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Acute, chronic, and behavioral toxicity of fluorine-free foams to earthworm species *Eisenia fetida* and *Dendrobaena veneta*

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HIGHLIGHTS

- Two F3 formulations showed widely different acute toxicity to earthworms.
- One F3 formulation was more acutely toxic than a common PFAS-containing product.
- Earthworms strongly avoided soils treated with F3 and PFAS-containing foams.
- Soil avoidance by earthworms indicates potential effects to terrestrial ecosystems.
- Chemical composition differences likely drive varying toxicity among foams.

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ABSTRACT

Aqueous film-forming foams (AFFF) containing per- and polyfluoroalkyl substances (PFAS) are widely used as fire suppressants. Due to rising health and environmental concerns, fluorine-free foams (F3) are being developed and tested as safer alternatives. However, the impact of these replacement products on ecologically important earthworms has not been adequately studied. This study addresses this gap by comparing the acute, chronic, and behavioral effects of two F3 formulations, BioEx Ecopol and Angus Fire Jetfoam, with a commonly used PFAS-containing AFFF, Buckeye, on two species of earthworms. Soil treated with BioEx Ecopol and Buckeye at the maximum 3% concentration did not cause significant acute mortality in *Eisenia fetida*, whereas Angus Fire Jetfoam resulted in 100% mortality at the same concentration. In a 28-day chronic test, weight loss was observed in *Dendrobaena veneta* worms exposed to soil treated with Angus Fire Jetfoam. Additionally, both *Eisenia fetida* and *Dendrobaena veneta* demonstrated almost complete avoidance of soils treated with any of the three foams at 3% concentration, indicating potential ecological effects despite lack of acute toxicity by two of the products. The differences in toxicity are likely due to the distinct chemical compositions and surfactant concentrations in these foams. BioEx Ecopol and Buckeye, despite their differing surfactant mixtures, exhibited lower acute toxicity, whereas Angus Fire Jetfoam's high mortality rate points to a potentially problematic chemical formulation. By understanding the toxicity of current formulations, this study supports the ongoing development and evaluation of new fire suppression products, supporting the creation of safer technologies.

1. Introduction

Aqueous film forming foams (AFFF) have been a widely used fire-fighting tool since the 1970s due to their ability to rapidly suppress liquid fuel fires. This film-forming function derives from AFFF's historical reliance on per- and polyfluoroalkyl substances (PFAS), which impart strong oleophobic and hydrophobic properties. However, health and environmental concerns surrounding PFAS have accelerated the transition fluorine-free foams (F3) (Ateia et al., 2023; Ateia and Scheringer, 2024). Research into the on F3 formulations is still emerging, with

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the first peer-reviewed environmental impacts studies published only in recent years (Kuperman et al., 2023; Yu et al., 2022). Currently, available ecotoxicological data on F3 formulations are limited with some studies finding that F3 formulations can exhibit equal or greater acute toxicity than C6 AFFF, particularly for aquatic species, which are often more sensitive than terrestrial species and mammals (East et al., 2023; Jones et al., 2022; Yu et al., 2022). This nascent body of research underscores the need for further investigation to fully comprehend the ecological risks of these foams, especially in comparison to the long-term effects of AFFF (Ateia et al., 2023; Sinclair et al., 2020).

The fate and transport of AFFF and F3 in the environment are primarily mediated through soil (Bizzotto et al., 2024; Yu et al., 2022). Consequently, soil invertebrates are among the first organisms to be exposed to these chemicals at potentially high concentrations. Understanding the toxicological impact on this group is crucial due to their ecological significance (Karnjanapiboonwong et al., 2018; Melo et al., 2022). Despite this importance, there is a notable scarcity of toxicological studies focusing on the effects of AFFF on terrestrial soil invertebrates, with even fewer studies addressing F3 formulations. Existing research has predominantly assessed the toxicity of AFFF on aquatic species. For instance, Jones et al. (2020) evaluated the lethal effects of seven F3 formulations and a PFAS-containing AFFF on 14 aquatic species. Their findings revealed that one F3 formulation, Avio Green, exhibited equal or greater toxicity to all species compared to the PFAS-containing AFFF (Jones et al., 2022).

The objective of this study is to address this gap by evaluating the toxicity of two F3 formulations (Angus Fire JetFoam and BioEx Ecopol) on earthworms. This evaluation uses a mix of acute, behavioral, and chronic endpoints and will compare the results with those obtained from a commonly used PFAS-containing AFFF (Buckeye). The behavioral aspect of the study provides a more comprehensive understanding of the ecological risks posed by both F3 and PFAS-containing AFFF, beyond what can be gained from acute and chronic tests.

2. Materials and methods

2.1. Test organisms

Eisenia fetida offers several advantages for soil ecotoxicity testing. It has been extensively studied, providing a wealth of data on its responses to various toxicants and soil conditions. This species is recognized by the European Union (EU), the Organization for Economic Cooperation and Development (OECD, 1984; 2016), and the U.S. Environmental Protection Agency (Greene et al., 1988) for screening toxicity at hazardous waste sites. As epigeic worms, both *Eisenia fetida* and *Dendrobaena veneta* inhabit the soil's surface layer and associated leaf litter, making them ideal for assessing areas impacted by spills and applications of aqueous film-forming foams (AFFFs). Although it's unclear if one species might be more sensitive to PFAS or PFAS-free AFFFs, testing multiple species is recommended to enhance the experiment's robustness, given known variations in species sensitivity (Latha and Basha, 2019). Sources of tested earthworms are listed in Section S1.

2.2. Characterization of soil and foams

The soil used for tests was a field-collected soil from a former farm field in New Richmond, OH, from a plot that hadn't been in use, and thus hasn't been treated with fertilizers or pesticides for ~50 years. Details on experimental conditions and characterization of soil samples are described in Section S1 and Table S1. To reflect environmentally-relevant concentrations, all foam concentrates were diluted to 3% concentration in distilled water, which is the set concentration for firefighting applications (Ateia et al., 2023). For sub-lethal concentrations, the solutions at 3% were further diluted in DI to 1.5% concentration. It should be noted that tested F3 formulations were not finalized and approved at the time of this study (i.e., formulations were in the development phase). For foam constituents with available analytical standards, the initial concentrations were measured using liquid chromatography–tandem mass spectrometry (LC-MS/MS) as described in Section S2. Other constituents were recoded as listed on the safety datasheet of each product (Table S2).

2.3. Avoidance, acute, and chronic tests

The earthworm avoidance test used here was developed by Yeardley et al. (1996) as a sensitive and fast tool to evaluate contaminated soils at hazardous waste sites, and due to its usefulness, has been incorporated an international standard (ISO, 2008). The 14-day acute tests were performed based on OECD (1984) and ASTM (2012) tests. Finally, the chronic test was, in essence, a modified acute test, with the same test parameters (temperature, light, no feeding) as the acute tests, and extended from 14 to 28 days. A weight loss element, which required depuration at test start, 14-days, and 28-days, was also added for the chronic test. Detailed description of all toxicity tests is listed in Section S3. For all tests, the starting concentration was based on using a 3% AFFF solution to hydrate the soils to 60% WHC. Hydrating the soils with a 3% solution was meant to represent a worst-case scenario, that would occur when the full-strength solution (3%) spilled directly on the ground and saturated it. For the acute tests, if we saw toxicity at 3%, we would re-test with more dilute solutions and calculate the lethal dose for 50% mortality (LD50).

3. Results and discussion

3.1. Foams and soil properties

The surfactant composition of firefighting foams significantly influences their toxicity, with F3 formulations typically containing nonionic, anionic, and amphoteric surfactants (Table S2) (Ateia et al., 2023; Hinnant et al., 2024). Nonionic surfactants like diethylene glycol butyl ether, used in the highest concentrations (up to 20%), enhance fire suppression by reducing bubble size and liquid drainage rate. Anionic surfactants such as sodium dodecyl sulfate and amphoteric surfactants like N,N-dimethyldodecyl ether can each constitute more than 3% of the foam's composition. Understanding these surfactants' roles and concentrations is crucial for assessing their environmental impact, particularly on soil organisms, as they affect both the chemical properties and biological health of the soil ecosystem.

From gravimetric tests (USE PA, 2020), the control and spiked soils used in all tests was approximately 32% sand, 67% silt and 1% clay. According to the soil texture triangle (Shirazi and Boersma, 1984), this would make it a silty loam. Water holding capacity (WHC), a soil characteristic important adjusting soil hydration in toxicity tests, was calculated following the methodology of Greene et al. (1989), and all soils were adjusted to 60% WHC. The particle size distribution was determined using a sieving process as specified in SOP #3953 (USEPA, 2020). These parameters are essential for understanding both the physical and chemical environment to which the earthworms were exposed during the tests. These properties are critical in determining the soil's capacity to retain moisture and nutrients, which can influence the bioavailability of toxicants. The organic matter content, which can affect both the chemical interactions within the soil and the health of test organisms, was assessed through the Ash-Free Dry Mass (AFDM) method. AFDM of our soil was found to be 5.3%, indicating a moderate level of organic content. This level of food content should not introduce stress on the organisms during testing.

3.2. Acute toxicity of F3 and AFFF

The initial tests were conducted using a 3% concentration for each foam to investigate the survival of earthworms (*Eisenia fetida*). The results demonstrated significant variations in toxicity among the foams

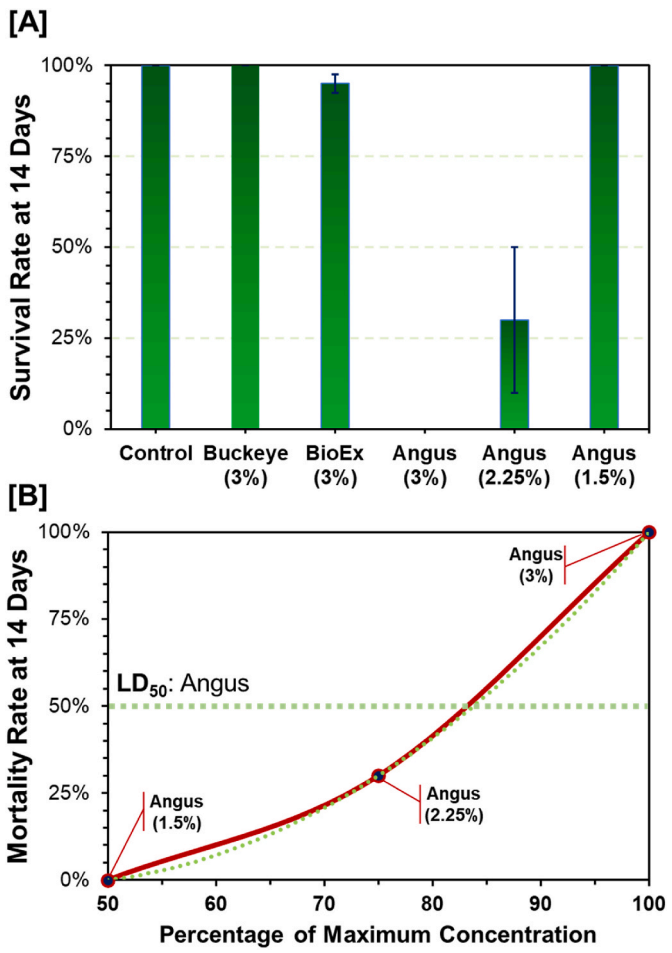

Fig. 1. **[A]** Results of Acute tests with *Eisenia fetida* with two F3 and one PFAS-containing AFFF product, including multiple concentrations of the Angus Fire AFFF. Error bars represent standard deviation. **[B]** The LD50 assessment with *Eisenia fetida* for the Angus Fire F3 formulation.

(Fig. 1A). The PFAS-containing Buckeye foam showed complete or near-complete survival at the 3% concentration, indicating relatively lower acute toxicity to *Eisenia fetida*. Similarly, the fluorine-free BioEx foam also resulted in high survival rates, suggesting that not all fluorine-free alternatives pose immediate toxicity risks. In stark contrast, the Angus foam, another fluorine-free product, exhibited 0% survival at the same concentration. This prompted further testing at reduced concentrations of 1.5% and 2.25% (50% and 75% of the initial concentration), where a polynomial model calculated the LD50 to be 2.52% (Fig. 1B).

These findings align with existing literature that suggests PFAS compounds, while persistent and toxic, do not always cause immediate acute toxicity at lower concentrations in soil organisms (Karnjanapiboonwong et al., 2018). However, the long-term environmental and health effects remain a significant concern due to their persistence and potential for bioaccumulation in food webs (Rich et al., 2015; Zhao et al., 2013). Conversely, the high acute toxicity observed in the Angus foam highlights that fluorine-free alternatives are not universally safer in terms of immediate toxicity. Yu et al. (2022) investigated the effects of F3 formulations on the nematode Caenorhabditis elegans (*C. elegans)*, and found that most tested foams, except NFD 20–391, reduced both growth and reproduction. They also noted a correlation between higher surfactant concentrations, particularly diethylene glycol butyl ether, and increased toxicity. Kuperman et al. (2023) extended these findings by examining the reproductive and lethal effects of F3 and PFAS-containing AFFF on the soil invertebrates *Enchytraeus crypticus* (potworm) and *Folsomia candida* (springtail). Their study indicated that the PFAS-free formulations Avio Green and Angus Fire JetFoam were equally or more toxic than the PFAS-containing Buckeye AFFF (Kuperman et al., 2023). This and previous studies underscore the need for careful evaluation of new formulations, as their environmental impact can vary significantly. This highlights the importance of comprehensive ecotoxicological assessments when developing and deploying firefighting foams. While fluorine-free foams are designed to reduce environmental persistence, their acute toxicity must also be considered to prevent adverse ecological effects.

3.3. Avoidance tests

Both *Eisenia fetida* and *Dendrobaena veneta* exhibited avoidance behavior in response to the three tested products, as the vast majority of earthworms migrated to the control side within 48 h (Fig. 2 and Fig. S1). Statistical analysis confirmed these findings, with extremely significant p-values for both species (<0.0001). Avoidance behavior is increasingly recognized as a sensitive endpoint for assessing soil contamination. Previous studies have highlighted its utility over traditional acute or sublethal endpoints (Hund-Rinke and Wiechering, 2001; Loureiro et al., 2005; Yeardley Jr et al., 1996). Yuan et al. (2017) specifically noted the heightened sensitivity of avoidance tests in soils contaminated with PFAS-containing AFFFs, a finding corroborated by our results (Cai et al., 2021; Yuan et al., 2017).

This avoidance behavior suggests potential ecological impacts, as earthworms play a crucial role in nutrient cycling and enhancing soil structure (Edwards and Lofty, 1977). Their absence, whether due to mortality or avoidance, can disrupt these processes, leading to long-term ecological consequences. The fact that Buckeye and BioEx foams were avoided despite low acute toxicity highlights a critical concern: substances that repel essential soil organisms can still pose significant risks to ecosystem health.

3.4. Chronic tests results

In the chronic toxicity tests, both survival and weight change of earthworms were evaluated over 14 and 28 days to assess the long-term effects of the tested firefighting foams. The findings indicated similarly high survival rates across all groups at both time intervals, suggesting that immediate mortality was not a significant concern (Fig. 3). However, a closer examination of weight changes revealed critical insights. Notably, the Angus AFFF group exhibited significant weight loss (Table 1), highlighting a stress response that, while not immediately lethal, could lead to mortality over extended exposure periods. This weight loss serves as an early indicator of sublethal stress, which aligns with findings from studies on PFAS toxicity, where chronic exposure often reveals adverse effects not evident in acute tests (Karnjanapiboonwong et al., 2018).

The observation that some earthworms survived the acute tests only to suffer weight loss in chronic conditions underscores the importance of evaluating both immediate and long-term impacts. This aligns with existing literature emphasizing that PFAS compounds, while not always causing acute mortality, can have significant chronic effects due to their persistence and bioaccumulative nature (Ateia and Scheringer, 2024; Rich et al., 2015). The Angus foam's chronic impact, despite initial survival, suggests that non-fluorinated surfactants may still pose significant ecological risks if their formulations are not carefully evaluated. The weight loss observed exclusively in the Angus AFFF group is particularly concerning, as it indicates that some fluorine-free alternatives may not be inherently safer. This emphasizes the need for comprehensive ecotoxicological assessments when developing new formulations. The avoidance behavior observed in both *Eisenia fetida* and *Dendrobaena veneta* further supports this, as even foams with low acute toxicity, like Buckeye and BioEx, were avoided by earthworms. Comprehensive evaluation, including both acute and chronic assessments, is crucial to ensure that new foam formulations do not

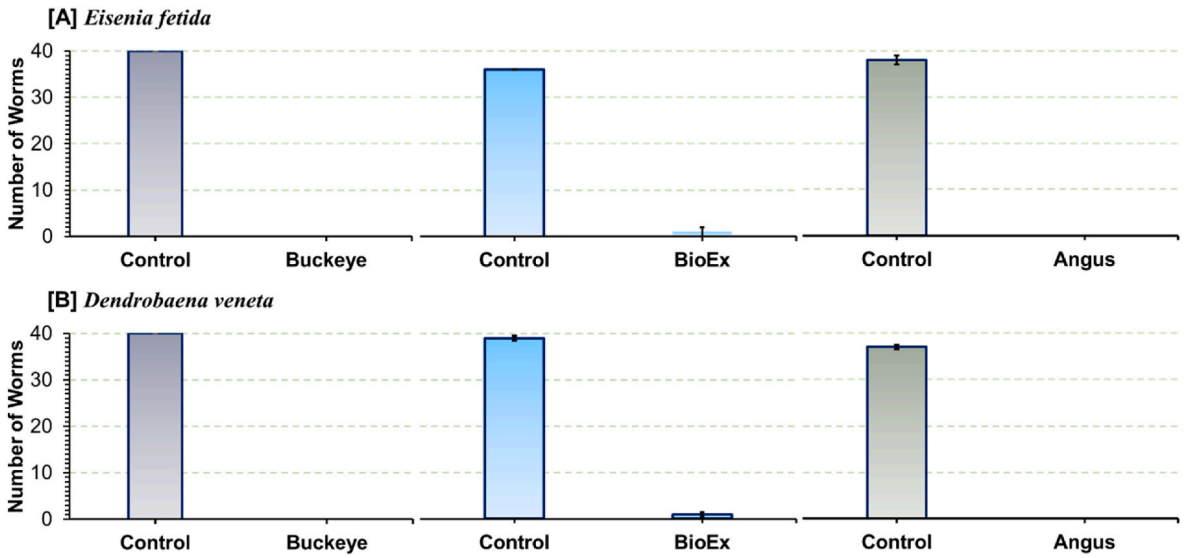

Fig. 2. Results of 48-h avoidance tests with **[A]** *Eisenia fetida* and **[B]** *Dendrobaena veneta.*

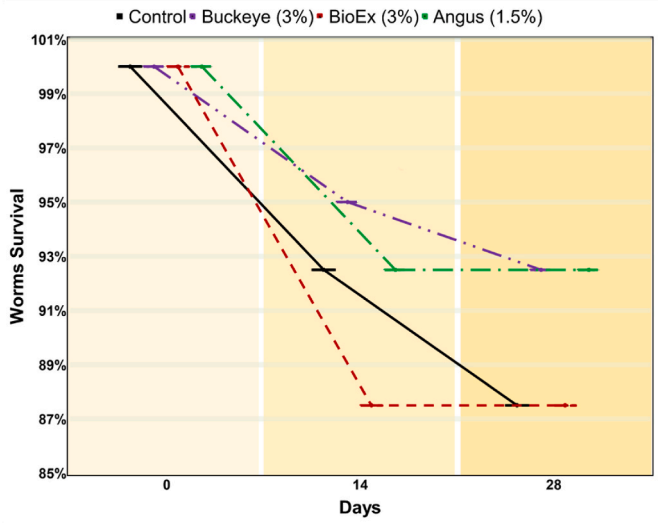

Fig. 3. Survival rates of *Dendrobaena veneta* earthworms from the 28-day acute/chronic tests using two F3 formulations and one PFAS-containing AFFF at sublethal concentrations.

Table 1
Weight change from 14 to 28 days, with *t*-test p values (indicating where change was statistically significant) and number or replicates used to assess weight change.

Treatment Group	Avg Wt. Change (g)	p (from *t*-test)	# Reps
Control	−0.069	0.235	2
Angus	−0.091	**0.001**	4
Buckeye	−0.052	0.149	3
BioEx	−0.089	0.075	4

inadvertently harm soil ecosystems.

4. Conclusions

This research provides a reminder that reformulating AFFFs to make them fluorine-free doesn't necessarily make them non-toxic to terrestrial invertebrates, or remove the potential for disruptive ecological effects. Of the 3 AFFFs tested, one F3, Angus Fire Jetfoam, was clearly more acutely toxic to two earthworm taxa (*Eisenia fetida* and *Dendrobaena veneta*) than an alternate F3 formulation (BioEx Ecopol) and a commonly used PFAS-containing AFFF (Buckeye). Angus Fire Jetfoam also was the only formulation that clearly showed weight loss in a 28-day chronic test. When tested for behavioral effects at concentrations where little to no mortality occurred, both taxa demonstrated almost complete avoidance of soils treated with any of the three products. Even foams with no acute toxicity can disrupt soil ecosystems by repelling essential organisms, leading to potential long-term ecological consequences. This study highlights the necessity of comprehensive ecotoxicological assessments that include both acute and behavioral endpoints. Transitioning to safer alternatives requires a nuanced understanding of their ecological risks, ensuring that new formulations do not inadvertently introduce new ecological hazards.

CRediT authorship contribution statement

Roger Yeardley: Writing – original draft, Validation, Formal analysis, Data curation, Conceptualization. **Michael Penrose:** Writing – review & editing, Methodology. **Paola Rodríguez Montoyo:** Investigation. **Mohamed Ateia:** Writing – review & editing, Visualization, Supervision, Funding acquisition, Conceptualization.

5. Disclaimer

This document has been subjected to the U.S. Environmental Protection Agency's review and has been approved for publication. Any mention of trade names, products or services does not imply an endorsement by the Agency. The Agency does not endorse any commercial products, services, or enterprises. Michael Penrose's Participation Program at the U.S. Environmental Protection Agency, administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy and EPA. Paola Rodríguez Montoyo was supported by the Department of Education Minority Science and Engineering Improvement Program (MSEIP). The views expressed in this article are those of the authors and do not necessarily represent the views or policies of the USEPA, ORISE, the Department of Education.

Declaration of competing interest

The authors declare that they have no known competing financial

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.143860.

Data availability

Data will be made available on request.

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