Pre-guideline literature data on bioaccumulation of CeO_2 nanoparticles in a REACH context – Conclusions, identified issues, and recommendations for standardised testing

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Introduction

Type of accumulation	Aquatic bioaccumulation	Terrestrial bioaccumulation
Mandatory under EU REACH?	YES, An IX	NO
Related key values and use in exposure/risk assesmsent	**Key BCF for fish** • Used for assessment of secondary poisoning, aquatic food chain • Used for man via the environment (MvE) assessment	**Key BCF for earthworms** • Used for assessment of secondary poisoning, terrestrial food chain **Key BSAF for terrestrial plants** • Used for MvE assessment
Test guidelines available?	OECD 305 (fish, aquatic/dietary) OECD 315 (sediment-dwelling benthic oligochaetes)	OECD 317 (terrestrial oligochaetes) No specific guideline for plants

1

Available guidelines are however not adapted for nanomaterials.

EU REACH update of CeO_2 dossier:

Literature data stem from experiments performed according to various, **non-standardised methodology,** hence various issues have been identified.

In absence of data generated according to standardised methodology, a **pragmatic approach,** considering **implications for exposure/risk assessment,** needs to be followed.

Main findings

Type of bioaccumulation	Aquatic	Terrestrial
# studies evaluated	33	104
# studies in WoE approach	10	8
Type of assays	Single-species / microcosm / mesocosm studies in water / sediment	Single-species exposure of terrestrial plants in soil / food chain experiment
Dosing via	Water / sediment / food	Soil / food
Bioaccumulation factors	BCF plants: 15.3-10917 L/kgww BCF/BAF aq inv: 1.8-1681 L/kgww BCF/BAF fish: 3.1-246 L/kgww BsedAF aq inv: 0.23-127 TTF: 0.05-0.58	BSAFs << 1 Root-to-shoot TF < 0.01 TTF < 1

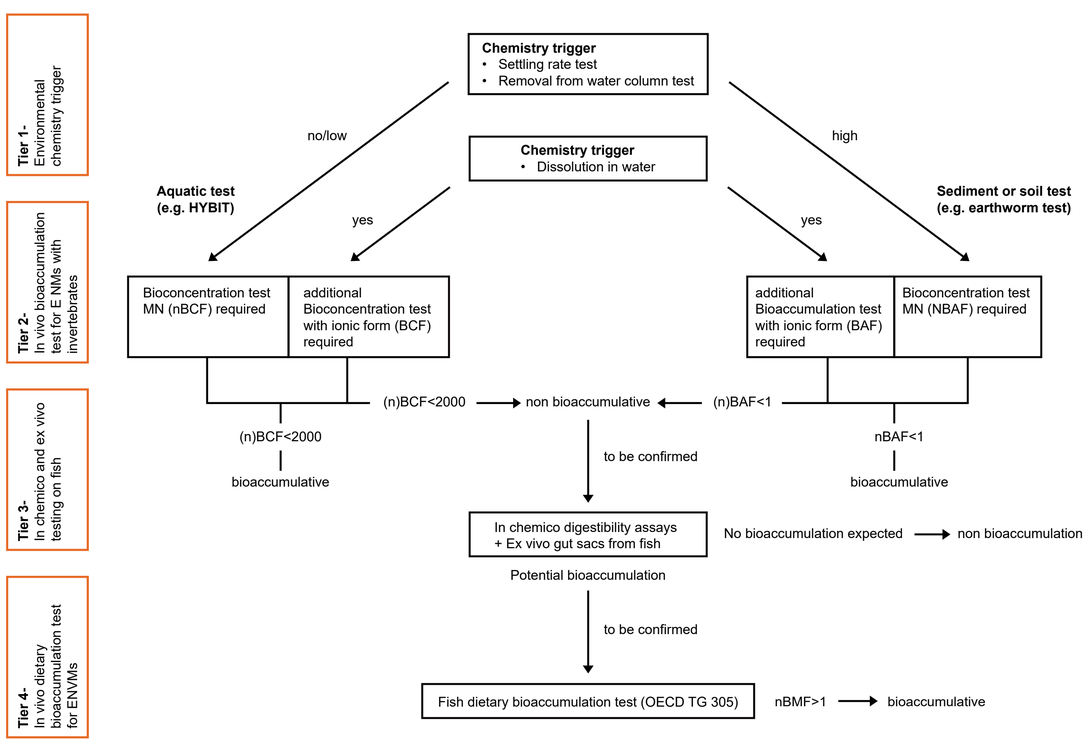
- **Bare CeO_2 NPs** are removed from the water column and **end up mainly in agglomerated state in the sediment.**
- **BCF/BAFs decrease** when **ascending the foodchain.**
- Overall, **bioaccumulation potential** is **low.**
- Trophic transfer factors are < 1, confirming **trophic dilution.**
- Bioaccumulation in aquatic organisms tends to decrease with increasing exposure concentration / duration (due to settling).
- **Accumulation** in **aquatic plants** occurs **mainly at the surface.**
- **Accumulation** in **fish and aquatic and terrestrial vertebrates** occurs **mainly at the surface** (incl. gills in fish) **or in the digestive tract.** Very limited transport to internal organs (potentially after transformation) was observed in some studies.
- Gut clearing results in reduced bioaccumulation factors.
- Bioaccumulation factors do not represent absorbed CeO_2 NPs → **mass-only bioaccumulation factors do not inform enough.**
- Specific excretion/elimination mechanisms: shedding (daphnids), pseudofeces (mussels).
- Reduction to Ce+III may partly occur in some organisms and cause transformation of CeO_2 to other harmless forms.
- Accumulation in **terrestrial plants**: roots >> stems > leaves >> fruits/seeds.
- CeO_2 NPs in **roots of terrestrial plants** are **mainly** found **in the epidermis** but are (though limited) also identified in endodermis, cortex, and xylem. **Passive transport through the cell wall** seems to occur to a limited extent.
- Majority stays CeO_2 in the roots, but also $CePO_4$ and carboxylates observed.
- Clusters of NPs are observed in terrestrial plants in parenchyma leaf tissues, in the stroma of the chloroplast and in vacuoles, but root-to-shoot translocation is very poor (factors typically < 0.01).
- **Soil properties affect bioaccumulation** in terrestrial plants, e.g. organic matter increases mobility and accumulation in roots, but decreases translocation to stems/leaves.
- **Shape affects accumulation** in terrestrial plants → CeO_2 nano-rods accumulate / translocate easier.

2

OECD has recently published a **scoping review** presenting a **tiered approach for reliable bioaccumulation assessment of manufactured nanomaterials** in the environment* in which a tiered assessment scheme is included with tiers for:

- Testing of physicochemical triggers (such as particle settling and dissolution)
- In vivo testing in invertebrates
- In chemico/ex vivo investigation
- OECD 305 testing in fish (as a final resort).

3



* Figure taken from: OECD Environment, Health and Safety Publications, Series on the Safety of Manufactured Nanomaterials No. 110, ENV/CBC/MONO(2024)2 - pdf (oecd.org)

Important questions/facts to consider when establishing test strategy/risk assessment strategy

Question/consideration	Implications for test strategy/risk assessment
How do NPs behave in the environmental compartment under consideration (dissolution rate, dispersion stability, transformation, …)?	For poorly soluble NPs which do not form stable dispersions (such as CeO_2 NPs), focus on sediment organisms.
Are there **different hazards** identified (separate DNELoral, PNECsec.pois.) for the **metal ion** and the **NPs?**	**Combined risk assessment might be needed** (and hence **separate bioaccumulation factors**) for the NP part and the 'dissolved' part released from NPs. If metal ion-based toxicity (and/or bioaccumulation) is dominant, **conservative risk assessment** using **total element-based bioaccumulation factors and metal ion-based DNELs/PNECs** may be sufficient.
Is there a need to distinguish between **true uptake** (of metal ion or NPs as such) through skin/ epidermis, gills, gut wall) and **adsorption/accumulation in the digestive tract?**	From a mechanistic point of view, this is always important to understand. For **secondary poisoning,** adsorption to skin or accumulation in digestive tract is equally important as absorbed fraction. For **MvE assessment,** bioaccumulation in **edible parts** is most relevant, therefore, bioaccumulation factors based on true uptake in fish / plant parts might be required for an accurate risk assessment.

Take-home messages (bare) CeO_2 NPs

Bare CeO_2 NPs mainly partition to sediment in the environment and **do not give rise to substantial amounts of dissolved Ce.**

Evaluation of **pre-guideline literature data** allows the **overall conclusion** that CeO_2 NPs show **poor bioaccumulation potential** and undergo **trophic dilution.**

Currently, based on hazard and bioaccumulation data, there is no need to perform exposure/risk assessment for secondary poisoning.

MvE assessment is mandatory for substances > 1000 tpy, but **no hazards have been identified after oral exposure.** Since there is no substantial dissolution, **total CeO_2-based bioaccumulation factors are sufficient to determine exposure of MvE.**

Taking into account all of the above, **no further bioaccumulation experiments are required** for CeO_2 NPs under EU REACH.