Zebrafish Embryo-Larval Assay as a New Approach Methodology for Teratogenicity Prediction

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INTRODUCTION: Nonclinical developmental and reproductive toxicity assessments are essential to support human clinical trials during pharmaceutical marketing authorization. However, traditional tests require the use of many animals, making them ethically challenging, costly and time-consuming. The recent revision of the ICH S5(R3) guideline encourages the use of non-mammalian in vivo assays as alternative assays to detect toxic effects of human pharmaceuticals on reproduction and development. Zebrafish (*Danio rerio*), a non-mammalian vertebrate, has emerged as a valuable model for assessing developmental toxicity due to its genetic and physiological similarities to humans. It offers several advantages over mammalian models, including small size, high reproductive, fast organogenesis, external development, embryo transparency and ease of genetic manipulation, making it ideal for cost-effective, high-throughput screening. OBJECTIVE: In this context, the present study assessed the zebrafish embryo-larval (CEUA/UFG Nº 072/20) model as a New Approach Methodology (NAM) to predict in vivo teratogenicity. MATERIALS AND METHODS: Fourteen chemical compounds, previously classified as teratogenic or non-teratogenic in rodents or humans, were tested using the Fish Embryo Toxicity (FET) test combined with bone mineralization analysis via alizarin red staining. Teratogenicity was evaluated based on a Teratogenic Index (TI), calculated as the LC_{50}/EC_{50} ratio, with $TI \geq 3$ indicating teratogenic potential. Sublethal concentrations ($LC_{50}/2$ and $LC_{50}/4$) were also assessed for skeletal effects. RESULTS: Five compounds were classified as teratogenic: cycloheximide, retinol, warfarin, propranolol (based on TI), and enalapril (based on bone defects). When compared to rodent data, the zebrafish model showed 50% sensitivity, 75% specificity, and 64.3% accuracy. Prediction aligned more closely with human data, reaching 57.1% sensitivity, 85.7% specificity, and 71.4% accuracy. The method was further applied to LQFM310, a candidate drug, which was classified as non-teratogenic in zebrafish. CONCLUSION: These findings support the potential of zebrafish as a reliable intermediate model in preclinical toxicology, bridging the gap between in vitro assays and traditional animal studies. The zebrafish embryo-larval assay associated with the bone mineralization analysis offers a promising, cost-effective alternative for early teratogenicity screening in drug development.

Keywords: Alternative method, developmental toxicity, skeletal damage.

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