OECD Ring Trial involving the NanoHYBIT: A Novel Method for Non-Vertebrate Regulatory Bioaccumulation Testing of Nanomaterials

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Background

Reliable, vertebrate-free methods to assess the bioaccumulation potential of engineered nanomaterials (ENMs) are still not covered by OECD Test Guidelines (TG). The freshwater amphipod *Hyalella azteca* has emerged as a promising model organism for determining bioconcentration (BCF) and biomagnification factors (BMF) for ENMs, with initial test protocols already established (Kuehr et al., 2021).
Within the projects NanoHYBIT* and NanoHYBIT 2.0** these protocols have been further refined, standardized, and successfully applied to a wider range of ENMs. To achieve regulatory acceptance of the protocols, robust validation is now essential. This includes demonstrating their inter-laboratory transferability, defining their applicability domain, and ensuring reproducibility and reliability of results.

Therefore, the Working Party of the National Coordinators of the OECD Test Guidelines Programme (WNT) has included a new project into its work plan, with the aim to get a new validated non-vertebrate protocol by expanding the OECD TG 321 (OECD; 2024) for the regulatory bioaccumulation assessment of ENMs. The first step involves the validation of the test system in the "NanoHYBIT" ring trial.

The NanoHYBIT ring trial

- Four selected ENMs will be tested for their bioaccumulation potential using both bioconcentration and biomagnification approaches based on the NanoHYBIT protocol.
- Test materials were selected to represent key ENM properties relevant for bioaccumulation (see Table 1).
- Participating laboratories are invited to conduct one or multiple tests.

Tabelle 1: Test materials of the OECD NanoHYBIT ring trial and their bioaccumulation-relevant properties

ENM	Characteristics
Au	Chemically stable / non-ion-releasing; well-dispersible
CuO	Ion-releasing; regulated metal uptake in the organism / metal with a physiological role; well-dispersible
CeO_2	Chemically stable / non-ion-releasing; metal without a physiological role; strongly agglomerating; rapidly sedimenting
^{14}C-Polystyrene	Chemically stable / non-ion-releasing; polymer-based; well-dispersible

What participants can expect:

- Initial online information session (June 2026) for all interested parties, including the opportunity to discuss the protocol and address questions
- Allocation of test materials and tests (BCF or BMF) in coordination with participants (i.e., which lab tests which materials in which tests: BCF and / or BMF tests)
- Provision of all test materials by the study coordinators
- Continuous support throughout the study, with coordinators available to assist with questions or technical issues
- A peer reviewed publication about the results with all participants

Interested?

Please feel free to contact us at the conference (Booth 43 – Fraunhofer IME) or send us a message at:

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Scan for further contact information

Timeline of the NanoHYBIT ring trial:

June 2026
Online meeting for all participants (not mandatory)

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Summer 2026 - Spring 2027
Ring trial

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Summer 2027 - Summer 2028
Evaluation of ring trail, validiation report, draft document & revision

Kuehr et al. (2021): Testing the bioaccumulation potential of manufactured nanomaterials in the freshwater amphipod *Hyalella azteca*. Chemosphere. Volume 263, 127961. DOI: 10.1016/j.chemosphere.2020.127961

OECD (2024): Test No. 321: Hyalella Azteca Bioconcentration Test. Paris: OECD Publishing. DOI: 10.1787/8ac30c4e-en

*funded by BMFTR under FKZ 16LW0289

**funded by BMFTR under FKZ 03WL0729 and supported by the Fraunhofer Internal Programs under Grant No. Attract 40-11859

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