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Short Report

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Considerations about the relationship of nanomaterial's physical-chemical properties and aquatic toxicity for the purpose of grouping

Short Report

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1 Summary

Manufactured nanomaterials (NMs) are being developed in many different variations such as size, shape, crystalline structure and surface modifications. So far the knowledge is still limited how the modifications affect ecotoxicity. To avoid the testing of each single nanomaterial modification, grouping and read across strategies for nanomaterials similar to classical chemicals are discussed. Grouping and read across aim to identify substance groups with analogous sets of properties or parameters that enable reasonable predictions of a NM hazard without additional testing. Whereas various approaches for grouping are proposed regarding human toxicity, the approaches and considerations regarding ecotoxicological grouping are limited.

The present project aimed at the development of a concept for the grouping of engineered NMs with regard to their ecotoxicological effects with focus on aquatic ecotoxicity. Following test organisms and test systems were selected by the German Environment Agency:

- Growth on green algae (OECD Guideline 201, 2011)
- Immobilization of daphnids (OECD Guideline 202, 2004)
- Fish embryo test (OECD Guideline 236, 2013)

The project was structured into five steps. First, fourteen nanomaterials were selected according to pre-defined criteria. The selected NMs were different subtypes of Ag, ZnO, TiO_2, CeO_2 and Cu. In a second step, their physico-chemical properties were determined in water and in all test media. Based on the results hypotheses regarding the expected ecotoxicity were formulated (third step). In a fourth step, the hypotheses were verified by testing the selected NMs in three ecotoxicological tests. Finally, step five consisted of the compilation of a grouping concept based on NM physico-chemical parameters which were identified as relevant for the emergence of a toxic effect in aquatic organisms.

Following nanomaterials had been selected together with the German Environment Agency. The list includes ion-releasing as well as "stable" NMs which differed in size and shape, crystalline structure, reactivity and zeta-potential. Specific surface modifications were excluded in order to avoid grouping dominated by surface specifics in this first attempt of grouping regarding ecotoxic effects.

Ag

- SRM 110525: wire, provided by the company „Rent a scientist"
- Batch 1340: nanowire, provided by the company „Rent a scientist"
- NM-300K: spherical nanomaterial, selected for the OECD Sponsorship Programme

TiO_2

- Doped with Eu (5 %), provided by IUTA
- Doped with Fe (5 %), provided by IUTA
- undoped, provided by IUTA

ZnO

- NM-110, selected for the OECD Sponsorship Programme
- NM-111, selected for the OECD Sponsorship Programme
- NM-113, selected for the OECD Sponsorship Programme

CeO_2

- Doped with Eu (5 %), provided by IUTA
- NM-211, selected for the OECD Sponsorship Programme
- NM-212, selected for the OECD Sponsorship Programme
- NM-213, selected for the OECD Sponsorship Programme

Cu

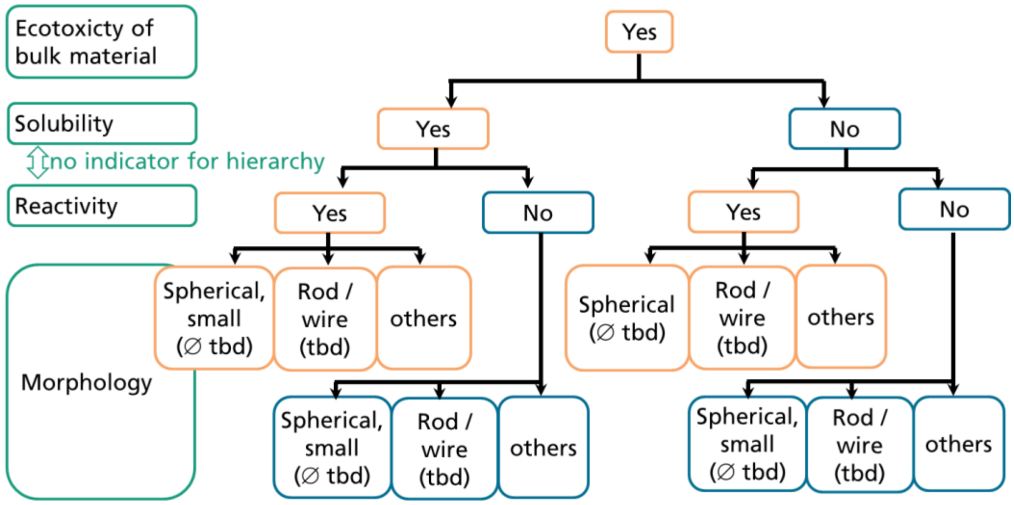
- Cu(0), provided by IUTA for comparison with other ion releasing NM with an expected high toxicity

Numerous statistical approaches were applied to the results of the comprehensive physico-chemical characterization as well as to a combination of these parameters and the EC50 values determined for the three test species. The combination of both was used to identify whether the grouping just on physico-chemical properties can be justified. If grouping with the two approaches (i) just on the PC-properties and (ii) on PC-properties and effect data result in the same groups, it can be concluded that the considered PC-parameters are the relevant ones.

Additionally, the NMs were grouped based on ecotoxicological data only by expert judgement.

A promising approach was a new flow-scheme, inspired by the ECETOC-approach (Arts et al., 2015; Arts et al., 2016). As the ECETOC approach was developed for the grouping regarding human toxicity, a specific scheme regarding ecotoxicity is required. In this scheme the physico-chemical parameters identified by the testing of the fourteen NMs are included. Additionally, as first step, the ecotoxicity of the bulk material or of NMs with the same chemical composition is considered. In the following the step-wise approach is presented for NMs with known ecotoxicological effect based on composition (Figure 1). The same scheme is applicable to NMs with known non-toxic chemical composition. If the information is missing, the NM has to be treated as toxic material.

Figure 1: Ecotox-scheme for the grouping of NMs (tbd = has still to be defined). The same scheme applies for materials with no toxicity of the bulk material.



In the following the findings and conclusions are summarized:

Grouping of the selected nanomaterials

- Based on the developed ecotox-scheme, six groups could be formed:
  - Ion releasing nanomaterials with DMPO/CPH reactivity [1] and other morphology: Ag NM-300K, Cu.
  - Ion releasing nanomaterials with DMPO/CPH reactivity, wire: Ag, Batch 1340.
  - Ion releasing nanomaterials without DMPO/CPH reactivity, wire: Ag, SRM 110525.
  - Ion releasing nanomaterials without DMPO/CPH reactivity, other morphology: the three investigated ZnO nanomaterials.
  - Non-ion releasing nanomaterials, without DMPO/CPH reactivity and other morphology: the investigated four CeO_2 nanomaterials.
  - Non-ion releasing nanomaterials, with DMPO/CPH reactivity and other morphology: the investigated three TiO_2 nanomaterials.
- With exception of silver, the differences between the sub-types of the nanomaterials with same chemical composition were too small, to result in different groups. It seems that the differences between the nanomaterials of one chemical composition must be more pronounced than the selected ones to result in a significant different ecotoxicity.

Physico-chemical (PC)-parameters

- The results for the PC-parameters of the tested NMs differed between the various test media (water, ISO water for fish embryo test, OECD-medium for algae, ADaM for daphnids). Thus, for predictions of the ecotoxicity towards a specific organism only values determined in the relevant test medium are suitable.
- Due to the low evidence regarding the relationships between PC-parameters and ecotoxicity we conclude that the usually discussed parameters are not sufficient to explain or even predict the ecotoxicity. For example, in the case of this study the solubility of the NMs as sole indicator is not suitable. ZnO was completely dissolved within a period of 72 h, whereas the dissolved ratio of Ag was much smaller. Nevertheless the toxicity of ZnO was as expected to be lower compared to that of Ag, due to the different toxicity of the chemical composition (Okamoto et al., 2015). Additionally, a comparison within one chemical group like the silver NM indicates that the NM with the highest solubility did not show the highest effects. Therefore, the effect data of the NM with the highest solubility cannot be used as worst case approach. The toxicity of the chemical substance and further parameters such as shape have to be considered.
- The influence of the zeta potential in an ecotoxicological test seems to be comparably small. Further indicators seem to be of higher relevance. Examples are the results with ZnO. Negative

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[1] *Reactivity measured with spin trap DMPO*

In the presence of hydrogen peroxide (H_2O_2) and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) hydroxyl radicals (OH·) generated via Fenton-type reactions are detected (Shi et al., 2003).

Reactivity measured with spin probe CPH

A possible (surface) reactivity of the material, caused by particle surfaces bound components and / or physicochemical particle properties, was established by measurements using the spin probe 1-hydroxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine hydrochloride (CPH) mixed with the chelator desferroxamine (0.1 mM) (Driessen et al., 2015; Papageorgiou et al., 2007). The (surface) reactivity is expressed by splitting of the H^+ of the CPH molecule or by generating an electron delocalization via binding. This effect is driven probably by directly active surfaces of the material.

and positive zeta potentials were determined but a relationship to ecotoxicity is not obvious. However, in the algae medium (OECD medium) we measured always high negative zeta potentials and observed always toxic effects. But especially in this test stability of the NMs seems to be of lower relevance as NMs and algae are in contact throughout the test due to extensive shaking.

- We assume *surface-reactivity* to be one reason for the observed ecotoxicity of the NMs TiO_2 and CeO_2. There is a need to identify or modify an existing method that the results correlate with the measured ecotoxicity which is assumed to be based on surface reactivity.
- The *morphology* of the NMs seems to be relevant. However, the differentiation in spherical and rods / wires is not sufficient (see also below: test species – daphnids). Threshold values have to be defined to indicate spheres and wires with a high toxic potential. A third group which comprises the remaining NMs is required.
- Several nanomaterials carried a *doping*. It is obvious that doping as sole information is not sufficient to indicate ecotoxicity. For TiO_2, doping modifies the crystalline structure followed by a modified ecotoxicity. For CeO_2 such a relationship is not obvious. The ecotoxicity of the Eu doped CeO_2 was comparable to the ecotoxicity of two non-doped CeO_2, but a third non-doped CeO_2 behaved differently.
- For NMs, obviously a set of parameters needs to be considered. The individual parameter has to be weighted taken the various test organisms and their behavior and the corresponding test media into account.
- Statistical analyses can be a useful tool to identify PC-parameters relevant for ecotoxicological effects. Statistical analyses provide only useful relationships if they are based on a large data set. The data set of this project is limited and has to be extended for robust conclusions.

Test species

- The test species behave differently regarding the ranking of the NMs. Therefore, they have to be treated separately and need to be considered separately in terms of reasoning for a certain grouping and read-across.
- The bioavailability of NM is linked to their behavior in the different test media. This actually means that data on toxicity of one NM in one test species cannot be used to forecast the toxicity of another NM of the same chemical substance for another test species.
- The *fish embryo test* turned out to be rather insensitive and for the assessment of NMs modifications such as dechorionation may be considered (e.g. (Bodewein et al., 2016); Henn and Braunbeck (2011)). Otherwise, chronic fish tests have to be performed even if animal testing should be minimized. However, it has to be considered that a test period of 72 h was used in the project (instead of 96 h).
- *Algae* turned out to be most sensitive test species. We do not assume that shading due to turbid test dispersions is the reason. The effects were observed even in low test concentrations without significant turbidity.
- *Daphnids* are sensitive to thin and long Ag wire NMs. The range of critical dimensions still has to be investigated.

Ranking / Grouping based on statistical analyses

- Although various correlations between the selected PC-parameters (agglomerate size, primary particle size, zeta potential, BET surface, solubility, reactivity based on CPH and DMPO) could be calculated using various statistical approaches, the cluster analyses resulted in inconsistent results although it was performed independently for every test medium. The groups are not comprehensible regarding the ecotoxicity as ion releasing and "stable" NMs are mixed in the groups although obvious differences can be observed in the ecotoxicity. Besides a larger volume of data (robust data set) and an improved selection of PC-parameters, a reason might

be the different conditions in the ecotoxicological testing and the determination of the PC-parameters. Influencing factors such as test organisms, their movement and exudates, illumination as well as turbulence such as shaking or stirring are not or to a lower extent considered in the determination of the PC-parameters.
Up to now it is not possible to predict the ecotoxicity based on a routine statistical analysis of PC-properties. Expert-knowledge regarding the interaction of the various parameters is still required.

Ranking / Grouping based on ecotoxicological profiles

- Grouping / ranking based only on ecotoxicological data and profiles resulted in only rough categories. However, ecotoxicological profiles support the identification and weighting of PC-parameters relevant for ecotoxicity. Ion-releasing and "stable" NMs can be differentiated. For ion-releasing NMs of different chemical nature the difference in ecotoxicity can mainly be related to the toxicity of the chemical substance.
- However, grouping solely based on ecotoxicological data will not support the rationale for *read across.* For the aim of read-across, data on PC properties for comparison are needed to waive data acquisition on ecotoxicity of every member of one group.

Ranking / Grouping based on PC-parameters and ecotoxicity data

- The utilization of existing approaches to group NM is not possible with respect to environmental conditions. Specification and adaptations to ecotoxicological data would be required for the grouping of NMs.
- The flow chart developed for an ecotoxicological grouping within this project would need specification with regard to some parameters, e.g. solubility and reactivity levels. Additionally, the shape of the rods / wires resulting in increased ecotoxicity to daphnids has to be defined. For a hazard ranking scientifically based but also pragmatic threshold values are required. In any case, next to correlations between PC parameters and ecotoxicity, always expert judgment will be an essential element to judge on the grouping on a case by case basis.

Recommendations

Several recommendations shall support the further development of the grouping / read across approach regarding ecotoxicity:

- Adaptation of methods characterizing reactivity which relates to ecotoxicity of surface reactive NMs.
- Increased number of robust datasets for statistical analyses (effect values (recommended: EC50) for the same test systems; PC-parameters for the NMs in each medium)
- Improved measurement of the exposure concentration (better recovery)
- A detailed investigation of the effect of the morphology by using different NM types with the same morphology like ZnO or TiO_2 rods / wires
- A detailed characterization of the kinetics of solubility, zeta potential, reactivity and agglomeration is necessary, as no clear relationship between the measured PC-parameters and toxicity was observed. It seems possible that (i) the toxic response is the result of a combination of parameters (ii) not all relevant parameters important for the ecotoxicity were measured and (iii) measurements of PC-parameters without organisms and at one time point (as performed in this study) can be misleading, as indicated by the results of the soluble fraction in the tests with algae.
- In this project the zeta potential was identified by the statistical analyses as a potential parameter which can be related to the observed effect. However, in the test media most of the NMs had a zeta potential in the range indicating instable dispersions (-15 to +15 mV) with focus on negative values. Therefore, no clear correlation between the zeta potential and the effect could be identified. To determine if the zeta potential has an effect on the toxicity it would be necessary to test further NMs with a negative and positive zeta potential outside of this range.
- Verification of the presented ecotox-scheme with further NMs.

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