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Systematic consideration of parameter uncertainty and variability in probabilistic species sensitivity distributions

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The simulation framework is available as a software package at <https://doi.org/10.5281/zenodo.3267194>

Abstract

The calculation of a species sensitivity distribution (SSD) is a commonly accepted approach to derive the predicted no-effect concentration (PNEC) of a substance in the context of environmental risk assessment. The SSD approach usually is data demanding and incorporates a large number of ecotoxicological values from different experimental studies. The probabilistic SSD (PSSD) approach is able to fully consider the variability between different exposure conditions and material types, which is of great importance when constructing a SSD for any chemical, especially for nanomaterials. The aim of our work was to further develop the PSSD approach by implementing methods to better consider the uncertainty and variability of the input

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data. We incorporated probabilistic elements to consider the uncertainty associated with uncertainty factors by using probability distributions instead of single values. The new PSSD method (named PSSD⁺) computes 10'000 PSSDs based on a Monte-Carlo routine. For each PSSD calculated, the HC5 was extracted to provide a PNEC distribution based on all data available and their associated uncertainty. The PSSD⁺ approach also includes the option to consider a species weighting according to a typically constituted biome. We applied this PSSD⁺ approach to a previously published data set on carbon nanotubes and silver nanoparticles. The evaluation of the uncertainty factor distributions and species weighting have shown that the proposed PSSD method is robust with respect to the calculation of the PNEC value. Furthermore, we demonstrated that the PSSD⁺ can handle both small and more comprehensive datasets as the PNEC distributions are a close representation of the data available. Finally, the sensitivity testing towards data set variations showed that the maximum variation of the mean PNEC was of a factor of about two, so that the method is relatively insensitive to missing data points as long as the most sensitive species is included.

Keywords: species sensitivity distribution, hazard assessment, predicted no-effect concentration, uncertainty

Key points

We incorporated probabilistic elements in SSDs to consider the uncertainty associated with uncertainty factors by using probability distributions instead of single values and species weighting according to a typical biome.

The HC5 extraction of all calculated PSSDs yields a PNEC distribution based on all data available and their associated uncertainty without omitting data points for species due to data aggregation.

The new PSSD⁺ approach avoids the fitting problem of conventional SSDs and can be used for small and bigger datasets.

Introduction

Environmental and ecological risk assessments of a substance require the comparison of an environmental concentration, which can be measured or predicted, with the predicted no-effect concentration (PNEC). The calculation of a species sensitivity distribution (SSD) is a commonly accepted approach to derive the PNEC of a substance (Monti et al., 2018). Generally, the SSD approach considers several environmental species that are assumed to be representative of the biological community of an environmental compartment. Substance- and compartment-specific data are collected and

evaluated, and a curve is built that adequately fits the data points (Posthuma et al., 2002, Hickey and Craig, 2012). Parametric and non-parametric approaches can be used to fit a curve to the dataset. On the SSD, the sensitivity of the species is represented in a cumulative order depending on the effect concentration associated with each of them. This allows one to determine an environmental concentration that would protect a given percentage of species in a specific compartment. In the European regulatory context, the PNEC value is usually derived from the HC₅ (hazardous concentration for 5% of species), protecting 95% of the biological community against the stressor in question (Aldenberg and Slob, 1993, Iwasaki et al., 2015, ECHA, 2008). Additionally, an assessment factor of one to five has to be applied to the HC₅ to consider the quality of the data set used (ECHA, 2008).

The first crucial step in the SSD approach is the selection of the experimental data. Thereby, the selection of the endpoint tested (e.g. growth, mortality etc.) is key to build a meaningful SSD (Smetanova et al., 2014). The toxicity values are reported in the literature as measures of the effect concentration (EC_x), inhibition concentration (IC_x) and lethal concentration (LC_x) at a certain percentage 'x' as well as the lowest observed effect concentration (LOEC) or the no-observed effect concentration (NOEC). Generally, EC_x or NOECs are preferred to create the SSD. The discussion on which measure is the most adequate is still ongoing (Green et al., 2013, Fox and Landis, 2016). The NOEC was criticized to have no biological meaning since it is not a measure of toxicity, but rather of non-toxicity. The NOEC considerably depends on the study design that can lead to higher variability (Laskowski, 1995, Jager, 2012, Fox and Landis, 2016, Monti et al., 2018, Isnard et al., 2001). Thus, the measure of EC_x was proposed to replace the NOEC, whilst the selected value for "x" depends on jurisdiction and risk management objectives (Isnard et al., 2001, Beasley et al., 2015). Still, the NOEC and EC₁₀ represent the conservative values for assessing the PNEC, which are proposed by the European regulation (Monti et al., 2018, ECHA, 2008).

In the European regulatory context, chronic data are preferred over acute datasets, since chronic studies include more life stages of the organisms and are therefore often more representative of their response towards the stressor in the environment (ECHA, 2008). However, acute data are more often available than chronic data and are representative of specific conditions such as pulsed exposures. It is often assumed that if more data are included in the SSD, the results are a better representation of reality, but this representation actually depends highly on the design of the studies included and on the way in which the data are reported (Dowse et al., 2013, Newman et al., 2000). Hence, both the data quantity and quality have to be considered in SSD assessments. Nevertheless, due to prevalent data scarcity, a trade-off between data quality and data quantity has to be made to foster the building of representative SSDs (Dowse et al.,

2013). To this aim, uncertainty factors (UFs) were proposed to convert acute data to chronic values (also named acute-to-chronic ratios) (Iwasaki et al., 2015, Harmon et al., 2017, Smetanova et al., 2014) and the various dose-descriptors reported (i.e. LOEC, EC₁₀, EC₂₅, EC₅₀ etc.) to NOEC or EC_x. Some UFs are generally recommended by the European regulation (ECHA, 2008) even though they can vary depending on the species and substances. The SSDs can then be calculated based on empirically derived chronic data combined with chronic data derived from acute datasets to enlarge the overall dataset.

The parametric approaches fit SSDs to the data presuming that the response of biological communities follows a given distribution (e.g. log-normal, log-logistic or Burr type III distribution (Wheeler 2002)). Yet, the biological community does not necessarily follow one single distribution (Xu et al., 2015). Non-parametrical SSD approaches have been developed to tackle this challenge (Newman et al., 2000, Wang et al., 2015, Monti et al., 2018). One of these approaches is the probabilistic species sensitivity distribution (PSSD) (Gottschalk and Nowack, 2013). The PSSD approach addressed the fitting problem by avoiding the use of pre-defined distributions to derive the PSSD with an empirical calculation using the complete dataset. Additionally, the inter-laboratory variability of data points stemming from experiments is considered in the approach. The result was one single PSSD that was representative for the dataset.

The parametric SSD approach includes only one value for each species (e.g. geometrical mean or lowest value) (Belanger et al., 2017), which does not represent the variability of the effect encountered in the whole environmental compartment. Semenzin et al. (2015) proposed to improve the data handling by addressing the species selection bias and data quality problem. These authors introduced weighting factors for data quality, trophic level and species relevance before the actual calculation was performed. However, the main advantage of the PSSD is that it considers the complete variability of all NOECs for each species and does not select only one value among all that are available. It therefore reflects much better the variability of responses that may occur in the environment. This PSSD approach has been applied to several compounds such as nanomaterials (Coll et al., 2016, Wang et al., 2016, Wang and Nowack, 2018, Giese et al., 2018, Wigger and Nowack, 2019).

The aim of this work was to develop further the probabilistic element of the existing PSSD method, providing a fully transparent methodology. By doing so, we have included the uncertainty associated with the UFs and extended the PSSD calculation to several PSSDs, which results in a closer representation of the data than the use of geometric means and more complete representation of their uncertainty than in previous methodologies such as in Gottschalk and Nowack (2013). Additionally, we included a weighting scheme with respect to a typical biological community in the freshwater

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compartment to assess the potential influence on the results and show the capability of the probabilistic species sensitivity distribution plus (PSSD⁺) approach to include species weighting. Based on these newly implemented probabilistic elements, a probability distribution for the PNEC is derived incorporating all associated uncertainties. The PSSD⁺ was applied to carbon nanotubes (CNT) and nano-Ag from two previously published datasets (Coll et al., 2016), representative for a case with few data (CNT) and one very rich in data (nano-Ag).

Material and methods

The dataset used and the PSSD⁺ approach are described in more detail in this section. It is aligned on the requirements of the Reach guideline R.10 provided by the European Chemical Agency (ECHA, 2008). Particularly, the data collection, the main requirements of the SSD and the application of UFs are described in this guideline, which we have followed as far as possible. Any exceptional deviations from this guideline are transparently discussed where it pertains in this article.

Selection of case studies, data collection and preparation

The datasets used to apply the PSSD⁺ method were based on those of Coll et al. (2016), detailed in Tables S1 and S2 of the Supplementary Information (SI). PSSDs were built for both nano-Ag and carbon nanotubes (CNTs). These case studies represent two cases of data availability, which was useful to test the robustness of our method. Many ecotoxicity data are available for nano-Ag while only a limited dataset could be obtained for CNTs.

The data were collected from peer-reviewed studies that characterized changes in reproduction, mortality, growth or any relevant metabolic process (e.g. photosynthetic activity, (ECHA, 2008)). If the study had reported chronic (i.e. long-term) as well as acute values, the first were preferred, while the latter were neglected. In total, 11 species and 24 data points were included in the CNT dataset, while that of nano-Ag contained 26 species and 173 data points. All data points were treated in the same way and combined in the substance-specific PSSDs. All REACH criteria on the occurrence of different types of species were met for nano-Ag, while three of them could not be met for CNTs (cf. Table 1). It has to be noted that we did not check the quality of the data (i.e. the experimental setup) used as this article is merely focused on the development of the method itself. The application of the PSSD⁺ approach should be accompanied by a detailed analysis of the studies used in the assessment.

Different types of dose descriptors were given in the studies analysed, from EC_x, LOECs, minimum inhibitory concentrations (MICs) to highest observed no effect

concentrations (HONECs) and NOECs. To comply with REACH guidance R.10 (ECHA, 2008) that recommends to use chronic NOECs or EC₁₀s if possible, all data points were converted into chronic NOECs by applying two UFs, for which uncertainty ranges were attributed (Tables 2 and 3). The first uncertainty factor (UF_t) considered the duration of the experiment and was used to convert acute measurements to chronic estimations. The second uncertainty factor (UF_{dd}) was used to convert a specific dose-descriptor to a NOEC. The assumptions and reasoning to derive the modal values of the UFs are shown in Tables 2 and 3. It has to be noted that UFs are critically discussed and no generic agreement is reached so far. Particularly, the conversion of different dose descriptors to obtain a NOEC is not yet regulated. An exception is the derivation of a NOEC based on a LOEC divided by 2 as described in the REACH guideline R.10 (ECHA, 2008). As a starting point for the transformation of EC/LC/IC₂₅ to EC/LC/IC₅₀, we have selected 10 as the modal value being used in the PSSD⁺ approach. All UF_{dd} probability distributions were triangular, with the minimum and maximum values being 50% and 150% of the modal value, respectively.

Some acute-to-chronic ratios (or UF_t) were calculated in the literature for nano-Ag (Harmon et al., 2017). The authors found values as low as 1.6. This was set as the minimum value of the probability distribution. Acute-to-chronic ratios usually used are 10, so this was set as the modal value of the probability distribution. To be conservative, we chose a maximum value of 100, which is the order of magnitude of the highest acute-to-chronic ratios calculated for metals (Raimondo et al., 2007). To the best of our knowledge, no acute-to-chronic ratios were reported for CNTs so far. Therefore, we used a triangular probability distribution with a modal value of 10, a minimum value of 5 (50% of 10) and a maximum value of 150 (150% of 10). This last probability distribution can be considered as generic, to be used when no more information is available, while the probability distribution for UF_t of nano-Ag is more specific. Two cases are therefore illustrated in this study, a generic one and a specific one.

The choices of UF_t, UF_{dd} and their probability distributions are discussed in the respective section of this manuscript. Tests were performed, which show the validity of our approach. However, we are aware that adaptations of the UFs used in this study could be made with new insights for appropriate UFs suggestions.

Building the probabilistic species sensitivity distributions

The PSSDs were built in three steps. First, ecotoxicological values were converted into chronic NOECs. Second, all NOECs of one species were combined together in a probability distribution. Finally, PSSDs were extracted. The calculations were

performed with a script programmed in R (R Core Team, 2013), which requires six items as inputs:

- A table of ecotoxicity values (data points, DPs) extracted from the literature
- A table of UFs associated with these values to correct for the exposure duration (UF_t)
- A table of UFs associated with the ecotoxicity values to convert them to NOECs (UF_{dd})
- A coefficient of variation that is unique to all data points, representing the inter-laboratory variability ($CV_{DP} = 30\%$, (Gottschalk and Nowack, 2013))
- A coefficient of variation that is unique to all uncertainty factors, representing the uncertainty associated with the use of non-substance-specific uncertainty factors ($CV_{UF} = 50\%$)
- A number of simulations

The following subsections describe the steps of the method used to obtain the PSSDs.

Conversion of ecotoxicological data to NOECs

The first step of the approach was to convert all ecotoxicity measurements (or data points, DP) extracted from the literature to chronic NOECs, based on the UF_t and UF_{dd} (Eq. 1):

$$NOEC = \frac{DP}{UF_t \times UF_{dd}} \quad (1).$$

Definition of species-specific probability distributions in generic cases (e.g. CNTs)

Second, a probability distribution was defined to represent the toxicity of the substance towards each species considered. The overall widths of these species-specific distributions were defined based on the range of NOECs obtained, and on the CVs defined above. The minimum and maximum of a species-specific distribution were determined by the uncertainties attributed to the lowest and largest calculated NOECs. These uncertainties were derived using error propagation, as in equation 2:

$$\sigma_{NOEC} = \sqrt{\sigma_{DP}^2 + \sigma_{UF_t}^2 + \sigma_{UF_{dd}}^2} \quad (2),$$

where σ_{NOEC} is the relative uncertainty attributed to the lowest or largest *NOEC*, and σ_{DP} , σ_{UF_t} and $\sigma_{UF_{dd}}$ are the relative uncertainties associated to the inter-laboratory variation of measurement, the UF used for the correction of chronic values UF_t and the UF used for the dose-descriptors UF_{dd} . Since our coefficients of variation

represent the maximum spread of triangular distributions, we used a factor of 2.45 to convert them into standard deviations and use error propagation (Eq. 4, based on (Kawecki et al., 2018)).

$$CV_{NOEC,CNT} = \sqrt{\left(\frac{CV_{DP}}{2.45}\right)^2 + \left(\frac{CV_{UF_t}}{2.45}\right)^2 + \left(\frac{CV_{UF_{dd}}}{2.45}\right)^2} \cdot 2.45 \quad (3).$$

Three different cases occurred when building species-specific NOEC distributions: (1) One data point was available for one species, (2) Two data points were available for one species and (3) Three or more data points were available for one species. Each case was treated separately, as follows.

Case 1: One data point available for one species (Figure 1A). When only one data point was available for a species, a triangular distribution was built around the NOEC calculated as in Eq. 1. The mode of the distribution was defined as the NOEC, while the minimum and maximum of the distributions were calculated as in Eq. 4 and 5, respectively:

$$\min = NOEC \cdot (1 - CV_{NOEC,CNT}) \quad (4)$$

$$\max = NOEC \cdot (1 + CV_{NOEC,CNT}) \quad (5)$$

Case 2: Two data points available for one species (Figure 1B). When two data points were available for one species, a trapezoidal distribution was built. Both modal values of the distribution were calculated as in Eq. 1. The calculations of the minimum and maximum values of the distribution were calculated based on Eq. 6 and 7, respectively:

$$\min = NOEC_1 \cdot (1 - CV_{NOEC,CNT_1}) \quad (6)$$

$$\max = NOEC_2 \cdot (1 + CV_{NOEC,CNT_2}) \quad (7)$$

Case 3: Three or more data points available for one species (Figure 1C). In this case, a more complex distribution was defined, following several steps. By default, an equal probability was assumed to all NOEC values. Uniform distributions were therefore built between each NOEC, as in (Gottschalk and Nowack, 2013), resulting in what we will call a base step distribution. Because this approach produced unrealistic distributions in the case where two NOECs were equal, it was defined that for each instance where two NOECs were equal, the probability for this NOEC was doubled. In all cases, we chose to double the highest probability value that could be attributed with the NOEC (p_{i1} in the example in Figure 1C). Similarly, when n NOECs were equal, a weight of n was attributed to this point in the probability distribution. Since in

the base step distribution, two different probabilities can be attributed to each NOEC value, one from the left and one from the right, only the largest of the two probabilities was considered (top panel in Figure 1C). Once the individual weights were set for each NOEC, the probability between two subsequent NOECs was defined as a linear function. This procedure then yielded one probability distribution for each species. Finally, to represent the uncertainties associated with inter-laboratory variability and the non-substance-specific evaluation of UFs, the same approach was used as in Case 1 and Case 2: Triangular distributions were added at the extrema of the step probability distribution (bottom panel in Figure 1C, Eq. 8 and 9, $NOEC_n$ being the highest NOEC obtained for the species):

$$\min = NOEC_1 \cdot (1 - CV_{NOEC,CNT_1}) \quad (8)$$

$$\max = NOEC_n \cdot (1 + CV_{NOEC,CNT_n}) \quad (9)$$

Definition of species-specific probability distributions for a specific case: nano-Ag

For nano-Ag, probability distributions were defined based on coefficients of variation for inter-laboratory variations and UF_{dd} , and on minimum and maximum values for UF_t . Therefore, a slightly different approach was used than for the CNT-case. The CV used to find the minimum or the maximum values of a species-specific probability distribution included only the variations due to inter-laboratory variation and UF_{dd} (Eq.10). The minimum obtained in this was then divided by 100, which is the maximum value of the probability distribution associated with UF_t (Eq. 11). To find the maximum of the species-specific probability distribution, the “interim” maximum found with the CV was divided by the lowest value of the probability distribution associated with UF_t (equal to 1), so remained as such (Eq. 12).

$$CV_{NOEC,Ag} = \sqrt{\left(\frac{CV_{DP}}{2.45}\right)^2 + \left(\frac{CV_{UF_{dd}}}{2.45}\right)^2} \cdot 2.45 \quad (10)$$

$$\min = NOEC_1 \cdot (1 - CV_{NOEC,Ag_1})/100 \quad (11)$$

$$\max = NOEC_2 \cdot (1 + CV_{NOEC,Ag_2}) \quad (12)$$

The minima and maxima obtained were then used in the same way as for CNTs to build the species-specific distributions.

Derivation of the probabilistic species sensitivity distributions

The PSSD graph represents the species sensitivity (or fraction of species affected) as a function of the NOEC. The ranges of species-specific NOEC distributions determined

the range of concentrations in which the PSSDs were built. In each simulation, i.e. for each PSSD, one value was sampled from each species-specific distribution. All the extracted NOEC values were combined in a cumulative distribution to give a PSSD. This process was repeated 10'000 times to obtain 10'000 PSSDs. The probability associated with each ecotoxicological value in the species-specific distributions represented the likelihood that it was sampled in one simulation. In this way, all species available in the dataset were represented with equal weight, and the structure of the community represented was that of the dataset available.

Typical freshwater communities generally consist of approximately 65% primary producers, 25% herbivores and 10% of carnivores (Forbes and Calow, 2002). The authors considered all invertebrates as herbivores and all fishes as carnivores. Since PSSDs should be representative of a general freshwater community, this typical structure was used to build generic PSSDs, although we acknowledge that this structure represents a very broadly simplified ecosystem. To this aim, the species available in the dataset were sampled randomly in each of the 10'000 simulations so that the structure of the community represented was typical of freshwaters. In each simulation, 65% of the NOEC species-specific distributions from which values were sampled were of primary producers, 25% of invertebrates and 10% of fishes. All species available for the most underestimated trophic level were always considered in the calculations of the PSSDs. This number was set to the corresponding typical representation of the trophic level, and the numbers of values sampled for the other two trophic levels were determined based on the number of values in the most underestimated trophic level and the typical proportions of the other levels. Even though the number of values sampled in each trophic level stayed the same for all simulations, the species corresponding to each of the originally overestimated trophic levels were chosen randomly so that they would all be represented in the graph.

Calculation of the predicted no effect concentration

As recommended by REACH guideline R.10 (ECHA, 2008), the PNEC was determined by extracting the fifth percentile of the hazardous concentration for each PSSD calculated. These 10'000 values represent the probability distribution of the PNEC. The REACH guideline also requires the use of an UF ranging from 1 to 5 depending on the quality of the dataset used. Since the purpose of this article was to develop further the probabilistic elements in the method, we applied here an UF of 1, although we are aware that future studies will need to define this factor to the best of their knowledge.

Test of robustness and sensitivity of the method

The robustness of the PSSD⁺ approach was assessed by comparing the PNECs calculated to already published datasets for CNT and nano-Ag. Its sensitivity to the used dataset was tested by removing one data point (NOEC) from the dataset at a time. The percentage of change from one PNEC to another was calculated to interpret the results.

Results and discussion

Further development of the PSSD approach

Table 4 describes the differences of the PSSD⁺ approach with that developed by Gottschalk and Nowack (2013) for each step towards the determination of a PNEC probability distribution. One of the main changes concerns the uncertainties considered to build probability distributions of individual NOECs. While the approach of Gottschalk and Nowack (2013) converts data points into NOEC values based on single values of UFs, our approach considers the uncertainties associated with the UFs. The other main change regards the number of PSSDs derived and the calculation of the PNEC distribution. Instead of applying a confidence interval to a single PNEC value, the probability distribution derived here is directly based on the multiple individual HC5s obtained from the PSSD data.

The PSSD⁺ approach has several advantages compared to conventional SSD methods, which relates to the handling of the data and uncertainties associated. Compared to conventional SSDs that are usually based on one data point per species (e.g. geometric mean or lowest value), the PSSD⁺ approach considers all values reported equally and thereby avoids a bias from data selection (Fox, 2015). Hence, this PSSD approach stays closer to the original data and avoids losing information by aggregating the data. It is therefore able to cope better with large datasets. Conventional SSDs also assume a predefined model that does not always accurately represent the ecotoxicity to all species, since it will be representative of a general trend only, and not of the individual responses of species. Here, since all data are considered in the final PSSD curves, no bias is entailed by curve fitting. The PSSD⁺ approach is therefore also preferable for small datasets. In the end, this method takes into account all elements of inter-test variation (Hickey and Craig, 2012): Inter-laboratory variations is taken into account explicitly with a coefficient of variation of 30%; intra-species variations are considered by the fact that we stay as close as possible to the raw data points, which are numerous for many species; experimental variations and those raised by the use of different models to derive the ecotoxicological values are taken into account implicitly

by not excluding any study based on such criteria: if they exist in the literature, all experimental conditions and modelling strategies are included in our work.

Case studies

The PSSDs of CNTs in freshwater were built on 39 ecotoxicity values reported in the literature and cover 10 species, while 158 values covering 21 species were available to build the PSSDs of nano-Ag (Figure 2). Both datasets provide a good representation of the trophic chain, from eukaryotes to fishes, but the dataset on nano-Ag is more complete since it includes plants, an insect and a snail, types of organisms for which the ecotoxicity of CNTs were not tested.

The PSSDs of both nano-Ag and CNTs shown in Figure 2 were built on the whole datasets. They represent the sensitivity of a community, which would have the structure of the dataset (Tables 5 and S4). The shaded areas represent various quantiles of the PSSDs, while the green points represent the NOECs that were calculated from the data available in the literature. These points are grouped per species and are reported on the graph only as a reference. The order in which the species appear on the y-axis is only defined by the geometric mean of their NOECs: the species with the lowest geometric mean of NOECs is considered as the most sensitive and therefore appears at the bottom of the graph. This is different from the order in which the species might appear on a PSSD, where they are ranked based on the NOEC values that were sampled in the calculation. The order of species is therefore not necessarily the same on each PSSD. This explains why, for example, the highest NOEC value found for *D. rerio* is considered on the highest levels of the PSSD, and not on the level on which all NOECs of *D. rerio* are reported.

Daphnia magna was shown to be the species most sensitive to CNTs; the lowest NOEC found for this organism was $13 \mu\text{g.L}^{-1}$ by Zhu et al. (2009). The amphibian *Ambystoma mexicanum* presented a NOEC of $10^5 \mu\text{g.L}^{-1}$ (Mouchet et al., 2007) and was the least sensitive species. The PNEC empirical probability distribution obtained from the PSSD had a mean of $86 \mu\text{g.L}^{-1}$ with 25th and 75th quantiles of 32 and $111 \mu\text{g.L}^{-1}$, respectively (Table 6).

Organisms were generally shown to be more sensitive to nano-Ag than to CNTs. Based on the geometric means of the NOECs, the species most sensitive to nano-Ag was found to be *Gammarus fossarum*, for which one NOEC was available (10 ng.L^{-1} , (Arce Funck et al., 2013)). However, the lowest NOEC of all was 1.5 ng.L^{-1} , which was obtained when exposing *Ceriodaphnia dubia* to nano-Ag (Angel et al., 2013). *Paramecium caudatum* was the least sensitive species, with a NOEC of 0.25 ng.L^{-1} (Kvitek et al., 2009). The range of the histogram of the simulated PNECs of nano-Ag

was 3 to 4 orders of magnitude lower than that of CNTs, with a mean of 77 ng.L⁻¹ and 25th and 75th quantiles of 11 and 126 ng.L⁻¹, respectively.

As with any model, the results rely heavily on the input data, here the NOECs, and in the way they are handled. These results are therefore only valid for the species included in this assessment, and not, as would be most often assumed, on all communities of species living in freshwater environments (Forbes and Calow, 2002). Fox (2015) underlines the fact that it is crucial to select species from all taxonomic groups relevant to the compartment under study. According to the REACH guidelines, all important taxonomic groups living in pelagic freshwater seem to be included in the data set for nano-Ag, while the occurrences of a higher plant, an insect and a family in a phylum other than Arthropoda or Chordata are missing for CNTs (Table 1). Regarding other guidelines, such as those of the US EPA (Stephen et al., 1985) and of the Canadian Council of Ministers of the Environment (CCME, 2007), criteria are still met for nano-Ag but not for CNTs (Table S3). Results could therefore certainly change with regard to CNTs if more data, especially on the types of organisms lacking, were included in the assessment. It has to be noted that future SSD as well as PSSD⁺ assessments should differentiate datasets according to the ecotoxicological endpoints tested, because the consideration of different endpoints such as mortality and growth inhibition introduces larger uncertainties to the results. Despite this fact seems to be acknowledged by researchers, some regulations allow such data aggregation as pointed out by Hickey and Craig (2012).

Testing the sensitivity of the PNEC results to data point variations

The sensitivity test of the PSSD⁺ approach was performed on the initial datasets, without any rearrangement of the structures of the biomes (Figure 3). Each graph shows the difference in the mean PNEC obtained when removing one specific NOEC from the overall distribution, these NOEC values being ordered ascendingly on the horizontal axis. It can be seen that both in the case of a lower number of ecotoxicity values available (CNTs) and that of a larger data set (nano-Ag), the maximum change in the calculated mean PNEC stays in all cases except one within a factor of 50% when any NOEC value is removed. This shows that, unlike fitting approaches, the PSSD⁺ can handle both small and large datasets.

The highest change of the mean PNEC for CNTs was observed when the lowest NOEC was removed from the PSSD (+40%). Removal of the 3^d, 5th, 8th and 14th NOECs also resulted in considerable changes of the PNEC, of about 20% or higher. All these NOECs were associated with the two most sensitive species, *D. magna* and *C. dubia*, which explains why they have the highest influence on the calculation of the 5th percentiles of the PSSDs. It is worth highlighting that both these species are

crustaceans, and that no other crustacean was found at lower toxicities. This might indicate that this type of organism is more sensitive than others to CNTs, although one should keep in mind the limited number of species for which data was available and therefore the potentially limited representation of the overall sensitivity of species towards CNTs.

Removing the lowest NOECs of nano-Ag induced a significant change in the resulting mean PNEC, but the highest change was observed only for the 6th NOEC of nano-Ag (+201%) corresponding to the removal of the NOEC of *G. fossarum*. The fact that only one data point was available for this species explains why its removal lead to such a high difference in the PNEC calculated, since *C. dubia* (2nd ranked in the complete nano-Ag dataset) becomes the most sensitive species and the lowest part of the PSSD is shifted to higher values (i.e. less toxic). Therefore, it appears that it is not only the values of the NOECs considered that influence the value of the PNEC, but also the overall sensitivity of the species to which these NOECs are associated. Other significant changes of the mean PNEC, higher than 30%, are due to the removal of points associated with *C. dubia*, the second most sensitive species.

A common criticism to SSDs is the basic assumption of exchangeability of the species selected allowing their “random sampling”. Craig et al. (2012) have shown that for some species, such as *Oncorhynchus mykiss* or *Carassius auratus*, this assumption is improper affecting the HC₅ derivation significantly, which is also visible in Figure 3. Some methods were provided to tackle this challenge (Craig et al., 2012, Belanger et al., 2017) and should be considered by environmental risk practitioners using the SSD and PSSD⁺ methods.

Generic assessment of a typical community structure

To better represent the sensitivity of a typical freshwater community to the chosen nanomaterials and not that of the community built by the availability of studies on specific species, PSSDs were built on the weighted datasets. Tables 5 and S4 show that, in the dataset for CNTs, the proportions of primary producers and herbivores were underestimated by about 15% and 5%, respectively, and that of carnivores was overestimated by 20%. The proportions of primary producers considered for nano-Ag was underestimated by about 25% and those of herbivores and carnivores were overestimated by about 15%. The PNEC of CNT obtained with a typical structure did not differ from the PNEC obtained with all species from which data could be obtained. For nano-Ag, the mean PNEC was smaller by a factor of 1.4 when a typical structure of ecological community was used in the calculations (Table 5).

Defining the range of uncertainty factors

The conversion of ecotoxicity values reported in the literature to chronic NOECs gives rise to uncertainties, associated with both UF_{dd} and UF_t . The use of uncertainty factors is recommended in regulation and test guidelines. However, these are neither species-specific nor substance-specific. Brix et al. (2001) showed that they are different among species for a same substance, while Chen et al. (2018) showed that they could be different for different nanomaterials. These authors evaluated the ratios of HC_5 derived from different dose descriptors (i.e. UF_{dd}) divided by the HC_5 derived from NOEC values for nano-Ag. They found HC_5-LC_{50}/HC_5-NOEC , HC_5-EC_{50}/HC_5-NOEC and $LOEC/NOEC$ equal to 10, 15.8 and 0.5. However, their calculations were based on five data points for LOECs and NOECs: even though they are a good indication, they cannot be considered as definitive. This might also explain why the HC_5 based on NOECs seems higher than that based on LOECs. Nevertheless, the ratios obtained by Chen et al. (2018) for LC_{50} s and EC_{50} s are in the range of uncertainty that were considered in the PSSD⁺ method. An uncertainty of $\pm 50\%$ around these uncertainty factors therefore seems reasonable.

The modal value of 10 used for the conversion of acute to chronic values (i.e. UF_t) could also be discussed. To this end, we took inspiration from Duboudin et al. (2004) and separated the calculations of PSSDs and PNECs probability distributions from chronic and acute datasets (Table 6). The resulting PNECs were compared, to see if the difference of their means would be around a factor of 10. Only the species for which both chronic and acute data were available were considered in these calculations. For CNTs, only *D. magna* could be included, so we compared the mean of the chronic NOECs available for this species to the mean of the acute NOEC. The chronic mean was lower than the acute mean, by a factor of 12.4. This factor was located at the higher end of the range of uncertainty attributed to the uncertainty factor (5 to 15). The use of such a range seems therefore rather conservative, as also illustrated by the fact that the PNEC calculated with the whole dataset was lower than the PNEC calculated with the chronic dataset only. However, the numbers of species included in the acute and chronic assessments of PNECs were low, so it is reasonable to keep an uncertainty range around the conversion factor. For nano-Ag, three species could be considered in the calculations so PNECs could be calculated. The chronic PNEC was lower than the acute PNEC, by a factor of 1.8. Again, this value is within the range of the probability distribution associated with UF_t , ranging from 1 to 100 with a modal value of 10. The PNEC of nano-Ag calculated with the whole dataset was lower than both the chronic and acute PNECs, which showed again that our method is rather conservative.

Conclusions

Ecotoxicological hazard assessments have to determine the PNEC value for a given substance to be used in a regulatory context. Unlike deterministic approaches that focus on single species of a compartment, SSD and PSSD methods are recommended to calculate a PNEC that is representative for the whole biological community because they allow the consideration of several species at the same time. In order to comply with regulations, chronic data on ecotoxicological effects are preferred in a regulatory context, although this preference may differ depending on the study and management objective. However, since the chronic data availability is more limited than that of acute data, UFs for acute-to-chronic conversion and also dose-descriptors are one potential mean to make chronic and acute data comparable. The adequacy of such data conversion is controversially discussed, as these UFs are particularly dependent on the species and substance considered. Also, the species relevance was discussed to have a critical impact on the PNEC values, when a weighting scheme is applied to consider key species in an environmental compartment.

In this light, we advanced the PSSD approach on three aspects to develop the PSSD⁺ tool. First, we incorporated additional probabilistic elements to this computational method in order to consider the uncertainty associated to UFs by using probability distributions instead of single values. By doing so, we cannot answer the question of the truly correct UF that should be used, but our approach can consider the uncertainty related to UFs for deriving the PNEC. Second, the new PSSD method computes 10'000 PSSDs based on a Monte-Carlo routine. For each PSSD calculated, the HC₅ was extracted to provide a PNEC distribution based on all data available and their associated uncertainty. Third, the novel PSSD approach is capable to consider a species weighting according to a typically constituted biome.

We tested the PSSD⁺ approach regarding the robustness and sensitivity based on published datasets on CNT and nano-Ag, representing two cases of small and bigger datasets. The evaluation of the UFs distributions and species weighting showed that the proposed PSSD method is plausible and indicated quasi-stability of the results when species weighting was applied. Furthermore, we demonstrated that the PSSD⁺ approach can handle both limited and larger datasets by avoiding the fitting problem of conventional SSDs as the resulting PNECs are a closer representation of the data available. The sensitivity testing towards data set variations showed that in all but one case, the results changed by a factor of less than 2. Therefore, the PSSD⁺ approach is relatively insensitive against missing data points as long as the most sensitive species is included. This underlines the need for adequate data rather than more data, which is why the application of the PSSD⁺ approach requires a sound preselection of studies by

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experts. Nevertheless, future developments of the PSSD⁺ can address this issue by incorporating data quality aspects. Finally, the PSSD⁺ approach can be not only applied to ENMs (Wigger and Nowack, 2019) but also to any other substance such as microplastics (Adam et al., 2019) or chemicals.

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Disclaimer

The authors declare no conflict of interests.

Data Availability Statement

The simulation framework is available as a software package at <https://doi.org/10.5281/zenodo.3267194>

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Figures

Figure 1: Approach used to define species-specific probability distributions of ecotoxicity in cases where (A) one data point was available, (B) two data points were available, (C) three or more data points were available, $NOEC_i$ appears twice in the dataset while all other $NOEC$ s appear once. The top panel of figure C represents the species-specific probability distributions without consideration of inter-laboratory variability and uncertainty associated to uncertainty factors. These variability and uncertainty are added on the bottom panel of figure C. Conc.: Concentration. p_{i1} and p_{i2} : first and second probability values that could be associated with $NOEC_i$.

Figure 2: Probabilistic species sensitivity distributions and histograms of the simulated predicted-no effect concentration of CNT (A) and nano-Ag (B) in freshwater

Figure 3: Differences in mean PNECs obtained when one NOEC value was removed from the probabilistic species sensitivity distribution. This calculation is based on the initial dataset that did not include any changes to biome structure.

Table 1: Characteristics of CNT and nano-Ag ecotoxicity datasets used to build the PSSDs. Criteria regarding occurrences of certain types of organisms are based on REACH guideline R.10 (ECHA, 2008)

Characteristic of dataset	CNT	Nano-Ag
Number of data points	39	158
Number of species	10	21
Proportion of chronic data points	49%	10%
Occurrence of fish	Yes	Yes
Occurrence of an algae	Yes	Yes
Occurrence of a higher plant	No	Yes
Occurrence of a crustacean	Yes	Yes
Occurrence of an insect	No	Yes
Occurrence of a second family in the phylum Chordata	Yes	Yes
Occurrence of a family in a phylum other than Arthropoda or Chordata (e.g. Rotifera, Annelida, Mollusca)	Yes	Yes
Occurrence of a family in any order of insect or any phylum not already represented	No	Yes

Table 2: Uncertainty factors used to convert acute to chronic values (UF_t). An UF_t of 10 was applied to values resulting from tests considered acute, while an UF_t of 1 was applied to values resulting from chronic bioassays

Type of organism	Exposure time (days)	UF_t modal value	Reference
Algae	< 3	10	(OECD, 2006a)
	≥ 3	1	
Plants ⁱ	< 28	10	(OECD, 2006b)
	≥ 28	1	
Crustacea ⁱⁱ	< 21	10	(OECD, 2004, OECD, 2012)
	≥ 21	1	
Mollusca	< 28	10	(OECD, 2016a, OECD, 2016b)
	≥ 28	1	
Fish	< 28	10	(OECD, 2000, OECD, 2009)
	≥ 28	1	

Table 3: Uncertainty factors used to convert dose descriptors into NOECs (UF_{dd}).

Type of dose-descriptor	UF_{dd} modal value	Reference / comment
LC ₂₅₋₅₀ ; EC ₂₅₋₅₀ ; IC ₂₅₋₅₀	10	Kim et al. (2011); Schwab et al. (2011)
LC ₂₀ ; EC ₂₀ ; IC ₂₀	2	Beasley et al. 2015; Azimonti et al. (2015)
LOEC	2	(ECHA, 2008), Table R.10-1
MIC	2	Equal to LOEC
LC ₁₀ ; EC ₁₀ ; IC ₁₀	1	Beasley et al. (2015); Azimonti et al. (2015)

HONEC	1	Equal to NOEC
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Table 4: Comparison of the PSSD⁺ with that of Gottschalk and Nowack (2013). Changes from one method to another are highlighted in bold.

Step	Gottschalk and Nowack (2013)	PSSD ⁺
1. Convert measurements to NOECs	NOECs are determined as probability distributions, based on the inter-laboratory variation (coefficient of variation = 30%) and single values of UF _t and UF _{dd} .	NOECs are determined as probability distributions, based on uncertainties associated with the measurement (coefficient of variation = 30%) and with UF_t and UF_{dd} (coefficient of variation = 50% when not more accurate information is available).
2. Combine all NOECs of one species in one probability distribution	In case of one measurement available for a species, a triangular distribution of the NOEC is built. In case of two measurements available for one species, a trapezoidal distribution is built. In case of three or more measurements available for one species, a step distribution is built.	In case of one measurement available for a species, a triangular distribution of the NOEC is built. In case of two measurements available for one species, a trapezoidal distribution is built. In case of three or more measurements available for one species, a complex distribution is built. The fact that an identical NOEC can occur several times for one species is considered.
3. Derive the PSSD(s)	A single PSSD is sampled from all species-specific distributions.	Multiple PSSDs are extracted from the species-specific probability distributions.
4. Calculate the PNEC probability distribution	The PNEC is calculated as the fifth percentile of the PSSD. A probability distribution is built from a confidence interval.	The PNEC probability distribution is derived directly from the fifth percentiles of all PSSDs.

Table 5: Structures of the communities corresponding to all species for which data was collected. The typical structure is as reported by Forbes and Calow (2002).

	CNT	Nano-Ag	Typical structure
Primary producers	50%	38%	65%
Herbivores	20%	38%	25%
Carnivores	30%	24%	10%

Table 6: Statistical values of the PNECs obtained with different methods and datasets. Q5, Q25, Q75 and Q95 correspond to the 5th, 25th, 75th and 95th percentiles.

CNT ($\mu\text{g.L}^{-1}$)						
Dataset	Q5	Q25	median	mean	Q75	Q95
Base dataset	13	32	61	86	111	257
Typical community	13	32	61	86	111	255
Chronic dataset	N.A.	N.A.	N.A.	125	N.A.	N.A.
Acute dataset	N.A.	N.A.	N.A.	1'551	N.A.	N.A.
Nano-Ag (ng.L^{-1})						
Dataset	Q5	Q25	median	mean	Q75	Q95
Base dataset	7	11	31	77	126	257
Typical community	3	6	9	57	20	337
Chronic dataset	606	1'082	1'562	1'606	2'081	2'785
Acute dataset	227	1'366	2'879	2'823	4'273	5'785

ⁱ For *Lemna* sp., the exposure duration distinguishing acute from chronic tests was taken as 2.5 days, which is the frond number doubling time required in OECD Test Guideline 221. This guideline suggests using an exposure duration of 7 days. The studies included in this work used exposure durations of 7 or 14 days, so the corresponding data points were all considered chronic.

ⁱⁱ The US EPA published a testing guideline specific to *C. dubia*, according to which chronic toxicity can be tested with exposure durations of 7 days. This threshold was therefore considered for this specific crustacean species.