**Title:** Acute and Chronic Toxicity of PFAS-Free Firefighting Foams and a Reference Aqueous Film-Forming Foams to Freshwater Aquatic Organisms

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**Abstract:** Amid concerns regarding the persistence, bioaccumulation, human and environmental impacts of per- and polyfluoroalkyl substances (PFAS), there is an urgent need to develop PFAS-free products across their diverse usage. Historically, aqueous film-forming foams (AFFF) used in firefighting contained PFAS, with recent legislation mandating the phase out of PFAS-containing AFFF and necessitating the development of fluorine-free foams (F3s). To support the implementation of novel F3s, this project aimed to test the toxicity of a suite of seven F3s and a reference short-chain PFAS-containing AFFF (Buckeye) to three freshwater aquatic species: the green algae, *Raphidocelis subcapitata*, the midge larvae, *Chironomus dilutus*, and the fathead minnow, *Pimephales promelas*. Both acute and chronic toxicity tests were performed with endpoints including survival, growth, and development. For the acute study, several of the tested F3s exhibited greater toxicity than the reference AFFF, with the Avio F3 ranked as very highly toxic based on the EPA’s Alternatives Assessment Criteria. Similar findings were observed for the chronic toxicity testing, with the reference AFFF among the least toxic of the tested products, and two foams characterized as highly toxic based on no observed effect concentrations (NOECs) for midge emergence. These findings are supported by a concurrent multi-laboratory effort focusing on the effects of F3s and a reference AFFF on a range of aquatic species including amphibians, crustaceans, and fish. The results of this study will be used to facilitate selection of an appropriate F3 that minimizes impacts on the environment.