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Morphological and Molecular Analysis of the Toxicity of Pharmaceutical-Derived Aquatic Contaminants (PPCPs)

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Contents of emerging concern (ECs) are ubiquitous aquatic contaminants and include pharmaceutical personal care products (PPCPs). Due to the exponential increase in pharmaceutical consumption in recent years, pharmaceutical compounds have been detected at elevated concentrations globally in surface water, finding their way into wetlands, streams, and sewage discharge containing unmetabolized drugs and improper drug disposal. As these medications are specifically designed to promote biological activity at low doses, there is increasing concern that pharmaceutical-derived aquatic contaminants could pose as potential endocrine disruptors to non-target organisms.

Due to their relative novelty, the toxicological implications of pharmaceutical-derived aquatic contaminants, especially in complex mixtures, are poorly understood. Previous research has largely focused on single-chemical exposures—however, field testing shows that this is not an accurate representation of the complex toxicity profile of PPCPs found in waters. Therefore, we present an overview of the toxicology of PPCPs in aquatic environments.

Purpose

The overarching objective of the study was to elucidate the toxicological implications of pharmaceutical-derived aquatic contaminants. Specifically, the study was to determine (1) quantitatively characterize toxicological responses to both single-chemical and mixtures of PPCPs in astacin embryos through morphological analysis and (2) conduct molecular analysis of PPCP toxicity in order to better understand potential mechanisms of toxicity.

Thus, the research question was: What are the toxicological implications of PPCPs both singly and in complex mixtures and what molecular analysis be applied to this specific class of contaminants for enhanced understanding of toxicity? It was hypothesized that contaminant compounds in a mixture would result in an additive toxic effect which could potentially induce measurable changes on the molecular level.

Experimental Compounds

Gemfibrozil and triamterene are pharmaceutical-derived contaminants that have been detected at elevated concentrations not just in the Puget Sound but globally. However, research regarding the aquatic toxicity of triamterene is limited and toxicologically relevant for this study; it was limited for gemfibrozil. The two drugs have distinct mechanisms of action, making them ideal compounds for accurately simulating endocrine disrupting conditions.

Gemfibrozil is a lipid-regulating fibrate used to treat hypertriglyceridemia.

Triamterene is a diuretic primarily used to treat hypertension and edema.

Morphometric Measurements

Gemfibrozil was used as a positive control due to its known cardiotoxic effects. Both drugs were assessed for the following endpoints: enlarged atrium, enlarged ventricle, hemorrhaging, blood degeneration, cardiacistle, tube heart, unroofed heart, pericardial blood pooling, and pericardial/peripheral edema. A binary (1, 0) system was used to indicate the presence of a specific condition and the resultant sum was used as a composite index of cardiac morphology/functioning (total score).

The response addition (RA) model was used to respond at the predicted mixture response based on single-chemical trials:

\[
\text{Mixture response} = \text{probability A} + \text{probability B} = (\text{probability A}) + (\text{probability B})
\]

where A and B are endpoints from single-chemical exposures.

Morphometric measurements were extracted from images of experimental embryos (48 hpf) for various toxicological endpoints (eye area, whole-body length, yolk sac size, cardiac abnormalities). Values were assessed for the following: endpoints: enlarged atrium, enlarged ventricle, hemorrhaging, blood degeneration, cardiacistle, tube heart, unroofed heart, pericardial blood pooling, and pericardial/peripheral edema. A binary (1, 0) system was used to indicate the presence of a specific condition and the resultant sum was used as a composite index of cardiac morphology/functioning (total score).

Conclusions/Discussion

Results indicate that gemfibrozil and triamterene induce sub-lethal toxic effects in astacin embryos.

Increased observed heart physiological abnormalities suggest that the triamterene is toxic to the developing fish heart in the presence of pericardial blood pooling being a sensitive measure of cardiotoxicity.

Gemfibrozil induced a similar dose-dependent increase in cardiac defects. The gemfibrozil-induced injury phenotype, however, also included extracardiac abnormalities, with a small eye phenotype (microphthalmic), enlarged yolk sac, and increased whole-body length.

The yolk sac area is an important variable to consider when developing methods for contaminants. The yolk sac area is a reverse variable and often used as a stress indicator. The yolk sac area is a key variable in development and early development stages.

Gemfibrozil induced similar dose-dependent increases in cardiac defects. However, with yolk sac area, observed toxicity is consistently above predicted values for all mixtures—this could be explained by differences in toxicity between the yolk sac area and the heart area. Moreover, yolk sac area values are not significantly different from predicted values, this trend is in the data indicating that the drugs may be interacting to produce greater than additive toxicity in terms of yolk sac enlargement.

An additional level of toxicity is not always following the toxic effect. For example, toxicity of the mixture is greater than the toxicity of each component. This is consistent with the observation that that the mixture does not interact with lipid metabolism.