

## DEVELOPMENT OF AN AUTOMATED MODEL FOR BEHAVIORAL ANALYSIS OF SEIZURE-INDUCING COMPOUNDS IN ZEBRAFISH

Barbara D. Fontana; Laura Blanco; Angela E. Uchoa; Mariana L. Müller; Falco L. Gonçalves; Kimberly Fontoura, Cássio M. Resmim; João V. Borba; Julia Canzian; Denis B Rosemberg.

*Universidade Federal de Santa Maria (UFSM) – Santa Maria – RS.*

**INTRODUCTION:** Neurotoxic substances, including pesticides, heavy metals, and pharmaceuticals, can induce seizures as an adverse effect, posing a risk to both human and environmental health. The zebrafish (*Danio rerio*) is a widely used model organism for assessing the effects of neurotoxic compounds due to its neurophysiological similarities to mammals and high sensitivity to seizure-inducing agents such as pentylenetetrazole (PTZ). However, manual analysis of seizure-related behavior is labor-intensive and prone to human error, leading to high variability in results. **OBJECTIVE:** To develop an automated machine learning-based model for accurate analysis of seizure-like behaviors induced by neurotoxic compounds in zebrafish. **MATERIALS AND METHODS:** Adult zebrafish were exposed to different concentrations of PTZ (2.5, 5.0, and 7.0 mM) to induce seizures and treated with two anticonvulsant drugs: diazepam (37.5  $\mu$ M - DZP) and valproate (1.5 mM - VALP) for pharmacological validation. A machine learning model using the Random Forest algorithm was trained to recognize behavioral patterns associated with seizures, classifying six distinct motor activity profiles (normal behavior, immobility, hypolocomotion, hyperlocomotion, clonic-like behavior, and tonic-like behavior). **RESULTS AND CONCLUSION:** The automated model accurately identified seizure-related behaviors induced by PTZ and distinguished the effects of the anticonvulsants, demonstrating that DZP and VALP exhibit distinct protective profiles. The developed model automates real-time detection of the neurotoxic effects of seizure-inducing molecules, improving analysis reproducibility and enabling an in-depth characterization of anticonvulsant drugs. Thus, the use of machine learning for behavioral analysis in zebrafish represents a promising tool for neurotoxic compound screening and risk assessment in experimental toxicology.

**Keywords:** neurotoxicity; seizures; machine learning.