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SPECIES SENSITIVITY WEIGHTED DISTRIBUTION (SSWD) FOR ECOLOGICAL RISK ASSESSMENT OF ENGINEERED NANOMATERIALS: THE N-TIO_2 CASE STUDY

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Abstract: Societal concerns about engineered nanomaterials (ENMs) environmental risks have increased over the last years, but nano ecological risk assessments (RAs) are constrained by significant gaps in basic information on e.g. long term effects and exposures. For this reason we propose an approach for ecological RA of ENMs that can operate in context of high uncertainty. It further develops the Species Sensitivity Weighted Distribution (SSWD) approach by including three weighting criteria (i.e. species relevance, trophic level abundance and nanotoxicity data quality) to address nano-specific needs (n-SSWD). Application of n-SSWD is illustrated for nanoscale titanium dioxide (n-TiO_2), available in different crystal forms, which was selected for its widespread use in consumer products (e.g. cosmetics) and ample availability of data from ecotoxicological studies in the literature (including endpoints for algae, invertebrates, bacteria, and vertebrates in freshwater, saltwater and terrestrial compartments). The n-SSWD application resulted in estimation of environmental quality criteria (Hazard Concentration affecting 5% and 50% of the species) and ecological risk (Potentially Affected Fraction of species), which were then compared to similar results from applying the traditional SSD approach to the same dataset. n-SSWDs were also built for specific trophic levels (e.g. primary producers) and taxonomic groups (e.g. algae), which helped to identify the most sensitive organisms. These results proved n-SSWD as a valuable risk tool, although further testing is suggested.

Keywords: Species sensitivity distribution, Ecological risk assessment, Nanomaterials, Data quality, Titanium dioxide

INTRODUCTION

Nanotechnology is an emerging field in the area of science and technology involving the design, production and use of structures at the nano-scale (i.e. from 1 to 100 nanometers) called nanomaterials [1, 2]. Presently there is no internationally harmonized definition of engineered nanomaterials (ENMs) [3], but according to the European Commission's Recommendation a nanomaterial is defined as "a natural, incidental or manufactured material containing particles in a unbound state or as aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimension is in the size range 1-100 nm" [4]. Due to their unique or enhanced physicochemical properties (e.g. tiny size, large surface area, surface reactivity, charge, shape), some ENMs are suitable for a wide variety of applications in many sectors (e.g. information technology, energy production, food, agriculture) [5]. In the last decade, there has been a significant increase in the development and widespread use of nano-products and at the same time societal concerns regarding the potential ecological and human health risks from ENMs have increased [6].

The OECD and its member countries concluded that "*the approaches for the testing and assessment of traditional chemicals are in general appropriate for assessing the safety of nanomaterials, but may have to be adapted to the specificities of nanomaterials*" (OECD communication available at http://www.oecd.org/env/ehs/nanosafety/). Although the scientific community is not unanimous in recognizing some specificities of nanomaterials [7], some non conventional tools have been recently proposed to address them (e.g., [8, 9]). Most of these tools address human health RA while there are only few specific ecological RA tools, which mainly focus on environmental exposure assessment [1, 10, 11]. This is mainly due to the fact that

ecological RA [12] presents a number of specific issues when applied to ENMs [1]. For example, scarcity of characterization data for ENMs in environmentally relevant media and quantitative toxicity models make it difficult to identify which physicochemical characteristics determine the effects documented in ecotoxicity studies. In addition to the difficulties in assessing the ecotoxicity of ENMs, there are various issues hampering the assessment of the environmental exposure including, but not limited to: i) environmental behaviour and fate of ENMs which is largely unexplored, and ii) lack of adequate methods and tools for effective monitoring of ENMs in the different media and distinguishing them from the background particles [13]. Another issue is that most of the ecotoxicological studies reported in literature have used non-standardized tests and test materials from different sources (responsible, in part, for the generation of non reproducible/comparable results) [14]; the OECD introduced standardised test guidelines for regulatory testing of chemicals to ensure global recognition of the used methods and is currently evaluating their applicability to nanomaterials (more information at [15]). Data quality is crucial for all risk assessment steps, in fact data gathered based on standard testing guidelines often offer the most robust and transparent information [16]. Therefore, the need to ensure quality control (validity, reliability) and relevance of the ecotoxicological data on ENMs [16] before using them for an ecological RA is highlighted.

Very recently, Gottschalk et al. [11] have proposed the first quantitative ecological RA approach for ENMs. The aim of their study was to quantify the environmental risk of ENMs by building Species Sensitivity Distribution (SSD) curves that were then compared to probability distributions of Predicted Environmental Concentrations (PEC) developed by Gottschalk et al. [17]. The approach was tested on five ENMs (i.e. n-Ag, n-TiO_2, n-Zn, carbon nanotubes and fullerenes), and results demonstrated a marginal risk of these ENMs in surface water and some

risk in sewage treatment plant effluent, while in the terrestrial compartment no risk was predicted except for a marginal value for n-TiO_2 in sludge treated soils [11]. In contrast to the traditional SSD approach [18], Gottschalk et al.'s [11] approach make use of all available ecotoxicological data in building the cumulative distribution curves. Not reducing the dataset to the number of tested species is indeed very positive, particularly in case of data scarcity (as it is for most nanomaterials). However, we deem that the integration of such dataset in a single SSD should take into account the relevance of the ecotoxicological data in representing the ecosystem of concern as well as their reliability, the latter being very critical for all chemicals and presenting some peculiarities for nanomaterials [19]. For this reason an ecological RA approach, called nano-Species Sensitivity Weighted Distribution (n-SSWD), able to critically integrate the available information and to update the risk estimation as soon as additional ecotoxicological data are available is proposed in the present paper. In n-SSWD emphasis was given to assess the quality of ecotoxicological data, according to the nano-specific method proposed by Card and Magnuson [19], in order to allow a more appropriate use of the available, mainly heterogeneous, ecotoxicological information in ecological RA.

The n-SSWD approach was compared to both the traditional SSD [18] and the "probabilistic" SSD proposed by Gottshalck and colleagues [11], by using nanoscale titanium dioxide (n-TiO_2), available in different crystal forms as well as an amorphous form, as case study (more background information on traditional SSD and SSWD approaches are included in Supplemental Data).

METHODS

Case study: the n-TiO_2

Nanoscale titanium dioxide (n-TiO_2) was selected as the case study because, due to its unique or enhanced physicochemical properties, it is suitable for a wide variety of applications in many sectors (e.g. cosmetics, household cleaning, paint). For this reason the market for n-TiO_2 has been exponentially growing, and in 2012 a worldwide production up to 10,000 t/year was estimated [20]. At the same time, societal concerns regarding the potential environmental and health risks from this material have increased and need to be addressed through robust risk analysis [6]. Numerous toxicological and ecotoxicological tests were carried out and published in order to better understand whether n-TiO_2 may cause adverse effects to the environment and the human health due to its enhanced reactivity. The studies conducted so far showed that the toxicity of n-TiO_2 is far higher than that of its bulk form in both aquatic and terrestrial environments and suggested that n-TiO_2 has size dependant toxic effects [21, 22]. Several studies showed the ability of n-TiO_2 to generate reactive oxygen species (ROS) if exposed to strong artificial source of ultraviolet radiation (UVR). These ROS are able to cause cytotoxicity in the test organism and for this reason oxidative stress mediated by photoactive n-TiO_2 is the likely mechanism of its toxicity [23]. However, no studies demonstrated so far that photoactivity is responsible for environmental toxicity of n-TiO_2 under natural levels of UVR [23], and that the effectiveness of n-TiO_2 interaction with ultraviolet (UV) light strongly depends on particle size [24] as well as crystal form of TiO_2. Regarding the fate and behaviour of ENMs and in particular of n-TiO_2 in environmental compartments, Gottschalk et al. [16] and Sun et al. [25] published environmental concentrations of different ENMs, including n-TiO_2, modelled for different Countries (U.S., Europe and Switzerland) in different environmental compartments (aquatic and terrestrial) [16, 25]. The results, although affected by uncertainties related to both input data (e.g.

emission estimates) and modelling assumptions, showed that n-TiO_2 can reach concentrations of 220 µg/L in freshwater compartment (i.e. sewage treatment plant effluent) [25] and 89.2 µg/kg in terrestrial compartment (i.e. sludge treated soil) [17].

Accordingly, it can be concluded that there are still too many uncertainties on toxicity, behaviour and fate of n-TiO_2 in the environment, and this uncertainty strongly influence ecological RA.

Nevertheless, as mentioned in the introductory section, very recently a study reporting a probabilistic ecological RA based on SSD for five ENMs (n-Ag, n-TiO_2, n-ZnO, carbon nanotubes (CNTs) and fullerenes) and four environmental compartments (surface freshwater, sewage treatment plant effluents, soils, and sludge-treated soils) was published [11]. In that work Gottschalk and colleagues [11] have collected 34 ecotoxicological endpoints in 23 papers for n-TiO_2 in different crystalline forms (e.g. rutile or anatase). With this dataset SSD curves were built by applying an innovative probabilistic approach and subsequently compared with PEC distributions for estimating the risk for both aquatic and terrestrial environments [11, 17]. The results of that work indicated that there is only a marginal risk from n-TiO_2 (<0.1%) to surface freshwater and to sludge-treated soil and some risk to sewage treatment plant effluent (39.7%).

In the present paper the ecotoxicological dataset on n-TiO_2 used by Gottschalk et al. [11] was supplemented by conducting a literature review. The search was carried out by consulting two scientific websites, Scopus and Web of Knowledge (ISI), using the keywords "toxicity nano TiO_2" and "nano TiO_2". It resulted in 128 articles concerning ecotoxicological data published in the period 2006-2015. Out of these 128 articles only 36 have been included in the database because they reported a standardized endpoint (e.g. mortality, inhibition of growth, inhibition of reproduction of the test organism). The endpoints collected included No Observed Effect

Concentration (NOEC), Lowest Observed Effect Concentration (LOEC), Effect Concentration of x% of species (ECx), Lethal Dose of x% of species (LDx), Lethal Concentration of x% of species (LCx), Inhibition Concentration of 25% species (IC25), Germination Index (GI), Microbial Toxic Concentration (MTC), Minimum Inhibitory Concentration (MIC), Threshold Effects Concentration (TEC), and Highest Observed No-Effect Concentration (HONEC) as defined by Gottschalk et al. [11].

From these 36 articles 213 single values to work with were extracted: 175 values in 29 papers for freshwater, 19 values in 3 papers for saltwater, 19 values in 7 papers for terrestrial compartment, related to the following taxonomic groups: unicellular organisms (bacteria), algae, invertebrates and vertebrates for the aquatic environment and plants, unicellular organisms, fungi and invertebrates for the terrestrial compartment. The obtained dataset supplements the one used by Gottschalk et al. [11] by including a set of 19 data for saltwater, 141 additional data for freshwater and 15 additional data for terrestrial compartment.

The collected ecotoxicological data were organized into a database according to the following five macro-themes:

data source: it reports information about the paper according to the following fields: authors, title, year of publication;

particle size: it contains information about the dimension of both (where available) pristine and tested n-TiO_2 particles and the name of the supplier companies;

material: it reports additional information on the n-TiO_2 according to the following fields: chemical composition/impurities (percentage purity of n-TiO_2), material phase of the nanoparticle (e.g. powder), solubility, crystal structure (type of crystal e.g. rutile), coating, surface area (m^2/g), shape, surface change (mV) and notes;

ecotoxicity: it contains information about the performed ecotoxicological test according to the following fields: standard test protocols (it reports if the protocol used is standardized); material phase of the n-TiO_2 used in the test (e.g. colloidal dispersion), dispersion type (e.g. physical, chemical), dispersion method of n-TiO_2 in the matrix (e.g. sonication), tested medium/matrix (e.g. freshwater), analytical monitoring of the particle size during the test (possible agglomeration), trophic level of tested organism (e.g. primary consumers), taxonomic group (e.g. vertebrates), taxonomic class (e.g. fish), species name, life stage/sex of the tested organism, test type (e.g. acute, chronic), test description (e.g. algal growth inhibition test), exposure time (hour), type of endpoint (e.g. EC_{50}), effect measured (e.g. mortality), value of the effect (tested concentration at which effects were found, mg/L) and notes.

The ecotoxicological data were then processed (i.e. converted from acute to chronic and from effect to no effect values) in order to obtain the input data (called "calculated NOEC") required to build the n-SSWD and SSD curves. According to the transformation reported by Gottschalk et al. [11], which follows the ECHA Guidance on information requirements and chemical safety assessment [26], two assessment factors were applied: the first one used to account for the difference between acute and chronic toxicity (AF_{time}, factor 10 to extrapolate short-to-long term effect) and the second one to extrapolate from various n-TiO_2 effect's endpoint (e.g. ECx) the no observed effect concentration required to build the SS(W)D curves ($AF_{no\text{-}effect}$).

Subsequently, data were sorted by the crystal structure of the tested ENM in order to identify any trend in the magnitude of the measured effects. Specifically, data were grouped as follows, according to the decreasing percentage of anatase (and therefore increasing percentage

of rutile or amorphous forms) in the tested material: 100%-99%; 98-80%; 79-60%; 50-0%. For each group minimum, median and maximum of the calculated NOECs were estimated.

n-SSWD for screening ecological RA of ENMs

In order to develop an ecological RA methodology for ENMs we deemed the work done by Duboudin et al. [27] on SSWD (see Supplemental Data for a description) a valuable starting point and we further refined it according to our specific goal (i.e. its application to ENMs), thus developing the nano-SSWD (n-SSWD) approach. More precisely, we have decided to refine the SSWD approach through the assignment of the following three weighting criteria:

Species relevance (W_s)

This criterion is the same proposed by Duboudin et al. [27], which aims to retain intraspecies variation while giving each species the same weight within the SSWD [27], in other words it is used to reduce the redundancy of data for each species. This weighting criterion (W_s) is calculated as 1/n, where n is the number of data available for a single species (e.g. $n_{s1}=5$ $W_{s1}=1/5$).

Trophic level abundance (W_t)

Unlike Duboudin et al. [27], who assign percentages of abundance to taxonomic groups (64% to algae, 26% to invertebrates and 10% to vertebrates, as explained in Supplemental Data), starting from the study by Forbes and Calow [28] the values 0.64, 0.26 and 0.10 were assigned to three trophic levels: primary producers, primary consumers, and secondary consumers, respectively. This way, it was possible to apply the weighting criterion W_t to both aquatic and terrestrial ecosystems and to distinguish between e.g. herbivorous and carnivorous invertebrates by assigning them to two different trophic levels (primary and secondary consumers, respectively).

Data quality (W_q)

In order to assess the quality of ecotoxicological data, the method proposed by Card and Magnuson [19] was applied. It is a 2-step method to assess the quality of nanotoxicity studies. The first step uses a publicly available tool (ToxRTool; see http://ecvam.jrc.it/; "Publications" section) to rank the reliability of the study based on adequacy of design and documentation of methods, materials, and results, providing a "study score" according to Klimisch et al. [29]. The second step determines the completeness of physicochemical characterization of the nanomaterial assessed within the study, providing a "nanomaterial score". According to the first step, the "study score" can result in one of the four following numbers: Reliable without restriction (1), Reliable with restriction (2), Not reliable (3) and Not assignable (4). In our approach the "study score" is scaled to [0,1] as follows: 1; 0.7; 0; 0.3, for the scores 1, 2, 3 and 4, respectively. In the second step the following 10 physicochemical parameters are considered: 1) agglomeration and/or aggregation; 2) chemical composition; 3) crystal structure/crystallinity, 4) particle size/size distribution; 5) purity; 6) shape; 7) surface area; 8) surface charge; 9) surface chemistry (including composition and reactivity); 10) whether any characterization was conducted in the relevant experimental media. Each parameter contributes 1 point to the total score and therefore a given study can be assigned a "nanomaterial score" of 0 (worst) to 10 (best). In our approach the "nanomaterial score" is scaled to [0,1] by dividing the original score by a factor of 10. Finally, "study score" and "nanomaterial score" are multiplied to obtain the data quality criterion (W_q), again in the 0-1 range.

The three criteria described above (W_s, W_t, W_q) are then multiplied in order to derive an overall weighting coefficient (in the 0-1 range) to be used into the weighted statistical method (i.e. weighted bootstrap) for the building of n-SSWD curves, the calculation of HCx (Hazard Concentration for x% of species) and the risk estimation in terms of PAF (Potentially Affected Fraction of species).

Environmental screening values and ecological risk

In the present study the proposed n-SSWD was applied to the described database of ecotoxicological data on n-TiO_2. Moreover, for comparison purposes, the traditional SSD was applied to same set of data. However, when the number of data was not sufficient to apply the geometric mean, the use of all the available data was preferred for both n-SSWD and SSD (in the latter case resulting in a distribution of available data rather than in a proper SSD curve).

SSD and n-SSWDs were used in two ways:

Inverse use. In order to extrapolate screening values corresponding to the Hazard Concentration (HCx), where x is the percentage of species that are not protected a safety value that allows to protect a cutoff percentage of species. The most used HCx is HC5, representing the 5th percentile of a chronic toxicity distribution which allows to protect the 95% of the species exposed to the chemical. The HC5 represent a threshold value, and concentrations higher than this value can be considered to pose an unacceptable risk. This approach is used to derive ecological quality criteria (EQCs) for different compartments. In the present study the environmental compartments are freshwater, saltwater and terrestrial. Obtained HC5 for freshwater was then compared with HC5 calculated by Gottschalk and colleagues [11] for a smaller n-TiO_2 ecotoxicological dataset.

Forward use. comparing the curve with PEC values or distributions in order to obtain a risk estimation for specific environmental compartments, which in this case study are freshwater, saltwater and terrestrial compartments. This comparison allowed the calculation of Potentially Affected Fraction (PAF) of species, the percentage of species at risk in the compartment studied. In the present study the PEC values used for comparison are those currently available for freshwater [25] and terrestrial [17] compartments. For saltwater, no PEC values have been estimated so far and therefore it was not possible to perform any risk estimation. Obtained PAFs for freshwater and terrestrial compartments were then comparted with risk estimations provided by Gottschalk and colleagues [11].

Finally, we used the proposed n-SSWD approach to build sensitivity distribution curves for each trophic level (i.e. primary producers, primary consumers and secondary consumers) and taxonomic group (e.g. algae, invertebrates) belonging to a specific environmental compartment, which in the case study were the freshwater, saltwater and terrestrial compartments, and to calculate specific HC5 and HC50. This allowed the identification of the most sensitive trophic level and taxonomic group for each environmental compartment studied. In cases when the number of available data did not allow to build the n-SSWD curves a statistical analysis of the no effect values (through the calculation of minimum, maximum and median values) was conducted in order to derive the most sensitive trophic level and taxonomic group.

RESULTS AND DISCUSSION

The methods presented above were applied to the n-TiO_2 database described in the Case study section. As a first step, the application of the specific assessment factors allowed to obtain the calculated NOECs which are reported in three tables for freshwater species (Table 1, 2 and 3

for primary producers, primary consumers and secondary consumers, respectively), in Table 4 for saltwater species and in Table 5 for terrestrial species. In these tables the three assigned weighting criteria (W_s, W_t, W_q) as well as the calculated weighting coefficient are also reported. As described in the method section W_q is composed by two scores (i.e. study score and nanomaterial score), both scaled to [0,1] and multiplied. It is interesting to note that in our case study, study scores were in general quite high while nanomaterial scores were responsible for a significant reduction of the final W_q value. In particular, the dataset was characterized by 86.12% of reliable data (study score equal to 1), 13.40% of data reliable with restriction (study score equal to 0.7), and only 0.48% of data for which a reliability score was not assignable (study score equal to 0.3). The nanomaterial score resulted =0.8 for 13.62% of the data, between 0.7 and 0.6 for 69.01% of the data, and =0.5 for 17.70% of the data. The most measured physicochemical properties were chemical composition and particle size/distribution, both of them measured in all the 213 studies, followed by agglomeration and or aggregation (197), crystal structure/crystallinity (168), surface area (155), whether any characterization was conducted in the relevant experimental media (140), and purity (114). The least measured properties were surface charge (49), surface chemistry (incl. composition and reactivity) (36), and finally shape (31).

For details regarding the applied assessment factors the reader should refer to Tables S1.1, S1.2, S1.3 for freshwater, Table S2.1 for saltwater and Table S2.2 for terrestrial compartment shown in Supplemental Data.

The subsequent analysis of the calculated NOECs sorted by crystal structure composition did not show any clear trend on the magnitude of the measured effects. As an example, for the large freshwater dataset, information about crystal structure/crystallinity were available for 150

out of 182 data and the minimum, median and maximum of the calculated NOECs in the four groups were: 0.001, 3.09, 30 mg/L (for the tested materials with 100%-99% of anatase); 0.002, 0.316, 10 mg/L (for 98%-80% of anatase); 0.185, 2.83, 100 mg/L (for 79%-60% of anatase); 0.160, 0.935, 1 mg/L (for 50%-0% of anatase). Since the analysis did not highlight any clear relationship between the crystal structure and the magnitude of ecotoxicity effects, it was decided to perform the ecological RA by using the complete dataset, regardless the composition of the tested ENM in terms of crystal structure.

By applying the presented approaches to the calculated NOECs, SSD and n-SSWD curves were obtained for freshwater, saltwater and terrestrial compartments, which are reported and discussed in the following paragraphs. Moreover, the obtained compartment-specific environmental quality criteria (i.e. HC5 and HC50, Hazard Concentration affecting 5% and 50% of the species, respectively), and ecological risk (i.e. PAF, Potentially Affected Fraction of species) are presented and discussed. Finally, the identified more sensitive trophic levels and taxonomic groups are also reported and discussed for each environmental compartment.

Aquatic environment – freshwater

SSD curves. Both log-normal and log-empirical traditional SSD curves were built for freshwater species (Figure 1). The log-normal SSD curve reported in Figure 1A has a high R^2 value (greater than 0.9) that indicates a good fitting of the ecotoxicological data. It also has a good KSpvalue (greater than 0.1), thus meaning that the log-normal distribution is appropriate to describe our set of data.

The range of distribution of the Best Estimate of both log-normal and log-empirical SSD (Figures 1A and 1B, respectively) goes from approximately 0.01 to 100 mg/L and their HC5 values (best estimate value with 50% confidence interval), reported in Table 6, are the same

(0.02 mg/L). HC50 values are only slightly different (0.57 mg/L for the log-normal curve and 0.54 mg/L for the log-empirical curve), thus highlighting an almost identical slope of the two curves.

In the two curves we cannot observe any particular trend in the distribution of the different trophic levels (i.e. primary producers, primary consumers and secondary consumers), in fact the data for all the trophic levels are uniformly distributed along each curve. Focusing on the part of the curve below HC5 (in which we find the 5% of affected species), both SSD curves show only the presence of primary consumers. This means that in case this HC5 is used as environmental quality standard we could expect only primary consumers to be partially affected.

n-SSWD curves. For freshwater species both log-normal and log-empirical n-SSWD curves were also built (Figure 2).

The log-normal distribution reported in Figure 2A has a high R^2 (greater than 0.9) but a not good KSpvalue (lower than 0.1), thus meaning that the log-normal distribution is not appropriate to describe the set of data and therefore from that curve reliable HCx cannot be obtained.

The range of distribution of the Best Estimate of both log-normal and log-empirical n-SSWD (Figures 2A and 2B, respectively) goes from approximately 0.001 to 100 mg/L. The HC5 and HC50 values obtained from the log-empirical curve (best estimate value with 50% confidence interval), reported in Table 6, are equal to 0.02 mg/L and 0.22 mg/L, respectively. The two curves do not show any particular trend in the distribution of the different trophic levels (i.e. primary producers, primary consumers and secondary consumers), in fact the data for all the trophic levels are uniformly distributed along each curve. In the part of the curve below HC5 (in

which we find the 5% of affected species) there is a majority of data related to primary consumers, but also secondary consumers can be found.

Comparison between SSD and n-SSWD curves. The two approaches do not show meaningful differences for the calculation of the environmental quality criteria HC5 while, considering the HC50, n-SSWD turned out to be the more conservative approach. However, the use of all the available data (i.e. avoiding the application of the geometric mean in n-SSWD) allowed a better assessment of the variability of the ecotoxicological response for different species.

Validation of environmental quality criteria and risk estimation. The log-empirical SSD and n-SSWD HC5 values were compared with HC5 (50% confidence interval) obtained by Gottschalk and colleagues [11]. As shown in Table 6 the value obtained in our study (0.02 mg/L), is more conservative than the HC5 value obtained by Gottschalk et al. [11] for freshwater (0.06 mg/L). This is due to the weighting coefficient that was introduced in the bootstrap procedure rather than to the larger dataset used in our study, including 145 additional data for freshwater. In fact, when applying our approach to the smaller dataset used by Gottshalck et al. [11], the resulting value for HC5 for SSD and n-SSWD was 0.02 mg/L (curves not shown) and thus exactly the value obtained by using the complete dataset. However, i) the complete curves should be compared (including e.g. HC50 values), and ii) a much larger and complete dataset should be used before confirming that, although following different methodological steps, the n-SSWD approach lead to a more conservative result.

Obtained HC5 (reported in Table 6) were also compared with the n-TiO_2 predicted environmental concentrations (PEC) proposed by Sun et al. [25] for surface water and Sewage Treatment Plant (STP) effluent in Europe (0.0014 mg/L and 0.11 mg/L upper percentile 0.85

estimation, respectively). Considering the PEC value for surface water, the comparison with both SSD and n-SSWD highlighted a marginal risk because the PEC value coincided with or was slightly lower than the lowest value in SS(W)D. Therefore, the use of a probabilistic PEC distribution could result in the estimation of a significant risk (>5% of affected species) for the freshwater compartment (higher than the marginal risk estimated by Gottschalk et al. [11]). Considering the PEC value for STP effluent, the comparison with both SSD and n-SSWD allowed the calculation of Potentially Affected Fraction (PAF) of species. Specifically, at the considered PEC value the affect effected percentages were: 20% of species (according to the best estimate curve; 10%-35% considering 5^{th} and 95^{th} percentiles, respectively) by using SSD, and 30% of species (28%-32%) by using n-SSWD. Therefore, the conventional SSD seemed to be less conservative than n-SSWD, showing, at the same time, a higher variability in the response value range (25% for SSD compared to 4% in n-SSWD).

Overall, our risk estimations for surface water and STP effluent appeared to be similar to Gottschalk et al.'s [11] estimations. However, since in the present study we used the higher PEC values proposed by Sun et al. [25] for Europe (400 times and 100 times higher for 0.85 upper percentile for surface water and STP effluent, respectively, compared to the PECs proposed by Gottschalk et al. [17] used in Gottschalk et al. [11]), it is expected that the use of the complete PEC distributions would result in a significantly higher risk estimation (i.e. >5% for surface water and >40% for STP effluent).

Trophic level and taxonomic group-specific n-SSWD curves. By applying the approach described in the Method section, specific n-SSWD curves were built for each trophic level (i.e. primary producers, primary consumers and secondary consumers) as well as taxonomic group

(i.e. algae, bacteria, invertebrates and vertebrates) represented in the ecotoxicological dataset for the freshwater compartment.

Obtained results are presented in Figures 3 and 4 and discussed in the following paragraphs.

The log-normal n-SSWD obtained for different trophic levels (Figures 3A, 3B, 3C) have high and very similar R^2 (equal or greater than 0.9), but none of them have a good KSpvalue (greater than 0.1). This means that the log-normal distribution is not adequate to represent the three dataset.

Primary consumers and secondary consumers have a wider range of distribution of the best estimate curves (approximately 0.001-1000 mg/L and 0.01-100 mg/L, respectively) than the primary producers (approximately 0.01-10 mg/L). The trophic level that shows by far the wider range of distribution, and therefore the higher ecotoxicological variability, is represented by the primary consumers. This wide variability can be explained by the fact that i) the majority of ecotoxicological tests published in peer reviewed literature is conducted using species belonging to this trophic level and ii) reported tests differ for several parameters such as the testing procedure, the type of n-TiO_2 tested (e.g. with different particles size, or purity), the exposure pathway (e.g. ingestion, dermal contact), the test duration (i.e. chronic, acute), and the pre-treatment of the sample.

The obtained HC5 values (reported in Table 6) differ by one order of magnitude (from 0.00 mg/L to 0.08 mg/L), and primary consumers result to be the more sensitive trophic level. By analysing HC50 values, results differ again by one order of magnitude (from 0.16 mg/L to 1.12 mg/L) but in this case primary producers obtained the lowest value. This means that for relatively low n-TiO_2 concentrations (around 10^{-3} mg/L) the first trophic level to be affected is

primary consumers, while for higher concentrations the most endangered trophic level is primary producers for which 70% to 85% of species (according to 5th and 95th centiles, respectively) is affected at 1 mg/L n-TiO_2 concentration (at the same environmental concentration approximately 50% and 2.5% to 10% of primary consumers and secondary consumers are affected, respectively).

Comparing the log-normal n-SSWD for different taxonomic groups, depicted in Figures 4A, 4B, 4C and 4D, only algae's, invertebrates' and vertebrates' n-SSWD curves have a high R^2 (close to or greater than 0.9), while the bacteria's n-SSWD has a lower R^2 (0.7648) but still acceptable. Only bacteria n-SSWD has a good KSpvalue (greater than 0.1), thus the log-normal distribution is adequate to represent this dataset.

Concerning the eight curves (both log-normal and log-empirical) reported in Figure 4, bacteria, invertebrates and vertebrates have a wider range of distribution of the best estimate curve (approximately 0.001-10 mg/L, 0.001-100 and 0.01-100 mg/L, respectively) than algae (approximately 0.01-10 mg/L). The taxonomic group that shows by far the wider range of distribution, and therefore the higher ecotoxicological variability, is represented by invertebrates.

According to the obtained HC5 reported in Table 6 the more sensitive taxonomic groups are bacteria and invertebrates, compared to algae and vertebrates. Moreover, *Vibrio fischeri* is the most affected species among bacteria, *Pseudokirchneriella subcapitata* among algae, *Daphnia Magna* among invertebrates and *Xenopus laevis* and *Danio rerio* among vertebrates.

As previously discussed, the trophic level of primary consumers resulted to be the most sensitive one and at the same time to have the higher variability in toxicological endpoints. The results obtained at taxonomic group level confirm this observation, in fact the more sensitive taxonomic group is invertebrates followed by bacteria, both of them belonging to the trophic

level of primary consumers. Moreover, as shown by obtained HC50 values, bacteria resulted to be also one of the most endangered taxonomic groups (together with algae) at relatively high n-TiO_2 concentrations (around 0.5 mg/L).

The species with the highest number of ecotoxicological data and at the same time showing the highest variability in the ecotoxicological endpoints is *Daphnia magna*. *Daphnia magna* is widely used to conduct ecotoxicological tests for various reasons, such as: i) there are several standardized protocols for this species; ii) the animal has a short life cycle that allows to conduct different types of test (e.g. inhibition of reproduction, chronic effects on offspring); iii) it is relatively easy breeding the animal in laboratory; iv) costs and execution time of the test are contained and v) the species is one of the most representative for the freshwater environment. Moreover, in the used dataset *Daphnia magna* was tested using different test conditions, thus resulting in the highest variability in ecotoxicological endpoints (as shown in Figure 4G).

A similar pattern (i.e. highest presence, highest variability in ecotoxicological response) is shown by some of the identified most sensitive species (*Vibrio fischeri* for bacteria, *Pseudokirchneriella subcapitata* for algae and *Xenopus laevis* for vertebrates).

Aquatic environment – saltwater

Both log-normal and log-empirical SSD and n-SSWD curves were built for saltwater species (Figure 5). In this case both curves were constructed using all available data because the application of geometric mean and minimum operators at species level resulted in only 6 data (i.e. 6 species), number not sufficient to build the traditional SSD curve (according to [30] the minimum acceptable dataset is 10-15). For the same reason it was not possible to build specific curves for different trophic levels and taxonomic groups.

The obtained log-normal SSD has a high R^2 (close to 0.9) as well as a high KSpvalue (higher than 0.1) while the log-normal n-SSWD curve has a lower R^2 (but still acceptable) and a not acceptable KSpvalue.

From this curves slightly different HC5 values were derived, ranging from 0.02 mg/L to 0.05 mg/L for SSD (log-normal and log-empirical curves, respectively), and to 0.06 mg/L for n-SSWD (Table 2.1 in SI). HC50 values resulted to differ more, being n-SSWD estimation (0.13 mg/L) more conservative than SSD results (0.23 mg/l and 0.37 mg/L for log-empirical and log-normal curves, respectively; Table S2.1 in Supplemental Data).

Taking into consideration the calculated NOECs for all the species in the different trophic levels, it can be observed that primary producers and primary consumers are equally sensitive trophic levels because their minimum calculated NOEC values are very close (0.07 and 0.04, respectively). However, at higher concentrations primary producers is the most sensitive trophic level because the median and maximum calculated NOEC values (0.09 and 0.70 mg/L, respectively) are lower than the correspondent values for the primary consumers (0.57 and 9.49 mg/L, respectively). Similar results were obtained by applying the geometric mean at species level, although more conservative (0.07, 0.14 and 0.26 mg/L as minimum, median and maximum values for primary producers compared to 0.68, 0.71 and 0.73 mg/L for primary consumers). Since primary producers are represented only by algae species and primary consumers are represented only by invertebrate species, algae resulted to be the most sensitive taxonomic group, and *Thalassiosira pseudonana*, *Dunaliella tertiolecta and Skeletonema costatum* the more sensitive species.

To conclude, due to the paucity of available data, it was not possible to draw a robust conclusion on the most sensitive trophic level, taxonomic group or species. Rather the need to

conduct further ecotoxicological tests on different species (belonging to the three trophic levels) for the saltwater environmental compartment is highlighted.

Validation of environmental quality criteria and risk estimation. For saltwater it was not possible to validate the obtained results because no previous studies were available which addressed n-TiO_2 environmental quality standards. Moreover, PEC values were not available, thus preventing any risk estimation for this environmental compartment.

Terrestrial environment

Both log-normal and log-empirical SSD and n-SSWD curves were built for terrestrial species (Supplemental Data, Figure S1.1). Also in this case both curves were constructed using all available data because the application of geometric mean and minimum operators at species level resulted in only 6 data (i.e. 6 species), not sufficient to build the traditional SSD curve. For the same reason it was not possible to build specific curves for different trophic levels and taxonomic groups.

The obtained SSD and SSWD curves, including 19 data, were characterized by an inadequate data fitting ($R^2 < 0.9$ and KSvalue < 0.1) and therefore they were judged as not reliable. As a consequence, instead of considering the extrapolated HC5 and HC50 values (reported in Table S2.2 in Supplemental Data), it was decided to draw some considerations on n-TiO_2 toxicity for terrestrial compartment based on the calculated NOEC values.

Considering the whole dataset of calculated NOEC values, it ranges from 0.0112 mg/Kg to 200 mg/Kg, with a median value of 100 mg/Kg. All the available data are referred to a few species (6) belonging to the trophic levels of primary producers (1 species), primary consumers (3 species) and secondary consumers (2 species). The majority of the data (68%) are referred to the same species (*Porcellio scaber*), showing a high variability in ecotoxicological response.

This variability is explained by the fact that the tests conducted on *Porcellio scaber* were characterized by different testing procedures, type of n-TiO_2 (e.g. particles size, purity), exposure pathway (e.g. ingestion, dermal contact), exposure time (e.g. chronic, acute test), and pre-treatment of the sample.

By applying the ECHA Guidance on information requirements and chemical safety assessment, Chapter R.10 [26] (using the Assessment Factors (AF) given for the terrestrial compartment) it was possible to estimate a PNEC of 0.00112 mg/Kg.

Taking into consideration the calculated NOEC values for the different trophic levels, it can be observed that primary producers is the most sensitive trophic level because the minimum, median and maximum calculated NOEC values (all equal to 0.0112 mg/Kg) are lower than the corresponding values for the primary (10, 200 and 200 mg/Kg, respectively) and the secondary consumers (0.80, 100 and 100 mg/Kg, respectively). Almost the same results are obtained when the geometric mean is applied at species level to primary producers (0.0112 mg/Kg as minimum, median and maximum values), primary consumers (10, 105 and 200 mg/Kg, respectively), and secondary consumers (0.80, 30, and 100 mg/Kg, respectively). Breaking down all data according to taxonomic groups, among primary consumers bacteria is more sensitive (10 mg/kg for minimum, median and maximum NOEC) than fungi (200 mg/kg for minimum, median and maximum NOEC); while primary producers are represented only by plants (0.0112 mg/kg for minimum, median and maximum NOEC), and secondary consumers are represented only by invertebrates. Invertebrates resulted to have the second lowest minimum calculated NOEC value (0.80 mg/kg) but median (30 mg/kg) and maximum (100 mg/kg) values are higher than the ones for bacteria. However, since for both plants and bacteria only one calculated NOEC is available no reliable conclusions can be drawn on the most sensitive taxonomic group.

To conclude, due to the paucity of available data, it was not possible to draw a robust conclusion on the most sensitive trophic level, taxonomic group or species. Rather, as for the saltwater compartment, the need to conduct further ecotoxicological tests on different species (belonging to the three different trophic levels) for the terrestrial compartment is highlighted.

Validation of environmental quality criteria and risk estimation. Due to the limited data available there was no validation of the results, because neither Gottschalk et al. [11] nor we have been able to calculate reliable HC5 values for n-TiO_2 in the terrestrial environmental compartment. It was only possible to carry out a risk estimation by comparing the calculated PNEC with the available predicted environmental concentrations (PEC) for soils and sludge treated soil in Europe proposed by Gottschalk et al. [17] (0.00445 mg/kg and 0.31 mg/kg upper percentile 0.85 estimation, respectively). Considering the PEC value for soil, the comparison with the calculated PNEC (0.00112 mg/L) resulted in a Hazard Quotient (HQ) equal to 3.97, thus suggesting a relevant risk (in contrast with Gottschalk et al. [11] that excluded any risk occurring in this environmental compartment). Considering the PEC value for sludge treated soil, the comparison with the same PNEC resulted in an extremely high HQ (equal to 276.77), more severe than the possible risk for this environmental compartment highlighted by Gottschalk et al. [11]. However, in order to draw more robust conclusions and eventually confirm these results, probabilistic distributions of both exposure and effect concentrations should be used.

CONCLUSIONS

Although conventional SSD and n-SSWD did not show substantial differences in the environmental quality criteria estimation, the proposed n-SSWD approach resulted to be a valuable tool for ecological RA because it allows to better evaluate the endpoint variability and

therefore identify more sensitive trophic levels and taxonomic groups.

The inclusion of a weighting coefficient composed by species relevance, trophic level abundance and data quality weighting criteria allows to make use of all the available ecotoxicological data and at the same time to account for their relevance in representing the studied environmental compartment as well as their reliability.

Moreover, the adoption of the method proposed by Card and Magnuson [19] allows to assess the quality of nanotoxicity studies by supplementing the Klimisch score [29] with a quantitative estimation of whether the physicochemical characterization provided for the tested nanomaterial is adequate to fully understand and trust the ecotoxicological value to be used in the ecological RA. In our dataset, it resulted that for several studies information about shape of the particles, surface charge as well as surface chemistry (including composition and reactivity) were missing, thus significantly reducing the reliability of those data and therefore their weight in the ecological RA.

The application of the available ecotoxicological dataset highlighted that n-TiO_2 is posing marginal risks to organisms exposed to freshwater (mainly primary consumers), and it is posing unacceptable risks to organisms exposed to sludge treated soil (HQ=19.39), soil (HQ=276.77), and STP effluent (around 26% PAF). Moreover, it can be expected that the use of the complete distributions of the PEC values provided by Sun et al. [25] will result in significantly higher risks for the freshwater environmental compartment. For the saltwater environmental compartment, since PEC values are currently not available, it was not possible to perform any risk estimation.

However, in order to confirm or refine the obtained results the need to test the approach with a more complete dataset, once available, was highlighted. Moreover, in order to further test the performance of the proposed approach, its application to other ENMs (e.g. CuO) is foreseen.

n-SSWD demonstrates the benefits derived from integrating a quantitative data quality evaluation, which is crucial given the unreliable datasets resulting of non-standardized testing and analytical methodologies, into a model for nano ecological RA. However, substantial input from subject matter experts is required for its application, which can introduce potential biases, thus increasing uncertainty in the analysis. Therefore we recommend that further work focuses on characterizing the uncertainties stemming from input data, expert judgment and model performance.

SUPPLEMENTAL DATA

Tables S1–S2.

Figure S1. (135 KB DOC).

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Data availability—Data, associated metadata, and calculation tools are included in the Supplemental Data accompanying the present study.

REFERENCES

1. Hristozov DR, Gottardo S, Critto A, Marcomini A. 2012. Risk assessment of engineered nanomaterials: a review of available data and approaches from regulatory perspective. *Nanotoxicology* 6:880-98.

2. British Standards Institution (BSI). 2007. PAS136 Terminology for nanomaterials. [cited 13 February 2013]. Available from: http://www.bsiglobal.com.

3. Lövestam G, Rauscher H, Roebben G, Klüttgen BS, Gibson N, Putaud J-P, Stamm H. 2010. Considerations on a Definition of Nanomaterial for Regulatory Purposes, No. EUR 24403 EN: Joint Research Center (JRC) of the EU Commission.

4. European Commission (EC) 2011. Commission Recommendation of 18 October 2011 on the definition of nanomaterial. (2011/696/EU). In: Official Journal of the European Union, E. Commission, Editor 2011, European Union: Luxemburg. p. 38-40.

5. Aschberger K, Micheletti C, Sokull-Kluttgen B, Christensen FM. 2011. Analysis of currently available data for characterising the risk of engineered nanomaterials to the environment and human healt- Lesson learned from four case studies. *Environment International* 37:143-1156.

6. Klaine SJ, Alvarez PJJ, Batley GE, Fernandes TF, Handy RD, Lyon DY, Mahendra S, McLaughlin MJ, Lead JR. 2008. Nanomaterials in the environment: Behavior, fate, bioavailability, and effects. *Environmental Toxicology and Chemistry* 27:1825–1851.

7. Donaldson K, Poland CA. 2013. Nanotoxicity: challenging the myth of nano-specific toxicity *Current Opinion in Biotechnology* 24:724–734.

8. Tervonen T, Linkov I, Figueira J, Steevens J, Chappell M, Merad M. 2009. Risk based classification system of nanomaterials. *Journal of Nanoparticle Research* 11:757-766.

9. Höck J, Epprecht T, Hofmann H, Höhner K, Krug H, Lorenz C, Limbach L, Gehr P, Nowack B, Riediker M, Schirmer K, Schmid B, Som C, Stark W, Studer C, Ulrich A, Götz NV, Wengert S, Wick P. 2010. Guidelines on the Precautionary Matrix for Synthetic Nanomaterials. Federal Office of Public Health and Federal Office for the Environment. Berne, Switzerland.

10. Dale AL, Lowry GV, Casman EA. 2013. Modeling Nanosilver Transformations in Freshwater Sediments. *Environmental Science & Technology* 47:12920-12928.

11. Gottschalk F, Kost E, Nowack B. 2013. Engineered nanomaterials in water and soils: a risk quantification based on probabilistic exposure and effect modeling. *Environmental Toxicology and Chemistry* 32(6):1278-1287.

12. Suter GW II. 2006. Ecological Risk Assessment. Second Edition. CRC press, Boca Raton, FL.

13. Kühnel D, Nickel C. 2014. The OECD expert meeting on ecotoxicology and environmental fate — Towards the development of improved OECD guidelines for the testing of nanomaterials. *Science of The Total Environment* 472:347-353.

14. Byrne HJ, Omen AG, Bos PMJ, Fernandes TF, Hund-Rinke K. Boraschi D, Aschberger K, Gottardo S, von der Kammer F, Kühnel D, Hristozov D, Marcomini A, Migliore L. 2014. Concern-driven integrated toxicity testing strategies for nanomaterials - Report of the NanoSafety Cluster Working Group 10. *Nanotoxicology* 14:195-216.

15. http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm [cited 2015, May 25].

16. Stone V, Pozzi-Mucelli S, Tran L, Aschberger K, Sabella S, Vogel UB, Poland C, Balharry D, Fernandes T, Gottardo S, Hankin S, Hartl M, Hartmann N, Hristozov D, Hund-Rinke K, Johnston H, Marcomini A, Panzer O, Roncato D, Saber AT, Wallin H, Scott-Fordsmand JJ. 2014. ITS-NANO - Prioritising nanosafety research to develop a stakeholder driven intelligent testing strategy. *Particle and Fibre Toxicology* 11:9.

17. Gottschalk F, Sonderer T, Scholz RW, Nowack B. 2009. Modeled environmental concentrations of engineered nanomaterials (TiO_2, ZnO, Ag, CTN, Fullerenes) for different regions. *Environmental Science and Technology* 43:9216-9222.

18. Posthuma L, Suter II GW, Traas TP. 2002. *Species sensitivity distributions in ecotoxicology*. Lewis publishers, Boca Raton, Florida, USA.

19. Card JW, Magnuson BA. 2010. A method to assess the quality of studies that examine the toxicity of engineered nanomaterials. *International Journal of Toxicology* 29(4):402–410.

20. Piccinno F, Gottschalk F, Seeger S, Nowack B. 2012. Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world. *Journal of Nanoparticle Research* 14:1109

21. Xiong D, Fang T, Yu L, Sima X, Zhu W. 2011. Effects of nano-scale TiO_2, ZnO and their bulk counterparts on zebrafish: Acute toxicity, oxidative stress and oxidative damage. *Science of the Total Environment* 409:1444-1452.

22. Wang H, Wick RL, Xing B. 2009. Toxicity of nanoparticulate and bulk ZnO, Al_2O_3 and TiO_2 to the nematode Caenorhabditis elegans. *Environmental Pollutution* 157:1171-1177.

23. Miller RJ, Bennett S, Keller AA, Pease S, Lenihan HS. 2012. TiO_2 nanoparticles are phototoxic to marine phytoplankton. PLoS ONE 7(1):e30321.

24. Zhang J, Wages YM, Cox YSB, Maul YJD, Li YY, Barnes ZM, Hope-Weeks ZL, Cobb GP. 2012. Effect of titanium dioxide nanomaterials and ultraviolet light coexposure on african clawed frogs (xenopus laevis). *Environmental Toxicology and Chemistry* 31(1):176-183.

25. Sun TY, Gottschalk F, Hungerbühler K, Nowack B. 2014. Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials. *Environmental Pollution* 185:69-76.

26. European Chemicals Agency (ECHA). 2008. Guidance on information requirements and chemical safety assessment Chapter R.10: Characterisation of dose [concentration]-response for environment. Helsinki, Finland.

27. Duboudin C, Ciffroy P, Magaud H. 2004. Effects of data manipulation and statistical methods on species sensitivity distributions. *Environmental Toxicology and Chemistry* 23(2):489-499.

28.Forbes VE, Calow P. 2002. Species sensitivity distributions revisited: A critical appraisal. *Human and Ecological Risk Assessment* 8(3):473-492.

29. Klimisch HJ, Andreae M, Tillmann U. 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regulatory Toxicology and Pharmacology* 25: 1–5.

30. Electricité de France (EDF), Institut de l'Environnement Industriel et des Risques (INERIS). 2003. Species Sensitivity Weighted Distribution, software user's guide. Version 1.0 – December 2003. Paris, France.

Figure 1. Log-normal (A) and log-empirical (B) traditional SSD curves for freshwater compartment. Wm.lg=mean value; wsd.lg=standard deviation value, R^2=multiple R-square coefficient; KSpvalue=Komogorov-Smirnov test value; PROD.=primary producers, CONS. 1=primary consumers; CONS. 2=secondary consumers.

Figure 2. Log-normal (A) and log-empirical (C) n-SSWD curves for freshwater compartment. Wm.lg=mean value; wsd.lg=standard deviation value, R^2=multiple R-square coefficient; KSpvalue=Komogorov-Smirnov test value; PROD.=primary producers; CONS. 1=primary consumers; CONS. 2=secondary consumers.

Figure 3. Log-normal (A,B,C) and log-empirical (D,E,F) n-SSWD curves for the different trophic levels in freshwater compartment. A,D) PROD.=primary producers (green), B,E) CONS. 1=primary consumers (orange), C,F) CONS. 2=secondary consumers (red). Wm.lg=mean value; wsd.lg=standard deviation value, R2=multiple R-square coefficient; KSpvalue=Komogorov-Smirnov test value.

Figure 4. Log-normal (A,B,C,D) and log-empirical (E,F,G,H) n-SSWD curves for the different taxonomic groups in freshwater compartment. ?Primary producers; ?Primary consumers; ?Secondary consumers. A&E) Algae: SKE=Skeletonema costatum, SCE=Scenedesmus sp., PSE=Pseudokirchneriella sub capitata, KAR=Karenia brevis, DES=Desmodesmus subspicatus, CHLO=Chlorella sp., CHLA=Chlamydomonas reinhardtii. B&F) Bacteria: VFI=Vibrio fischeri, MIC=11 microbial species, ECO=Escherichia coli, AVA=Anabaena variabilis. C&G) Invertebrates: TPL=Thamnocephalus platyurus, OLA=Oryzias latipes, HAT=Hydra attenuata, GFO=Gammarus fossarum, DPU=Daphnia pulex, DPS=Daphnia similis, DMA=Daphnia magna,

CSP=Chydorus sphaericus, CDU=Ceriodaphnia dubia. D&H) Vertebrates: XLA=Xenopus laevis, PPR=Pimephales promelas, OMY=Onchorynchus mykiss, DRE=Danio rerio.

Figure 5. Log-normal (A) and log-empirical (B) SSD and log-normal (C) and log-empirical (D) n-SSWD for the saltwater compartment. Wm.lg=mean value; wsd.lg=standard deviation value, R^2=multiple R-square coefficient; KSpvalue=Komogorov-Smirnov test value; PROD.=primary producers, CONS. 1= primary consumers.

TABLES

Table 1: Calculated NOEC values for freshwater primary producers and related weighting criteria and weighting coefficient used to build the n-SSWDs. For each ID, reference is reported in Table S1.1 in the Supplemental Data

Freshwater (FW) – primary producers (PP)								
ID	Toxicological endpoint	Taxonomic group	Test organism	Calculated NOEC (mg/L)	Ws[a]	Wt[b]	Wq[c]	Weighting coefficient
FW_PP_1	EC50	Algae	Chlamydomonas reinhardtii	0.1	1	0.64	0.6	0.384
FW_PP_2	NOEC	Algae	Chlorella sp.	16	0.2	0.64	0.7	0.090
FW_PP_3	EC30	Algae	Chlorella sp.	30	0.2	0.64	0.7	0.090
FW_PP_4	EC50	Algae	Chlorella sp.	1.2	0.2	0.64	0.7	0.090
FW_PP_5	EC50	Algae	Chlorella sp.	0.1612	0.2	0.64	0.9	0.115
FW_PP_6	NOEC	Algae	Chlorella sp.	0.89	0.2	0.64	0.9	0.115
FW_PP_7	EC50	Algae	Desmodesmus subspicatus	0.44	1	0.64	0.28	0.179
FW_PP_8	EC50	Algae	Pseudokirchneriella subcapitata	0.0583	0.042	0.64	0.3	0.008
FW_PP_9	EC20	Algae	Pseudokirchneriella subcapitata	0.905	0.042	0.64	0.3	0.008
FW_PP_10	NOEC	Algae	Pseudokirchneriella subcapitata	0.984	0.042	0.64	0.3	0.008
FW_PP_11	IC25	Algae	Pseudokirchneriella subcapitata	100	0.042	0.64	0.21	0.006
FW_PP_12	IC25	Algae	Pseudokirchneriella subcapitata	1.5	0.042	0.64	0.5	0.013
FW_PP_13	EC10	Algae	Pseudokirchneriella subcapitata	1.65	0.042	0.64	0.7	0.019
FW_PP_14	EC20	Algae	Pseudokirchneriella subcapitata	7.25	0.042	0.64	0.7	0.019
FW_PP_15	EC50	Algae	Pseudokirchneriella subcapitata	2.41	0.042	0.64	0.7	0.019
FW_PP_16	EC10	Algae	Pseudokirchneriella subcapitata	7.75	0.042	0.64	0.7	0.019
FW_PP_17	EC20	Algae	Pseudokirchneriella subcapitata	13.1	0.042	0.64	0.7	0.019
FW_PP_18	EC50	Algae	Pseudokirchneriella subcapitata	0.711	0.042	0.64	0.7	0.019
FW_PP_19	EC10	Algae	Pseudokirchneriella subcapitata	9	0.042	0.64	0.7	0.019
FW_PP_20	EC20	Algae	Pseudokirchneriella subcapitata	18.45	0.042	0.64	0.7	0.019
FW_PP_21	EC50	Algae	Pseudokirchneriella subcapitata	1.45	0.042	0.64	0.7	0.019
FW_PP_22	EC50	Algae	Pseudokirchneriella subcapitata	0.501	0.042	0.64	0.7	0.019
FW_PP_23	EC50	Algae	Pseudokirchneriella subcapitata	3.162	0.042	0.64	0.7	0.019
FW_PP_24	EC50	Algae	Pseudokirchneriella subcapitata	1.59	0.042	0.64	0.7	0.019

Freshwater (FW) – primary producers (PP)								
ID	Toxicological endpoint	Taxonomic group	Test organism	Calculated NOEC (mg/L)	Ws[a]	Wt[b]	Wq[c]	Weighting coefficient
FW_PP_25	EC50	Algae	Pseudokirchneriella subcapitata	0.316	0.042	0.64	0.7	0.019
FW_PP_26	EC50	Algae	Pseudokirchneriella subcapitata	0.316	0.042	0.64	0.7	0.019
FW_PP_27	EC50	Algae	Scenedesmus sp.	0.212	0.5	0.64	0.9	0.288
FW_PP_28	NOEC	Algae	Scenedesmus sp.	1.2	0.5	0.64	0.9	0.288
FW_PP_29	EC50	Algae	Pseudokirchneriella subcapitata	1	0.042	0.64	0.28	0.008
FW_PP_30	LC50	Algae	Pseudokirchneriella subcapitata	0.16	0.042	0.64	0.8	0.022
FW_PP_31	LC50	Algae	Pseudokirchneriella subcapitata	0.21	0.042	0.64	0.8	0.022
FW_PP_32	LC50	Algae	Pseudokirchneriella subcapitata	0.61	0.042	0.64	0.8	0.022
FW_PP_33	LC50	Algae	Pseudokirchneriella subcapitata	0.87	0.042	0.64	0.8	0.022
FW_PP_34	EC50	Algae	Karenia brevis	0.1069	1	0.64	0.6	0.384
FW_PP_35	EC50	Algae	Skeletonema costatum	0.0737	1	0.64	0.6	0.384
FW_PP_36	EC50	Bacteria	Anabaena variabilis	1.398	0.2	0.64	0.9	0.115
FW_PP_37	EC50	Bacteria	Anabaena variabilis	0.062	0.2	0.64	0.9	0.115
FW_PP_38	EC50	Bacteria	Anabaena variabilis	0.015	0.2	0.64	0.9	0.115
FW_PP_39	EC50	Bacteria	Anabaena variabilis	0.116	0.2	0.64	0.9	0.115
FW_PP_40	EC50	Bacteria	Anabaena variabilis	0.04	0.2	0.64	0.9	0.115

[a] W_s=species relevance criterion

[b] W_t=trophic levels abundance criterion

[c] W_q=data quality criterion

Table 2: Calculated NOEC values for freshwater primary consumers and related weighting criteria and weighting coefficient used to build the n-SSWDs. For each ID reference is reported in Table S1.2 in the Supplemental Data

Freshwater (FW) – primary consumers (PC)								
ID	Toxicological endpoint	Taxonomic group	Test organism	Calculated NOEC (mg/L)	Ws[a]	Wt[b]	Wq[c]	Weighting coefficient
FW_PC_1	LD50	Bacteria	Escherichia coli	11.048	1	0.26	0.3	0.078
FW_PC_2	MTC50%	Bacteria	11 microbial species	1	1	0.26	0.21	0.055
FW_PC_3	IC25	Bacteria	Vibrio fischeri	10	0.167	0.26	0.21	0.009
FW_PC_4	EC50	Bacteria	Vibrio fischeri	0.0112	0.167	0.26	0.7	0.030
FW_PC_5	EC50	Bacteria	Vibrio fischeri	200	0.167	0.26	0.14	0.006
FW_PC_6	NOEC	Bacteria	Vibrio fischeri	2000	0.167	0.26	0.14	0.006
FW_PC_7	MIC	Bacteria	Vibrio fischeri	1000	0.167	0.26	0.14	0.006
FW_PC_8	EC50	Bacteria	Vibrio fischeri	1	0.167	0.26	0.28	0.012
FW_PC_9	LC50	Invertebrates	Ceriodaphnia dubia	0.1	0.017	0.26	0.7	0.003
FW_PC_10	LC50	Invertebrates	Ceriodaphnia dubia	0.076	0.017	0.26	0.5	0.002
FW_PC_11	IC25	Invertebrates	Ceriodaphnia dubia	8.5	0.017	0.26	0.5	0.002
FW_PC_12	LC50	Invertebrates	Daphnia magna	0.6	0.014	0.26	0.35	0.001
FW_PC_13	LC50	Invertebrates	Oryzias latipes	0.085	0.33	0.26	0.35	0.030
FW_PC_14	EC50	Invertebrates	Ceriodaphnia magna	0.42	0.017	0.26	0.7	0.003
FW_PC_15	HONEC	Invertebrates	Ceriodaphnia dubia	4	0.017	0.26	0.8	0.004
FW_PC_16	LC50	Invertebrates	Chydorus sphaericus	1	1	0.26	0.28	0.073
FW_PC_17	EC50	Invertebrates	Daphnia magna	0.038	0.014	0.26	0.42	0.002
FW_PC_18	EC50	Invertebrates	Daphnia magna	0.0024	0.014	0.26	0.42	0.002
FW_PC_19	LC50	Invertebrates	Daphnia magna	0.00016	0.014	0.26	0.7	0.003
FW_PC_20	LC50	Invertebrates	Daphnia magna	200	0.014	0.26	0.14	0.001
FW_PC_21	LC50	Invertebrates	Daphnia magna	0.055	0.014	0.26	0.4	0.001
FW_PC_22	LOEC	Invertebrates	Daphnia magna	0.2	0.014	0.26	0.4	0.001
FW_PC_23	NOEC	Invertebrates	Daphnia magna	0.1	0.014	0.26	0.4	0.001
FW_PC_24	HONEC	Invertebrates	Daphnia magna	1	0.014	0.26	0.49	0.002
FW_PC_25	LC50	Invertebrates	Daphnia magna	0.298	0.014	0.26	0.49	0.002
FW_PC_26	LC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.8	0.003

Freshwater (FW) – primary consumers (PC)								
ID	Toxicological endpoint	Taxonomic group	Test organism	Calculated NOEC (mg/L)	Ws[a]	Wt[b]	Wq[c]	Weighting coefficient
FW_PC_27	LC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.8	0.003
FW_PC_28	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_29	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_30	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_31	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_32	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_33	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_34	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_35	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_36	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_37	EC10	Invertebrates	Daphnia magna	4.56	0.014	0.26	0.6	0.002
FW_PC_38	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_39	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_40	EC10	Invertebrates	Daphnia magna	0.185	0.014	0.26	0.6	0.002
FW_PC_41	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_42	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_43	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_44	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_45	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_46	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_47	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_48	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_49	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_50	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_51	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_52	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_53	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_54	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_55	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_56	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002

Freshwater (FW) – primary consumers (PC)								
ID	Toxicological endpoint	Taxonomic group	Test organism	Calculated NOEC (mg/L)	Ws[a]	Wt[b]	Wq[c]	Weighting coefficient
FW_PC_57	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_58	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_59	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_60	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_61	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_62	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_63	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_64	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_65	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_66	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_67	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_68	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_69	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_70	EC10	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_71	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_72	EC10	Invertebrates	Daphnia magna	3.82	0.014	0.26	0.6	0.002
FW_PC_73	NOEC	Invertebrates	Daphnia magna	3	0.014	0.26	0.6	0.002
FW_PC_74	LOEC	Invertebrates	Daphnia magna	10	0.014	0.26	0.6	0.002
FW_PC_75	EC50	Invertebrates	Daphnia magna	2.66	0.014	0.26	0.6	0.002
FW_PC_76	EC10	Invertebrates	Daphnia magna	2.51	0.014	0.26	0.6	0.002
FW_PC_77	NOEC	Invertebrates	Daphnia magna	30	0.014	0.26	0.6	0.002
FW_PC_78	LOEC	Invertebrates	Daphnia magna	100	0.014	0.26	0.6	0.002
FW_PC_79	EC50	Invertebrates	Daphnia magna	6.61	0.014	0.26	0.6	0.002
FW_PC_80	EC10	Invertebrates	Daphnia magna	15.75	0.014	0.26	0.6	0.002
FW_PC_81	NOEC	Invertebrates	Daphnia magna	5	0.014	0.26	0.6	0.002
FW_PC_82	EC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_83	LC50	Invertebrates	Daphnia magna	1	0.014	0.26	0.6	0.002
FW_PC_84	NOEC	Invertebrates	Daphnia magna	0.01	0.014	0.26	0.6	0.002
FW_PC_85	EC50	Invertebrates	Daphnia magna	0.0162	0.014	0.26	0.6	0.002
FW_PC_86	LC50	Invertebrates	Daphnia magna	0.0202	0.014	0.26	0.6	0.002

Freshwater (FW) – primary consumers (PC)								
ID	Toxicological endpoint	Taxonomic group	Test organism	Calculated NOEC (mg/L)	Ws[a]	Wt[b]	Wq[c]	Weighting coefficient
FW_PC_87	EC50	Invertebrates	Daphnia magna	0.0046	0.014	0.26	0.6	0.002
FW_PC_88	LC50	Invertebrates	Daphnia magna	0.0262	0.014	0.26	0.6	0.002
FW_PC_89	LC50	Invertebrates	Daphnia pulex	0.1	0.5	0.26	0.7	0.091
FW_PC_90	LC50	Invertebrates	Daphnia pulex	0.092	0.5	0.26	0.5	0.065
FW_PC_91	EC50	Invertebrates	Gammarus fossarum	0.002	1	0.26	0.35	0.091
FW_PC_92	LC50	Invertebrates	Oryzias latipes	1.55	0.33	0.26	0.49	0.042
FW_PC_93	LC50	Invertebrates	Oryzias latipes	0.0219	0.33	0.26	0.49	0.042
FW_PC_94	LC50	Invertebrates	Thamnocephalus platyurus	1	0.33	0.26	0.21	0.018
FW_PC_95	LC50	Invertebrates	Thamnocephalus platyurus	200	0.33	0.26	0.14	0.012
FW_PC_96	NOEC	Invertebrates	Thamnocephalus platyurus	2000	0.33	0.26	0.14	0.012
FW_PC_97	HONEC	Invertebrates	Daphnia similis	10	0.125	0.26	0.7	0.023

[a] W_s=species relevance criterion

[b] W_t=trophic levels abundance criterion

[c] W_q=data quality criterion

Table 3: Calculated NOEC values for freshwater secondary consumers and related weighting criteria and weighting coefficient used to build the n-SSWDs. For each ID reference is reported in Table S1.3 in Supplemental Data

Freshwater (FW) – secondary consumers (SC)								
ID	Toxicological endpoint	Taxonomic group	Test organism	Calculated NOEC (mg/L)	Ws[a]	Wt[b]	Wq[c]	Weighting coefficient
FW_SC_1	EC50	Invertebrates	Hydra attenuata	0.55	1	0.1	0.21	0.021
FW_SC_2	LC50	Vertebrates	Danio rerio	0.1	0.33	0.1	0.7	0.023
FW_SC_3	LC50	Vertebrates	Danio rerio	0.1	0.33	0.1	0.7	0.023
FW_SC_4	LC50	Vertebrates	Danio rerio	1.245	0.33	0.1	0.8	0.026
FW_SC_5	TEC	Vertebrates	Onchorynchus mykiss	0.55	0.33	0.1	0.21	0.007
FW_SC_6	LC50	Vertebrates	Onchorynchus mykiss	1	0.33	0.1	0.8	0.026
FW_SC_7	LC50	Vertebrates	Onchorynchus mykiss	1	0.33	0.1	0.8	0.026
FW_SC_8	LC50	Vertebrates	Pimephales promelas	5	0.5	0.1	0.5	0.025
FW_SC_9	IC25	Vertebrates	Pimephales promelas	45.2	0.5	0.1	0.5	0.025
FW_SC_10	NOEC	Vertebrates	Xenopus laevis	9.02	0.034	0.1	0.6	0.002
FW_SC_11	LC50	Vertebrates	Xenopus laevis	2.102	0.034	0.1	0.6	0.002
FW_SC_12	NOEC	Vertebrates	Xenopus laevis	3.09	0.034	0.1	0.6	0.002
FW_SC_13	NOEC	Vertebrates	Xenopus laevis	3.09	0.034	0.1	0.6	0.002
FW_SC_14	NOEC	Vertebrates	Xenopus laevis	7.77	0.034	0.1	0.6	0.002
FW_SC_15	NOEC	Vertebrates	Xenopus laevis	28.18	0.034	0.1	0.6	0.002
FW_SC_16	NOEC	Vertebrates	Xenopus laevis	3.09	0.034	0.1	0.6	0.002
FW_SC_17	NOEC	Vertebrates	Xenopus laevis	0.95	0.034	0.1	0.6	0.002
FW_SC_18	NOEC	Vertebrates	Xenopus laevis	7.77	0.034	0.1	0.6	0.002
FW_SC_19	NOEC	Vertebrates	Xenopus laevis	7.77	0.034	0.1	0.6	0.002
FW_SC_20	LC50	Vertebrates	Xenopus laevis	2.951	0.034	0.1	0.6	0.002
FW_SC_21	NOEC	Vertebrates	Xenopus laevis	7.77	0.034	0.1	0.6	0.002
FW_SC_22	NOEC	Vertebrates	Xenopus laevis	0.001	0.034	0.1	0.6	0.002
FW_SC_23	NOEC	Vertebrates	Xenopus laevis	7.77	0.034	0.1	0.6	0.002
FW_SC_24	NOEC	Vertebrates	Xenopus laevis	0.95	0.034	0.1	0.6	0.002
FW_SC_25	LC50	Vertebrates	Xenopus laevis	0.579	0.034	0.1	0.6	0.002
FW_SC_26	NOEC	Vertebrates	Xenopus laevis	0.95	0.034	0.1	0.6	0.002

Freshwater (FW) – secondary consumers (SC)								
ID	Toxicological endpoint	Taxonomic group	Test organism	Calculated NOEC (mg/L)	Ws[a]	Wt[b]	Wq[c]	Weighting coefficient
FW_SC_27	NOEC	Vertebrates	Xenopus laevis	7.77	0.034	0.1	0.6	0.002
FW_SC_28	NOEC	Vertebrates	Xenopus laevis	28.18	0.034	0.1	0.6	0.002
FW_SC_29	NOEC	Vertebrates	Xenopus laevis	3.09	0.034	0.1	0.6	0.002
FW_SC_30	LC50	Vertebrates	Xenopus laevis	0.696	0.034	0.1	0.6	0.002
FW_SC_31	NOEC	Vertebrates	Xenopus laevis	3.09	0.034	0.1	0.6	0.002
FW_SC_32	NOEC	Vertebrates	Xenopus laevis	0.95	0.034	0.1	0.6	0.002
FW_SC_33	NOEC	Vertebrates	Xenopus laevis	28.18	0.034	0.1	0.6	0.002
FW_SC_34	NOEC	Vertebrates	Xenopus laevis	7.77	0.034	0.1	0.6	0.002
FW_SC_35	LC50	Vertebrates	Xenopus laevis	2.676	0.034	0.1	0.6	0.002
FW_SC_36	NOEC	Vertebrates	Xenopus laevis	7.77	0.034	0.1	0.6	0.002
FW_SC_37	NOEC	Vertebrates	Xenopus laevis	3.09	0.034	0.1	0.6	0.002
FW_SC_38	NOEC	Vertebrates	Xenopus laevis	7.77	0.034	0.1	0.6	0.002

[a] W_s=species relevance criterion

[b] W_t=trophic levels abundance criterion

[c] W_q=data quality criterion

Table 4: Calculated NOEC values for saltwater primary producer and primary consumers and related weighting criteria and weighting coefficient used to build the n-SSWDs. For each ID reference is reported in Table S1.4 in Supplemental Data

Saltwater (SW)									
ID	Toxicological endpoint	Trophic level	Taxonomic group	Test organism	Calculated NOEC (mg/L)	Ws[a]	Wt[b]	Wq[c]	Weighting coefficient
SW_1	NOEC	Primary producers	Algae	Dunaliella tertiolecta	0.5	0.64	0.6	0.192	0.5
SW_2	HONEC	Primary producers	Algae	Dunaliella tertiolecta	0.5	0.64	0.6	0.192	0.5
SW_3	NOEC	Primary producers	Algae	Isochrysis galbana	0.5	0.64	0.6	0.192	0.5
SW_4	NOEC	Primary producers	Algae	Isochrysis galbana	0.5	0.64	0.6	0.192	0.5
SW_5	HONEC	Primary producers	Algae	Skeletonema costatum	0.5	0.64	0.6	0.192	0.5
SW_6	HONEC	Primary producers	Algae	Skeletonema costatum	0.5	0.64	0.6	0.192	0.5
SW_7	NOEC	Primary producers	Algae	Thalassiosira pseudonana	0.5	0.64	0.6	0.192	0.5
SW_8	HONEC	Primary producers	Algae	Thalassiosira pseudonana	0.5	0.64	0.6	0.192	0.5
SW_9	EC50	Primary consumers	Invertebrates	Haliotis diversicolor supertexta	0.333	0.26	0.7	0.061	0.333
SW_10	EC50	Primary consumers	Invertebrates	Haliotis diversicolor supertexta	0.333	0.26	0.7	0.061	0.333
SW_11	NOEC	Primary consumers	Invertebrates	Haliotis diversicolor supertexta	0.333	0.26	0.7	0.061	0.333
SW_12	EC50	Primary consumers	Invertebrates	Artemia salina	0.125	0.26	0.8	0.026	0.125
SW_13	EC50	Primary consumers	Invertebrates	Artemia salina	0.125	0.26	0.8	0.026	0.125
SW_14	EC50	Primary	Invertebrates	Artemia salina	0.125	0.26	0.8	0.026	0.125

Saltwater (SW)									
ID	Toxicological endpoint	Trophic level	Taxonomic group	Test organism	Calculated NOEC (mg/L)	Ws[a]	Wt[b]	Wq[c]	Weighting coefficient
		consumers							
SW_15	EC50	Primary consumers	Invertebrates	Artemia salina	0.125	0.26	0.8	0.026	0.125
SW_16	EC50	Primary consumers	Invertebrates	Artemia salina	0.125	0.26	0.8	0.026	0.125
SW_17	EC50	Primary consumers	Invertebrates	Artemia salina	0.125	0.26	0.8	0.026	0.125
SW_18	EC50	Primary consumers	Invertebrates	Artemia salina	0.125	0.26	0.8	0.026	0.125
SW_19	EC50	Primary consumers	Invertebrates	Artemia salina	0.125	0.26	0.8	0.026	0.125

[a] W_s=species relevance criterion

[b] W_t=trophic levels abundance criterion

[c] W_q=data quality criterion

Table 5: Calculated NOEC values for terrestrial primary consumers and secondary consumers and related weighting criteria and weighting coefficient used to build the n-SSWDs. Ws=species relevance criterion; Wt=trophic levels abundance criterion; Wk=data quality criterion. For each ID reference is reported in Table S1.5 in Supplemental Data

Terrestrial (TR)									
ID	Toxicological endpoint	Trophic level	Taxonomic group	Test organism	Calculated NOEC (mg/kg)	Ws[a]	Wt[b]	Wq[c]	Weighting coefficient
TR_1	GI	Primary producers	Plant	Lactuca sativa, Cucumis sativus, Solanum lycopersicum, Spinacia oleracea	0.0112	1.000	0.64	0.7	0.448
TR_2	EC50	Primary consumers	Bacteria	mix of soil bacteria	10	1.000	0.26	0.28	0.073
TR_3	EC50	Primary consumers	Fungi	Saccharomyces cerevisiae	200	0.500	0.26	0.4	0.052
TR_4	EC50	Primary consumers	Fungi	Saccharomyces cerevisiae	200	0.500	0.26	0.4	0.052
TR_5	LC50	Secondary consumers	Invertebrates	Caenorhabditis elegans	0.799	1.000	0.1	0.8	0.080
TR_6	EC50	Secondary consumers	Invertebrates	Eisenia fetida	100	1.000	0.1	1	0.100
TR_7	HONEC	Secondary consumers	Invertebrates	Porcellio scaber	100	0.077	0.1	0.7	0.005
TR_8	HONEC	Secondary consumers	Invertebrates	Porcellio scaber	100	0.077	0.1	0.7	0.005
TR_9	HONEC	Secondary consumers	Invertebrates	Porcellio scaber	100	0.077	0.1	0.7	0.005
TR_10	HONEC	Secondary consumers	Invertebrates	Porcellio scaber	100	0.077	0.1	0.7	0.005
TR_11	HONEC	Secondary	Invertebrates	Porcellio	100	0.077	0.1	0.7	0.005

Terrestrial (TR)									
ID	Toxicological endpoint	Trophic level	Taxonomic group	Test organism	Calculated NOEC (mg/kg)	Ws[a]	Wt[b]	Wq[c]	Weighting coefficient
		consumers		scaber					
TR_12	HONEC	Secondary consumers	Invertebrates	Porcellio scaber	100	0.077	0.1	0.7	0.005
TR_13	HONEC	Secondary consumers	Invertebrates	Porcellio scaber	100	0.077	0.1	0.7	0.005
TR_14	HONEC	Secondary consumers	Invertebrates	Porcellio scaber	100	0.077	0.1	0.7	0.005
TR_15	HONEC	Secondary consumers	Invertebrates	Porcellio scaber	100	0.077	0.1	0.7	0.005
TR_16	HONEC	Secondary consumers	Invertebrates	Porcellio scaber	100	0.077	0.1	0.7	0.005
TR_17	HONEC	Secondary consumers	Invertebrates	Porcellio scaber	100	0.077	0.1	0.7	0.005
TR_18	HONEC	Secondary consumers	Invertebrates	Porcellio scaber	100	0.077	0.1	0.7	0.005
TR_19	HONEC	Secondary consumers	Invertebrates	Porcellio scaber	30	0.077	0.1	0.7	0.005

[a] W_s=species relevance criterion

[b] W_t=trophic levels abundance criterion

[c] W_q=data quality criterion

Table 6: HC5 and HC50 values for log-normal and log-empirical SSD and n-SSWD curves and HC5 value calculated by Gottschalk et al. [11] for freshwater compartment followed by HC5 and HC50 values for the log-normal and log-empirical n-SSWD curve for the different trophic levels and taxonomic groups in the freshwater compartment

FRESHWATER COMPARTMENT		SSD		n-SSWD		Gottschalk et al. [11]			
HCx[a] (mg/L)		5%	50%	5%	50%	5%			
Log-normal	Best-Estimate (C.I.[d] 50%)	0.02	0.57	n.r.[b]	n.r.[b]	n.a.[c]			
Log-empirical		0.02	0.54	0.02	0.22	0.06151 (empirical median value)			
FRESHWATER'S TROPHIC LEVELS		PRIMARY PRODUCERS		PRIMARY CONSUMERS		SECONDARY CONSUMERS			
HCx(mg/L)		5%	50%	5%	50%	5%	50%		
Log-normal	Best-Estimate (C.I.[d] 50%)	n.r.[b]	n.r.[b]	n.r.[b]	n.r.[b]	n.r.[b]	n.r.[b]		
Log-empirical		0.04	0.16	0.00	0.99	0.08	1.12		
FRESHWATER'S TAXONOMIC GROUPS		ALGAE		BACTERIA		INVERTEBRATES		VERTEBRATES	
HCx(mg/L)		5%	50%	5%	50%	5%	50%	5%	50%
Log-normal	Best-Estimate (C.I.[d] 50%)	n.r.[b]	n.r.[b]	0.00	0.14	n.r.[b]	n.r.[b]	n.r.[b]	n.r.[b]
Log-empirical		0.07	0.21	0.01	0.08	0.00	0.96	0.07	1.23

[a] HCx=Hazard Concentration for x% of species

[b] n.r.=not reliable

[c] n.a.=not available

[d] C.I. 50%=confident interval of 50%

FIGURES

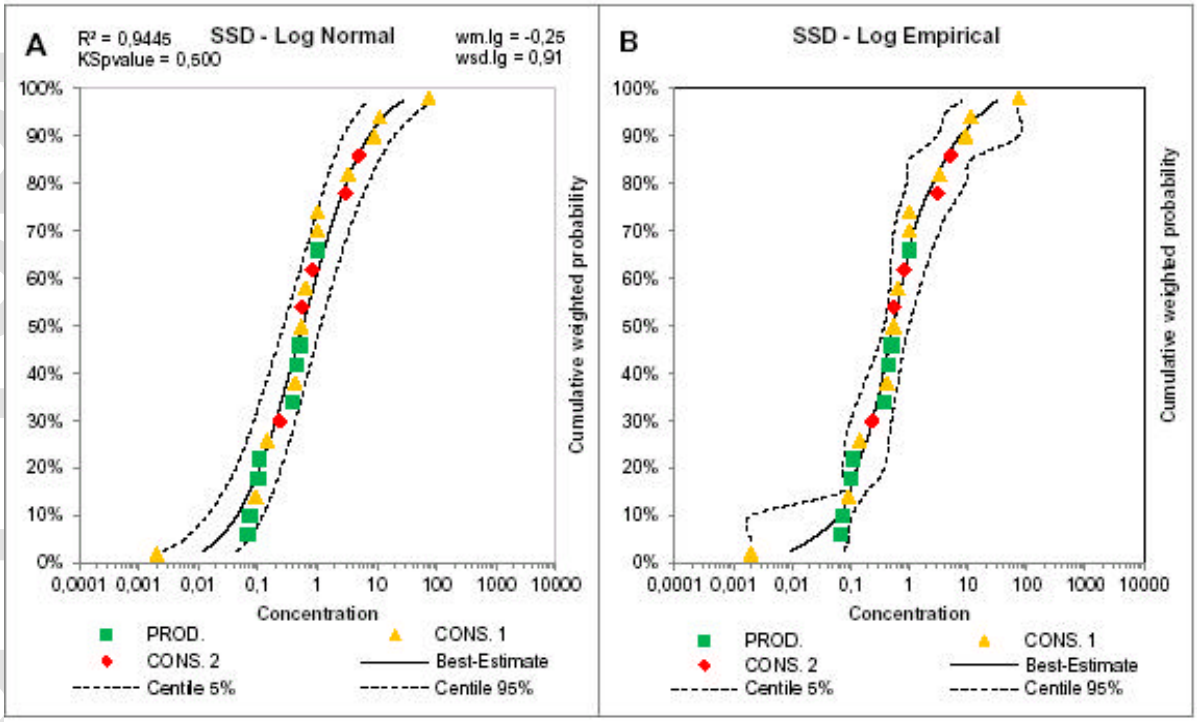

Figure 1: Log-normal (A) and log-empirical (B) conventional SSD curves for freshwater compartment. Wm.lg=mean value; wsd.lg=standard deviation value, R^2=multiple R-square coefficient; KSpvalue=Komogorov-Smirnov test value; PROD.=primary producers, CONS. 1=primary consumers; CONS. 2=secondary consumers.

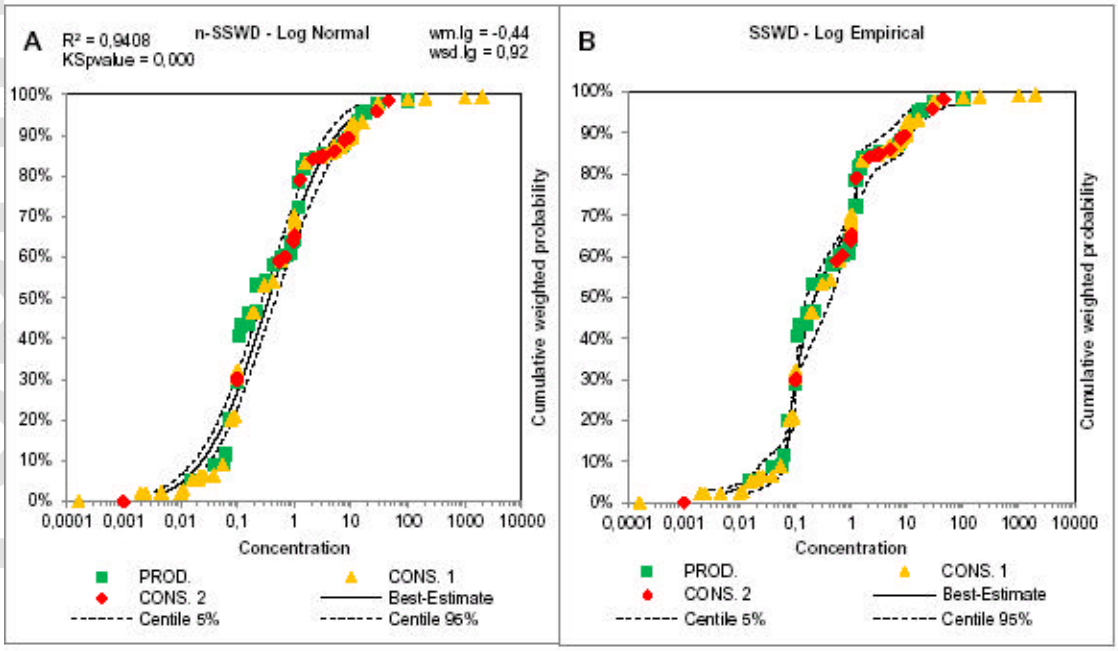

Figure 2: Log-normal (A) and log-empirical (B) n-SSWD curves for freshwater compartment. Wm.lg=mean value; wsd.lg=standard deviation value, R^2=multiple R-square coefficient; KSpvalue=Komogorov-Smirnov test value; PROD.=primary producers; CONS. 1=primary consumers; CONS. 2=secondary consumers.

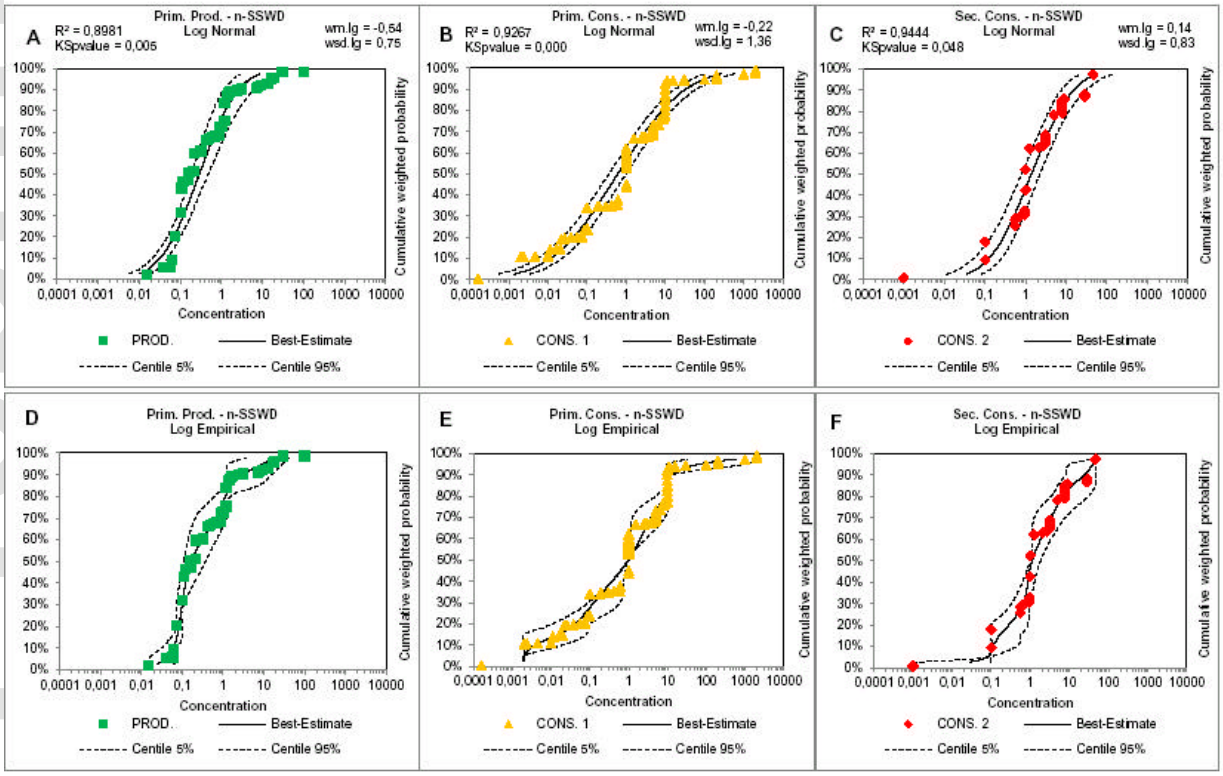

Figure 3: Log-normal (A,B,C) and log-empirical (D,E,F) n-SSWD curves for the different trophic levels in freshwater compartment. A,D) PROD.=primary producers (green), B,E) CONS. 1=primary consumers (orange), C,F) CONS. 2=secondary consumers (red). Wm.lg=mean value; wsd.lg=standard deviation value, R^2=multiple R-square coefficient; KSpvalue=Komogorov-Smirnov test value.

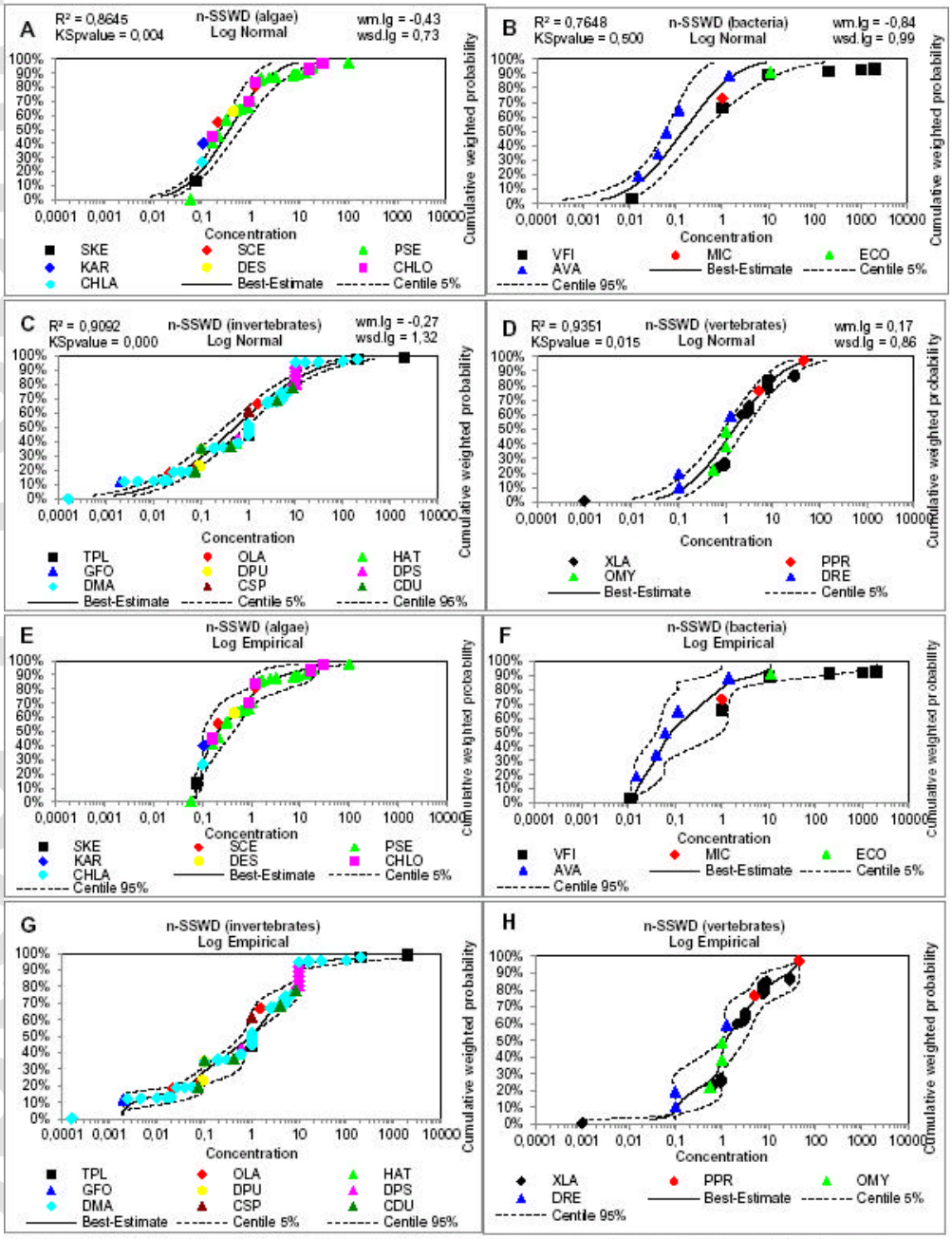

Figure 4: Log-normal (A,B,C,D) and log-empirical (E,F,G,H) n-SSWD curves for the different taxonomic groups in freshwater compartment. □Primary producers; ΔPrimary consumers; ◊Secondary consumers. A&E) Algae: SKE=Skeletonema costatum, SCE=Scenedesmus sp., PSE=Pseudokirchneriella sub capitata, KAR=Karenia brevis, DES=Desmodesmus subspicatus, CHLO=Chlorella sp., CHLA=Chlamydomonas reinhardtii. B&F) Bacteria: VFI=Vibrio fischeri, MIC=11 microbial species, ECO=Escherichia coli, AVA=Anabaena variabilis. C&G) Invertebrates: TPL=Thamnocephalus platyurus, OLA=Oryzias latipes, HAT=Hydra attenuata, GFO=Gammarus fossarum, DPU=Daphnia pulex, DPS=Daphnia similis, DMA=Daphnia magna, CSP=Chydorus sphaericus, CDU=Ceriodaphnia dubia. D&H) Vertebrates: XLA=Xenopus laevis, PPR=Pimephales promelas, OMY=Onchorynchus mykiss, DRE=Danio rerio.