

# AZITHROMYCIN, NIMESULIDE, AND THEIR COMBINATION ALTER BEHAVIOR IN ADULT ZEBRAFISH

Paola Aparecida de Almeida<sup>1</sup>; Wagner Antonio Tamagno<sup>2</sup>; Francieli Ubirajara India Amaral<sup>1</sup>; Luciane Dallazari da Silva do Prado<sup>1</sup>; João Antônio Duarte Lampugnani<sup>3</sup>; Gabriela Witkowski Rutikoski<sup>3</sup>; Leonardo José Gil Barcellos<sup>1,3,4\*</sup>

<sup>1</sup>Bioexperimentation Graduate Program, University of Passo Fundo, Passo Fundo, RS, Brazil.

<sup>2</sup>School of Health Science, Purdue University, West Lafayette, IN, United States of America.

<sup>3</sup>University of Passo Fundo, Passo Fundo, RS, Brazil.

<sup>4</sup>Pharmacology Graduate Program, Federal University of Santa Maria, Santa Maria, RS, Brazil.

**INTRODUCTION:** The increased use of various pharmaceuticals is evident in modern society, and the presence of these residues in the environment has become a global concern. Improper disposal of drugs into aquatic environments can result in water contamination, bioaccumulation of chemical compounds, and adverse effects on aquatic organisms. Among the widely used classes, antibiotics and anti-inflammatory drugs such as azithromycin (AZT) and nimesulide (NIME) stand out, both of which have already been detected in aquatic ecosystems. However, their effects on non-target species are still poorly understood. **OBJECTIVE:** To evaluate the behavioral effects on adult zebrafish (*Danio rerio*) exposed to environmental doses of AZT and NIME, both individually and in combination, using zebrafish as an animal model due to its high translational relevance. **MATERIALS AND METHODS:** A population of 60 fish (1:1 female/male) was used, distributed across 5 experimental groups (Control, DMSO, AZT, NIME, AZT+NIME). The experiments were conducted in glass aquariums (4L, density of 1 fish.L<sup>-1</sup>). The groups were exposed to azithromycin (12.5 µg.mL<sup>-1</sup>) and nimesulide (0.4 µg.mL<sup>-1</sup>) concentrations, both individually and in combination, for 15 minutes per day over 4 consecutive days. This strategy was repeated weekly until reaching n=12 per treatment. On the 5th day, the behavior of the fish was analyzed using the Novel Tank Test, Social Preference Test, and Light-Dark Test. The concentrations were confirmed by high-performance liquid chromatography (HPLC) in the water. **RESULTS AND CONCLUSION:** Exposure to AZT and NIME, both individually and in combination,

induced behavioral responses similar to anxiety in adult zebrafish. Notably, exposure to NIME alone caused hypomotility. These findings highlight the ecotoxicological risks associated with these drugs and emphasize the need for more stringent regulations regarding their use and disposal. The observed behavioral changes may compromise critical aspects of species survival, such as reproduction, growth, and immune functions, potentially leading to significant ecological imbalances.

**Keywords:** Antibiotics; Anti-inflammatories; Environmental contaminants; Behavioral tests; *Danio rerio*.

**Funding source:** National Council for Research and Technology (CNPq): Universal Notice 2023 (403048/2023-0) and Productivity Scholarship in Research of LJGB (302167/2022-6).