# Grouping of nanomaterials regarding their ecotoxicity – The ECOTOX BOND tool

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## Introduction

- The large variety of nanomaterials (NM) represent a challenge for scientists and regulators in assessing the potential hazard.
- Risk assessment on a case-by-case basis is not practicable for the many different varieties of NM.
- A tool for grouping and read-across for NM based on existing knowledge on similar NMs is required for hazard prediction.

→ The development of a grouping option for nanomaterials (NM) with regard to their ecotoxicity is one goal of the nanoGRAVUR project. Grouping of NM requires the identification of relationships between physicochemical properties of the nanomaterials and their ecotoxicological behaviour.

**Table 1: NM selected for the literature study, the parameters considered most relevant for ecotoxic effects, and the test set selected.** Grey: no data available.

| NM selected for literature study | Most relevant parameters for ecotoxicity | NM test set |
|---|---|---|
| $Fe_xO_y$, Ag, CNT, Carbon Black, Cu/CuO, $C_{60}$, Au, Quantum Dots, ZnO, Graphen, $TiO_2$, $Al/Al_2O_3$, Kaolin, $CeO_2$, $SiO_2$ | • Coating/Surface modification<br>• Shape/size (->physical effects)<br>• Release of ions | ➢ $SiO_2$ untreated and with various surface modifications<br>➢ $Fe_2O_3$ and $CeO_2$ with different size and shapes<br>➢ Cu/CuO and ZnO |
| $CaCO_3$, granulated cinder, cement | | |

## Results

The first evaluation shows that several of the initial grouping hypotheses can not be confirmed. However, it also shows that inert and ion-releasing NM need to be considered differently. We conclude that an approach based on single physical-chemical parameters is not sufficient for a robust grouping.

| | | |
|---|---|---|
| Ecotoxicity of bulk material (EC50 < 100 mg/L / 1000 mg/kg) | YES → 1 | NO → 0 |
| Form: Wires Dimensions? | YES → 1 | NO → 0 |
| Release of ions | YES → 1 | NO → 0 |
| Reactivity | YES → 1 | NO → 0 |

| Sum | Group |
|---|---|
| 0+0+0+0 | 1 |
| 0+0+0+1 | 2 |
| 0+0+1+1 | 3 |
| 0+1+1+1 | 4 |
| 1+1+1+1 | 5 |

**Figure 1: The ECOTOX BOND combines the toxicity of the bulk material with 3 physical-chemical parameters which are considered as relevant.**

**Table 3: Assignment of the NM tested to specific risk groups and toxicity values**

| 1 | | 2 | | 3 | | 4 | | 5 |
|---|---|---|---|---|---|---|---|---|
| NM (prediction) | Ecotox [mg/L] (measured) | NM (prediction) | Ökotox [mg/L] (measured) | NM (prediction) | Ökotox [mg/L] (measured) | NM (prediction) | Ökotox [mg/L] (measured) | |
| $Cu_{phtalo\_halogen}$ | --- | $Cu_{\_phtalo\_nano}$ | 100 | ZnO NM111 ZnO NM110 | 0,1 | CuO | 0,2 | |
| $Fe_2O_3$ nanoA, $Fe_2O_3$ nanoB, $Fe_2O_3$ larger | 27 – 51 | $SiO_2$ untreated, $SiO_2$ amino, $SiO_2$ Phosphonat | 86 – 128 | | | Cu Ag NM-300K Ag Batch 1340 Ag Batch SRM 110525 | 0,002 – 0,04 | |
| $TiO_2$ uncoated $TiO_2$ - Eu $TiO_2$ - Fe | 0,38 – 3,6 | $CeO_2$ – Eu $CeO_2$ NM211 $CeO_2$ NM212 $CeO_2$ NM213 | 3,2 – 43,8 | | | | | |

## Method

For this purpose, the physico-chemical parameters relevant for an ecotoxicological effect were identified by means of a literature research in which 18 different NMs were taken into account (Table 1). Based on these results, three parameters were identified to be relevant for ecotoxicty. A NM test set was then selected for testing in various ecotoxicological test organisms (Table 2) involving several subtypes of a given NM, which differed in the parameter considered as relevant for ecotoxicity. The identified toxicities were then compared with the initial grouping hypotheses in order to check their validity and to specify required adaptations.

**Table 2: Test organisms taken for the ecotoxicity tests performed on the NM**

| | Test organisms / Test |
|---|---|
| Terrestrial Tests | Microbial ammonium oxidation |
| | Earthworm reproduction test |
| Aquatic Tests | *Desmodesmus subspicatus* (algae) |
| | *Daphnia magna* (chronic) |
| | *Daphnia magna* (acute) |
| | *Danio rerio* Embryo (Zebrafish) |

## Tool: ECOTOX BOND

A tool was developed combining the toxicity of the bulk material with three physical-chemical parameters, the ECOTOX BOND (Fig. 1). To allow a grouping of NM for which there is little prior information on toxicity and properties, the focus was on 4 criteria. These represent a combination of the toxicity of the bulk material with 3 physico-chemical particle parameters recognized as particularly relevant. The 4 criteria contribute equally to the determination of a numerical parameter with values ranging from 1-5 (see Figure 1). A grouping in group 1 would mean that a NM is considered to be non-toxic, while group 5 indicates a high toxicity. This approach is in accordance with the environmental behaviour assessment approach, which is also being developed within the framework of nanoGRAVUR, so that both can be combined for a risk assessment. Since the ion release and the reactivity are dependent on the composition of the medium in which the NM is present during the toxicity test, these parameters should be measured in the corresponding medium in order to increase the reliability of the prediction.

### Testing the ECOTOX BOND

For the testing of the ECOTOX BOND, characterization and toxicity data were available for 8 NM in 23 modifications. The effect concentration ($EC_{50}$) for the most sensitive test organism was used as the basis for assignment. The NMs were initially assigned to the corresponding groups based on the ECOTOX BOND. In order to check the prediction, the toxicity values were then assigned as well. From this assignment of the data into the ECOTOX BOND, it is obvious that most NMs are assigned to the defined groups (Fig. 1) according to the actually observed toxicity (Table 3). However, some assignments are questionable ($TiO_2$-, $CeO_2$-; $Fe_2O_3$ to a lesser extend; marked in red in Table 3). As in these cases the alga *D. suspicatus* was the most sensitive species, ecotoxicity may be caused by shading by turbidity and/or attachment to the algal cell wall. This phenomenon is investigated in more detail in a master thesis at the UFZ and may be considered in future as an additional parameter in the ECOTOX BOND tool.

Funded by:

Federal Ministry of Education and Research

nanoGRAVUR

Nanostructured materials – grouping for occupational health, consumer and environmental protection and risk mitigation

Fraunhofer IME

Umwelt Bundesamt

HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH – UFZ