TOPIC N° 1 – METHODS, TOOLS, AND TECHNOLOGIES FOR SSBD PURPOSES

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

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Nanoparticles (NPs) and nano-enabled products (NEPs) emerged as novel antimicrobial agents with proven efficacy against antimicrobial resistant (AMR) bacteria, which can be found in water bodies associated to fish and animal farming [1]. Nevertheless, in line with the concept of Safe and Sustainable by Design, their safety and sustainability must be evaluated at an early phase of the innovation process [2]. In fact, the increase in nanomaterials (NMs) manufacture and use may lead to inappropriate disposal into the aquatic environment, representing a potential risk to non-target species [3]. In this context, the EU project AMROCE aims at reducing the spread of AMR bacteria in the aquatic environments through a platform of novel nano-antimicrobial products. Among the several NMs with antimicrobial properties, CuO NPs have been extensively used as bactericidal agent [4], yet their potential toxicity to cells and organisms is recognized [3]. In order to contribute to a safe development of the new nano-biocidals, this work aims at evaluating the nanosafety of CuO-based NMs in exploitation scenarios by using zebrafish (*D. rerio*), a promising model organism for high-throughput developmental and behavioral screening [4].
In this study, sonochemically synthesised water-based CuO NPs (wCuO) in the framework of the ERA-NET project AMROCE were investigated, in comparison with sonochemical Zn-doped CuO (ZnCuO) NPs, already developed under the EU-H2020 project PROTECT for coating water depuration membranes. This comparison was conducted to investigate any differences in response based on the NPs physico-chemical structures and identify CuO-induced adverse outcomes, to be used to predict the hazards from antimicrobial NEPs that will be further developed.
NPs suspensions were characterized by TEM, DLS and ICP-OES, while the aquatic toxicity potential was assessed by the Fish Embryo acute Toxicity (FET) test (OECD n. 236). Zebrafish embryos were exposed to NPs at increasing concentrations (0.1, 1, 10, 100 mg/L) for 96 hours and were screened every 24 hours for lethal and sub-lethal endpoints. Acute toxicity was analyzed by calculating the LC50 and EC50. No significant lethal effects were found. The morphometric analyses revealed significant differences in the NPs-treated embryos' length, eyes size and distance and yolk area. A complete lack of hatching was already evident at the lower concentrations for wCuO, while this effect decreased in ZnCuO-treated embryos, as testified by the higher effect concentrations, suggesting that the modulation of the NPs physico-chemical structure, including metal doping, may contribute to the safety profile of such effective antimicrobial NPs. According to these results, in addition to the behavioral assessment in zebrafish larvae at the end of the FET test through the EthoVision XT Software (Noldus Information Technology©), the spontaneous tail coiling is also being analysed in pre-hatching embryos, by the use of the DanioScope Software (Noldus Information Technology©). This will allow the definition of additional hazard response functions for sublethal effects, including hatching efficiency and neurological dysfunctions, at low exposure concentrations to be implemented in the Life Cycle Assessment studies performed in parallel.

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