Deliverable 2.3 – Bioaccumulation and persistency evaluation of selected NMs

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Public Summary

This summary of Deliverable D2.3 presents findings from the HARMLESS persistency and "point of entry" bioaccumulation evaluations performed within Task 2.3 by project partners DTU, INIA and BASF. It also provides a proposal for a "HARMLESS Principles for point-of-entry bioaccumulation and persistency evaluation in a Safe and Sustainable by Design (SSbD) context" according to an assessment of the overall applicability of performed persistency tests and the HARMLESS "point of entry" approach. According to the proposed HARMLESS "point of entry" approach, materials were screened for their potential for interaction and accumulation in lower trophic level organisms (algae and daphnia), since these will act as gatekeepers for an actual biomagnification to occur. Bioaccumulation studies in fish were then used as confirmatory tests to validate the "point of entry" approach for bioaccumulation screening being proposed and the possibilities of biomagnification. Then, as nanomaterial (NM) persistency plays a key role in bioaccumulation, by determining the availability of NMs for organisms the persistency of materials to stay as NMs was evaluated using dissolution and stability monitoring studies under simulated environmental conditions. Finally, this information was used to inform the development of SbD principles and risk matrices (WP4).

Description of Task

To deliver data for the "point-of-entry" bioaccumulation as input to the HARMLESS SSbD approach, first algae-NM interaction quantification tests were performed and used as a proxy for the biomagnification potential of NM. Then in a similar way, tests with Daphnia were performed and assessed for use and applicability. Confirmatory bioaccumulation studies performed in fish (*Oncorhynchus mykiss*) using a dietary exposure route (OECD TG 305) (OECD 2012) provided a technically feasible and appropriate method to test these materials with poor stability and susceptibility to dissolution in environmentally relevant media. Thereafter to characterise the extent to which materials were susceptibility to dissolution and overall dispersion stability tests using OECD TG 318 (OECD 2020) approaches and continuous flow systems were used.

Point-of-entry bioaccumulation studies with algae and applicability

Algae –NM interaction tests were performed with the freshwater green algae *Raphidocelis subcapitata* and CeO_2 NPs. Single-cell inductively coupled plasma time-of-flight mass spectrometry (sc-ICP-MS-TOF) was used to determine the elemental "algal cell fingerprint" and the incidence of this fingerprint with Ce signals was used as evidence of NM-algal cellular interaction that could potentially be transported further up the food chain. Results from these studies have been published and can be found in the article "Effect of Exposure Concentration and Growth Conditions on the Association of Cerium Oxide Nanoparticles with Green Algae" by the authors Mackevica A, Hendriks L, Meili-Borovinskaya O, Baun A, Skjolding LM (Mackevica et al 2023). Aswell as providing quantitative data on the different NM interactions, results provided evidence for the influence on growth. Algae appear to have an ability to shed NPs when actively growing and thus experiments performed under non-growth conditions may

overestimate bioaccumulation potential. The need to use such analytical equipment that is not readily available in general laboratories limits this “point of entry” approach using algae for first stages in an early approach. However, it is still seen as useful in later stages to assess potential point-of-entry on multiple lower trophic levels.

Point-of-entry studies with daphnids and applicability

Tests with daphnids were performed following the method of Skjolding et al. (2014) with some modifications and using a 24-hour uptake and a 24-hour depuration period. *Daphnia magna* cultures were exposed to a range of lanthanum-cobalt-nickel (LaCoNi) structure perovskite advanced materials (AdMa) with different SbD versions (LaCoNi(5), LaCoNi(8), LaCoNi(16), LaNi(22), LaCoNiPd, LaCoNiPt) in Elendt M7 medium and the levels of particle specific uptake and depuration were analysed using single particle (SP)-ICP-MS. Analysis during different sampling times (1, 2, 4, 8, and 24h) allowed uptake and depuration kinetics/rates to be calculated and these were used to derive “apparent kinetic bioconcentration factors”. These factors were used to distinguish SbD versions with higher/lower bioaccumulation potential. The point of entry studies performed with daphnia did not require advanced exposure and characterization instrumentation and provided a feasible and a cost-effective way for providing point-of-entry bioaccumulation data for informing SbD decisions (for example in a screening method in the initial stage of a SSbD approach).

Confirmatory bioaccumulation studies with fish and applicability

Rainbow trout (*Oncorhynchus mykiss*) juveniles were exposed to LaCoNi and the doped material LaCoNiPd perovskite spiked diets (OECD TG 305-III: Dietary Exposure Bioaccumulation Fish Test) for 14 days. Samples were taken for analysis using traditional ICP MS and SP-ICP-MS to quantify whole fish uptake as well as the presence of particles in fish tissues. Samples were also taken during a depuration phase to assess depuration rates. The dietary exposure route represents the route of natural trophic food chain transfer, and thus served as a confirmatory test to validate the “point of entry” approach for bioaccumulation. According to this test, a biomagnification factor (BMF) is generated and is calculated as the ratio of concentration of a substance in a predator (fish) relative to the concentration in the predator's prey (or food source, e.g. spiked feed) at steady state. BMF values >1 would suggest a material is bioaccumulative and thus values calculated can be used to compare with the results from the lower level trophic organism tests and validate the “point of entry” approach

Persistency evaluation – dissolution and agglomeration testing and applicability

Tests to analyse dissolution and agglomeration behaviour based on the OECD TG 318 (OECD 2017) and guidance document (GD) 318 (OECD 2020) were developed. The methods used included continuous flow system and analytical ultracentrifugation. These methods were applied to assess the LaCoNi, LaCoNiPd and LaCoNiPt perovskite materials in a range of aquatic test media and results have been published in the peer review article “Behaviour of advanced materials in environmental aquatic media – dissolution kinetics and dispersion stability of perovskite automotive catalysts (Di Battista et al. 2024). Aswell as providing important transformation rate data, results showed that while natural organic matter (NOM) increased stability it also increased the fraction of leachable ions at a given ionic strength and water hardness of the test media. The combination of the methods for dissolution and dispersion stability assessments provide a useful pre-screening tool for scientists working in the environmental hazard assessment of AdMa; as well being relevant for establishing design principles in SSbD practices (see Figure 1).

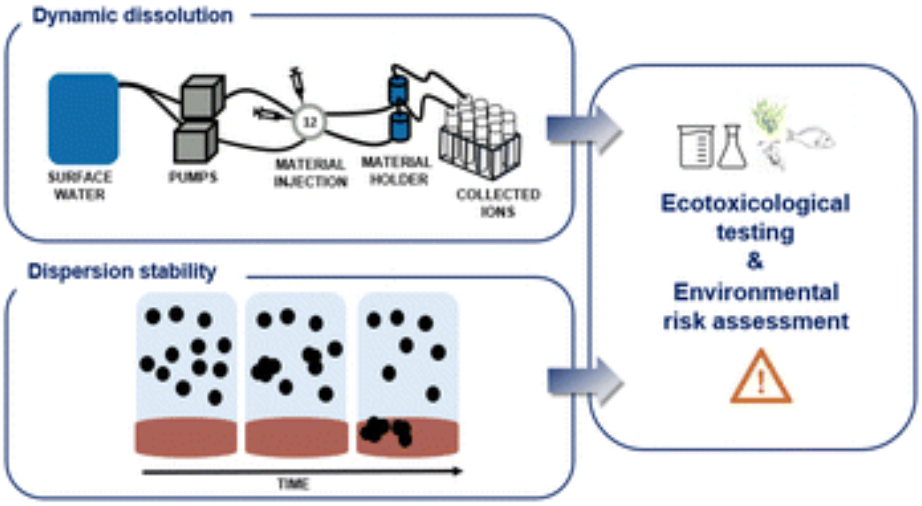

Figure 1. Graphical Abstract depicting approach used for persistency evaluation (*Battista et al. 2024).*

HARMLESS Principles for point-of-entry bioaccumulation and persistency evaluation in a SSbD context

All the generated information together with the specific characterisation of persistency through dissolution and stability studies was compiled into principles in the HARMLESS SSbD approach using perovskites as the case study materials. This includes a workflow firstly involving a characterization of persistency evaluation, secondly using daphnia testing as a screening test, and thirdly to expand to algal interaction tests and fish bioaccumulation confirmatory tests using a dietary exposure route (OECD TG 305). This tiered approach to data generation was linked to the stage-gate model as it takes both cost and level of testing difficulty into account. Consistent results between tier one daphnia testing and the final tier fish confirmatory test proved the usefulness of such a point of entry approach in screening.

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