COMBINING EXPOSURE AND EFFECT MODELING INTO AN INTEGRATED PROBABILISTIC ENVIRONMENTAL RISK ASSESSMENT FOR NANO PARTICLES

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Abstract: There is a growing need for good environmental risk assessment of engineered nanoparticles (ENPs). Environmental risk assessment of ENPs has been hampered by lack of data and knowledge about ENPs, their environmental fate, and their toxicity. This leads to uncertainty in the risk assessment. To deal with uncertainty in the risk assessment effectively, probabilistic methods are advantageous. In the present study, the authors developed a method to model both the variability and the uncertainty in environmental risk assessment of ENPs. This method is based on the concentration ratio and the ratio of the exposure concentration to the critical effect concentration, both considered to be random. In this method, variability and uncertainty are modeled separately so as to allow the user to see which part of the total variation in the concentration ratio is attributable to uncertainty and which part is attributable to variability. The authors illustrate the use of the method with a simplified aquatic risk assessment of nano-titanium dioxide. The authors’ method allows a more transparent risk assessment and can also direct further environmental and toxicological research to the areas in which it is most needed.

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INTRODUCTION

There is a growing need for good environmental risk assessment of engineered nanoparticles (ENPs). The increased production and use of ENPs and derived products result in relevant release of ENPs into the environment, which may pose a potential risk in the environment [1–3]. Environmental risk assessment of ENPs has been hampered by large uncertainty. This uncertainty may be the result of lack of data and knowledge about ENPs, their environmental fate, their toxicity [4], and how to apply standard methods [5]. Part of the uncertainty may also come from artificial results [6]. In traditional risk assessment procedures, the problem of uncertainty is commonly addressed by making use of conservative or worst-case scenarios.

Using conservative scenarios to deal with uncertainty, however, is not desirable for 3 reasons. First, a conservative scenario is by definition unrealistic, to be on the safe side. This may result in an overconservative risk assessment, leading to unnecessarily stringent regulation on the use of nanotechnology. Second, the transparency of the risk assessment is compromised in that it is nearly impossible to explicitly quantify how conservative the risk assessment is. Third, in a deterministic conservative risk assessment, it is not possible to differentiate between uncertainty and variability. Uncertainty is, in principle, the reducible variation that exists because of lack of data and knowledge [7]. Variability, on the other hand, is the inherent variation that is present in all natural processes and living organisms and, therefore, is not reducible [7]. To improve a risk assessment, the effect of uncertainty on risk assessment needs to be studied and, if necessary, reduced. This is possible only if we separately quantify uncertainty and clearly follow the path to its sources. A deterministic risk assessment does not allow for such a separation in a transparent way. This hampers focused research on areas of high uncertainty because these cannot be identified. Probabilistic methods are a way forward to effectively deal with uncertainty in the risk assessment.

A literature search on the words “probabilistic risk assessment” in Scopus, the world’s largest abstract and citation database, covering more than 21,000 peer-reviewed journals, produced more than 10,000 results. Figure 1 illustrates the massive increase in the number of publications in the last 15 yr. Adding the word “nano” to the search, however, only gives a meager 60 results (20 February 2016). The difference is evident in Figure 1 and underlines the need for more research into probabilistic methods for the risk assessment of ENPs. This need is echoed by Koelmans et al. [8], who call for probabilistic modeling when dealing with uncertainty.

Probabilistic methods for the risk assessment of ENPs include Monte Carlo analysis and Bayesian networks [9–12]. Although these methods quantify the variation in the various components of the risk assessment, this variation is referred to as “uncertainty” only in the mentioned publications. Some of this variation, however, is also attributable to variability.

In the present study, we use integrated probabilistic risk assessment (IPRA) to model both the variability and the uncertainty in environmental risk assessment of ENPs. The IPRA method was developed for the risk assessment of human health effects caused by chemicals [13,14] and has found many applications [15–20]. It has also been applied to nanosilica in
food [21]. To the best of our knowledge, this is the first time that IPRA has been used for environmental risk assessment of ENPs. We use the method that was developed by Gottschalk and Nowack [23] and Coll et al. [10] to study the case of nano-titanium dioxide (nanoTiO2). The case study is presented as an illustration of our proposed method, and it should be noted that a full risk assessment of nanoTiO2 is outside the scope of the present study.

Risk assessment consists of exposure assessment, hazard assessment, and risk characterization. For environmental exposure assessment, we use the multimedia fate model SimpleBox4Nano (SB4N) [22] to predict exposure concentrations of nanoTiO2 in the aquatic compartment. By extending the model with uncertainty and variability distributions, we can quantify the variability of predicted exposure concentrations in a cumulative distribution function with confidence bands that quantify the uncertainty.

For environmental hazard assessment, we start from the probabilistic species sensitivity distribution (pSSD) model of Gottschalk and Nowack [23] and Coll et al. [10] and adjust it to separately quantify variability and uncertainty. Similar to the exposure assessment, our method allows the variability in critical effect concentrations to be quantified in a cumulative distribution function with confidence bands that quantify the uncertainty. Finally, the exposure and hazard assessment are combined into the concentration ratio. Besides being designed for the separate quantification of variability and uncertainty, our method also allows us to study the contribution of the different uncertainty sources to the total uncertainty in the concentration ratio.

In the Methods section, we provide the background of the SB4N and pSSD models and describe the IPRA method. In the Results section, we provide the results of applying our method to nanoTiO2 in water. In the Discussion section, we discuss the results and our method and its limitations.

METHODS

Background

Exposure assessment. SimpleBox4Nano is a multimedia fate model that simulates the environmental fate of ENPs [22]. It is a modification of the original SimpleBox model [24–26] used for chemical exposure assessment in the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) regulation. SimpleBox4Nano models the fate of ENPs in 4 compartments: atmosphere (including rain), surface water, sediment, and soil (including soil porewater). Within each compartment, ENPs can occur in different physical–chemical forms: freely dispersed (free), hetero-aggregated with natural colloidal particles, or attached to larger natural coarse particles that are prone to gravitational forces in aqueous media [22]. Using a mass balance modeling system [27,28], SB4N obtains the masses (in kilograms) of ENPs in each of the 4 compartments and for each of the 3 forms. These can be converted to concentrations by dividing the mass by the total water volume.

The SB4N model performs a deterministic exposure risk assessment. It takes single-value inputs and returns single-value masses. In the section Quantifying variability and uncertainty in exposure, we place SB4N in a 2-dimensional (2D) Monte Carlo structure to feed the model with variability and uncertainty data and obtain the variability and uncertainty distributions of the exposure concentrations of ENPs. For easier implementation in IPRA, we coded SB4N (which is an Excel model) in R software [29] in an object-oriented way.

Hazard assessment. Different species have different sensitivities. Sensitivities are quantified in the form of what we call limit concentrations, such as the no-observed-effect concentration (NOEC), 10% lethal concentration (LC10), 50% lethal concentration (LC50), 10% effect concentration (EC10), and 50% effect concentration (EC50). A statistical distribution describing the differing sensitivities among a group of species is called a species sensitivity distribution (SSD). Gottschalk and Nowack [23] developed the pSSD method, which, in addition to quantifying the variability in species’ sensitivity, includes the variation within a species caused by different experimental conditions. This method was extended to include further uncertainty about the data points and the assessment factors used [10,11].

The pSSD method was developed on data from literature. The data were collected [10] according to selection criteria in accordance with REACH guidance [30]. First, only effects on survival, growth, reproduction, and changes in significant metabolic processes (e.g., photosynthesis [10]) were included. Second, only toxicity studies on living organisms were included (i.e., no tissue or in vitro experiments). Third, if chronic and acute limit concentrations were available, the chronic one was chosen. Fourth, only 1 limit concentration per study was used. Finally, all different limit concentrations from tests that used different particle types, particle sizes, or media were included. For the specific case of nanoTiO2 in the aquatic compartment, there were 73 limit concentrations for 31 species from 5 taxonomic groups (Supplemental Data, Table S5) [10].

To incorporate all of the different limit concentrations into 1 SSD, the limit concentrations are transformed to species sensitivity values by making use of 2 assessment factors [10,11]. In the present study, we refer to these species sensitivity values as chronic critical effect concentrations. The first assessment factor transforms the limit concentration to a critical effect concentration. An assessment factor of 1 was used for the NOEC and the highest-observed-no-effect concentration (HONEC); an assessment factor of 2 was used for the LC10, 20% lethal concentration (LC20), EC10, 20% effect concentration (EC20), lowest-observed-effect concentration, and lowest effective dose; and an assessment factor of 10 was used for the 25% lethal concentration (LC25), LC50, 25% effect concentration (EC25), and EC50 values. The second assessment factor transforms from short-term to long-term effects. An assessment factor of 1 was used for long-term experiments, and an assessment factor of 10 was used for short-term experiments. The exposure time needed to classify an experiment as long-term or short-term varies according to the taxonomic group [10].
After the data-transformation step, the SSD is constructed in 2 steps. In the first step, a single empirical SSD for each species is constructed using a Monte Carlo routine [11]. In the second step, all of the single-SSDs are combined into 1 empirical SSD.

Risk assessors are often interested in a predicted-no-effect concentration (PNEC), which generally is the 5th percentile of the SSD, also referred to as the 5% hazard concentration. Coll et al. [10] extended the pSSD method to include uncertainty on the assessment factors and extra uncertainty on the endpoints, which, in a Monte Carlo simulation, provides an uncertainty distribution for the PNEC.

The pSSD method quantifies uncertainty and variability. The constructed SSD, however, contains both the variability of species sensitivity and the uncertainty from experimental differences within a single species. In addition, the uncertainty distribution of the PNEC contains the uncertainty of the assessment factors and only partially the uncertainty of the limit concentrations. This is because the experimental uncertainty was modeled together with variability in the constructed SSD, thereby combining variability and uncertainty in a single SSD. It is, therefore, not possible to study the effect of uncertainty on the effect assessment nor to study the contribution of the different sources of uncertainty to the hazard assessment and ultimately the concentration ratio. In the section Quantifying variability and uncertainty in hazard, we adjust the pSSD method to allow for the separate quantification of variability and uncertainty.

Integrated probabilistic risk assessment

Integrated probabilistic risk assessment uses a 2D Monte Carlo scheme to quantify uncertainty and variability distributions separately in the risk assessment, as illustrated in Figure 2. Details on the exposure, hazard, and risk aspects of the model are discussed in the sections Quantifying variability and uncertainty in exposure, Quantifying variability and uncertainty in hazard, and Integrated probabilistic risk assessment, respectively. Integrated probabilistic risk assessment is available in the Monte Carlo Risk Assessment software [31] in the context of human health; for our environmental risk-assessment application, however, it was coded in R software [29].

Quantifying variability and uncertainty in exposure. To define variability and uncertainty in exposure concentrations, it is important to define the unit at risk of the risk assessment. The SB4N model is designed to predict exposure concentrations on the regional scale, where regions are defined as spatial units of 200 × 200 km. Variability in exposure is, therefore, defined as the naturally occurring variation in exposure concentrations between regions.

The SB4N model has many input variables, which may be variable between regions, uncertain, or both. To keep the number of variables manageable, we made a selection of the most important variables on which to apply the 2D Monte Carlo algorithm. Meesters et al. [32] conducted a sensitivity analysis to determine which variables play a large role in determining the nanoparticle masses in the various compartments. In the present study, we only considered aquatic risk assessment and are, therefore, only interested in the nanoparticle masses in the aquatic compartment. From the sensitivity analysis of Meesters et al. [32], we selected those variables that had a large influence on the nanoparticle masses in the aquatic compartment.

For each of the selected variables, we obtained ranges of possible values from the literature (see Supplemental Data, Tables S1–S4, column 7). We assumed that the main source of variation for each variable was the result of either variability or uncertainty. Moreover, the available information in the literature was not sufficient to determine which part of the range of values was the result of variability and which part was the result of uncertainty. Therefore, we assumed that the reported variation was attributable completely to either uncertainty or variability. The selected variables are indicated by a “V” (for a variable input) or a “U” (for an uncertain input) in Supplemental Data, Tables S1 through S4, column 4. The remaining variables (indicated by a “C” in Supplemental Data, Tables S1–S4, column 4) were given the default value.

First, we look at variability in greater detail. In terms of the final output of the exposure assessment—namely, predicted environmental concentration—variability is the naturally occurring variation in environmental concentration between regions. The distributions should, therefore, quantify the natural variation of that variable between regions.

As an example, consider the variable “water depth,” which is a system dimension variable of SB4N. It is obvious that “water depth” is variable when looking at a collection of water bodies. Considering the regional scale of SB4N, however, the “water depth” variable does not represent the depth of an individual water body but rather the average water depth of all water bodies in a 200 × 200 km region. The variability distribution for “water depth” should, therefore, quantify the variability in average water depth between regions. More concrete, this means capturing the variability in average water depth between 200 × 200 km regions in The Netherlands, Italy, and Norway.

Figure 2. A schematic diagram of uncertainty and variability loops in the 2-dimensional Monte Carlo scheme used in integrated probabilistic risk assessment. SimpleBox4Nano (SB4N) is a multimedia fate model that simulates the environmental fate of engineered nanoparticles producing exposure concentrations from input variables. DLVO = Derjaguin-Landau-Verwey-Overbeek theory [33,34] to calculate attachment efficiencies.
for example. This variability is quantified by providing “water depth” with a variability distribution—namely, a log-normal distribution with a mean of 3 and a standard deviation of 0.237 (Supplemental Data, Table S1).

Similarly, variability distributions were applied to all the variables from the preselection considered to vary as a result of variability. These variables are indicated by a “V” in Supplemental Data, Tables S1 through S4. The choice of distributions was based on experimental or expert knowledge from the literature (see references in Supplemental Data, Tables S1–S4). To keep the method simple, we assumed that the variability distributions were fully known (i.e., the distribution parameters are assumed to be known and not subject to uncertainty).

For 1 variable, “invkdebye,” we obtained an empirical variability distribution. “invkdebye” is the debye length used to calculate attachment efficiencies between engineered and natural nanoparticles with the Derjaguin-Landau-Verwey-Overbeek theory [33,34]. Experimental debye length values were obtained by Hammes et al. [35]. Because of the large number of values (808), it was possible to quantify the variability via an empirical distribution.

Next, we consider uncertainty. A variable that is subject to uncertainty only is in theory considered to be a constant.

As an example, consider the variable “diameterenp,” which represents the mean nanoTiO2 particle size. We assume a similar nanoTiO2 production between regions and, therefore, similar particle size distributions between regions. In a perfect world where everything is known, the mean particle size would be a known constant. In practice, however, we are uncertain about what this average particle size actually is. This uncertainty is quantified by providing “diameterenp” with an uncertainty distribution—namely, a log-normal distribution with 2.5th percentile equal to 1 and 97.5th percentile equal to 100 (Supplemental Data, Table S2).

All variables of the preselection considered to be uncertain are given an uncertainty distribution. These distributions are based on experimental or expert knowledge from the literature (see references in Supplemental Data, Tables S1–S4). As was the case for variability, there is a variable, “prodvol,” for which we obtained an empirical uncertainty distribution. Prodvol is the production volume of nanoTiO2. We obtained production volumes from a Monte Carlo simulation study [36] from which we created the empirical distribution.

The variability and uncertainty distributions applied to the variables are given in Supplemental Data, Tables S1 through S4. These distributions are the inputs for the 2D Monte Carlo algorithm [37], in which we generated 200 draws from the joint distribution of the uncertain variables and, given these draws, thus for each row 1000 draws from the joint distribution of the variables that cause variability in the exposure (see Supplemental Data for detailed algorithm). The values obtained for each combination of uncertainty and variability draws are used as input to SB4N, resulting in an exposure concentration that thus represents a draw from the exposure distribution for a particular draw of the uncertain variables.

The output of the algorithm is thus a 200 × 1000 matrix with exposure concentrations, where each row represents the variability distribution of the exposure given a particular draw from the joint uncertainty distribution. If each row of the 200 × 1000 exposure concentration matrix is sorted from small to large, then the value in the 10kth column of each row is an estimate of the kth percentile of the exposure distribution for that particular row. Consequently, each column then represents the uncertainty distribution of 1000 equally spaced percentiles of the exposure distribution.

Quantifying variability and uncertainty in hazard. Building on the pSSD method [10,11,23], we develop a 2D Monte Carlo method to separately quantify uncertainty and variability. Again, it is necessary to define the unit at risk, which for effect distributions is commonly taken to be the species.

For the SSD, we need chronic critical effect concentrations (CEC_{chronic}). These, however, are often not directly available for the species we want to include in the SSD. In this case, they are calculated as

$$CEC_{chronic} = \frac{CONC}{AF_{time} \times AF_{no-effect}}$$

where CONC is the limit concentration (e.g., LC10 or EC20) obtained from a toxicological study, AF_{time} is the assessment factor to extrapolate from acute to chronic studies, and AF_{no-effect} is the assessment factor to extrapolate from the limit concentration to the critical effect concentration.

Variability in chronic critical effect concentrations refers to the natural variation in critical effect concentrations between species. This variability is quantified by defining a distribution over chronic critical effect concentrations for different species. In practice, such a distribution is often taken as the log-normal distribution [38–41], assuming the species critical effect concentrations are normally distributed on the log scale. We also use log-normal distributions in our method.

The 3 variables used to calculate the chronic critical effect concentration (the limit concentration and 2 assessment factors in Equation 1) can all be subject to uncertainty.

Uncertainty in the limit concentration is the result of differences between toxicity studies within a species. To quantify this uncertainty, we divide the limit concentrations into groups per species. The uncertainty distribution for each species is taken to be a log-normal distribution with the 2.5th (97.5th) percentile equal to the minimum (maximum) concentration in that group divided (multiplied) by an uncertainty factor of 2. This uncertainty factor is based on the assumption that, as a result of uncertainty, the limit concentration can be a factor 2 lower or higher than the measured experimental limit concentration value(s). This factor of 2 is similar to the 50% coefficient of variation used in the pSSD method [11].

Within 1 species, however, there can be different experimental duration types (2 types: short or long) and limit concentration types (3 types: NOEC, HONEC; LC10, LC20, EC10, EC20; LC25, LC50, EC25, EC50). An example of such a species is Danio rerio, as shown in Table 1. We cannot combine these into 1 uncertainty distribution because each group needs to

| Table 1. Nano–titanium dioxide effect data for Danio rerio [10] |
|------------------|-------------|-------------|---------|---------|
| Limit concentration type | Limit concentration (µg/L) | Exposure time (h) | AF_{time} | AF_{no-effect} |
| HONEC | 500 | 4320 | 1 | 1 |
| LC50 | 124500 | 96 | 10 | 10 |
| LC50 | 156000 | 24 | 10 | 10 |
| LC50 | 300000 | 24 | 10 | 10 |
| HONEC | 500000 | 96 | 10 | 1 |

AF = assessment factor; HONEC = highest-observed-no-effect concentration; LC50 = 50% lethal concentration.
have different assessment factors applied to it. For these species, we sample in each uncertainty run 1 group with probability equal to the number of concentration values divided by the total number of concentration values for that species. For the example in Table 1, we would sample 1 of the groups with probabilities 0.2, 0.6, and 0.2 for the 3 groups. The log-normal species uncertainty distribution is then assumed for that group as explained.

The uncertainty distribution for the assessment factors is centered around the nominal values, as explained in the section Hazard assessment and given in Supplemental Data, Table S5, columns 6 and 7. As in the case of the limit concentrations, we again use an uncertainty factor of 2 below and above each assessment factor value. This is similar to the 50% deviation used in the pSSD method [10]. The obtained lower and upper bounds are again equated to the 2.5th and 97.5th percentiles of a log-normal distribution.

In each uncertainty run, 1 limit concentration is drawn from each of the 31 species uncertainty distributions. Each of these limit concentrations is then divided by a value drawn from the corresponding uncertainty distribution of each assessment factor. The resulting 31 chronic critical effect concentration values are used to estimate the mean and standard deviation for the log-normal distribution of the variability (i.e., the SSD). A detailed algorithm can be found in the Supplemental Data.

Similar to the exposure assessment, the output of the algorithm is a 200 × 1000 matrix with critical effect concentration values, where each row represents the variability distribution of the critical effect (i.e., the SSD) for a particular draw from the joint uncertainty distribution. If each row of the 200 × 1000 critical effect concentration matrix is sorted from small to large, then the value in the 10th column of each row approximately is an estimate of the kth percentile of the critical effect distribution for that particular row. Consequently, each column then represents the uncertainty distribution of 1000 equally spaced percentiles of the critical effect distribution or SSD.

**Integrated probabilistic risk assessment.** In this section, we discuss the integration of the exposure and hazard assessments into the risk characterization. For this, we use the concentration ratio (CR), given by

\[ CR = \frac{\text{ExpC}}{\text{CEC}_{\text{chronic}}} \]  

A concentration ratio less than 1 indicates that the exposure concentration is lower than the chronic critical effect concentration of the species and, therefore, indicates a safe situation. A concentration ratio greater than 1, however, indicates a possibly unsafe situation.

Combining the units of the exposure and the effect models, we obtain the unit at risk as a species in a 200 × 200 km region. The variability distribution, therefore, describes variation between random species in random regions.

The matrix of concentration ratio values is obtained by dividing the (unsorted) exposure matrix by the (unsorted) critical effect matrix element-wise. Each row represents the variability distribution of concentration ratio given a particular draw from the joint uncertainty distribution. If each row of the 200 × 1000 concentration ratio matrix is sorted from small to large, then the value in the 10th column of each row is an estimate of the kth percentile of the concentration ratio distribution for that particular row. Consequently, each column then represents the uncertainty distribution of 1000 equally spaced percentiles of the concentration ratio distribution.

A simple graphical representation of both variability and uncertainty of the concentration ratio can be given in the form of a so-called concentration ratio bar graph (similar to the IPRA bar graphs in Jacobs et al. [21] and van der Voet et al. [14]). In a concentration ratio bar graph, a box represents the variability distribution of the concentration ratio between specified percentiles. These can be particular percentiles (denoted by px for the xth percentile; e.g., p0.1 and p99.9, p1 and p99, or p5 and p95, depending on the level of protection required). Whiskers are used to represent the 5% lower and 95% upper uncertainty limits of these percentiles. A dot on the bar indicates the median of the variability distribution.

We also calculate the risk, \( R = P(\text{CR} > 1) \), together with its uncertainty bounds.

To study the extent to which sources of uncertainty contribute to the total uncertainty present in a certain percentile of interest, we implement a probabilistic uncertainty analysis [13]. We group all sources of uncertainty into just 2 groups: exposure-related and effect-related uncertainties. This results in a 22 factorial design where sampling from the uncertainty distributions for each group is turned on and off. For a given percentile, \( 2^2 = 4 \) values are obtained in each uncertainty run, resulting in 4 distributions, which are summarized by their variance. An additive model is then fitted to the 4 variances. When this model explains most of the variance, which is usually the case, the coefficients of the main effects can indicate the contribution to the total variation [21]. The intercept term represents the additional uncertainty from Monte Carlo sampling when the 2 input group uncertainty sources are turned off. Without any uncertainty in the inputs, there is still variation in output from the random Monte Carlo sampling of variability. Results are illustrated by means of a bar graph.

**RESULTS**

In this section, we describe the results obtained from an application of the method that we propose in the present study to an aquatic risk assessment of nanoTiO2. Hereby, we illustrate what kind of information can be obtained from our method and how our method can be used to gain insight into the roles that variability and uncertainty play in nanoparticle risk assessment.

The variability and uncertainty distributions applied to variables of the SB4N model are provided in Supplemental Data, Tables S1 through S4.

Figure 3 illustrates the total exposure and the critical effect distributions, with uncertainty bands. The exposure distribution is plotted as an exceedance (1−cumulative distribution function) curve, indicating the percentage of regions that exceed the concentration on the x axis. The amount of overlap of the curves is an indication of the amount of risk and is related to the expected risk concept [42] and the area under the joint probability curve [42,43].

Figure 4 shows the concentration ratio bar graph plotted for various forms of nanoparticle exposure. For each bar, a different exposure concentration (as indicated by the labels) was used to calculate the concentration ratio. The 5 bars represent the variability distribution of the concentration ratio between the 1st (p1) and the 99th (p99) percentiles. For each bar, the whiskers represent the 5% lower and 95% upper uncertainty limits of these percentiles. The dot on each bar indicates the median of the variability distribution. Except for the free nanoparticle exposure concentrations, all the exposure concentrations caused to some extent a concentration ratio greater than 1. The
implications of this are further discussed in the Discussion section.

Figure 5 illustrates the uncertainty distribution of the risk, \( R = P(CR > 1) \), using total exposure. The vertical line indicates the nominal risk value (0.111), which is \( R = P(CR > 1) \) calculated using only the variability distribution of the concentration ratio without any uncertainty. The risk distribution specifies variation between species and between regions. Note that this can correspond with many different situations, such as, as extremes, 11.1% of species being at risk in all regions or all species being at risk in 11.1% of the regions. A discussion on this double interpretation and its drawbacks can be found in Verdonck et al. [40].

It is important to determine how the uncertainty in the percentiles of the concentration ratio is affected by the different uncertainty sources. Figure 6 indicates the relative contribution of each source of uncertainty to the total uncertainty in 4 upper percentiles of the concentration ratio distribution—namely, p90, p95, p97.5, and p99. We note that the contribution of Monte Carlo uncertainty is negligible for all the percentiles; therefore, our choice for 1000 Monte Carlo iterations to describe the variability seems sufficiently high. The uncertainty in the critical effect concentration is the main contributor to the total uncertainty for all percentiles, increasing for the more extreme percentiles. To further study the exact source of this uncertainty, one could perform a similar uncertainty analysis on the individual uncertainty sources that contribute to the uncertainty in the exposure and critical effect concentrations.

**DISCUSSION**

In this section, we discuss the results on the use of free nanoparticles, ENP hetero-aggregates with natural colloid particles, and ENPs attached to natural coarse particles in risk assessment and model uncertainty of the exposure and effect models.

Although Figure 4 may lead one to believe that nanoTiO\(_2\) poses some risk to the aquatic environment, care should be taken in its interpretation. The concentration ratio is calculated using the chronic critical effect concentration, which is assumed to be a no-effect concentration. This concentration is extrapolated from some limit concentration by an assessment factor, \( AF_{no-effect} \). The true no-effect concentration is not known. Keeping this in mind, a concentration ratio greater than 1 does not indicate a negative effect with certainty but rather a potentially unsafe situation. We can no longer exclude a possible risk.
Another point to consider is the quantification of variability in the exposure assessment. All of the distributions used (as given in Supplemental Data, Tables S1–S4) are motivated by the literature as the possible range the variable can take. In the case of variability, however, these distributions do not always necessarily reflect the realistic variability. This is because in the literature one usually finds the possible range of individual values that a certain variable can take. The variability distribution, however, should quantify the variability of the mean value of a variable in a region (200 × 200 km). From the central limit theorem, we know that the standard deviation of the mean is σ/n, where σ is the standard deviation of the individual values and n the sample size [44]. We would, therefore, expect the true variability distributions to be narrower than those we used. In the case of the exposure, this would result in a narrower exposure concentration distribution. This will work into the concentration ratio distribution in Figure 4 and may cause the gray bars to be less wide, resulting in a less extreme upper percentile.

Variability in exposure, even when possibly overestimated, does not, however, seem to be the major source of variability in the concentration ratios. The large contribution of effect variability is clearly illustrated in Figure 7, which shows the variability distributions (p1–p99) of the various exposure concentrations and the critical effect concentrations. The distribution of the critical effect is much wider than that of the exposure. The large variability in the effect is the result of the large variation in critical effect concentrations among species. Some species are very much more sensitive to nanoTiO2 than others.

Although we might be able to reduce the total variability of the concentration ratio distribution by more accurate specification of the variability distributions in the exposure assessment, the large amount of variability in the effect concentrations will prevent any significant reduction.

In the present study, we applied the IPRA method to the aquatic risk assessment of nanoTiO2. This method, however, is not limited to the aquatic compartment. SimpleBox4Nano is a generic model, modeling the fate of nanoparticles for the environmental compartments of air, water, soil, and sediment [22]. Our method can, therefore, be applied to any of these 4 compartments, provided there are sufficient critical effect data available for that compartment.

**Exposure of free, hetero-aggregated, and attached ENPs**

The ratios between exposure and critical effect concentrations suggest safe concentrations only for the free forms of nanoTiO2 but not for exposure to hetero-aggregates with natural...
colloid particles, ENPs attached to natural coarse particles, or the sum of all ENP forms (Figure 4). That does not directly indicate that aquatic organisms are at risk. This is complex to assess because it is not yet known to what extent the relevant exposure concentration should include ENPs that are attached to natural particles [8]. There are no approaches designed to quantify predicted exposure concentrations into bioavailable exposure estimates [45] because the fate and exposure of ENPs are not incorporated in aquatic toxicity tests [46]. The current risk-assessment frameworks, such as REACH, do not consider the fraction of chemicals or metals that is associated with suspended particles to contribute to environmental exposure because free metal species are “far more bioavailable than most complexed metal species” [47]. Under REACH, the free (dissolved) concentration of a metal (oxide) is defined as “the fraction of a metal that passes through a filter of 450 nm” [47]. Following this definition for ENPs would mean that the sum of dissolved/ionic, free pristine nanoparticulate forms and hetero-aggregates (<450 nm) is considered to be the bioavailable fraction. Moreover, aggregation might even increase the uptake and bioavailability of ENPs. Depending on the feeding mechanism of the organism at stake, (hetero-)aggregated ENPs may have grown to a size so that they no longer pass the filtering apparatus of filter feeders [8,48]. On the other hand, an aggregated state probably yields higher effect thresholds because particle toxicity would be lowered by aggregation or encapsulation of the nanoscale particles [8,48]. The critical effect concentrations that are applied in the concentration ratio are based on toxicity testing of free and homo-aggregated ENPs [10] and do not account for such possible reduction of the toxicity of the hetero-aggregated species. Hence, the concentration ratios for nanoTiO2 that do not ensure safe concentrations (Figure 4) are still conservative estimates and should thus be interpreted with caution. Nonetheless, the results emphasize the relevance of the debate over whether aquatic toxicity of ENPs should be tested in their freely dispersed and unaggregated state or in a more environmentally realistic state that includes ENPs present as aggregates [8]. The concentration ratios prove that only including free ENPs and excluding the hetero-aggregated ENPs may lead to supposed safe but in reality unsafe concentrations.

From the above discussion, we see that the nanoparticle form in current toxicity tests is not compatible with the nanoparticle form to which species are exposed in the environment. Toxicity testing is performed on a substance (free and homo-aggregated ENPs) to which species are hardly exposed (see Figure 7). At the same time, we do not know the toxicity of the substance (hetero-aggregated ENPs) to which species are exposed in reality. This incompatibility between toxicity and exposure data constitutes extra uncertainty, which, if not resolved, could possibly be modeled.

Moreover, possible cumulative exposure to natural and engineered nanoTiO2 is not considered in our case study, serving as a proof of concept for the IPRA approach. Such natural background concentrations are derived as elemental Ti concentrations in field samples filtered for submicron particles for <450 nm, which are found to typically range between 0.02 g/L and 2.3 g/L in rivers [49,50]. Hence, these measured concentrations are actually the sum of the elemental mass of Ti in dissolved, free, and aggregates of nanoparticles able to pass through a filter of <450 nm [49,50]. The current measurement techniques are not able to quantify the different forms of Ti in these field samples [51] so that considering the cumulative exposure of natural and engineered particles is only possible for the predicted concentrations that reflect the sum of free and hetero-aggregated nanoTiO2 (Figure 7). Indeed, there is some overlap between the range of typical natural concentrations and of the concentrations calculated for engineered nanoTiO2 but only at the lower end of the exposure distribution. Moreover, the natural Ti concentrations hardly surpass the critical effect concentrations so that cumulative exposure of natural and engineered nanoparticles would only contribute to a minor extent in the environmental risk assessment of nanoTiO2. Nonetheless, the possibility of such cumulative exposure again emphasizes the need for consensus on what forms of ENPs should be interpreted as the relevant exposure concentration and their compatibility with effect concentrations determined in the current toxicity testing protocols [8].

**Exposure model uncertainty**

The simulations of environmental fate and concentrations of nanoTiO2 are performed within the context of the chemical safety assessment guidelines of REACH [52]. Within this context, environmental exposure models are considered a means to determine whether manufacture, import, or uses of a substance do not lead to concentrations that are not safe for the environment [52]. In a first-tier approach, nonspatial multimedia fate models such as SimpleBox suffice, but further iteration is required if the conservative estimates for exposure levels are not below PNEC values [52].

The SB4N model is a screening-level model that is designed for exposure assessment of background concentrations on a regional or continental scale [8,53]. Simplifications in environmental exposure modeling are inevitable but acceptable if they can be justified scientifically [54]. As such, SB4N is a generic model that is not temporal or spatially explicit, whereas complex chemical reactions between ENPs and environmental matrices are only implicitly included in the calculations of speciation [22]. Multimedia fate models that are spatially explicit, however, only yield better estimates if data on spatial variability in emission intensities are available [55]. To our knowledge, such data are only available for nanomaterials by extrapolation of the global and US production volume data in proportion to the gross domestic product of the region [36,56,57]. Including temporal explicitness in exposure estimation also does not yield better estimates. The exposure concentrations are calculated for a steady state, but recalculation of the progress over time in reaching the simulated steady state predicted exposure concentrations in surface water (see Supplemental Data) shows this only leads to an overestimation of insoluble ENPs that are attached to natural coarse particles (see Supplemental Data).

Furthermore, chemical transformation processes such as functionalization, oxidation, sulfidication, phosphorization, and adsorption of natural organic carbon are considered to be too complex to explicitly include in a screening-level exposure model such as SB4N [8,22,58]. These complex transformations of ENPs in the environment, however, are not disregarded in SB4N. Rather, they are indirectly included through their contribution in ENP dissolution rates and the interaction between ENPs with natural particles (see Supplemental Data). Hence, the simplification in chemical speciation modeling and the lack of spatial and temporal detail do not hamper the evaluation of the exposure of aquatic species to nanoTiO2 as long as the SB4N model outcomes are interpreted on a generic screening level (i.e., conservative and first-tier) [8].
Effect model uncertainty

One of the problems in hazard assessment is how to deal with more than 1 critical effect concentration per species. The REACH regulation suggests using the geometric mean for each species with equivalent data on the same toxic endpoint [30]. The geometric mean, however, favors small values because it shifts the SSD to the left [23]. This may lead to lower critical effect concentrations and a possibly overconservative risk assessment [23]. The pSSD method solves this problem by using all the available data to construct single-species SSDs, which are then combined into a single SSD. The pSSD method, however, does not differentiate whether these single-species SSDs quantify uncertainty or variability. In our method, we assume that under identical experimental test conditions and using identical test species, repeated experiments would, in theory, result in the same limit concentration (i.e., no variability). From this assumption, we deduce that the observed differences in limit concentrations for the same species should be attributed to uncertainty. The next question is then, how this uncertainty should be quantified. In our method, we assumed a log-normal distribution.

Another source of model uncertainty is usage of the assessment factors. Gottschalk et al. [11] applied the 2 assessment factors (AFtime and AFno-effect) according to REACH guidelines as explained in the section Hazard assessment. To apply AFtime, we need to know which studies are acute and which are chronic. Gottschalk et al. [11] provide a detailed description of the choice of AFtime for different taxonomic groups. For algae, for example, limit concentrations were considered chronic from an experiment duration of 72 h and more, whereas an experiment duration of 21 d was considered chronic for vertebrates. Such choices, although based on recommendations from the literature, are ultimately subject to varying levels of uncertainty. This also holds for the choice of applying a value of 1, 2, or 10 for AFno-effect. This uncertainty was not quantified in our method. It is, however, possible to extend our method to include additional uncertainty sources. These can be added as extra uncertainty factors.

CONCLUSION

In the present study, we developed an integrated probabilistic risk-assessment method and applied it to nanoTiO2 in the aquatic environmental compartment. This method allows for separate quantification of the variability and uncertainty in the risk assessment. In this way, we can see which part of the total variation in the concentration ratio is the result of uncertainty and which part is the result of variability. Variability was found to contribute the most. This was mainly because of the large variability in the critical effect concentrations. Furthermore, the uncertainty contribution of the exposure and critical effect to the total uncertainty in the concentration ratio was studied. We found that the uncertainty in the critical effect is by far the greatest contributor. This conclusion is, of course, dependent on the choice of uncertainty distributions.

We do need to caution that the results do not constitute a fully comprehensive risk assessment. They should, therefore, be interpreted in the context of model development and not as an authoritative aquatic risk assessment of nanoTiO2. As discussed in the section Exposure of free, hetero-aggregated, and attached ENPs, there is a need to broaden the scope of nanoparticle forms used in toxicity tests to include hetero-aggregated nanoparticles. This constitutes an important future research area.

We conclude that a probabilistic risk assessment in which variability and uncertainty are quantified separately adds to a more transparent risk assessment. Such a method allows for easy identification of variability and uncertainty sources, which in turn can direct further environmental and toxicological research to the areas in which it is most needed.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3476.

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