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Assessing the toxicity of herbicides mixtures used in sugarcane culture in Brazil using zebrafish

A.M. Moura¹, I. Domingues², R. Oliveira², A.M.V.M. Soares³, K.R. Medeiros², C.M. Jonsson⁴, A.J.A. Nogueira²¹Instituto Biológico, Campinas, Brazil²Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal³University of Aveiro & CESAM, Aveiro, Portugal⁴Embrapa Meio Ambiente, Jaguariúna, Brazil

Brazil is a pioneer in large-scale use of ethanol, a renewable fuel that contributes to the reduction of greenhouse gas emissions by combustion of fossil fuels. The high productivity of sugarcane crops demands the intensive use of equipment and inputs that can contaminate water bodies adjacent. Herbicides are the most common pesticide used in this crop, but the impact of this practice on non-target organisms is not well known. Moreover, possible interactions between different herbicides used simultaneously are not understood. Most studies into herbicides ecotoxicology are related to temperate ecosystems thus, there are still many gaps about its effects in a tropical climate. The aim of this work was to evaluate the effects of the mixture of ametryn (AMT) and diuron (DIU), herbicides widely used in sugarcane crop, to the tropical zebrafish (*Danio rerio*). Binary combinations of DIU and AMT were tested as follows: 0, 4.8, 6.8, 9.6, 13.6, 19.2 mg L⁻¹ of DIU and 0+ (solvent control); and 0, 0+, 9.7, 13.7, 19.4, 27.4 and 38.8 mg L⁻¹ of AMT. The trials were based on the OECD guideline on Fish Embryo Toxicity Test. Several sub-lethal and lethal endpoints were evaluated. The activity of the biomarkers CHE, LDH and GST was determined after 96 h of embryos exposure to the binary mixture: 0, 0+, 2.5, 3.5, 5.0, 7.1 and 10.01 mgL⁻¹ DIU; and 0, 0+, 5.1, 7.2, 10.1, 14.2 and 20.0 mgL⁻¹ of AMT. Sigma Plot 10.0 package was used for statistical analyses and ToxCalc spreadsheet were used to calculate LC50-96h and determine the model that best describes the mixture. LC50-96h values for zebrafish exposed to AMT and DIU were, respectively, 48.46 (2.2) mgL⁻¹ and 23.93 (1.24) mgL⁻¹. The model which best describes the mixture was the independent action, with toxicity dose level-dependent: synergism in low and antagonism in high dose. GST activity was induced by AMT. However, when the mixture had higher concentrations of DIU we observed a reduction in activity. We observed an inhibition of CHE in higher concentrations of the two components of the mixture and no influence on LDH activity. We determined that the model which best describes biomarkers activity for these enzymes are concentration



addition. Our results show that there is a synergism between ametryn and diuron, which enhances its effects on *Danio rerio*. Risk assessment which uses data from the single substances may underestimate the real risk of these substances, as it is almost impossible to found them isolated in nature.

