DGMBE
F3 6	0	100%	SDS
F3 6	7	16%	SDS
F3 6	14	15%	SDS
F3 6	21	11%	SDS
F3 6	28	12%	SDS
F3 6	0	100%	DMDA N-O
F3 6	7	0%	DMDA N-O
F3 6	14	0%	DMDA N-O
F3 6	21	0%	DMDA N-O
F3 6	28	0%	DMDA N-O
F3 7	0	100%	DGMBE
F3 7	7	0%	DGMBE
F3 7	14	0%	DGMBE
F3 7	21	0%	DGMBE
F3 7	28	0%	DGMBE

Table A27. Degradation of chemical constituents over the 28 day biodegradation study.

Foam	Day	C/C_0	Chemical
F3 7	0	100%	SDS
F3 7	7	0%	SDS
F3 7	14	0%	SDS
F3 7	21	0%	SDS
F3 7	28	0%	SDS

Notes:
C/C_0 is the concentration relative to starting.
DGMBE = Diethylene glycol monobutyl ether
SDS = Sodium dodecyl sulfate
DMDA N-O = N, N-Dimethyldecylamine N-oxide
HG = Hexylene glycol

APPENDIX B LIST OF SCIENTIFIC/TECHNICAL PUBLICATIONS

Peer Reviewed Articles

Fuller, N., Suski, J. G., Lanasa, S., Chanov, M. K., Jones, D. K., Haskin, D. L., Quinlin, K. A., Wigren, M. A., … & Wirth, E. F. (2024). Chronic Toxicity of PFAS-Free Firefighting Foams to Aquatic Organisms. *Environmental Toxicology and Chemistry*, *43(11).* 2436-2454.

Modiri Gharehveran, M. M., Walus, A. M., Anderson, T. A., Subbiah, S., Guelfo, J., Frigon, M., ... & Suski, J. G. (2022). Per-and polyfluoroalkyl substances (PFAS)-free aqueous film forming foam formulations: Chemical composition and biodegradation in an aerobic environment. *Journal of Environmental Chemical Engineering*, *10*(6), 108953.

Hossain, F., Dennis, N. M., Subbiah, S., Karnjanapiboonwong, A., Guelfo, J. L., Suski, J., & Anderson, T. A. (2022). Acute Oral Toxicity of Nonfluorinated Fire-Fighting Foams to Northern Bobwhite Quail (Colinus virginianus). *Environmental Toxicology and Chemistry*, *41*(8), 2003-2007.

Hossain, F., Dennis, N. M., Karnjanapiboonwong, A., Subbiah, S., Longwell, A. S., Suski, J. G., ... & Anderson, T. A. (2024). Evaluation of the Chronic Reproductive Toxicity of a Fluorine-Free Firefighting Foam and a Short-Chain Fluorinated Foam to Northern Bobwhite Quail (Colinus virginianus). *Environmental Toxicology and Chemistry*, *43*(1), 211-221.

Jones, D. K., Quinlin, K. A., Wigren, M. A., Choi, Y. J., Sepúlveda, M. S., Lee, L. S., ... & Hoverman, J. T. (2022). Acute toxicity of eight aqueous film-forming foams to 14 aquatic species. *Environmental Science & Technology*, *56*(10), 6078-6090.

Conference Proceedings

Hossain, F. Analytical Methods for Assessing Exposure to Fluorine-Free Firefighting Foams: Implications for Determining Tissue-Specific Toxicity Reference Values (2022). Oral Presentation, Society for Environmental Toxicology and Chemistry (SETAC) North American Meeting. Pittsburgh, PA, 13-17th November 2022.

Hossain, F. Chronic Reproductive Toxicity of Fluorine-Free Firefighting Foams to Northern Bobwhite Quail (*Colinus virginianus)* (2022). Oral Presentation, Society for Environmental Toxicology and Chemistry (SETAC) North American Meeting. Pittsburgh, PA, 13-17th November 2022.

Hossain, F. Chronic Reproductive Toxicity of Five Fluorine-Free Firefighting Foams and a Short Chain Fluorinated Foam to Northern Bobwhite Quail (*Colinus virginianus*) (2023). Poster Presentation, Society for Environmental Toxicology and Chemistry (SETAC) North American Meeting. Louisville, Kentucky, 12-16th November 2023.

Hudson, S. An Evaluation of Aquatic Receptor Sensitivities to Novel Fluorine-Free Firefighting Foam Versus Short Chain Per- and Polyfluoroalkyl Substances Aqueous Film Forming Foam Products (2022). Poster Presentation, Society for Environmental Toxicology and Chemistry (SETAC) North American Meeting. Pittsburgh, PA, 13-17th November 2022.

Hudson, S. An Evaluation of Aquatic Receptor Sensitivities to Novel Fluorine-Free Firefighting Foam Versus Short Chain PFAS AFFF Products (2023). Poster Presentation, Society for Environmental Toxicology and Chemistry (SETAC) North American Meeting. Pittsburgh, PA, 13-17th November 2022.

Modiri Gharehveran, M. Testing Biodegradability of Aqueous Film Forming Foam Formulations in an Aerobic Environment (2022). Oral Presentation, Society for Environmental Toxicology and Chemistry (SETAC) North American Meeting. Pittsburgh, PA, 13-17th November 2022.

Modiri Gharehveran, M (2022). Variable Importance Analysis of Toxic Constituents in PFAS-Free Aqueous Film Forming Foam Formulations: An Application of Machine Learning Approaches. Poster Presentation, SERDP Winter Symposium 2022. Arlington, VA, 29th November – 2nd December 2022.