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Identifying ecotoxicological descriptors to enable predictive hazard assessments of nano-TiO_2 from a meta-analysis of ecotoxicological data

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Abstract

Hazard assessments for ENMs are made more difficult by the multitude of different nano-forms that have to be tested if a case-by-case approach is applied. Predictive hazard assessments are currently being developed to streamline the environmental risk assessment of ENMs. The present study compiled an ecotoxicological dataset for nano-TiO_2 and aimed to identify potential descriptors for the prediction of toxicological effects induced by different nano-forms based on their material properties and experimental conditions. We collected 219 nano-TiO_2 data points (*in vivo*), of which 205 were from freshwater studies. Only 23 of the 65 data points for *Daphnia magna*—the most investigated species—were considered as high-quality according to the DaNa2.0 criteria. Nano-TiO_2's EC_{50} was predicted using a multiple linear regression (MLR) model for six selected features including intrinsic (primary particle size, crystal composition) and extrinsic parameters (exposure duration, UV and non-UV illumination, concentrations of divalent cations). The EC_{50} was found to form two main clusters according to the type of illumination, with experiments conducted under UV light resulting in a lower EC_{50}. Nano-TiO_2's toxicity to *D. magna* could be predicted with an R^2 of 0.95 (p = 0.15) for the UV dataset and an R^2 of 0.55 (p = 0.19) for the non-UV dataset. This was a better performance than the full dataset MLR model which had an R^2 of 0.29 (p = 0.41). A one-factor-at-a-time sensitivity analysis identified the share of anatase and the exposure duration as the most sensitive parameters triggering adverse effects. The main impediment to the development of better predictive models is the lack of high-quality datasets with coherent sets of parameters. Future studies will have to overcome several challenges in order to enable a comprehensive approach for measuring and reporting critical parameters and making comparisons between studies.

Keywords: nanomaterials; titanium dioxide; meta-analysis; ecotoxicology, prediction, *D. magna*

Introduction

The environmental risk assessment of engineered nanomaterials (ENMs) still has several challenges to overcome before it can offer adequate risk management and governance for this promising key field of technology. ENMs are versatile in terms of their use in applications, their material configurations, and their surface modifications (Warheit, 2018). Hazard assessments, therefore, have to deal with a multitude of nano-forms which must be tested separately if the case-by-case testing paradigm is followed. Although modes of action have been partially identified, such as the formation of reactive oxygen species (ROS) (Bundschuh *et al.,* 2018), hazard assessments are hampered by the heterogeneous data available from the variety of nano-forms tested, different testing strategies, and the differing exposure conditions applied (Hjorth *et al.,* 2017; Sayre *et al*., 2017). Standard operational procedures, reference materials, and testing guidelines were introduced to align data generation and thereby decrease data variability (Rasmussen *et al*., 2016; Sayre *et al*., 2017). Physicochemical parameters such as particle size distribution, zeta potential, solubilization, crystalline phase, morphology, and surface chemistry are often discussed as being toxicologically relevant particle characteristics (Rasmussen *et al.*, 2018). Moreover, these parameters should not be considered individually when evaluating potential adverse effects (Hund-Rinke *et al*., 2018; Fernández-Cruz *et al*., 2018). A minimal set of reported physicochemical parameters has yet to be agreed upon, although ECHA (2017) has proposed a preliminary list that ought to be considered when using grouping and read-across approaches (Fernández-Cruz *et al.*, 2018).

Faced with the challenge of testing multitudes of ENM forms, grouping, read-across, and computational predictive (eco)toxicological approaches are being put into place. Although the term *grouping* is not yet commonly defined (Mech *et al.*, 2018), grouping concepts generally aim to pool different nano-forms of different parent ENMs, or different nano-forms of the same ENM, by common parameters. The underlying assumption is that grouped nano-forms exhibit similar adverse effects driven by the same physicochemical parameters. Consequently, grouping is an initial step towards enabling read-across, in which data gaps about one nano-form can be filled in by using data available on another nano-form in the same group (Lamon *et al.*, 2018a).

Several grouping concepts have been proposed, each differing in the extent to which their assessments consider intrinsic and extrinsic parameters (Lamon *et al*., 2018a; Arts *et al*., 2014; Oomen *et al*., 2015; Braakhuis *et al*., 2016; Hund-Rinke *et al*., 2018). Most of the currently available grouping concepts are still in the developmental stage, and practical applications to case studies are still limited (Arts *et al*., 2016; Aschberger *et al*., 2018; Lamon *et al*., 2018b; Park *et al*., 2018). Furthermore, different methods have been proposed to determine the similarity of two (or more) nano-forms. For example, Park *et al.* (2018) developed a similarity index based on a pragmatic scoring system to overcome the current data gaps linked to analytical challenges in determining similarities. Aschberger *et al.* (2018) used the hierarchical clustering technique and principal component analysis to assess different nano-forms of multiwalled carbon nanotubes. In this case, functionalization and impurities were not the main drivers of endpoint genotoxicity. However, these methods require complete datasets on ENMs' physicochemical properties, and this limits their applicability.

Computational (nano)toxicology attempts to predict adverse effects by relating *in vitro* to *in vivo* phenomena based on a set of molecular or atomic descriptors (Chen *et al*., 2018; Puzyn *et al*., 2018). Concepts are named differently depending on the outcome desired: quantitative (nano)structure activity relationship (QSAR/QNAR), quantity structure property relationship (QSPR), quantitative structure toxicity relationship (QSTR) (Roy *et al*., 2018; Burello, 2017; Chen *et al*., 2016), or quantity property-property relationship (QPPR) (Quik *et al.*, 2018). These concepts use different kinds of descriptors to relate molecular or atomic descriptors to the activity/property (e.g., toxicity). The descriptors include morphological structural properties, physicochemical properties, or other theoretical descriptors such as constitutional properties, electronic properties, and others (Ying *et al*., 2015; Basei *et al*., 2019). The modeling approaches using these descriptors range from conventional multiple linear regression (MLR) models to non-linear (un)supervised machine learning algorithms such as artificial neuronal networks, support vector machines, decision trees, principal component analyses, dosimetry models, hazard ranking, clustering analyses, and others (Puzyn *et al*., 2018; Basei *et al*., 2019; Chen *et al*., 2017; Liu *et al*., 2015a; Liu *et al*., 2015b; Harper *et al*., 2015). Sizochenko and Leszczynski (2016) made a comprehensive review of the QSARs developed for inorganic nanomaterials, and they identified approximately fifty QSAR models focusing more on the *in vitro* toxicity than on the *in vivo* toxicity towards bacteria, cell lines, and microorganisms. Such models generally require substantial datasets (of similar substances, like different metal oxides) to obtain statistically significant predictions (Raies and Bajic, 2016). These modeling approaches are then applied to a dataset comprising several ENMs, using the selected descriptor to predict the activity or property (e.g., toxicity) of similar substances for which information is missing from the dataset (Sizochenko *et al.*, 2018). The challenge for the QSAR approach to ENMs is the development of meaningful descriptors, as they not only depend on the intrinsic material parameters but also on other extrinsic parameters in a dynamic system (Ying *et al.*, 2015). However, a general acceptance of nano-QSAR models is still being debated because there are questions as to whether sufficient data will be available in short-term perspectives (Gajewicz, 2017). Consensus models may improve the accuracy of individual models, however, as shown by Roy *et al.* (2018).

When examining data availability, many of the last decade's *in vivo* studies on the ecotoxicological effects of ENMs have described different parent materials and nano-forms. Meta-analyses of these studies, performed using species sensitivity distributions, have shown that the range of ecotoxicological values can span up to six orders of magnitude, depending on the nano-forms and test organisms involved (Wigger and Nowack, 2019; Gottschalk *et al*., 2013; Wang and Nowack, 2018). This large variability may be attributed to the varied exposure conditions and nano-forms used in the experiments. For instance, Wigger and Nowack (2019) showed that the predicted no-effect concentrations for nano-TiO_2 varied by up to a factor of six when different crystal forms (i.e., anatase and rutile) were considered. Furthermore, the relevance of intrinsic material properties is stressed by the fact that anatase is known to be more photoreactive than rutile. Anatase is therefore used primarily in photocatalytic applications (e.g., air and water purification), whereas rutile is employed in applications requiring photostability (e.g., sunscreens) (Wigger and Nowack, 2019). Anatase can produce ROS in sunlight, causing several detrimental effects to organisms, such as cell injury and oxidative damage (Jovanović, 2015). This large variability in ecotoxicological values triggered our question about whether it is possible to predict ecotoxicological effects based on the intrinsic properties of different nano-forms and the exposure conditions (extrinsic properties) provided in published studies. Since nano-TiO_2 is one of the most widely used ENMs, with a production volume of approximately 10,000 metric tons per year in Europe (Sun *et al.*, 2014), it is also one of the most often-tested ENMs. Consequently, the data available on nano-TiO_2 was expected to be one of the most extensive, helping to answer our research question.

The present study therefore aimed first to compile an ecotoxicological dataset for nano-TiO_2 based on published research and then to assess the quality of the data points collected. The species with the most reported ecotoxicological values was selected to identify the most relevant intrinsic (primary particle size, crystal composition) and extrinsic parameters (exposure duration, UV and non-UV illumination, concentrations of divalent cations) driving ecotoxicological effects. We considered the intrinsic and extrinsic properties of nano-TiO_2 because they are interlinked and either might trigger the observed effect.

Material and Methods

The meta-analysis was based on a three-step analysis of the nano-TiO_2 case study. To build an ecotoxicological database for nano-TiO_2, we first identified relevant studies by using a keyword-based query on the Web of Science (WoS) platform. Second, parameters relevant to nano-TiO_2 toxicity were determined, and the quality of the database was evaluated. Only data points that fulfilled our quality criteria were considered. Third, we applied both single correlation and multiple linear regression (MLR) models to the selected dataset to identify relevant parameters and enable the prediction of nano-TiO_2 toxicity. MLR results were evaluated statistically using T- and F-tests. Additionally, we applied a one-factor-at-a-time (OAT) sensitivity analysis to identify the most sensitive parameters in the MLR prediction models.

Data collection for nano-TiO_2

Studies published before 2014 were taken from Coll *et al.* (2016), who provided a complete listing of the published ecotoxicological *in vivo* studies on nano-TiO_2. Based on this study, a literature search was conducted in the WoS to identify studies published after 2014. We used the query "(nano* AND toxic* AND titanium *oxide) OR (nano* AND toxic* AND titania) OR (nano* AND toxic* AND TiO_2) NOT (in vitro)", which was modified from the keywords used by Juganson *et al.* (2015). A total of 3,444 records were revealed for the period from January 2014 to December 2018, but only 41 papers were identified as relevant due to false-negative and false-positive hits. We considered toxicological studies on organisms that were exposed to nano-TiO_2 in freshwater, soil, and sediment, focusing on the endpoints of mortality, growth, reproduction, and change in significant metabolic processes (e.g., photosynthesis), as per the approach proposed by Coll *et al.* (2016). Finally, 96 publications—the 41 papers from the WoS search and 55 publications taken from Coll *et al.* (2016)—were considered as relevant during the timeframe from 1990–2018.

The data points were extracted using the approach developed by Coll *et al.* (2016). To avoid a bias towards studies reporting more than one data point, only one endpoint concentration was collected from each experimental study, as an EC_{50} calculated based on the nominal concentration. If a study reported several endpoints, only those considering different nano-forms of TiO_2, exposure media, or illumination conditions were selected. We omitted the effective dose, as this was not calculated statistically based on a dose-response curve. In all, 219 data points were extracted from 96 publications, including 205 data points for the freshwater compartment and 14 for the soil compartment. *Daphnia magna* (*D. magna)* was the most frequently reported species, with 65 data points in 28 studies (Table 1). We later excluded the soil compartment from the assessment due to the limited number of studies.

Table 1. Number of publications and data points collected.

Overall	Environmental compartment	Species
96 studies (n = 219)	Freshwater: 85 studies (n = 205)	*D. magna*: 28 studies (n = 65) *Pseudokirchneriella subcapitata*: 7 studies (n = 14)
	Soil: 11 studies (n = 14)	*Eisenia fetida*: 4 studies (n = 4) Soil microbial community: 3 studies (n = 3)

Quality evaluation of the experimental studies identified

To evaluate the preselected studies and establish a comprehensive, high-quality database for nano-TiO_2, we used the DaNa2.0 criteria catalogue (DaNa, 2016), which recommends a minimal set of parameters that should be reported in experimental studies (Table 2). This catalogue was also used for evaluating the quality of the OECD's nano-silver dossier (Schmutz *et al.*, 2017). Its minimal set of parameters not only ensures that a particular study is understood but also that its experimental results are evaluated with regard to their quality.

Table 2. Parameters considered for checking the quality of the studies collected, modified from DaNa (2016).

Category	Parameters
Characterization	pristine form: manufacturer, purity, composition, primary particle size, specific surface area
	in an aqueous system: medium, concentration, hydrodynamic diameter, zeta potential
Sample preparation	medium of the stock suspension, dispersion method
Testing parameters	protocols, duration, illumination conditions, pH, charged ions, natural organic matter, conductivity
Toxicity descriptor	endpoint concentration

Since *D. magna* had the greatest amount of data available, later assessment focused solely on this species. Additionally, *D. magna* plays an important role in regulatory chemical testing, has been included in several international standards (OECD, 2004; OECD 2012; ISO, 2012), and is therefore commonly used in toxicological tests on ENMs. Regarding exposure media, standard testing guidelines recommend the use of the OECD medium (OECD, 2004) or reconstituted water (ISO, 2012), without natural organic material (NOM), in which *D. magna* is then exposed to the substance being tested in suspension. Depending on the study aims, experimental duration is usually set to 48 hours for an acute test (OECD, 2004) and to 21 days for a chronic test (OECD, 2012). The OECD acute test guideline also recommends a cycle of 16 hours of light and 8 hours of dark, although without indicating light intensity. The chronic test guideline proposes 16 hours of illumination with an intensity below 15–20 $\mu E \bullet m^{-2} s^{-1}$.

The quality of the experimental studies on *D. magna* was investigated, and results are shown in Table 3. The most frequently reported parameters, in more than 95% of the 65 data points, were exposure duration, the manufacturer's name, and primary particle size. The medium's electrical conductivity was the least reported parameter, available in only 20% of studies. It should be noted that this relative share, even though at a high percentage, fails to reflect the fact that none of the studies (65 data points out of 28 studies) reported all of the parameters of the criteria in list in Table 3. These omissions in the reporting of nano-TiO_2's intrinsic properties and exposure conditions made the consideration of all the relevant parameters for constructing predictive models of ecotoxicological effects more difficult.

Table 3. The quality parameters tested and the percentage rate of reporting in the nano-TiO_2 dataset for D. magna (65 data points from 28 studies).

Category	Parameters	Percentage
Material characterization	Manufacturer	97%
	Purity	54%
	Crystal composition	91%
	Primary particle size*	97%
	Specific surface area	74%
Material characterization in aqueous suspension	Test medium	83%
	Test concentration	65%
	Hydrodynamic diameter	88%
	Zeta potential	57%
Sample preparation	Medium for the stock suspension	85%
	Dispersion method for preparing the aqueous suspension	83%
Experimental condition	Exposure duration	100%
	Protocols	74%
	Illumination	78%
	pH	85%
	Composition of exposure medium/charged ions	92%
	Natural organic matter	95%
	Conductivity	20%
Endpoint	Quality (i.e., statistical analysis)	66%

*When the size of the primary particle being measured was available, this was preferred. Otherwise, the nominal size was considered. However, both cases were considered to be *reported*.

Feature selection, data harmonization, and data selection

To compile a high-quality dataset from the limited number of available data points, we had to determine the most critical features. Omitting relevant parameters could not be avoided due to low amounts of data. We oriented our approach around the appendix of REACH guideline R6.1 (ECHA, 2017), which recommends twelve key physicochemical parameters that should be considered in QSARs, grouping, and read-across concepts (Table 4). Even though zeta potential is a relevant parameter, it could not be considered due to insufficient available data. We therefore included the sum of the concentrations of Ca^{2+} and Mg^{2+} in mg/L as an alternative parameter closely related to zeta potential and affecting particles' colloidal properties. Ca^{2+} and Mg^{2+} are the two most abundant divalent cations in the standard medium used for the *D. magna* toxicity test (OECD, 2004; EPA, 1994), and they are known to strongly affect the agglomeration behavior of ENMs. Besides the parameters listed in Table 4, pH and the concentration of NOM are often regarded as two of the most influential factors on the toxicological effects of nano-TiO_2. However, we had to omit the pH value because it was mostly reported as a range over the entire experiment. Together with the relatively small range of pH-values reported (pH 6.5–8.6), a consideration of pH would have introduced too much uncertainty into our assessment. Moreover, we also attempted to include NOM in our initial assessment as it is known to be the main driver for agglomeration processes in aquatic media (Zhang *et al*., 2009; Wormington *et al*., 2017). However, it was impossible to include

NOM because only one of the 28 studies on *D. magna* was carried out in the presence of NOM. Standard test guidelines do not require the use of NOM in *D. magna* tests (OECD, 2004). Furthermore, our assessment included the duration of exposure because a longer exposure time affects the total exposure of organisms to stressors (Rozman, 2000).

Table 4. Feature selection based on the ECHA list (ECHA, 2017).

Category	ECHA list	Selection	Comment
Chemical parameters	Crystalline structure	Yes	The percentage of anatase was selected.
	Impurities	No	The reporting rate was relatively low (54%), and 70% of these studies reported purities above 99%.
Physical parameters	Particle size	Yes	Primary particle size was preferred over hydrodynamic diameter since the latter was determined in different media at various concentrations and time points, and these were incomparable between studies.
	Shape	No	Nano-TiO_2 was spherical in all the studies selected.
	Surface area	No	The reporting rate was relatively low (74%); surface area is implicitly included in the particle size parameter.
Behavior	Solubility	No	Nano-TiO_2 has low solubility in water.
	Hydrophobicity	No	Not available in the studies selected.
	Zeta potential	No	Despite being a relevant parameter, it was not regularly reported (54%).
	Dispersibility	No	Hardly reported.
	Dustiness	No	This parameter is not relevant for aquatic exposure.
Reactivity	Biological/surface reactivity	No	Normally not reported in the studies selected.
	Photoreactivity	Yes	Illumination conditions were considered.

The six features of primary particle size, crystal composition, UV and non-UV illumination conditions, exposure duration, and the presence of cations in the exposure medium were selected as the input values for the meta-assessment. Twenty-three data points for *D. magna* reported on all these parameters and were consequently used in our assessment for predicting nano-TiO_2's potential toxicity towards *D. magna*.

Preparing and transforming the database

The studies selected reported illumination conditions differently, which required data harmonization. Most reported one of three illumination conditions: simulated solar radiation (SSR), laboratory light, and darkness. The energy received on the test system's surface was determined since it is one of the most important factors influencing the toxicity of photocatalytic materials such as nano-TiO_2. To compare the energy received from different light sources, units of energy were converted into mWh/m^2 using Equation (1), where *I* is the irradiance [mW/m^2], *t* is the duration [h], and *E* [mWh/m^2] is the energy received at the water's surface.

$$E = I * t \qquad Equation\ (1)$$

Because UV radiation contains more energy than non-UV radiation, the total energy of (light) irradiation was broken into two parts: UV (260–400 nm wavelengths) and non-UV (400–800 nm wavelengths). Assumptions were then made to estimate the energy received from UV or non-UV light (See SI). Briefly, we estimated the share of UV and non-UV light from SSR according to the spectral power distribution provided by the company which provided the test chamber (Q-Lab, 2011). Moreover, for experiments that were conducted under ordinary laboratory conditions, the use of non-UV light irradiance was assumed according to the OECD guideline (OECD, 2012).

Multiple linear regression analysis

Nano-TiO_2's EC_{50} was predicted using a multiple linear regression (MLR) model of six selected features. MLR was used to assess each feature's influence on toxicity, as shown in Equation (2):

$$\mathrm{EC}_{50} = INT + a * ANA + b * SIZE + c * UV + d * nonUV + e * Time + f * CAT \qquad Equation\ (2)$$

Where:

$\boldsymbol{INT}$: *Intercept [-]*

$\boldsymbol{ANA}$: *Percentage of anatase [%]*

$\boldsymbol{SIZE}$: *Primary particle size [nm]*

$\boldsymbol{UV}$: *Energy received from UV light at the water surface [mWh/cm^2]*

$\boldsymbol{nonUV}$: *Energy received from non-UV light at the water surface [mWh/cm^2]*

$\boldsymbol{Time}$: *Duration of exposure [h]*

$\boldsymbol{CAT}$: *Concentration of cations [mg/L]*

All the MLR models were built using the "Fitting linear models" function in R software (version 3.4.3) (Team, 2018). The algorithm's output is the sum of the weighted variables. Three MLR models were built for three datasets: M-Full for all of the data points (n = 23); M-UV for data points with UV illumination (n = 9); and M-non-UV for those without UV illumination (n = 14). The models' input values can be found in Table S3. Finally, statistical T- and F-tests were performed to determine the significance of the model and each parameter.

Sensitivity analysis

Six independent parameters drove the model to predict the toxicity of nano-TiO_2. An OAT sensitivity analysis was performed to evaluate each parameter's influence on the MLR results. This analysis was done by varying a single parameter by ± 10% while keeping the other inputs at their mean values. The percentage change of output *y* as a function of the changing input *x* was calculated using Equation (3):

$$\text{Change in } \% = \frac{y_{x\pm 10\%} - y}{y} * 100\% \qquad Equation\ (3)$$

Results and discussion

Correlation of single parameters with the EC_{50}

To assess the influence of the six selected features on the EC_{50}, we first plotted each parameter against the EC_{50}, as shown in Figure 1. Datasets were divided into two groups based on the type of illumination applied during the experiments. Experiments run under UV light tended to result in a lower EC_{50}. The other intrinsic (percentage of anatase, primary particle size) and extrinsic parameters (exposure duration, non-UV light irradiance, concentration of cations in the medium) demonstrated no clear correlation with the EC_{50}. However, in each independent parameter evaluation, two main clusters formed linked to the UV and non-UV datasets. This led us to the conclusion that UV light was the main factor affecting the toxicity of nano-TiO_2. Variations in all the other parameters showed no correlations with the EC_{50}. To improve the assessment, we applied the MLR method to simultaneously evaluate all the selected features influencing the EC_{50}.

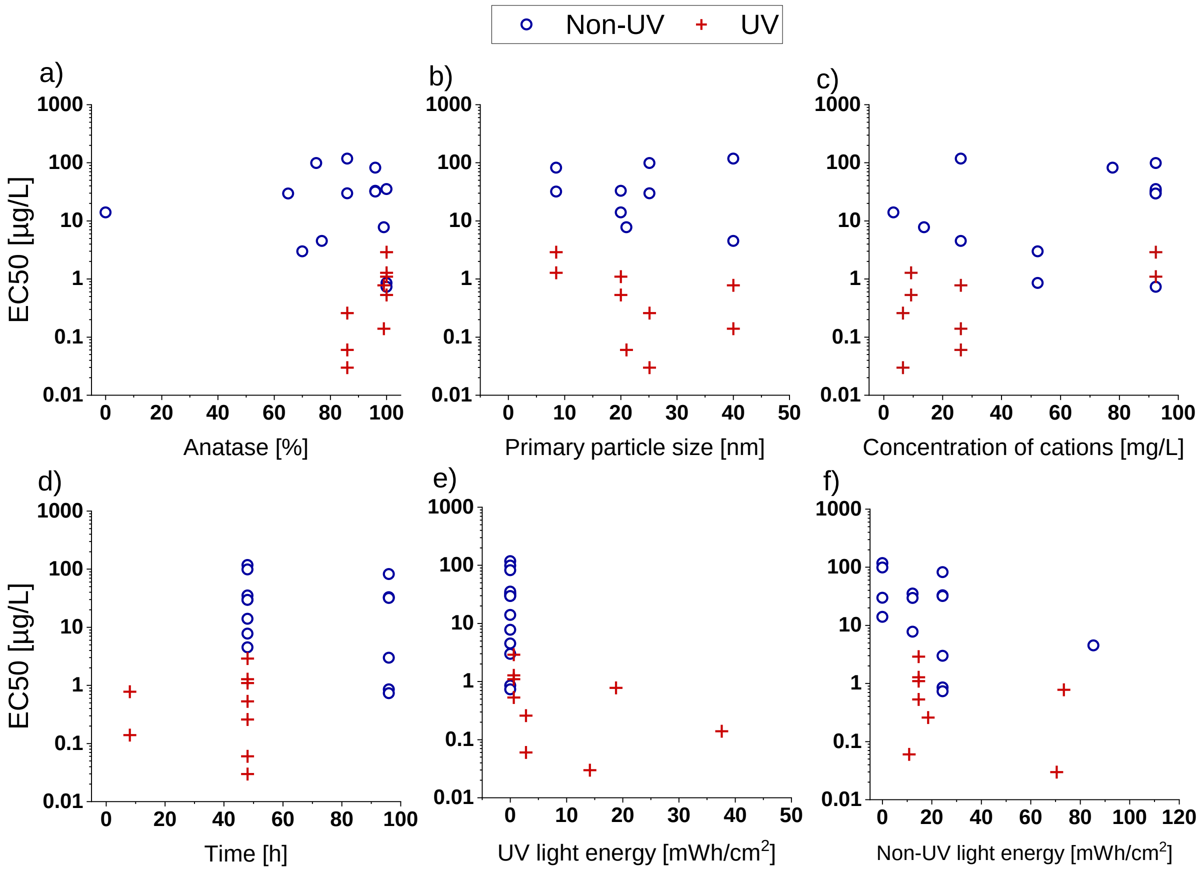

Figure 1. Correlation plots between single parameter evaluations and the reported EC_{50}: a) share of anatase; b) primary particle size; c) concentration of cations; d) experiment duration; e) energy received from UV illumination; f) energy received from non-UV light. EC_{50} was calculated based on the nominal concentration.

Multiple linear regression models and sensitivity analysis

Three MLR models (M-full, M-UV, and M-non-UV) were built for the datasets including the six selected features, and results are shown in Figure 2, Table 5, and Table S3. The MLR obtained a correlation coefficient (R^2) of 0.29 for the complete dataset, indicating an insignificant correlation. This was confirmed by the F-test, resulting in a

p-value of 0.41. This low significance is depicted in Figure 1, where most of the parameters demonstrated no clear correlation with the EC_{50}. It is also depicted in Figure 2a, showing two main clusters that represent the non-UV (blue) and UV (red) datasets.

The UV and non-UV datasets were then analyzed separately, resulting in a very good R^2 value of 0.95 for the UV dataset (Figure 2b) and a lower R^2 of 0.55 for the non-UV dataset (Figure 2c). The F-test returned p-values of 0.15 and 0.19 for the M-UV and M-non-UV models, respectively. This means that although the R^2 and p-values were better than those of the M-Full dataset, none of the models was statistically significant at $p < 0.05$. Nevertheless, in the context of predictive hazard modeling, an $R^2 \geq 0.64$ has been suggested as good for *in vivo* data analysis within QSARs (Puzyn *et al.*, 2011).

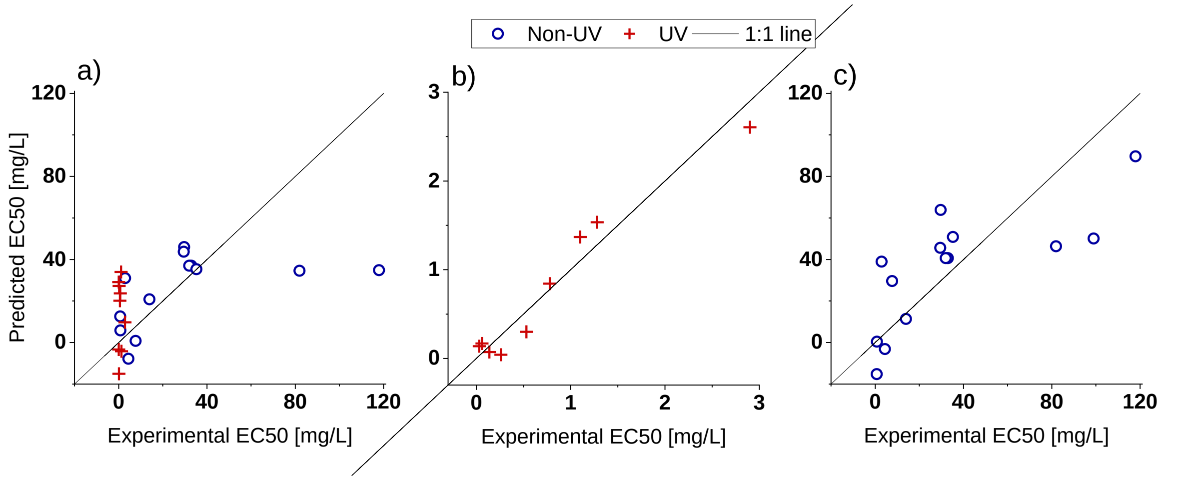

Figure 2. Multiple linear regression models: a) Full for the full dataset (n = 23); b) UV for the UV dataset (n = 9); and c) non-UV dataset (n = 14). The black line is the 1:1 line and not a fit to the data.

Table 5. Parameters calculated for the three M-full (full dataset), M-UV (UV dataset), and M-non-UV (non-UV dataset) models based on the multiple linear regression (MLR) method and the F-test.

Model	INT [-]	ANA [%]	SIZE [nm]	TIME [h]	UV [mWh/cm²]	non-UV [mWh/cm²]	CAT [mg/L]	R^2	*p*
M-Full	-51	0.22	2.1	0.38	1.6	-0.95	0.17	0.29	0.41
M-UV	12	-0.05	-0.11	-0.10	-0.33	0.07	0.01	0.95	0.15
M-non-UV	-145	1.3	5.1	0.63	-	-2.1	-0.39	0.55	0.19

Abbreviations: intercept (INT); percentage of anatase (ANA); primary particle size (SIZE); energy received from UV (UV); energy received from non-UV light (non-UV); concentration of cations (CAT); correlation coefficient (R^2); P-value in the F-test (P).

An OAT sensitivity analysis was applied to the three MLR models and the relative output changes calculated are shown in Table 6. The values indicate the changes in the results (i.e., EC_{50}) if one parameter is varied by ±10% at a time. A negative value indicates a decrease in the EC_{50} (i.e., an increase in toxicity) with an increase in input. All the MLR models showed symmetrical behavior in their sensitivity analysis, whether single parameters were increased or decreased. Nevertheless, parameters showed different sensitivities depending on the model investigated. The primary particle size was the most sensitive parameter in the M-Full model, whereas the relative share of anatase was the most sensitive feature in the M-UV and M-non-UV models. The concentration of cations was predicted to have the lowest sensitivity in all three models.

Table 6. Results of the sensitivity analyses for the six parameters in the Full, UV, and non-UV multiple linear regression (MLR) models. The table only shows the relative change in y when increasing x by 10%. The full sensitivity analysis can be found in Table S4.

Model	ANA [%]	SIZE [nm]	Time [h]	UV [mWh/cm²]	non-UV [mWh/cm²]	CAT [mg/L]
M-full	8.7%	20%	10%	2.6%	-12%	4.0%
M-UV	-65%	-32%	-51%	-37%	40%	5.4%
M-non-UV	29%	28%	12%	-	-11%	-7.2%

Abbreviations: percentage of anatase (ANA); primary particle size (SIZE); energy received from UV (UV); energy received from non-UV light (non-UV); and concentration of cations (CAT).

Predicting nano-TiO_2's toxicity to D. magna

We observed the interesting fact that the separate models for the UV and non-UV datasets demonstrated considerably better performance than the model for the M-Full dataset combining both the other complete datasets. In general, nanoparticle toxicity was reported to correlate with physicochemical parameters nonlinearly, as did the relative share of anatase (Marcone *et al.*, 2012) and the primary particle size (Wyrwoll *et al.*, 2016). Using linear models to predict toxicity may therefore lead to inaccurate results. However, our assessment showed that the UV dataset could acceptably predict toxicity towards *D. magna* with an R^2 of 0.95. The main reason that the UV dataset performed better than the other two models was the thorough data preparation and thus the exclusion of data points that had been evaluated under non-UV conditions. This was also supported by the sensitivity analysis, which showed both the relevance of UV exposure and the share of anatase triggering adverse effects. Another reason for UV dataset's better fit could be that the M-UV model only comprised photocatalytic nano-TiO_2, whereas the M-non-UV model included different nano-forms of TiO_2. The share of anatase used in our assessment does not seem to be the best predictor for the crystal form of a diverse set of nano-TiO_2 particles.

M-UV dataset

Most of the parameters predicted by the MLR for the M-UV dataset had a negative correlation with the reported EC_{50}, suggesting that an increase in any one parameter increased toxicity. Two exceptions were the energy received from non-UV light and the concentration of cations, which were positively correlated with the EC_{50}. Furthermore, the MLR's results and the sensitivity analysis suggested that intrinsic and extrinsic properties exhibited different influences on the overall toxicity to *D. magna*.

Anatase content showed the highest sensitivity in the M-UV model, with -65%, although this parameter was less sensitive in the non-UV dataset (29%). This result seems reasonable because UV exposure and the share of anatase are crucial for the production of ROS (Jovanović, 2015). Nano-TiO_2 is known for its synergistic photocatalytic effects that crystalline mixtures of anatase and rutile generally outperform pure crystal forms with regard to their photoactivity. Consequently, anatase–rutile mixtures increase toxicity due to their greater production of free radicals during UV illumination (Pfeifer *et al*., 2013; Luttrell *et al*., 2014). The study by Wigger and Nowack (2019) also concluded that P25/NM105, with an anatase share of approximately 80%, exhibited greater toxicity than the pure anatase or rutile. However, we did not observe this effect from the MLR model which might due to the limited anatase/rutile mixtures data in our study (either 80% or 100% shares of anatase, see Figure 1a).

The exposure duration was the second most sensitive parameter (-51%) in the M-UV dataset. The model predicted a decreasing trend for the EC_{50} as exposure time lengthened, which is in accordance with the current knowledge about how exposure duration affects the substance's total exposure (Rozman, 2000): longer exposure duration leads to higher cumulative exposure resulting in higher toxicity.

Regarding the percentage of anatase and exposure duration, both UV light and non-UV light energy showed lower sensitivity in the M-UV dataset, at -37% and +40%, respectively. The M-UV model predicted that nano-TiO_2's toxicity to *D. magna* increased with higher UV light energy. This is not only in agreement with the observation in Figure 1e but also in line with results from several experimental studies (Ma *et al*., 2012; Li *et al*., 2014; Clemente *et al*., 2014). On the other hand, it was surprising that UV light and non-UV light energy exhibited similar parameter sensitivity. Non-UV light energy was expected to have a lower influence on toxicity than UV light since the spectrum of non-UV light does not have enough energy to excite electrons from the valence band, causing radicals. The reason for this unexpected result is likely related to the data harmonization, which assumed the use of the standard UV-lamp spectrum in cases of missing information. Ideally, as Haynes *et al.* (2017) pointed out, researchers should use specific programs to calculate the actual light field present at the organism's surface, which is usually influenced by the composition of the natural media. However, the studies available did not provide the required information with which to apply a more sophisticated approach to calculating the true UV-illumination on the organism.

Primary particle size also showed low sensitivity, at 32%, although it is commonly assumed that particle size matters a lot in the context of ecotoxicological effects (Seitz *et al.*, 2014; Wyrwoll *et al.*, 2016). The M-UV model predicted that the EC_{50} decreased with higher particle sizes (Table 5). Indeed, many studies have reported that the toxicity of nano-TiO_2 may not decrease linearly with bigger primary particle size (Wyrwoll *et al*., 2016; Metzler *et al*., 2011). Nano-TiO_2 particles sized between 20 nm and 30 nm exhibited the highest toxicity to *D. magna* in the experiment by Wyrwoll *et al.* (2016). As mentioned before, hydrodynamic diameter would be a more appropriate parameter, representing the actual stressor on the organism, including potential transformations (e.g., agglomeration) of ENMs in their respective media (Maguire *et al.*, 2018). However, this parameter was only available in a small subset of studies and could not be included in our meta-analysis.

Finally, the concentration of Ca^{2+} and Mg^{2+} was the least sensitive parameter, suggesting their relatively low influence on the EC_{50} in this medium. The EC_{50} values were predicted to be positively correlated with the concentrations of the two divalent cations, meaning that higher concentrations of cations would result in lower toxicity. This result is in agreement with the fact that divalent cations lead to a higher likelihood of agglomeration/aggregation, which reduces the intracellular uptake of ENMs by organisms and thus subsequent toxicity (Zhang *et al*., 2009; Tan *et al*., 2017).

M-non-UV dataset

The results from the M-non-UV model exhibited a lower correlation coefficient ($R^2 = 0.55$) than those of the M-UV model. Surprisingly, the mathematical terms for the exposure duration and cation concentration parameters had values opposite to those expected. For instance, a longer exposure time led to lower toxicity, which was the opposite of the expected outcome. We analyzed the dataset in detail in order to find an explanation for this result. The M-non-UV dataset included more studies, different nano-forms of TiO_2 (i.e., crystal forms), and experiments with different exposure duration and illumination conditions (i.e., darkness). Consequently, the results from the non-UV dataset included greater variability due to the addition of heterogeneous data from different studies. For instance, it has been shown that different crystalline phases of anatase and rutile can have antagonistic and/or additive effects (Li *et al*., 2017; Iswarya *et al*., 2015), which is why the consideration of different crystal forms may have been conflicting in this regard. However, removing rutile data points did not significantly improve the results from the M-non-UV dataset.

Limitations and challenges

The present study started with a broad search for all the published studies addressing the toxicity of nano-TiO_2 on living organisms in several environmental compartments, focusing on the endpoints of mortality, growth, reproduction, and changes in significant metabolic processes. We found 219 data points covering all species and 65 data points for *D. magna*. Due to reasons of data availability and quality, only 23 of the 65 data points could be used in the final model. This suggests that although many studies have been published with a focus on the toxicity of nano-TiO_2, high-quality data with a complete characterization of materials and experimental conditions used are still scarce. Indeed, our study has revealed the current challenges related to reporting on time-dependent parameters that change dynamically throughout an experiment. For instance, hydrodynamic diameter is usually regarded as an important parameter influencing the toxicological behavior of nano-TiO_2 in aqueous suspensions. It is a better descriptor than primary particle size since the hydrodynamic diameter is the actual size of the particle to which an organism is exposed. However, the studies selected determined the hydrodynamic diameter in different media at various concentrations and time points before or during the experiment, which made them incomparable. Another example was pH, which is often reported as a range throughout an experiment without indicating the time of measurement: it thus could not be considered in the model. These missing descriptors influenced the predictive model's accuracy. Coherent approaches for measuring and reporting hydrodynamic diameters and pH throughout experiments are urgently needed. These parameters should be monitored and recorded continuously during the exposure experiment in the test medium and be parts of the published article.

Moreover, for some frequently reported parameters, descriptions lack important details regarding the experimental set-up. Thus assumptions were necessary for their consideration in our study. For example, illumination conditions were often reported as types of illumination (UV, non-UV) with their corresponding duration, but the type and spectra of the UV exposure were not reported. We thus assumed the use of a commonly used UV lightbulb, which introduced additional uncertainty to one of the most crucial parameters. A similar problem was found for studies carried out without UV illumination. Although the OECD acute test guidelines for *D. magna* mention the requirements for illumination (OECD, 2004), they do not specify how much energy the organism should be exposed to. Actual non-UV light intensities during experiments were rarely reported, and we had to estimate this parameter based on the standard conditions in the chronic test guidelines for *D. magna* (OECD, 2012). We acknowledge that this might have been the source of additional uncertainties but disregarding such data points would have decreased our small dataset even further. The relevance of the experimental setup and reporting were emphasized by Haynes *et al.* (2017) and Jovanović (2015). Future experimental studies (and test guideline revisions) should address these issues, especially for photocatalytic materials, and they should report illumination conditions in detail to enable comparisons between studies.

Furthermore, most of the ecotoxicological studies in our assessment only reported nominal concentrations of the nanomaterial that were either calculated at the beginning or during the experiment at specific points in time. However, the nominal concentration is not the actual concentration to which the organism is exposed. Therefore, measured concentrations would be better descriptors of ecotoxicological effects, and we can only encourage other authors to report on this in future articles.

There are three additional aspects which make environmental hazard assessments more difficult with regards to the availability of data and the nano-forms tested. Firstly, we could not include NOM in the model due to the lack of sufficient studies conducted in the presence of NOM. The addition of NOM has generally been found to reduce nano-TiO_2 ecotoxicity due to the reactive oxygen quenching process (Li *et al.*, 2016). Secondly, the majority of the data available related to pristine nanomaterials, which are likely not present under relevant environmental conditions (Maurer-Jones *et al*., 2013; Nowack *et al*., 2016). It is known that the ENMs released from products into the environment may transform there, and their transformation may affect the composition of the materials measured (Nowack *et al.*, 2016). Also, since ENMs are often incorporated into products, they can be released as ENMs bound in a matrix (Froggett *et al.*, 2014). Future ecotoxicological studies should try to represent the realistic forms of ENMs found in the environment. The need for predictive approaches is highlighted by the fact that there are continuously new nano-forms which need to be tested. Thirdly, the available data are heterogeneous and it is therefore challenging to compare them as many of their parameters are defined as ranges, which may differ significantly between studies despite standard procedures being followed (Park *et al.*, 2018).

Finally, it should be kept in mind that the model presented in this study only addressed the effects of one photocatalytic, non-soluble ENM on one invertebrate. Thus the model may not apply to non-photocatalytic or soluble ENMs. The study's results are nevertheless relevant to other freshwater species despite their focus on one species. Although *D. magna* is a model organism and a good indicator of toxicity, it can only provide a partial insight into broader ecotoxicity. Other species may be less prone to ENMs and results may not be transferrable to higher organisms such as fish or the organisms living in the other environmental compartments. Nevertheless, the challenges requiring the development of a coherent approach to measuring and reporting some of the parameters critical to nano-TiO_2 ecotoxicity, as revealed by this study, are likely also true for other ENMs and species.

Conclusion and outlook

Computational hazard assessments using QSARs can lead to the prediction of adverse effects due to exposure to ENMs. Streamlining hazard assessments could obviate the need for case-by-case examinations. To date, such models have mainly focused on datasets that included different parent ENMs and nano-forms to identify potential descriptors for the prediction of toxicity in similar ENMs. The present study attempted to predict ecotoxicity by using an *in vivo* dataset focusing on one nanomaterial—nano-TiO_2—and the freshwater organism, *Daphnia magna*. We considered the intrinsic and extrinsic parameters of the experimental setup and applied a multilinear regression analysis. Our work was partially successful, as it used the available data to elucidate the influence of selected intrinsic (primary particle size, crystal composition) and extrinsic parameters (exposure duration, UV and non-UV illumination, concentrations of divalent cations), especially for the M-UV dataset, which demonstrated a correlation coefficient of 0.95. Our results revealed that UV illumination was the most important factor in determining the toxicity of nano-TiO_2, which is also influenced by other factors such as crystal form, primary particle size, exposure duration, and the concentration of cations. The biggest challenge to the development of predictive models is the availability of high-quality datasets. Although hundreds of studies have been published to address the toxicity of nano-TiO_2, those that have reported data on a broad, coherent set of parameters are still scarce. Future studies should develop a coherent approach to measuring time-dependent parameters throughout the experiment, provide a complete description of the illumination conditions applied, especially for photocatalytic materials, and report measured exposure concentrations rather than nominal concentrations.

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