# INVESTIGATING ZEBRAFISH (*Danio rerio*) DEVELOPMENT AND BEHAVIOR TO ASSESS THE HAZARD OF ANTIMICROBIAL CuO NANOPARTICLES

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Nanoparticles (NPs) and nano-enabled products emerged as novel antimicrobial agents with proven efficacy against antimicrobial resistant (AMR) bacteria, which can be found in water bodies associated to fish farming. CuO NPs have been extensively used as bactericidal agent, yet their potential toxicity to cells and organisms is recognized. This work aims at evaluating the nanosafety of CuO-based nanomaterials in exploitation scenarios by using zebrafish (*D. rerio*), a promising model organism for high-throughput developmental and behavioral screening. Sonochemically synthesised water-based CuO (wCuO) NPs were studied, in comparison with sonochemical Zn-doped CuO (ZnCuO) NPs to investigate any differences in response based on the NPs physico-chemical structures and identify CuO-induced adverse outcomes. NPs suspensions were characterized by TEM, DLS and ICP-OES. The aquatic toxicity potential was assessed by the Fish Embryo acute Toxicity test (OECD n. 236). Zebrafish embryos were exposed to NPs at increasing concentrations (0.1, 1, 10, 100 mg/L) for 96 hours and screened every 24 hours for lethal and sub-lethal endpoints, to calculate LC50 and EC50. No significant lethal effects were found. The morphometric analyses revealed significant differences in all of the NPs-treated embryos' parameters with respect to controls. A complete lack of hatching was already evident at the lower concentrations for wCuO, while this effect decreased in ZnCuO-treated embryos. To investigate the mechanism preventing the hatching, the spontaneous tail coiling was analysed in pre-hatching embryos by using the DanioScope Software, but no significative differences were found compared to control. Collectively, these results suggest that the modulation of the NPs physico-chemical structure (i.e., metal doping) may contribute to their safety profile and that the mechanism responsible for the hatching delay induced by this class of NPs needs to be investigated at further levels (e.g., molecular).

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