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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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different species were then plotted against the cumulative probability which reflects the proportion of species affected at a certain concentration.

In the study, lognormal distributions of species sensitivity were fitted using the 'fitdistr' function of the MASS package in the R statistical software (version 3.3.1). This function generates a maximum-likelihood fitting of univariate distributions, allowing parameters to be held fixed if desired (Venables and Ripley, 2002). The 95% CI of the fitted regressions was also estimated by employing the strategy of parametric bootstrap. The HC5 values of the SSDs were extracted by the 'quantile' function in the R software (Hyndman and Fan, 1996).

3. Results

We firstly analyzed the data availability for the preparation of building SSDs for the metal-based ENMs (Table 1). Before constructing separate SSDs, the SSDs for Ag, CeO_2, CuO, TiO_2, and ZnO ENMs were generated with all available data for the corresponding ENMs (see Fig. S1 as provided in the Supplemental Information). LOEC and NOEC data for Ag and CuO ENMs are available for only five species. These data were nevertheless included in the analysis to allow for a more comprehensive comparison. Separate SSDs were afterwards obtained for Ag ENMs grouped by surface coating, size, shape, and exposure duration (Fig. 1); for CuO and ZnO ENMs grouped by size (Fig. S2); and for TiO_2 ENMs grouped by size and light exposure (Fig. S2). SSDs based on different toxicity endpoints were compared (Fig. 3). The significance of difference between relevant HC5s was discussed (Fig. 2, Fig. 4, and Fig. S3). All the calculated HC5 values with corresponding CI were listed in a Microsoft Excel spreadsheet (see Supplemental Information). The lists of species that were used to build SSDs were also presented in the Supplemental Information. Examples of building SSDs in the present study using LC50, EC50, LOEC, and NOEC datasets were presented in the Supplemental Information.

3.1. Data availability for generating SSDs

The information in the newly collected dataset includes but is not limited to: characteristics of ENMs (core, size, surface coating, shape, surface area etc.), experimental conditions (exposure duration, light exposure etc.), tested species, detected biological effects, type of toxicity endpoints, and values of nanotoxicity. The studied ENMs cover a wide range of types of ENMs such as Ag, CeO_2, CuO, FeO_x, NiO, SiO_2, TiO_2, ZnO ENMs etc. The toxicity endpoints that are potentially useful for building SSDs are LC50, EC50, LOEC, and NOEC, as data availability of other endpoints is very limited. In order to develop SSDs, the number of species was analyzed for which data with regard to each type of ENMs and with respect to each type of the endpoint was available. The results of this analysis are shown in Table 1. ENMs for which data for each endpoint were available for no more than three species were included in the group 'Others'.

Table 1
Number of species tested for Ag, CeO_2, Cu, CuO, Ni, TiO_2, ZnO and other ENMs. The species are from seven groups of organisms, namely algae, bacteria, crustacean, fish, nematodes, protozoa and yeast. ENMs with species number less than four (for every type of endpoint) are in the group 'Others'.

ENMs	LC50	EC50	LOEC	NOEC
Ag	17	20	5	5
CeO_2	2	6	2	8
Cu	4	1	0	0
CuO	9	10	5	5
Ni	4	4	0	0
TiO_2	10	16	2	17
ZnO	8	13	6	11
Others	10	14	4	10

The analysis showed that Ag, CeO_2, Cu, CuO, Ni, TiO_2, and ZnO ENMs have received the most research attention among all the metallic ENMs. Ag ENMs have been shown to be generally studied for their lethal toxicity to different taxa (17 species), as well as its sub-lethal biological effects (20 species for which EC50 values were reported). CuO, TiO_2, and ZnO ENMs were also widely tested on various species, which provided toxicity data for respectively 9, 10, 8 species on LC50, and 10, 16, 13 species on EC50. For CeO_2 ENMs, 6 and 8 data points are available on EC50 and NOEC respectively. For Cu and Ni ENMs, the retrieved data for constructing SSDs is very limited based on both LC50 and EC50. Based on this analysis, we subsequently developed SSDs for the ungrouped Ag, CeO_2, CuO, TiO_2, and ZnO ENMs (Fig. S1) and the ENMs differentiated by surface coating, size, shape, exposure duration, light exposure, and type of endpoint.

3.2. Separate SSDs by ENM characteristics and experimental conditions

Within the first constructed SSDs, uncoated, polyvinylpyrrolidone (PVP)- and sodium citrate- coated Ag ENMs were separated (Fig. 1a). The SSD of ungrouped Ag ENMs is also enclosed for comparison. As can be observed from this figure, the SSD of the PVP-coated Ag ENMs shifted to the left compared with that of the uncoated Ag ENMs, which means that a PVP coating may considerably enhance the toxicity of Ag ENMs to most species. This agrees with the results obtained by Garner et al. (2015). Similarly, the sodium citrate-coated Ag ENMs also showed increased toxicity at high concentrations compared with the uncoated ones. As reported, both PVP and citrate are able to significantly reduce the aggregation and deposition to surfaces, and thus increase the bioavailability and toxicity (Gutierrez et al., 2015). The SSD of ungrouped Ag ENMs showed little statistical difference from that of the uncoated Ag ENMs. This could possibly be due to the counteraction of the influences of all kinds of surface coatings on the toxicity of Ag ENMs. The estimated HC5 value of uncoated Ag ENMs is 0.0063 mg/L, with the 95% CI ranging from 0.00098 to 0.068 mg/L. The HC5 of ungrouped Ag ENMs is 0.0036 mg/L (0.00064–0.029 mg/L). The HC5 of PVP-coated Ag ENMs is 0.0011 mg/L (0.00012–0.031 mg/L), and that of the sodium citrated-coated Ag ENMs is 0.0030 mg/L (0.00040–0.050 mg/L).

Grouped according to different size clusters of 1–20, 20–50, and 50–100 nm, the data were also ranked to create SSDs for Ag ENMs of different sizes (no surface coating reported), as shown in Fig. 1b. Only minor differences were seen between the three SSDs especially at low concentrations, even though ENMs with smaller sizes are expected to act differently (Auffan et al., 2009, 2010). The SSD of ungrouped Ag ENMs unsurprisingly lies between those of Ag ENMs of 1–20 and 50–100 nm, which is nearly identical to the SSD of Ag ENMs with sizes ranging from 20 to 50 nm. The difference in behavior and/or effect is seen according to the separate SSDs when the exposure concentration increases; the group of smallest Ag ENMs tends to be relatively more toxic compared with the other two groups. One possible explanation for this observation is that the biological effects triggered by Ag ENMs are most likely to result from the release of Ag^+ ions (Juling et al., 2016). Therefore regardless of sizes, the mode of action of Ag ENMs of different sizes at low concentrations may be similar. As concentration rises, the proportion of the particle form significantly increases and ENM characteristics like size may start to play a role in affecting the toxicity. The study of Xiao et al. (2015) showed that the relative contribution of the particle forms of Cu ENMs to the accumulation

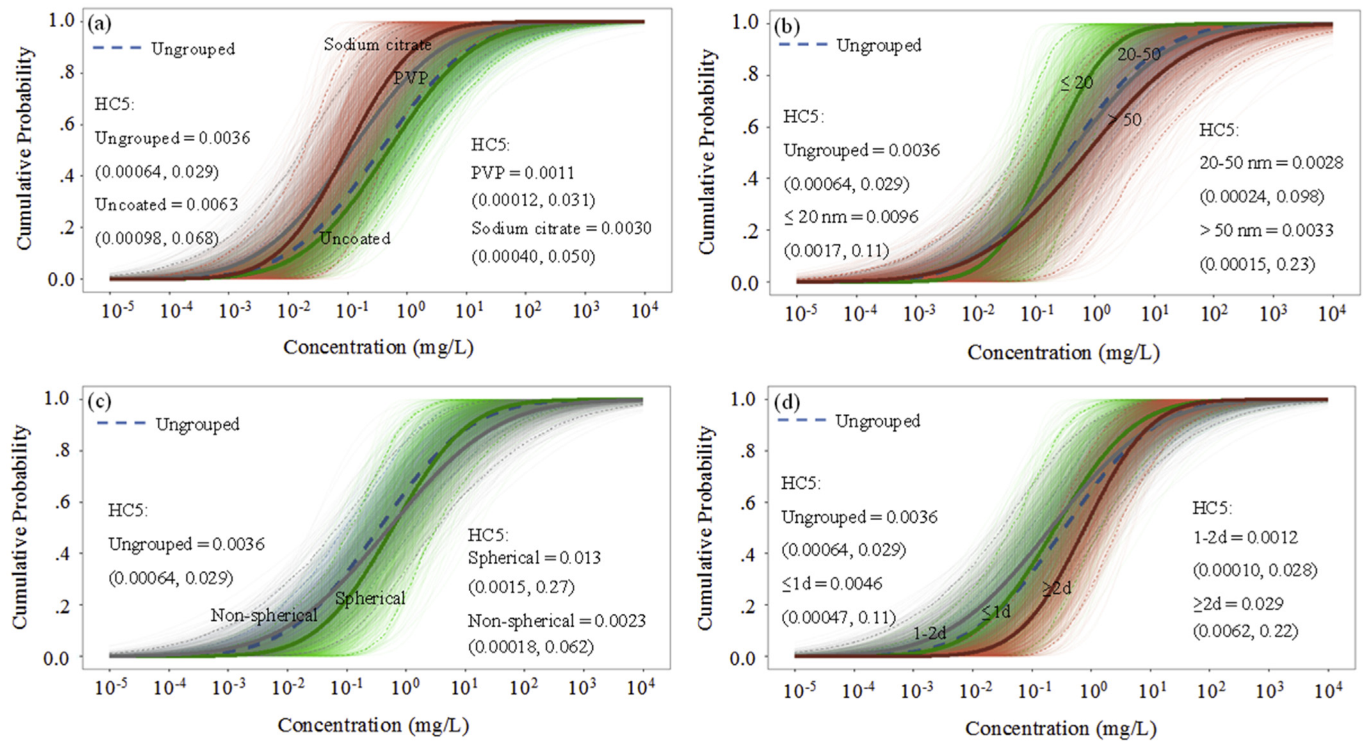

Fig. 1. SSDs of Ag ENMs distinguished by (a) surface coating; (b) size; (c) shape; and (d) duration of toxicity exposure, based on LC50 values. The SSD of ungrouped Ag ENMs was also depicted in each figure for the comparison (dashed blue line). The shaded region along each curve shows the 95% confidence interval. The Ag ENMs in figures (b), (c) were not reported as being surface coated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

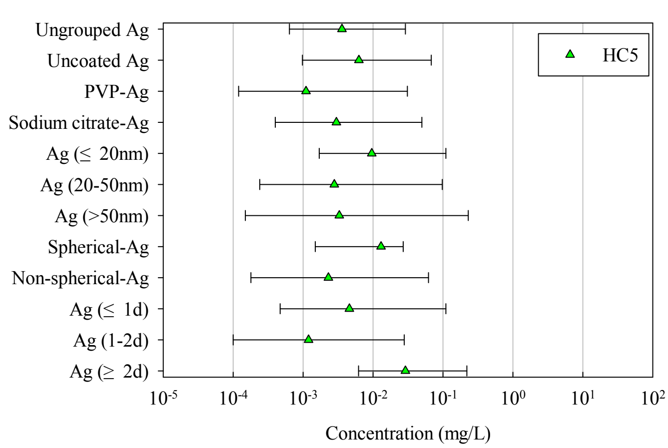

Fig. 2. Comparison of HC5 values derived from SSDs of Ag ENMs differentiated by surface coating, size, shape, and exposure duration. Error bars show the 95% confidence interval of HC5s.

in *Daphnia magna* increased from 48% to 72% when the concentrations of ENM suspensions increased from 0.05 to 0.1 mg/L. The same applies for the ZnO ENMs, as the relative contribution of their particle forms increased with the rise of concentrations of ZnO ENM suspensions (from 47% to 64% as concentration rised from 0.5 to 1 mg/L). The HC5 value of Ag ENMs ranging from 1 to 20 nm is 0.0096 mg/L (0.0017–0.11 mg/L). For Ag ENMs of 20–50 and 50–100 nm, the established HC5s are 0.0028 mg/L (0.00024–0.098 mg/L) and 0.0033 mg/L (0.00015–0.23 mg/L), respectively. SSDs of ENMs with different ranges of sizes were also derived for CuO, TiO_2, and ZnO ENMs as shown in Fig. S2. The SSDs of CuO and TiO_2 ENMs distinguished by size highly overlap with those of the corresponding ungrouped ENMs within 95% CI. The SSD developed for ZnO ENMs of 50–100 nm also overlaps with that of the ungrouped ZnO ENMs especially at low concentrations.

Grouped within different shapes of ENMs, the data was also ranked to create SSDs. On the basis of the available data, only for spherical-shaped Ag ENMs (no reported coatings) a sufficient number of data points is available for the modeling. We therefore grouped the Ag ENMs as spherical and non-spherical Ag ENMs to determine if there are major differences between the distributions, as shown in Fig. 1c. A comparison shows that the SSDs for spherical- and non-spherical- shaped Ag ENMs are nearly identical and the differences are minimal within corresponding 95% CI. Also the 95% CI of the ungrouped Ag ENMs heavily overlaps with those of the ENMs grouped by shape. This similarity could be possibly caused by the physical-chemical transformations of the particles in the medium, of which aggregation, agglomeration, and dissolution are the most important processes that alter the behaviors of ENMs and thereby the interactions of ENMs with biota (Chen et al., 2015; Hua et al., 2016). In this context, the shape of Ag ENMs seems to play a less important role in influencing the toxicity of the materials. The calculated HC5 of non-spherical Ag ENMs is 0.0023 mg/L with the 95% CI ranging from 0.00018 to 0.062 mg/L. The HC5 value of the SSD of spherical Ag ENMs is equal to 0.013 mg/L (0.0015–0.27 mg/L).

The exposure duration used in the toxicity testing (Fig. 1d) and light exposure (Fig. S2) were also considered when constructing SSDs for metallic ENMs. No major statistical differences were seen between the ungrouped SSDs and the SSDs with distinct groups of species ranked as being exposed for ≤1 d and 1–2 d. Even so, at high concentrations (particularly above 10 mg/L) the three distributions highly overlap. HC5s derived from the SSDs of exposure duration ≤1 d and in between 1 and 2 d are 0.0046 mg/L (0.00047–0.11 mg/L) and 0.0012 mg/L (0.00010–0.028 mg/L),

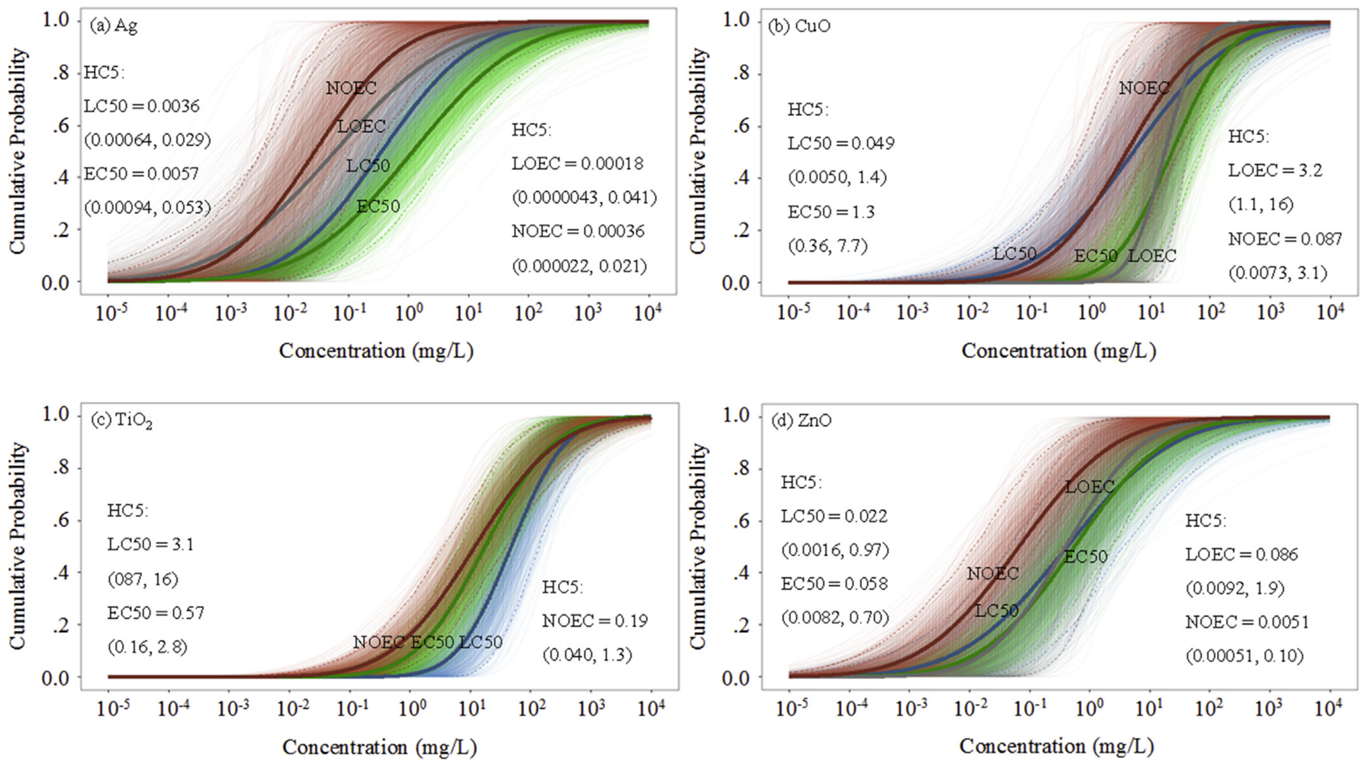

Fig. 3. SSDs of (a) Ag; (b) CuO; (c) TiO_2; and (d) ZnO ENMs based on respectively LC50, EC50, LOEC, and NOEC data. The shaded region along each curve depicts the 95% confidence interval.

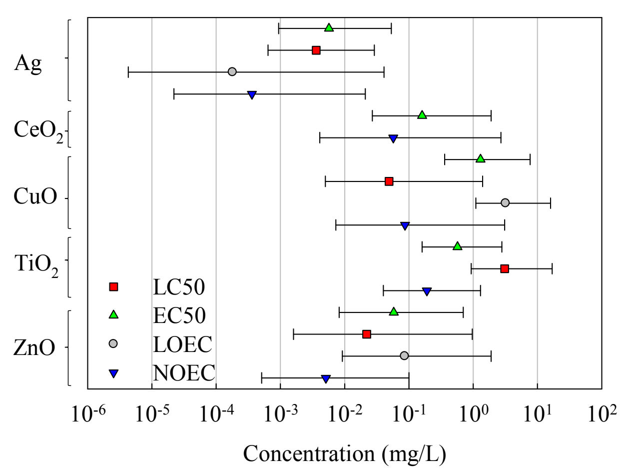

Fig. 4. Variation of HC5 values of Ag, CeO_2, CuO, TiO_2, ZnO ENMs based on respectively LC50, EC50, LOEC, and NOEC data. The 95% confidence interval is also given as well as the HC5 values.

respectively. The HC5 generated from SSD of ≥2 d is 0.029 mg/L (0.0062–0.22 mg/L). For the toxicity of ENMs under different light exposures, most experiments followed standardized protocols such as OECD 202 (2004) and US EPA (2002) which recommend a 16/8 h-light/dark-cycle for the toxicity testing. However, different lighting regimes were found to be applied for the toxicity test of TiO_2 ENMs due to their photoactivated toxicity. Sufficient data points based on EC50 (six species) were obtained only for UV exposed TiO_2 ENMs, and these were used in building the relevant SSD together with the SSD of ungrouped TiO_2 ENMs based on EC50 (Fig. S2). As can be observed from the figure, the 95% CI of the SSD for UV exposed TiO_2 ENMs is much wider given the much smaller number of data points, which almost fully covers the 95% CI of the SSD for ungrouped TiO_2 ENMs (16 species). The HC5 value with respect to the ungrouped TiO_2 ENMs based on EC50 is 0.57 mg/L (0.16–2.8 mg/L), the HC5 estimated for the UV exposed TiO_2 ENMs is 1.5 mg/L with a 95% CI of 0.24–21 mg/L.

For the purpose of environmental risk assessment of ENMs, the variation of the obtained HC5s with 95% CI is of interest, as depicted in Fig. 2 for Ag ENMs. As observed, most of the values of HC5s fall within the range of 10^{-3} to 10^{-2} mg/L with established 95% CI mainly ranging from 10^{-4} to 10^{-1} mg/L. Almost all the calculated 95% CIs of the HC5s highly overlap. This indicates that there are actually no statistically significant differences between the estimated HC5s from the SSDs of grouped or ungrouped Ag ENMs. The obtained HC5s of CuO, TiO_2, and ZnO ENMs were also depicted in Fig. S3. Also no statistically significant differences were observed between the HC5s of relevant grouped and ungrouped ENMs.

3.3. SSDs based on different toxicity endpoints

To compare the SSDs of certain ENMs based on different endpoints, the fitted distributions in Fig. S1 were reorganized according to the type of ENM (Fig. 3). Unexpectedly, only the SSDs of TiO_2 ENMs exhibited a reasonable order of NOEC < EC50 < LC50 at low concentrations. For Ag ENMs the difference is minimal between the NOEC- and LOEC-based SSDs, and also between the LC50- and EC50- based SSDs when the concentration is low. As concentration rises an order of NOEC < LOEC < LC50 < EC50 is seen. In the case of ZnO ENMs, major differences only appeared between the NOEC-SSDs and the SSDs based on other endpoints. The SSDs of ZnO ENMs based on EC50, LOEC, and NOEC showed no significant difference. This also applied for the NOEC- and LC50- SSDs, and the LOEC- and EC50- SSDs of CuO ENMs. The HC5s derived from these

SSDs were calculated and compared in Fig. 4. Based on LC50 (also see Fig. S1), HC5s of the ENMs in an ascending order is Ag (0.0036 mg/L) < ZnO (0.022 mg/L) < CuO (0.049 mg/L) < TiO_2 (3.1 mg/L); For the HC5s based on EC50, it is Ag (0.0057 mg/L) < ZnO (0.058 mg/L) < CeO_2 (0.16 mg/L) < TiO_2 (0.57 mg/L) < CuO (1.3 mg/L); the order of LOEC-HC5s is Ag (0.00018 mg/L) < ZnO (0.086 mg/L) < CuO (3.2 mg/L); and in the case of NOEC the order is Ag (0.00036 mg/L) < ZnO (0.0051 mg/L) < CeO_2 (0.057 mg/L) < CuO (0.087 mg/L) < TiO_2 (0.19 mg/L).

Interestingly, in all cases the HC5s of Ag and ZnO ENMs were shown to be lower than those of the other ENMs considered, whereas the ranking of the toxicity of CuO and TiO_2 ENMs differs when considering different toxicity endpoints. The predicted HC5s of Ag ENMs are always the lowest, and the toxicity of TiO_2 ENMs is commonly the lowest as can be concluded from the HC5 values (Garner et al., 2015; Coll et al., 2016; Gottschalk et al., 2013). As can be seen from Fig. 4, the 95% CI of Ag ENMs is clearly significantly different from that of TiO_2 ENMs with no overlap of 95% CI with respect to any endpoint considered. This situation changes for the 95% CI of Ag and CuO ENMs which appear to be significantly different on the basis of EC50 and LOEC, but overlap when LC50 and NOEC are used. The conclusions of comparing HC5s (with 95% CI) of different ENMs vary when different endpoints are employed for modeling SSDs. For each ENM, no significant difference was found when comparing the NOEC-based HC5s with the HC5 values based on LC50, EC50 and LOEC, even though the NOEC-HC5s tend to be the lowest as concluded from the cases of CeO_2, TiO_2, and ZnO ENMs. Additionally, the ratios of LC50-HC5/NOEC-HC5, EC50-HC5/NOEC-HC5, and LOEC-HC5/NOEC-HC5 were calculated as listed in Table S1. The ratio of LC50-HC5/NOEC-HC5 ranges from 0.6 (CuO ENMs) to 16.3 (TiO_2 ENMs). The ratio of EC50-HC5/NOEC-HC5 was found to range from 2.8 (CeO_2 ENMs) to 15.8 (Ag ENMs). With respect to LOEC-HC5/NOEC-HC5, the values vary from 0.5 (Ag ENMs) to 36.8 (CuO ENMs).

4. Discussion

4.1. Data availability

Even though a large dataset (more than 1800 records) has been retrieved from >300 publications or scientific reports, it seems like so far only a limited number of ENMs were thoroughly investigated with regard to their toxicity to only a limited number of test species (Chen et al., 2015). When developing SSDs for the grouped ENMs, the data availability becomes even scarcer because of the lack of the data on, for example, ENM surface coatings, sizes, shapes, experimental conditions, etc. which are crucial for distinguishing the ENMs. The absence of these data could be due to the lack of data in original articles, or the missing of data when extracting information from publications to databases. In the present study, SSDs could only be developed for Ag, CeO_2, CuO, TiO_2, ZnO ENMs based on all possible endpoints. According to the study of Bondarenko et al. (2013), Ag, CeO_2, CuO, TiO_2, and ZnO ENMs are indeed among the ENMs that are produced at the highest amounts, together with AlO_x, FeO_x, and SiO_2 ENMs. It would benefit the risk assessment of ENMs if all these metallic nanomaterials that are produced in high amounts were comprehensively evaluated for their safety, as they are all considered to inevitably enter into the environment and potentially pose impacts on human beings and environmental species (Echegoyen and Nerín, 2013). Developing SSDs for those ENMs of concern is one of the keys to manage the risks brought by the marketed nanomaterials. This nevertheless requires more types of ENMs to be tested, and also more relevant reliable models to be developed to reduce the time consumption and accelerate the process of risk evaluation. For the previously studied ENMs, toxicity data covering a wider range of taxa and trophic levels other than only standard species are also of significant importance to minimize the variabilities and levels of uncertainties.

In part, the data availability in developing SSDs also depends on firstly if the experimental results derived from a wide variety of protocols should be combined for building one SSD; and secondly, on the required minimum number of data points (number of species) to generate an SSD. Ideally, a distribution of species sensitivity ought to be generated from experiments that employed consistent protocols, for example, by using toxicity data reflecting the inhibition of growth or reproduction, or mortality, etc (Garner et al., 2015). In this context, only experimental results reflecting exactly the same biological effects should be grouped and used for the development of SSDs. This unquestionably largely reduces the available data for the modeling. According to the standardized toxicity testing protocols, different effects are recommended to be assessed for different standard test species, e.g., growth inhibition for *Pseudokirchneriella subcapitata* (OECD 201), immobility (OECD 202) and reproduction inhibition (OECD 211) for *Daphnia magna*, lethality for *Oryzias latipes* (OECD 203) and *Danio rerio* embryo (OECD 236), etc. (OECD, 1992, 2004, 2011, 2012, 2013). Given the scarcity of data, it is as yet technically infeasible to include most of the species tested so far in one single SSD on the basis of one consistently measured effect level other than lethality. Therefore, data manipulation was adapted in previous studies so as to combine data representing different biological effects and to perform regression analysis (Coll et al., 2016; Garner et al., 2015; Gottschalk et al., 2013). Additionally, the minimum number of data points to build an SSD also determines whether a dataset with a very limited number of species can be used for modeling. Although it was proposed by Garner et al. (2015) that a minimum of four species is needed to construct SSDs, Cedergreen et al. (2004) stated that at least six to eight species must be represented. Therefore, assuming that only four data points are required for the SSD derivation, the SSDs for Cu and Ni ENMs could also be built based on LC50 data (Table 1). This will however induce a quite broad CI.

4.2. Comparison of SSDs and relevant HC5s

Given the relatively high amount of data, SSDs could be built for Ag ENMs distinguished by coating, range of size, shape, and exposure duration. Although a few of the distributions (e.g., SSDs in Fig. 1b) at high concentrations showed some variations, the HC5s that were derived from the developed SSDs do not differ significantly. This means that, on the basis of the currently available data, all kinds of Ag ENMs entering into the environment are supposed to share similar maximum acceptable concentrations, regardless of surface coatings, shapes, sizes, exposure durations, or even other structural characteristics. This similarity could possibly result from either or both of the two major reasons. The first is the physical-chemical transformation of Ag ENMs in the aquatic media which can completely change the structural properties of ENMs (Chen et al., 2015). Despite the fact that the structural parameters of ENMs have been formerly linked to the toxicity of ENMs (Chen et al., 2016), it is still difficult to quantify the relationship between the characteristics of pristine ENMs (e.g., size, surface coating, shape, etc.) and the behaviors of ENMs in a medium. This behavior may alter the mobility, bioavailability and ultimately the toxicity of the nanomaterials, and thus is of vital significance to understand the mechanisms governing nanotoxicity. The second reason is the general mechanism of toxicity of nano-, micro-, and bulk- Ag releasing metal ions. As known, one of the major mechanisms of Ag-induced toxicity is the leaching of Ag^+ ions. Therefore especially at low concentrations, Ag ENMs with varied structural

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properties tend to exhibit analogous biological activities. But as concentrations increase, the proportion of the nanoparticulate Ag will as well rise and differences would probably emerge between the SSDs of Ag ENMs with different structural properties. As for the influence of light exposure, the SSD could only be developed for the UV exposed TiO_2 ENMs which is incomparable. The different is not significant either between the SSDs of UV exposed and ungrouped TiO_2 ENMs based on EC50 (Fig. S2).

Assessment factors are commonly used when deriving the predicted no observed effect concentrations from the HC5s. For instance, a factor of 10 was used by Gottschalk et al. (2013) to calculate the predicted no observed effect concentrations from LC50 and EC50, while a factor of 2 was applied to generate this value from LOEC. In the study of Coll et al. (2016), a factor of 10 was used for LC50 and EC50, and a value of 1 was employed for LOEC and NOEC. Based on our results, the ratio of HC5s of L(E)C50/NOEC ranges from 0.6 to the highest 16.3 with a median value of 10 (Table S1). For the combination of LOEC/NOEC the three values are 0.5 (Ag ENMs), 16.9 (ZnO ENMs), and 36.8 (CuO ENMs). Although the limited number of data points of Ag and CuO ENMs (only five data points for both LOEC and NOEC data, see Table 1) will cause larger uncertainties, the value of 16.9 (LOEC-HC5/NOEC-HC5) for ZnO ENMs with a relatively sufficient number of data (respectively 6 and 11 for LOEC and NOEC data) does not seem to be close to a factor of 2. With respect to the SSDs built on different toxicity endpoints, the NOEC-SSDs were not as expected significantly lower than that based on LC50, EC50, and LOEC except for the case of ZnO ENMs. Neither did the LOEC-SSDs always appear in between the NOEC-SSDs and the EC50-SSDs, as expected on forehand. Given the situation that NOEC should always represent the most sensitive case, the ratio of L(E)C50-HC5/NOEC-HC5 and LOEC-HC5/NOEC-HC5 was actually also not considered to be lower than 1. This was however observed for the LOEC-HC5/NOEC-HC5 of Ag ENMs (0.5) and for the LC50-HC5/NOEC-HC5 of CuO ENMs (0.6). Together with the discussed discrepancies of SSDs in Fig. 3, we understand that this might be attributed to the fact that the data used were retrieved from a variety of sources with varying data quality. The limited sample sizes of Ag and CuO ENMs based on respectively LOEC and NOEC also resulted in the wide CI and low statistical power. These uncertainties could only be diminished by future increase of data quality and availability.

4.3. Most sensitive species and organism groups

Based on the developed SSDs, we listed the most sensitive species of every SSD in Table S2. Despite that no single species was found to be always the most susceptible, a few species were constantly observed to be the most vulnerable to metallic ENMs. These species include *Ceriodaphnia affinis*, *Ceriodaphnia dubia*, *Daphnia magna*, *Daphnia pulex*, *Escherichia coli*, and *Pseudokirchneriella subcapitata*. Most of these species are crustaceans which account for 26 out of 32 of the most sensitive species in the SSDs developed. This indicates that crustaceans are more likely to be the organism group that is affected by the metal-based ENMs at the lowest concentrations of ENMs. This observation is in line with the study of Garner et al. (2015), in which the most sensitive species to metallic ENMs were all crustaceans, namely *Ceriodaphnia dubia* (in SSDs of uncoated and PVP-coated Ag, Al_2O_3, Cu, and TiO_2 ENMs), *Daphnia pulex* (CuO ENMs), *Daphnia similis* (CeO_2 ENMs), and *Thamnocephalus platyurus* (ZnO ENMs). Since the HC5 represents a concentration where only 5% of the species could be affected, it seems that the crustaceans would be those that are within the 5% of the species. Therefore in the case of a generic risk assessment, it may be important to include at least a few representative species from the crustacean group in the SSDs such as *Ceriodaphnia dubia*, *Daphnia magna*, and *Daphnia pulex*.

4.4. Conclusions

To conclude, reliable information on the characteristics of ENMs that govern toxicity and the experimental conditions are needed for the development of separate SSDs. More data on the highly produced ENMs such as AlO_x, CeO_2, CuO, FeO_x, SiO_2, TiO_2, and ZnO ENMs are favorable for a comprehensive evaluation of the environmental risks of ENMs. Sufficient data on Ag ENMs enabled a comparison between the SSDs constructed for the grouped Ag ENMs. For the Ag ENMs grouped by shape and exposure duration, the separate SSDs of Ag ENMs showed no statistically significant difference. For the Ag ENMs of different size ranges, differences in behavior and/or effect were only seen at high exposure concentrations. The PVP- and sodium citrate- coatings on the surface of Ag ENMs enhance the nanotoxicity as the SSDs shifted to the left compared to the SSD of the uncoated Ag ENMs. The derived HC5s for all the grouped Ag ENMs do not differ significantly, which implies that only the intrinsic chemical toxicity of Ag ENMs greatly affected the corresponding SSDs. HC5s generated from the SSDs of ungrouped Ag, CeO_2, CuO, TiO_2, and ZnO ENMs based on respectively LC50, EC50, LOEC, and NOEC were also compared. Median values of 10 for the ratio of L(E)C50-HC5/NOEC-HC5, and of 16.9 for the ratio of LOEC-HC5/NOEC-HC5 were obtained. An analysis of the most sensitive species in every SSD showed that no single species was consistently the most sensitive, however crustaceans as an organism group tend to be extra vulnerable to metal-based ENMs. Due to the limitations caused by data quality and availability, it should be noticed that uncertainties still exist associated with our results. For the developed SSDs, such uncertainties could be reduced if reliable toxicity information of sufficient species became available which could represent a comprehensive ecosystem. Despite these considerations, we believe the present study is helpful in gauging the SSDs of ENMs grouped by individual ENM properties and other important factors, and in enabling the further development of SSDs for metallic ENMs.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

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Transparency document

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