## Title

New Approach Methodologies: Physiological Responses of Daphnids to Pharmaceutical Mixtures

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## Abstract

The levels of pharmaceuticals have been continuously increasing in aquatic ecosystems over the last decade due to their extensive use in human and veterinary medicine. Traditional water monitoring methods have been proven insufficient to capture pollution due to pharmaceuticals because of sensitivity limits, inability to monitor every pollutant, and their failure to provide mechanistic insight into the actions of pollutants. To combat this, New Approach Methodologies (NAMs) are increasingly introduced as supplementary tools that can provide sensitive metrics for pollution assessment. In addition, NAMs can bridge the gap in ecotoxicological information regarding mixtures of pharmaceuticals, which are more commonly found in aquatic ecosystems versus pharmaceuticals existing alone. In this context, freshwater sentinel species, such as the water flea known as daphnids, were used to assess the acute effects of a typical pharmaceutical mixture on various physiological and molecular responses. In addition to mortality, phenotypic and molecular endpoints such as ingestion rate and enzyme activity were measured to determine the impact of a cocktail of commonly prescribed pharmaceuticals. Specifically, diclofenac, metformin, gabapentin, amoxicillin, trimethoprim, and erythromycin were mixed in equal amounts and tested at 0.1, 1, and 10 mg/l. Results showed differences in mortality, a decrease in feeding, and changes in enzyme activities, thus supporting the distinct pattern in physiological responses of daphnids.