Zebrafish early-life stage as an alternative model to predict acute oral toxicity *in vivo*

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Zebrafish (*Danio rerio*) early-life stages offer a complex and multicellular system integrating various tissues and differentiation processes. In addition, the zebrafish embryos are structurally and functionally similar to humans. Although the Fish Embryo Acute Toxicity (FET) test with the zebrafish is not included in the normative of the Council for the Control of Animal Experimentation (CONCEA), it is already worldwide recognized as an alternative model for ecotoxicological assessment. This organism model can fill the gap between conventional *in vitro* and *in vivo* tests for extrapolation of data for humans. Based on this, the present study aimed to develop an alternative method for assessing acute oral toxicity of chemicals using zebrafish early-life stage. Initially, 15 substances belonging to different Global Harmonization System (GHS) categories were evaluated by FET test (OECD 236). Lethal and sublethal effects were assessed after 96 h of exposure to determine the LC_{50} and EC_{50} values. Subsequently, LC_{50} values were compared with rodent acute oral toxicity data (LD_{50} values) from literature. Then, a linear regression-model using the -log toxicities, pLC_{50} and pLD_{50}, respectively, was generated for the prediction of acute toxicity. This model resulted in the following equation pLD_{50} (mg/kg) = 0.5749 x pLC_{50} (mg/L) + 1.284. The method domain of application was of 53.3% and R-squared close to 0.7. Sublethal effects indicated that substances more toxic presented more abnormalities. Finally, this alternative method was used to assess the acute toxicity of the drug prototype LQFM021 (Category 4 *in vivo* GHS). The DL_{50} of the LQFM021 predicted with FET was of 408.5 mg/kg, which also classified it as Category 4. Therefore, our results suggested that early-life stages of zebrafish could be at least a refinement for assessing acute oral toxicity *in vivo*, being as an intermediary in the preclinical assessment between *in vitro* and *in vivo*.

Keywords: Zebrafish, acute oral toxicity, DL_{50}, alternative-testing methods, Global Harmonization System.