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# Developing species sensitivity distributions for metallic nanomaterials considering the characteristics of nanomaterials, experimental conditions, and different types of endpoints

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A B S T R A C T

A species sensitivity distribution (SSD) for engineered nanomaterials (ENMs) ranks the tested species according to their sensitivity to a certain ENM. An SSD may be used to estimate the maximum acceptable concentrations of ENMs for the purpose of environmental risk assessment. To construct SSDs for metal-based ENMs, more than 1800 laboratory derived toxicity records of metallic ENMs from >300 publications or open access scientific reports were retrieved. SSDs were developed for the metallic ENMs grouped by surface coating, size, shape, exposure duration, light exposure, and different toxicity endpoints. It was found that PVP- and sodium citrate- coatings enhance the toxicity of Ag ENMs as concluded from the relevant SSDs. For the Ag ENMs with different size ranges, differences in behavior and/or effect were only observed at high exposure concentrations. The SSDs of Ag ENMs separated by both shape and exposure duration were all nearly identical. Crustaceans were found to be the most vulnerable group to metallic ENMs. In spite of the uncertainties of the results caused by limited data quality and availability, the present study provided novel information about building SSDs for distinguished ENMs and contributes to the further development of SSDs for metal-based ENMs.

## 1. Introduction

Over the last decade, products that incorporate nano-structured materials have been rapidly introduced to the market. In 2014, the value of the global market regarding nanotechnology products was estimated to be $26 billion, and is expected to reach about $65 billion by 2019 (Winkler, 2016). While the benefits of nanotechnology are beyond debate, the concern is increasing about the safe use and subsequent environmental impacts of engineered nanomaterials (ENMs). Evaluating the environmental risks of ENMs is essential to manage relevant risks and ensure the safety of these manufactured materials (Piperigkou et al., 2016; Toropova and Toropov, 2013). One of the well-established approaches assisting risk assessment of ENMs is the development of species sensitivity distributions (SSDs) (Gottschalk and Nowack, 2013). SSDs rank the species based on their sensitivity to a certain ENM, and reflect the potentially affected fraction of species under an exposure concentration of interest (Garner et al., 2015). From the SSD, among others the 5th percentile of the fitted distribution (HC5) can be derived. The HC5 is commonly used as the basis for environmental risk assessment of chemicals and is assumed to be the concentration that is sufficiently protecting ecosystems following addition of an extra safety factor that ranges in between 1 and 5 (European Chemicals Agency, 2008). Risk quantification is usually performed by dividing the predicted environmental concentration by either the predicted no observed effect concentration in case of specific species or by the HC5 in case of generic risk assessment. When the risk quotient is greater than or equals 1, a potential risk of the nanomaterials exists and further assessment is required, including the option of additional toxicity testing; when the risk quotient is less than 1, environmental risks are not expected.

Previously, a few examples of SSDs have been presented for different ENMs based on a limited set of laboratory derived toxicity data. To quantify the environmental risks of nano-Ag, nano-$TiO_2$, nano-ZnO, carbon nanotubes and fullerenes in four environmental compartments (surface water, sewage treatment plant effluents, soils, and sludge-treated soils), SSDs were generated for the five

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ENMs (Gottschalk et al., 2013). The SSDs reflecting the no observed effect concentrations were then compared with the distributions of predicted environmental concentrations in the four environmental compartments. The results indicated marginal risks of Ag and $TiO_2$ ENMs to surface water species and a low level of risk caused by Ag, $TiO_2$ and ZnO ENMs in sewage treatment plant effluents. SSDs for the same five metallic ENMs were also generated by Coll et al. (2016) for different taxa. The risk quotients that are closest to 1 for both ZnO and $TiO_2$ ENMs among others indicated the highest priority of these materials to be studied in more depth. In another study, SSDs for seven types of metallic ENMs were built including Ag, $Al_2O_3$, $CeO_2$, Cu, CuO, $TiO_2$, and ZnO ENMs (Garner et al., 2015). The HC5 values with 95% confidence interval (CI) of each ENM were calculated and compared with those of the corresponding ionic and bulk counterparts. The SSDs of PVP-coated and uncoated Ag ENMs were separately modeled, allowing to conclude about the influence of surface coatings on SSDs. As first attempts of developing SSDs for ENMs, those developed SSDs have provided significant information of the potential environmental impacts of ENMs, and contributed to the derivation of HC5 values as policy measures of the ENMs of concern. The further interest of the development of SSDs for ENMs would be, ideally, to cover more types of ENMs to comprehensively evaluate the risks of all the widely applied ENMs; and to include the large variety of environmental species in order to build robust and reliable SSDs. Meanwhile better estimates could be obtained when specific attention is paid in SSD development to specific ENM properties such as surface coating, size, and shape, and also to the dynamic behaviors of ENMs in the exposure media (Garner et al., 2015; Gottschalk et al., 2013). The consideration of ENM characteristics in developing SSDs may also provide hint messages for the safe-by-design of ENMs, if the SSDs of ENMs separated by certain characteristics were found to shift significantly compared with that separated by other properties. The implementation of the research needs mentioned here, is however strongly limited by the quality of published raw data from the ecotoxicity assays and to a lower extent by the limited availability of suited exposure and effect data.

In response to the above-mentioned challenges, the present study aims to investigate the availability of currently published ecotoxicity data of ENMs for their suitability in developing SSDs for metal-based ENMs; and secondly to build SSDs for ENMs considering the structural characteristics (e.g. surface coating, size, shape), experimental conditions and also different types of toxicity endpoints. All together more than 1800 ecotoxicity records of metallic ENMs from >300 publications or open access scientific reports were retrieved from the databases of Chen et al. (2015), Juganson et al. (2015) and the online chemical modeling environment (OCHEM) (Sushko et al., 2011). The toxicity endpoints in the collected dataset include the lethal concentration (LC), the effect concentration at a specific effect level (ECx), the lowest observed effect concentration (LOEC), and the no observed effect concentration (NOEC). The studied species originated from seven widely investigated organism groups namely algae, bacteria, crustacean, fish, nematodes, protozoa, and yeast. Based on the analysis, the development of SSDs focuses on Ag, $CeO_2$, CuO, $TiO_2$, and ZnO ENMs due to relatively sufficient information availability. Different SSDs were generated for the Ag ENMs grouped by surface coating, size, shape, and exposure duration. The SSD for UV exposed $TiO_2$ ENMs was also derived. To determine whether and to what extent the shape of the SSD curve might alter and the HC5s may vary based on different toxicity endpoints, these topics were also considered in the development of SSDs in the present study. To discuss the vulnerability of different organism groups and species to the metallic ENMs, the most sensitive species in each developed SSD was analyzed as well.

## 2. Methods

### 2.1. Datasets

Experimental data of ENM ecotoxicity were assembled from three databases. The first database is that developed by Chen et al. (2015) consisting of 886 records of toxicity endpoints of various metal-based ENMs. The second database is the NanoE-tox database listing in total 1518 EC50 (the concentration at which 50% of the test species is affected), LC50 (median lethal concentration), and NOEC values regarding eight ENMs including carbon nanotubes and fullerenes, Ag, $CeO_2$, CuO, $TiO_2$, ZnO, and $FeO_x$ nanomaterials (Juganson et al., 2015). The third data source is the OCHEM platform which explicitly provided 244 LC50 values and 170 EC50 values of different metallic ENMs (Sushko et al., 2011). After removing duplicate information, the newly developed dataset counts all together more than 1800 values of metallic ENMs from >300 publications or open access scientific reports. This information was afterwards filtered by the following conditions: a) toxicity of metal-based ENMs solely; b) tested organisms are algae, bacteria, crustacean, fish, nematodes, protozoa, and yeast only; c) toxicity endpoints are LC, EC, LOEC, and NOEC. In the dataset, units of all toxicity values were unified into mg/L, and the endpoints larger than 10000 mg/L were excluded as these are considered to be irrelevant from a toxicological point of view.

As for certain ENMs, the toxicity data was separated by the characteristics of the ENMs (i.e. surface coating, size, shape), experimental conditions (duration of exposure, light exposure), and type of different endpoints (LC, EC, LOEC, NOEC), respectively. The number of species in each sub-dataset is required to be at least six in order to construct a reliable SSD (Cedergreen et al., 2004). SSDs for the uncoated and differently coated ENMs were modeled. With regard to grouping ENMs by size, it was suggested by Garner et al. (2015) to divide the data in size ranges in between 1-10, 10–50, and 50–100 nm. Here, we adapted the division of sizes as 1–20, 20–50, and 50–100 nm, as it was stated that nanoparticles with size <20 nm may have significantly increased surface reactivity and behave differently than larger particles (Auffan et al., 2009, 2010), whereas nanomaterials of 20–50 nm appear to be taken up more rapidly than particles of other sizes (Iversen et al., 2011; Jin et al., 2009). When generating SSDs based on data separated by the size and shape, ENMs with reported surface coatings were excluded. The exposure duration was determined as $\leq 1$ d, 1–2 d, and >2 d, to investigate if over time the shape of SSD-curve might shift as result of both the dynamic changes of ENMs in the media and the increased length of the life cycle of an organism. The experimental condition of light exposure was also considered in the study as nanomaterials like $TiO_2$ ENMs were reportedly able to catalyze reactions under UV radiation and cause phototoxicity (Yin et al., 2012; Sanders et al., 2012).

### 2.2. Modeling algorithm

Data was grouped regarding LC50 value and ranked from lowest to highest by the following equation (US EPA, 1998):

$$\text{Proportion} = \frac{Rank - 0.5}{Number\ of\ species}$$

For the toxicity data relating sub-lethal effects of ENMs (i.e. EC50, LOEC, NOEC), the median toxicity values based on a certain biological effect to a species were initially calculated per reported effect. The obtained medians of different effects to that species were afterwards compared and the lowest median value was used in ranking the species sensitivities. The ranked median values of

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