SAFE AND SUSTAINABLE NANO-ENABLED ANTIMICROBIALS TO REDUCE THE PRESENCE OF CONTAMINANTS OF EMERGING CONCERN IN THE AQUATIC ENVIRONMENTS

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Contaminants of emerging concern (CECs), such as antibiotics and antimicrobial resistant (AMR) bacteria associated to intensive fish and animal farming, represent a great threat to environmental and human health. Nanoparticles (NPs) and nano-enabled products (NEPs) emerged as novel antimicrobial agents with proven efficacy against AMR bacteria, nevertheless, their safety and sustainability must be evaluated at an early (design) phase. Indeed, uncertainties persist about their safety and environmental sustainability, as evidenced by toxicological and Life Cycle Assessment (LCA) studies. Most importantly, 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.

In this context, the AMROCE project[1] aims at reducing the spread of AMR bacteria in the aquatic environments through a platform of novel nano-antimicrobial products. Specifically, the project will develop antimicrobial/antibiofilm fish cage nets and wastewater filtration membranes through polymer nano-engineered bulk- and surfaces with metal-oxide NPs (e.g., CuO). Marine-derived nano-formulated antimicrobial agents and antibiofilm enzymes are proposed as an alternative to antibiotics for fish and animals. To contribute to the development of safe and sustainable by design nano-biocidals, the project investigates their efficacy and assesses their associated risk in exploitation scenarios through the implementation and integration of both *in vivo* and *in silico* methodologies.

Evaluation of the safety and sustainability of water-based copper oxide (wCuO) NPs and wCuO-based NMs, such as coated, co-extruded polymers and filtration membranes, along with nano-formulations of marine-derived NPs (i.e., oils from microalgae) and quorum quenching enzymes (i.e., acylase) are investigated.

Both NPs suspensions and leachates obtained from the polymeric materials were characterized by means of TEM, DLS and ICP-OES. Nanosafety of the novel nanotechnology-embedded products was assessed by using zebrafish (*D. rerio*), a promising model organism for high-throughput developmental and behavioural screening. The aquatic toxicity potential was assessed by the Fish Embryo acute Toxicity (FET) test (OECD n. 236) by exposing zebrafish embryos to increasing NPs concentrations or to polymers-derived leachates for 96 hours and screened every 24 hours for lethal and sub-lethal endpoints. Acute toxicity was analysed by calculating the Lethal Concentration 50 (LC_{50}) and the Effective Concentration 50 (EC_{50}). To further investigate the toxic potential at sub-lethal levels, morphometric analyses were performed following the FET test, as well as through behavioural evaluations on embryos at earlier development stages.

Dedicated LCA studies are performed to assess the NMs environmental sustainability. Within the inventory phase, AMROCE partners provided primary data and information about the NPs and the NMs synthesis/production processes, which were further modelled. Secondary data were derived by the Ecoinvent 3.7 database. NPs and NMs associated impacts were assessed with the CML 2001 impact method by using the OpenLCA software. In line with the safe and sustainable by design approach, a comparative assessment was performed by increasing NPs concentrations in the NMs formulations.

The suggested integration of novel nano-eco toxicology assessment and standard LCA studies represents a potentially effective methodology to provide a comprehensive and harmonised framework supporting decision-making at design stage for safe and sustainable nano-enabled antimicrobials within the field of fish and animal farming.

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