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Freshwater ecotoxicity characterisation factor for metal oxide nanoparticles: a case study on titanium dioxide nanoparticle

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Abstract

The Life Cycle Assessment (LCA) methodology is widely applied in several industrial sectors to evaluate the environmental performance of processes, products and services. Recently, several reports and studies have emphasized the importance of LCA in the field of engineered nanomaterials. However, to date only a few LCA studies on nanotechnology have been carried out, and fewer still have assessed aspects relating to ecotoxicity. This is mainly due to the lack of knowledge in relation on human and environmental exposure and effect of engineered nanoparticles (ENPs). This bottleneck is continued when performing Life Cycle Impact Assessment, where characterization models and consequently characterization factors (CFs) for ENPs are missing. This paper aims to provide the freshwater ecotoxicity CF for titanium dioxide nanoparticles (nano-TiO₂). The USEtox™ model has been selected as a characterisation model. An adjusted multimedia fate model has been developed which accounts for nano-specific fate process descriptors (i.e. sedimentation, aggregation with suspended particulate matter, etc.) to estimate the fate of nano-TiO₂ in freshwater. A literature survey of toxicity tests performed on freshwater organism representative of multiple trophic levels was conducted, including algae, crustaceans and fish in order to collect relevant EC₅₀ values. Then, the toxic effect of nano-TiO₂ was computed on the basis of the HC₅₀ value. Thus, following the principle of USEtox™ model and accounting for nano-specific descriptors a CF for the toxic impact of freshwater ecotoxicity of 0.28 PAF day m³ kg⁻¹ is proposed.

1. Introduction

The advent of nanotechnology is considered as one of the greatest innovations since the beginning of industrial engineering (Som et al., 2010). Nanotechnology is used in a rapidly increasing number of products available to industries and private consumers: electronics, cosmetic, nutrition, medical drug

designing and other. The term engineered nanoparticles (ENPs) refers to a subset of nanomaterial which is a material with at least one external dimension in the size range from 1 and 100 nm (ISO, 2008).

The increasing use of ENPs in consumer and industrial products has also increased the concerns on their adverse effect on human health and ecosystems (Alvarez et al., 2009; Klaine et al., 2012), Therefore considerable effort has been made to assess the impacts of ENPs to humans and the environment. Among the several tools available, the European Commission encouraged life cycle-based methods to assess the sustainability of nanotechnology (UNEP, 2011). Life cycle assessment (LCA) is regulated by the international standards ISO 14040 series (ISO, 2006 a,b). LCA has been proposed and adopted as essential tool to analyse, evaluate, understand and manage the environmental and health effects of the ENMs (Hischier, 2014). Despite all, the LCA studies often do not cover the complete life cycle of ENMs (Hischier and Walser, 2012; Gavankar et al. 2012; Miseljic and Olsen, 2014). Hence, most of the studies are cradle-to-gate and the environmental impacts primarily reflect the energy and material flows for the extraction of raw materials and manufacturing phases, whereas the environmental impacts related to the release of ENPs into the environment are rarely assessed. This is due to gaps in knowledge concerning both the release of ENPs (Life Cycle Inventory) and their potential effects on the environment and humans (Life Cycle Impact Assessment). The latter of these is expressed by the so-called eco/toxic characterisation factors (CFs) describing and quantifying the cause-effect chain of an emission of a substance to the environment. The development of freshwater-ecotoxicity CFs for ENPs is still in its infancy due to the scarce knowledge of the exposure and effects to aquatic organisms. More fate and transport models (F&T) to assess the concentration of ENPs in the environmental media are yet to emerge (Liu and Cohen, 2014; Gottschalk et al., 2010; Gottschalk et al., 2013). It is evident that the lack of ecotoxicity CF of ENPs impedes the evaluation of ecotoxicological impacts caused by their emissions into the environment. As far, only two studies have calculated the freshwater- and seawater-ecotoxicity CF of ENPs: Eckelman et al., 2012 and Walser et al., 2012 calculated the CF for carbon nanotubes (CNT) and silver nanoparticles, respectively. Many metal containing materials, particularly metal oxides, belong to the class of ENPs: zinc oxide (ZnO), titanium dioxide (TiO₂), cerium dioxide (CeO₂), chromium dioxide (CrO₂), molybdenum trioxide (MoO₃), bismuth trioxide (Bi₂O₃) and binary oxides such as, lithium Cobalt dioxide (LiCoO₂), indium tin oxide (InSnO) (Bhatt and Tripathi, 2011). The estimated worldwide production of nano-TiO₂ is 3,000 tons/year for 2010 (Piccinno et al., 2012). The environmental n-TiO₂ release into aquatic system can occur through wastewater treatment plant effluents, from exterior facades or accidents during transport (Gottshalck F, Scholz RW, Nowack B, 2010) This study proposes a method for calculating the freshwater-

ecotoxicity characterization factors of metal oxide ENPs. In particular, nano-TiO₂ ENPs have been chosen as representative substance based on their extensive application, the availability of data regarding their ecotoxicological effect and on their environmental behaviour.). Based on the USEtox™ model and on the multimedia fate models for organic chemicals, this paper develops an adjusted model which includes nano-specific descriptions to estimate the fate of nano-TiO₂ in freshwater and thereby calculates a CF for freshwater ecotoxicity.

2. Materials and methods

2.1 Characterization model

Several characterization models are available for the ecotoxicity impact category and often the final results vary substantially amongst the models. It must be noted that the quantification of the ecotoxicity impacts is one of the most debatable items in LCA (Hischier and Walser, 2012). The variability in model outcomes has been reduced thanks to the USEtox™ model, recently developed by an international collaboration of leading LCIA specialists (Rosenbaum et al., 2008). The USEtox™ provides CFs for organic and inorganic substances for both human toxicity and aquatic freshwater ecotoxicity. However, it does not yet consider marine ecosystems or sediments (where sedimentation is considered a removal process). The International reference for Life Cycle Data System (ILCD) Handbook (JRC-IES, 2011) recommends using USEtox™ to model impacts related to ecotoxicity and human toxicity. Thus, in this study, the USEtox™ model has been selected as characterisation model and its framework has been applied to calculate the CF for n-TiO₂. Since it was developed for organic chemicals, the application of the USEtox™ to model ENPs has some inherent difficulties. As will be explained in section 2.5.2 ENPs show different environmental behaviours (and thus descriptors), relative to their bulk phases and other chemicals. Therefore, environmental fate modelling has to be adapted to ENP's specific fate processes and to the physicochemical properties governing them.

2.2 Characterization Factor calculation

The USEtox™ model estimates the CF of a substance for the impact category of freshwater ecotoxicity, as:

$$CF = EF \cdot FF \cdot XF \quad (1)$$

where EF (PAF m³ kg⁻¹) is the effect factor that represents the ecotoxicity of the substance and it is expressed in term Potentially Affected Fraction of species-PAF, FF (day) is the fate factor and expresses

the residence time of a substance in a particular environmental compartment (such as freshwater) and XF [dimensionless] is the exposure factor. The development of each factor for nano-TiO₂ is discussed below. The CF for n-TiO₂ was then calculated, with units describing the temporal and volumetrically integrated potentially affected fraction of aquatic organisms per unit mass of released n-TiO₂ (PAF m³ day kg⁻¹).

2.3 The Effect Factor calculation

USEtox™ adopts a PAF (Potentially Affected Fraction of species) based approach to calculate the EF for aquatic ecotoxicity of a substance (Larsen et al., 2007(a,b); Rosenbaum et al., 2008). The PAF is the fraction of species exposed to a concentration above their EC₅₀ (Klepper et al., 1998). The EF is defined as:

$$EF = \frac{0.5}{HC50_{EC50}} \quad (2)$$

where, HC50_{EC50} represents the concentration at which 50% of species is exposed above their chronic EC₅₀ and 0.5 is the working point (PAF=0.5) on the PAF curve. At least three EC₅₀ values from three different phyla are required to reflect the variability of the physiology and to ensure a minimum diversity of biological responses (Henderson et al., 2011). USEtox™ suggests to calculate the HC50_{EC50} as the geometric mean of the available single species EC₅₀ for organisms representative of three trophic levels: algae, crustaceans and fish. In this study, the EF of n-TiO₂ was estimated from toxicity values reported in previous studies on freshwater organism representative of the three trophic levels recommended by the USEtox™ model (algae, crustaceans and fish).

As highlighted by the literature, the toxicity of n-TiO₂ is influenced by: i) type of n-TiO₂: crystalline structure, nominal size, content of impurities (Crane et al., 2008; Navarro et al., 2008; Ji et al., 2011; Seitz et al., 2013; Seitz et al., 2014); ii) procedure followed to conduct the toxicity test, i.e. suspension preparation method with use of solvent, sonication, filtration, (Clement et al., 2013; Arouja et al., 2009, Handy et al., 2008,); and iii) mode of exposure to organism i.e. the time of exposure, UV exposure (Zhu et al., 2010; Dabrunz et al., 2011; Seitz et al., 2013; Ma et al., 2012). In order to increase the reliability of the estimated EF criteria have been applied to select the toxic value involved into the EF calculation. The toxic values applied are reported in Tables S.1–S.3. The criteria are listed below and aim account for the main sources of variability

i) Chemical tested: concerning crystalline structure, anatase form seems more toxic than rutile. Toxicity tests performed with nano-TiO₂ composed mainly of anatase have been preferred to those conducted

with nano-TiO₂ composed mainly of rutile. Also, toxicity results are selected from toxicity studies that uses the same type of n-TiO₂ (e.g. P 25).

ii) Toxicity tests: to avoid as much variation as possible of the toxicity of nano-TiO₂ originating from different treatments, the EC₅₀ values were extrapolated from toxicity tests in which similar treatments to nano-TiO₂ were applied. Thus, toxicity tests where chemical solvents (e.g. THF) were applied or where test units were pre-irradiated by UV light, or where the organisms were exposed under simulation of solar radiation have been avoided. Indeed, several researchers have found that the use of solvents to produce water-soluble compounds may significantly influence their toxicity (Oberdorster et al., 2007; Zhu et al., 2009). Toxic values higher than the highest concentration tested are not included into the toxic value dataset (Table S.1–S.3). Chronic toxicity test are best representative so are preferred to acute toxicity test. More of the exposure time of the toxicity test affects the toxicity of nano-TiO₂ (Kim et al., 2010; Dabrunz et al., 2011). Thus, acute toxicity tests, where the exposure time have been extended (i.e. up to 96-h for acute toxicity test on *Daphnia magna*) are included and applied into the EF calculation. Following the USEtox guidelines (USEtox user manual, 2014; Larsen and Hauschild, 2007b), chronic toxicity values are prioritized and endpoint of mortality (for crustacean and secondary/tertiary consumer) or immobility (crustacean) and of inhibition of growth (primary producers-algae) are preferred. The toxic values have been collected only on the freshwater aquatic species reported in the USEtox manual (USEtox user manual, 2014). For primary producers (algae) there are evidences that testing insoluble nanoparticles is a challenge due to the formation of aggregates in solution and algae-particle interactions. As investigated by Hartmann et al., 2013 the fluorimetric method is the most suitable measuring technique. For this reason toxicity tests where the reading of the sample has been performed with this technique are here preferred. USEtox™ prioritizes chronic toxicity values (EC₅₀chronic) and, as recommended by the USEtox™ model authors (USEtox user manual, 2014), an acute-to-chronic ratio (ACR) of 2 is applied when chronic data were unavailable.

2.4 The Exposure Factor calculation

In USEtox™ the Exposure Factor, XF_w [dimensionless], is the fraction of a chemical dissolved in freshwater (FR_{w,w}) and it is calculated as:

$$FR_{w,w} = \frac{1}{1+(K_p * SUSP + K_{DOC} * DOC + BCF_{fish} * BIOMASS) * 10^6} \quad (3)$$

Where K_p is the partitioning coefficient between water and suspended solid ($L\ kg^{-1}$), $SUSP$ is the suspended matter concentration in freshwater, K_{doc} is the partitioning coefficient between dissolved organic carbon and water, DOC is the dissolved organic carbon concentration in freshwater, BCF_{fish} is the bioconcentration factor in fish ($L\ kg^{-1}$) and $BIOmass$ is the concentration of biota in water. The eq.3 is applied for organic substances. In contrast to organic chemicals, evidence shows that the partitioning coefficients are not valid for ENPs due to the low solubility, low vapour pressure, high surface reactivity (Mackay, 2006; Praetorius et al., 2014). So, in the present case study, the method proposed by USEtoxTM for calculating the XF is not applicable. A precautionary approach of setting XF equal to 1 has been applied which does not weight the final results of the model based on the exposure of the organism to the TiO_2 ENP.

2.5 The Fate Factor calculation

2.5.1 The USEtoxTM and nested-multimedia models

USEtoxTM adopts the principles of the nested-multimedia mass balance modelling, simulating the behaviour of chemicals released from the technosphere to the environment as the net result of mass flows between a suite of well-mixed and homogeneous compartments (Henderson et al., 2011). USEtoxTM uses physicochemical properties of substances and of the environment as input parameters to model transport among different phases as well as loss rates. Losses can occur through adsorption to suspended solids and subsequent sedimentation (since toxicity in sediment has not yet been included), volatilization, degradation, and advection. From a mathematical point of view, the USEtoxTM model is structured in a matrix framework composed of a series of matrices combining fate with exposure and effect (Rosenbaum et al., 2007). The fate matrix (\overline{FF}) links the quantity released into the environment to the chemical mass (or concentration) occurred in a given compartment. The fate matrix is defined as \overline{FF} equals the negative inverse of the transfer rate coefficient matrix, (\overline{k}). The size of the fate matrix is determined by the number of environmental compartments (n_m) and the number of source compartments (n_i). Since every destination compartment can also be a source compartment ($n_m = n_i$) and it can also be written as ($n_i \times n_i$). The elements of \overline{k} are the inverse of the residence time of the substance, having units of 1/day. The off-diagonal elements ($k_{i,j}$), reflect intermedia or advective transport from environmental compartment i to j (i.e. air, water, soil), while the diagonal elements ($-k_{i,tot}$) represent the negative of the total removal rate coefficient for compartment i including biotic/abiotic degradation, advective and intermedia removal (for further details see Rosenbaum et al., 2008).

2.5.2 Application problem of nested-multimedia model to ENPs

Nested-multimedia fate models have been used to simulate the transport and transformation of organic chemicals through a range of different environmental media (e.g. air, water, soil) for 30 years (MacLeod et al., 2010). The environmental fate modelling of organic compounds accounts physicochemical properties of substances as vapour pressure, solubility and partitioning values. Due to the particular chemical and physical properties of ENPs (low solubility, low vapour pressure, size, structure, high surface reactivity, catalytic, magnetic and optical properties), the classic approach and prior experience with organic chemicals may be irrelevant or not applicable to ENPs (Mackay et al., 2006; Praetorius et al., 2014). As highlighted in previous works, the fate of ENPs dispersed in freshwater is comparable with those of the colloid (Handy et al., 2008; Ardvisson et al., 2011). In these cases, the colloidal science is applied to develop fate model specific for ENPs.

2.5.3 Definition of an adjusted nested-multimedia model for ENPs

From the basis of the USEtox™ and of the multimedia fate models for organic chemicals, an adjusted model has been developed which accounts for nano-specific descriptors and estimates the fate factor of nano-TiO₂ in freshwater. The environmental fate processes considered in this model are described in Fig. 1. The strong and fast aggregation observed in natural water samples (Sillanpää et al., 2011; Keller et al., 2010) highlights that persistence (following the USEtox™ referred in term of day) of ENPs in freshwater may be strongly affected by sedimentation processes. Additionally, the sediment compartment may be greatly affected by the sedimentation of ENPs such as nano-TiO₂. Thus, the model accounts two environmental compartment, freshwater and sediment, at the continental geographic scale. The landscape parameters at the continental scale (i.e. depth of sediment, height of water column) are derived from the USEtox™ model (Table S.4). The ENPs are likely to undergo i) transformation, ii) interaction with SPM and iii) transport (Praetorius et al., 2012)

i) The transformation process can be described by dissolution and aggregation, where the aggregation process can either be homo- or heteroaggregation. Homoaggregation has not been accounted for in this case because particles are unlikely to be in high enough concentrations to interact with each other. However, heteroaggregation is readily expected, where ENPs can be associated with ambient particles in the matrix. The understanding of dissolution process is of great importance for fate exposure assessment, however little is known about solubility and rates of dissolution of ENPs in water. Thus, modelling dissolution remains highly speculative (Quik et al., 2011); the dissolution of ENPs can be

described as a surface controlled process where a first order dissolution rate constant k_{diss} reflects the local hydrodynamic condition near nanoparticle-water interface (Praetorius et al., 2012). While a second order dissolution rate constant may better represent the most realistic scenario in the environment, in absence of adequate data for dissolution of particles generally, first order kinetics as a simplified approach to model dissolution of ENPs is considerable acceptable. As Quik et al. (2011) have previously described, this is an acceptable simplification where in water the rate was in the range of $0\text{-}10^{-5} \text{ s}^{-1}$.

ii) SPM in freshwater has been reported as both continuous size distributions and a log-normal size distributions with a concentration that can vary depending on the specific body of water. The interaction of ENPs with SPM in water is described by the hetero-aggregation process and quantified by the hetero-aggregation rate constant ($k_{\text{het-agg}}$). Aggregation is dependent on the collision rate and the attachment efficiency of the particles. It has been shown that the attachment efficiency is largely affected by the ionic strength and natural organic matter (NOM) content of natural waters (Arvidsson et al. 2011; Keller et al. 2010). The $k_{\text{het-agg}}$ is calculated by multiplying the ENPs' collision rate, k_{coll} , by the attachment efficiency for hetero-aggregation $\alpha_{\text{het-agg}}$ (Eq.S.1). The collision rate k_{coll} accounts for the main mechanisms leading to collision of the particles as brownian motion (perikinetic aggregation), fluid motion (orthokinetic aggregation) and differential settling. Not all collisions successfully produce aggregates; the fraction of successful collisions is called the attachment efficiency (α). The tendency of particles to aggregate is described within the colloidal science in the Derjagiu Landau Verwey Overbeek (DLVO) theory of colloidal stability. The DLVO theory describes the total interaction energy experienced by a particle when approaching another particle (in the case of aggregation) (Petosa et al., 2010). According to the DLVO theory, attachment efficiency depend from the repulsive and attractive interaction energies between two colliding particle. Currently, the attachment efficiency has not been successfully predicted by DLVO theory. The repulsive forces between ENPs and natural colloids are not easy to determine with the DLVO theory and therefore the attachment efficiency is preferably obtained from experimental work (Meester et al., 2014). However, to our knowledge, no measurements of the attachment efficiency for the hetero-aggregation of ENPs and SPM currently exist in the literature. Therefore in our studies, two different values of α (1 and 0.001) were considered, in order to cover two different aggregation efficiency scenarios. The rate constant of aggregation is a second order rate constant (m^3s^{-1}). Here, it is assumed as first order, therefore it is multiplied by the particle concentration C_{SPM} (m^{-3}) in water (Praetorius et al., 2012).

iii) ENPs are affected by transport processes (i.e. advection and sedimentation) which predict mobility within and between the environmental compartment and the concentration in the water and sediment

compartment. The sedimentation velocity (ms^{-1}), or settling rate $v_{\text{sed},i}$ (Eq S.3) is derived from Stokes' law for gravitational settling of particles. The sedimentation velocity is applied in Eq.S.7, so a first order rate constant for sedimentation $k_{\text{sed},i}$ is calculated by dividing with the height of the freshwater compartment (m). Also, ENPs are affected by transport process with moving water by advection (k_{adv}). In this study, the water outflow (adv,flow; m^3s^{-1}) from freshwater at the continental geographic scale to continental sea water has been accounted for and the rate constant of advection $k_{\text{adv}}(\text{s}^{-1})$ (Eq.S.2) as the ratio between the water outflow and the volume of the freshwater has been calculated. In addition to this, sediment resuspension (k_{resusp}) (Eq. S.9), horizontal bed load transfer ($k_{\text{sed,transfer}}$) (Eq.S.10) and burial in the deep sediment (k_{burial}) (Eq.S.8) are taken into account.

2.5.4 The fate matrix

In this study a fate matrix of 2x2 has been developed, which describes the environmental compartment of freshwater (w) and sediment (sed):

$$\overline{FF} = \begin{pmatrix} FF_{w,w} & FF_{w,sed} \\ FF_{sed,w} & FF_{sed,sed} \end{pmatrix} \quad (4)$$

The element on the diagonal ($FF_{w,w}$) describes the residence time (day) in the respective compartment. In this study, looking for the calculation of a CF for the toxic impact of freshwater ecotoxicity, only the element $FF_{w,w}$ is of our interest since impacts in the sediment is not yet included in the USEtoxTM. Indeed we aim to calculate the persistence of nano-TiO₂ in the freshwater column “*that can be seen as measure of the length of the exposure to which the aquatic organisms are exposed*”. As previously described \overline{FF} is calculated as the negative and the inverse of the rate coefficient matrix (\overline{k}):

$$\overline{k} = \begin{pmatrix} -k_{w,w} & k_{w,sed} \\ k_{sed,w} & -k_{sed,sed} \end{pmatrix} \text{ for } (n_{\text{sizes}}^{\text{TiO}_2} \text{ class} = 1..5) \quad (4.1)$$

$k_{w,w}$ and $k_{sed,sed}$ elements represent the total removal rate constant in the water column (calculated as the negative sum of the first-order rate constant, s^{-1} , for the processes of advection, hetero-aggregation, dissolution, sedimentation) and in sediment (as the negative sum of the rate constant, s^{-1} , burial, resuspension, bed load transfer), respectively.

And so, $k_{w,w}$ is:

$$k_{w,w} = -(k_{w,adv} + k_{sed} + k_{dis} + k_{w,heter-agg}) \text{ for } n_{\text{sizes}}^{\text{TiO}_2} = 1 \dots 5 \quad (5)$$

And $k_{sed,sed}$ is

$$k_{sed,sed} = -(k_{burial} + k_{resusp} + k_{sed,transf}) \quad (6)$$

$k_{w, sed}$ and $k_{sed, w}$ are the inter-media exchange between water and sediment and are described by the rate constant of sedimentation from freshwater to sediment and by the rate constant of resuspension from sediment to freshwater respectively (for further details about the model equations, see Supplementary material).

In this study, in order to calculate the FF the following assumptions are applied: i) a direct release of uncoated nano-TiO₂ into freshwater compartments from wastewater was assumed; ii) in accordance with Praetorius et al. (2012) a size distribution of nano-TiO₂ aggregated in freshwater was assumed and set to be log-normally distributed with the mode at 300 nm; the particles in distribution to 5 size classes were assigned, ($n_{sizes\ class}^{TiO_2} = 1 \dots 5$). The size class parameters are reported in Table S.5 (for more details see Praetorius et al., 2012). Four scenarios are analysed assuming the hereunder conditions: a SPM particle concentration of $3.7 \cdot 10^{10} \text{ m}^{-3}$ and of $1.1 \cdot 10^{11} \text{ m}^{-3}$ both with a density of 2 g cm^{-3} (Praetorius et al., 2012) and the attachment efficiency has been set to equal to 1 and to 0.001.

The $FF_{w,w}$ is calculated considering the size distribution of nano-TiO₂ ($n_{sizes\ class}^{TiO_2} = 1 \dots 5$) in freshwater. Indeed, for each one of the 5 size classes a $FF_{w,w,i}$ ($n_{sizes\ class}^{TiO_2} = 1 \dots 5$) is calculated. Then, $FF_{w,w}$, as the weighted average of $FF_{w,w,i}$, is computed.

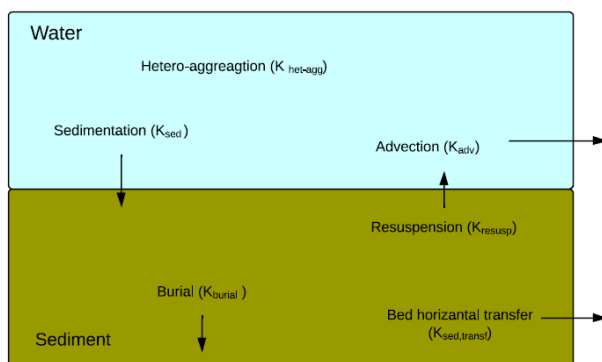


Figure 1: The "box" model and the environmental processes with the corresponding rate constant accounted in this study.

3. Results

3.1 The Effect Factor

Tables S.1-S.3 show the ecotoxicity dataset created through the literature research selecting only the freshwater aquatic species listed in the USEtox manual. The dataset counts 32 studies (time period 2006-

2013): 11 studies for algae, 17 for crustaceans and 4 for fish. As it is possible to observe, a high variability, by several order of magnitude, of the EC₅₀ of nano-TiO₂ occur among the aquatic organisms representative of each trophic level.

The criteria mentioned in the paragraph 1.3 have been applied on the dataset of the EC₅₀ values collected from the literature survey in order to reduce the variability. The EC₅₀ selected and used to calculate the EF (Eq.2) are reported in Tables S.1-S.3. Adopting the criteria proposed, 17 toxic values for crustaceans, 11 toxic values for algae and only 2 toxic values for fish have been taken into account for the EF calculation. USEtox™ calculates the HC50_{EC50} as the geometric mean of the EC₅₀ values on species level (GM-species level). Alternatively, first the EC₅₀ for each trophic level is computed as the geometric mean of the values of all the species belonging to the trophic level; then, the HC50_{EC50} is computed as the geometric mean of the EC₅₀ values of the trophic levels (GM-trophic level) (Larsen and Hauschild, 2007a,b). The HC50_{EC50} values for nano-TiO₂ calculated on the basis of the two approaches are listed in Table 1; the HC50_{EC50chronic} value obtained adopting GM species-level results in 12.8 mg L⁻¹ whereas the value obtained through GM trophic-level is 17.8 mgL⁻¹.

Table 1: HC50_{EC50acute/chronic} and Effect factor (EF) values for n-TiO₂

| | GM Species-level | GM Trophic-level |
|---|------------------|------------------|
| HC50_{EC50chronic}(mgL⁻¹) | 12.8 | 17.8 |
| EF (PAF m³ kg⁻¹) | 39.1 | 28.1 |

Larsen and Hauschild (2007b) indicated an approach based on the GM trophic-level as the best practice to calculate the HC50_{EC50}. In our study, the approach based on the GM trophic-level is adopted, as it avoids the bias caused by the unequal distribution of toxic values among the three trophic levels, and an EF of 28.1 (PAF m³ kg⁻¹) for n-TiO₂ is proposed.

Finally, in order to examine the importance of applying criteria for selection of datasets from the literature survey (Tables S.1-S.3), additional ecotoxicological studies were included adopting the following modifications to the selection criteria explained in paragraph 1.3: i) no rules on the treatment of the test solution are considered (i.e. filtration-Lovern and Kapler 2006); ii) all the endpoint are considered (USEtox™ suggest to only consider endpoint as the mortality or the immobilization for the trophic level of crustacean); iii) toxic value equal to the highest concentration tested are included; iv) phototoxicity tests are included This leads to the inclusion of 14 additional toxic values (crustacean toxicity values pass from

17 to 29 and algae tests from 11 to 14, fish toxicity values from 2 to 4). As a result, the HC50_{EC50chronic} (GM-trophic level) shows a little reduction (from 17.8 mg/L to 15.6mg/L) and consequently the EF undergoes a slight change (from 28.1 to 32.1PAF m³ kg⁻¹).

3.2 The Fate Factor in freshwater

The Table 2 shows the element of the fate factor matrix for the Scenario 1 ($\alpha=1$, $C_{SPM} = 3.70 \cdot 10^{10} \text{ m}^{-3}$, $\rho=2.0 \text{ g/cm}^3$) and for each of the 5 size class distribution of n-TiO₂ in freshwater ($n_{sized\ class}^{TiO_2} = 1 \dots 5$).

Table 2:The components of the FF matrix (day);FF_{w,w,i} expresses the residence time (day) in freshwater for each size class of aggregates. FF_{w,sed,i} is calculated from the intermedia rate coefficient (sedimentation from water to sediment), it expresses the time of the particle to sediment; the FF_{sed,w,i} represents the time of resuspension as well; FF_{sed,sed} expresses the residence time (day) in sediment for each size class of aggregates.

| $n_{sized\ class}^{TiO_2}$ | 1 | 2 | 3 | 4 | 5 |
|----------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Radius | 8 nm | 106 nm | 204 nm | 302 nm | 400 nm |
| FF _{w,w,i} | $5.9 \cdot 10^{-1}$ | $5.3 \cdot 10^{-1}$ | $3.6 \cdot 10^{-2}$ | $4.7 \cdot 10^{-3}$ | $1.1 \cdot 10^{-3}$ |
| FF _{w,sed,i} | $4.5 \cdot 10^{-1}$ | $4.0 \cdot 10^{-1}$ | $2.7 \cdot 10^{-2}$ | $3.5 \cdot 10^{-3}$ | $8.0 \cdot 10^{-4}$ |
| FF _{sed,w,i} | $8.0 \cdot 10^3$ | $1.0 \cdot 10^3$ | $1.8 \cdot 10^3$ | $1.7 \cdot 10^3$ | $1.6 \cdot 10^3$ |
| FF _{sed,sed,i} | $9.8 \cdot 10^2$ | $1.8 \cdot 10^3$ | $2.4 \cdot 10^3$ | $2.3 \cdot 10^3$ | $2.1 \cdot 10^3$ |

A low persistence of the particle in the freshwater column is observable (Table 2). The FF_{w,w,i} nano-TiO₂ range from 10⁻¹ day (for radius equal to 8 nm) to 10⁻³ day (for radius equal to 400 nm). In order to obtain a fate factor representative of size distribution of nano-TiO₂ aggregated, a FF_{w,w} calculated as the weighted average of FF_{w,w,i} of all size classes is computed (Table 2.1).

Table 2.1: FF_{w,w} as weighted average of FF_{w,w,i} in each size class; Scenario 1 with $\alpha=1$, particle concentration of SPM equal to $3.70 \cdot 10^{10} \text{ m}^{-3}$ and with $\rho=2.0 \text{ g/cm}^3$

| Radius (nm) | weight | FF _{w,w,i} weighted |
|-------------|--------|------------------------------|
| 8-105 | 0.3 | $1.9 \cdot 10^{-1}$ |
| 106-203 | 0.4 | $1.6 \cdot 10^{-2}$ |
| 204-301 | 0.1 | $6.6 \cdot 10^{-4}$ |
| 302-400 | 0.02 | $2.4 \cdot 10^{-1}$ |

| | | |
|-------------------------|---|---------------------|
| sum | 1 | $2.1 \cdot 10^{-1}$ |
| FF_{w,w} | | $5.3 \cdot 10^{-2}$ |

Thus, for the Scenario 1 a FF_{w,w} of $5.3 \cdot 10^{-2}$ day is obtained. The FF_{w,w,i} and the respectively F_{w,w} for scenario 2-3-4 are reported in the Supporting Material, Tables: from S.6 to S.8. The FF_{w,w} (day) values for each of the four scenario are summarized in Table 3.

Table 3: FF_{w,w} calculated for each of the four scenario proposed

| Scenario | $\alpha=1, \rho=2.0 \text{ g/cm}^3$ | $\alpha=0.001, \rho=2.0 \text{ g/cm}^3$ | $\alpha=1, \rho=2.0 \text{ g/cm}^3$ | $\alpha=0.001, \rho=2.0 \text{ g/cm}^3$ |
|------------------------------|--|--|--|--|
| | $C_{\text{SPM}}=3.70 \cdot 10^{10} \text{ m}^{-3}$ | $C_{\text{SPM}}=3.70 \cdot 10^{10} \text{ m}^{-3}$ | $C_{\text{SPM}}=1.10 \cdot 10^{11} \text{ m}^{-3}$ | $C_{\text{SPM}}=1.10 \cdot 10^{11} \text{ m}^{-3}$ |
| FF_{w,w}(day) | $5.3 \cdot 10^{-2}$ | $7.7 \cdot 10^{-2}$ | $3.2 \cdot 10^{-2}$ | $7.7 \cdot 10^{-2}$ |

3.3 Freshwater ecotoxicity Characterisation Factor for n-TiO₂

The characterization factor for freshwater ecotoxicity is calculated as $CF_w = FF_{w,w} \times XF_w \times EF_w$. Where, an EF_w for n-TiO₂ of 28.1 (PAF m³ kg⁻¹) is proposed, an XF set equals to 1 is assumed. Here, a FF_{w,w} which represents the average residence time of a size distribution of the nano-TiO₂ aggregated in freshwater is applied. The FF_{w,w} applied has the same order of magnitude among the four scenario. Thus, a FF_{w,w} of 10⁻² (day) is used. In such way, the CF_w results in 0.28 PAF day m³ kg⁻¹.

4. Discussion

4.1 The Effect Factor for nano-TiO₂

A large variation of the EC₅₀ values for nano-TiO₂ is observable and reported in Table S.1-S3. In our case of study the large variation in the toxicities values for nano-TiO₂ can be the major impediment for making a robust estimation of the relative EF. The same issue is reported by Eckelman et al., 2012. The author evaluated the EF for CNT following the framework of USEtoxTM. Due to the scarce number of toxic values reported in literature and due to the large variation among them, the author applied several assumptions. As for example, assuming no distinction between multi-walled and single-walled nanotubes even if it might affect their fundamental toxicity Although several research show as nano-TiO₂ is capable of causing toxicity in multiple aquatic species, it is proved that the effect concentrations (e.g. EC₅₀) for

nanoparticles is influenced by several aspects (Li et al., 2014; Seitz et al., 2013; Dabrunz et al., 2011; Gottschalk et al., 2013) as: the crystalline structure of nano-TiO₂, the treatments of the sample to test (i.e. filtered or unfiltered, use of solvent), the combination of different materials with varying testing condition, the exposure time and the exposure under UV radiation. And more, aquatic toxicology of nano-TiO₂ is challenged by methodological difficulties arising from highly dynamic and poorly understood behaviour of ENPs in biological test systems (Hartmann et al., 2013). One target of application of nano-TiO₂ is photochemical degradation of contaminants and photo-killing of microbes due to its strong photoreactivity. Generally, the phototoxicity of nano-TiO₂ is mainly assessed for antimicrobial applications, where higher organisms are not included (Ma et al., 2012). To date only few phototoxicity studies on freshwater organisms have been carried out. Indeed, the ecotoxicity studies are mainly performed in lab conditions and in absence of UV exposure (Li et al., 2014). Even if the influence of these factors to the toxicity test results are reported and discussed in literature (Zhu et al., 2009; Dabrunz et al., 2011; Seitz et al., 2013, 2014; Li et al., 2014), a systematic assessment of their importance is still missing. This leads to a difficult comparison of the toxicity values among the studies and a scarce understanding of the magnitude of the influence of different factors on the toxicity results.

As mentioned in Section 2.3, the set of criteria proposed to select the EC₅₀ values aims to increase the reliability of the ecotoxicity dataset and, consequently, of the EF estimated. Applying these criteria the number of the available toxicity values decreases from 47 to 30 but the variation of the EC₅₀ values reduces significantly increasing the comparability of the results. In any case, it is important to highlight that some studies are filtered out and this may result in negligence of some effects that are not exerted in standard test.

The two different HC₅₀EC₅₀chronic values obtained adopting GM species-level (12.8 mg L⁻¹) and GM trophic-level (17.8 mg L⁻¹) approach confirm that the choice of the averaging approach is a relevant issue as to the evaluation of the CF. A study by Larsen and Hauschild (2007b) indicates that unequal representation of EC₅₀ values from different taxonomy groups at three trophic levels may introduce a bias in the estimation of the effect factor when applying the GM-species level approach. This is the case in our study. Indeed, the unequal distribution of the EC₅₀ values among the trophic levels (two EC₅₀ values for fish, 17 and 11 EC₅₀ values for crustaceans and algae, respectively) will put more weight on the trophic levels with many measured values. For this reason in this study, an approach based on GM-trophic level is adopted for the calculation of the EF and a value of 28.1 PAF m³ kg⁻¹ is proposed.

The test to examine the importance of applying criteria for dataset definition carried out omitting some criteria led to EFs very similar (30.1 vs 27.7 PAF m³ kg⁻¹). That is mainly because only in one of the three trophic levels (crustaceans) there is a strong increase, in term of number of toxicity values collected. That is mainly because only in one of the three trophic levels (crustaceans) there is a strong increase, in term of number of toxicity values collected

More, the addition of the phototoxicity studies slightly increases the toxicity on nano-TiO₂. It is remarkable that the main contribute to this increasing, is the addition of toxicity values reported by Ma et al., 2012. The author assessed the phototoxicity of nano-TiO₂ by exposing *D.magna* and *Ozyzias latipes* under simulated solar radiation. An acute 48-h LC₅₀ of 29.8 µg/L and acute 96-h LC₅₀ of 2.2 mg/L were calculated respectively. The toxic value reported by Ma et al., 2012 is the lowest toxic value collected in our literature survey both for the trophic level of crustacean and fish. The authors report that the toxicity of nano-TiO₂ was enhanced by two to four orders of magnitude under simulated solar radiation compared to standard laboratory lighting (i.e. 29.8 µg/L vs 500 mg/L for *D.magna*). Here, the EF is affected by a slight increase because the inclusion of the toxic values higher than the highest concentration tested clearly overlaps the lower toxic values reported within the phototoxicity tests. However, the choice of the averaging approach to calculate the EF is confirmed to be significant both when applying the selection criteria and without. Indeed, without the application of the criteria, the EF calculated on the basis of GM-species approach is 31.7 PAF m³ kg⁻¹ whereas with a GM-trophic one the EF is 32.1 PAF m³ kg⁻¹. On the other hand, when applying the selection criteria the EF ranges from 39.1 (GM-species) to 28.1 (GM-trophic) PAF m³ kg⁻¹. Thus, the EF based GM-trophic level remains more stable.

4.2 The Exposure Factor for nano-TiO₂

The procedure of calculation of XF proposed by USEtox is not applicable due to type of chemical investigated here. This is mainly due to the portioning approach which is not valid for ENPs. Moreover, ENPs such as nano-TiO₂, which are generally insoluble, will not dissolve. Aggregation and sedimentation processes govern the behaviour of poorly soluble ENPs. The aggregation process promotes the sedimentation of the particles and decreases the surface area of the ENPs. Therefore the aggregation might reduce the bioavailability of ENPs along the water column to freshwater organism and decrease toxic responses mediated by the surface area (e.g. ROS generation and dissolution of free ions). On the other hand, it increases the persistence of the ENPs, decreasing the rate of dissolution or degradation (Hotze et al., 2010). Under environmental conditions a multimodal size distribution of ENPs is expected and

organisms may be exposed to different size ranges of aggregates (Keller et al., 2010; Lin et al., 2010; Sillanpää et al., 2011; Praetorius et al., 2012). Where, the smallest particles in the size distribution of nano-TiO₂ may easily penetrate inside the organism and thus promote accumulation in tissues. Few studies have been focused on bioaccumulation and bioavailability of ENPs (Dalai et al., 2013). Therefore, the bioavailability and bioaccumulation of aquatic organism are still under debate. And, on our knowledge, no bioaccumulation and bioavailability y factor that could describe and replaced the XF have been proposed in literature. For all these reasons, a precautionary approach of setting XF equal to 1 has been adopted which does not weight the final results of the model based on the exposure of the organism to the TiO₂ ENPs.

4.3 The Fate Factor for nano-TiO₂

The resulting FFs show a low persistence of the particle in the freshwater column independently by size, attachment efficiency and SPM concentration: the model predicts a very fast removal of nano- TiO₂ from the freshwater column. For example, as far as concern size, Table 2 shows that in the Scenario 1 FF_{w,w,i} range from $5.9 \cdot 10^{-1}$ day (for radius equal to 8 nm) to $1.1 \cdot 10^{-3}$ day (for radius equal to 400 nm).

The attachment efficiency shows a similar behaviour, as it is possible to observe comparing Scenario 1 (CSPM = $3.70 \cdot 10^{10} \text{ m}^{-3}$, $\alpha = 1$) and Scenario 2 (CSPM = $3.70 \cdot 10^{10} \text{ m}^{-3}$, $\alpha = 0.001$) in Table 3. In Scenario 1, FF_{w,w} results in $5.3 \cdot 10^{-2}$ while in Scenario 2, FF_{w,w} results in $7.7 \cdot 10^{-2}$: the values are very similar even if the attachment

efficiencies are different by three order of magnitude. Finally, we can appreciate the influence of SPM comparing, for example, Scenario 1 versus Scenario 3 which differ in the SPM concentration (see Table 3). In Scenario 1 (CSPM = $3.70 \cdot 10^{10} \text{ m}^{-3}$), FF_{w,w} results in $5.3 \cdot 10^{-2}$ while in Scenario 3 (CSPM = $1.10 \cdot 10^{11} \text{ m}^{-3}$), FF_{w,w} results in $3.2 \cdot 10^{-2}$ also in this case, although the SPM concentration differ by one order of magnitude, the FFs are very close. In any case, even if the differences in FFs are small, the speed of removal of the nano-TiO₂ from freshwater column is positively related to size, attachment efficiency and SPM concentration in water.

The model has indirectly provided information on sediment compartment; the extent of transfer from freshwater to sediment has been calculated as net result of competition between the removal mechanisms from freshwater: aggregation, sedimentation, leaching to deeper layers. From Table 2, for example, it is possible to appreciate that FF_{sed,w,i} and FF_{sed,sed,i} are several orders of magnitude higher than FF_{w,w,i} and FF_{w,sed,i}, indicating that the sediment compartments act as a sink and may be greatly affected by the sedimentation of ENPs such as nano- TiO₂. The sedimentation of nano-TiO₂ poses a risk to benthonic

organisms but the toxicity of nano-TiO₂ to benthonic organisms it is not been included in this study. Indeed, to date the sediment compartment is not considered in USEtox™. However, the sediment compartment seems to be strongly affected and, in the opinion of the authors, further research should be carried out to include the impact on the sediment compartment in USEtox™.

Finally, the application of the colloidal science to study the fate of nano-TiO₂ and the attention paid to ENPs propensity to form aggregates have allowed the calculation of FFs dependent on the size distribution of the aggregated nano-TiO₂ in freshwater ecosystems. This approach aims to be environmentally realistic (Praetorius et al., 2012) and is innovative in an LCIA scenario. Indeed, i) the fate factor may be expressed as dependent on a size class of ENPs; if the effect factor depending on the size of particles is also known, then the exposure scenario may be correctly assessed, ii) the framework allows to apply a size distribution of ENPs or SPM for site-dependent conditions. However, concerning the calculation of the fate factor several points have to be taken into consideration:

i) abiotic degradation processes are not taken into account in this method; ii) the landscape data (depth of sediment, volume of water, height of water column) are extrapolated from the USEtox™ and have been estimated as average on a continental scale and iii) the average size distribution of nano-TiO₂ has been referred for Rhine river (Praetorius et al., 2012). This may be leading to a bias in the evaluation of the fate factor and to scarce relevance of the estimated FF respect to different environmental conditions. Since that, the bioavailability of ENPs and the mechanism of toxicity (e.g. size dependent toxicity or surface area dependent toxicity) are still under debate and the aquatic organisms in freshwater ecosystem are exposed to different size range of aggregates a FF_{w,w} based on the weighted average of the FF_{w,w,i} of all the size classes is proposed.

4.4 Characterisation Factor for nano-TiO₂

The Usetox provides CFs for organic

The USEtox™ provides CFs for organic substance whereas emerging substance as ENPs are still not included in the chemical database for which CF can be calculated. Eckelman et al., 2012 applied the USEtox™ to calculate the CF for CNT for the impact category of freshwater ecotoxicity. The authors investigated the FF factor for CNT without considering the important colloidal mechanism as aggregation and sedimentation. The FF for CNT was calculated following the approach of the USEtox™ based on the portioning of the substance. However, as declared by the author, a more detailed kinetic model would improve the applicability of the model.

In our study the framework of the USEtox™ has been adopted. But several aspects have been implemented, in order to account nano- specific aspect, as the application of the colloidal science and of kinetic equations to describe the environmental fate processes of ENPs in fresh- water. More, a size distribution of nano-TiO2 aggregates in freshwater is considered and for four scenarios are applied. Thus, a CFw for a specific size class of nano-TiO2 may be calculable. For example, considering the size class of nano-TiO2 with the smallest radius (8 nm, $\alpha = 1$, $CSPM = 3.70 \cdot 10^{10} \text{ m}^{-3}$, $\rho = 2.0 \text{ g/cm}^3$) a FFW,w,i of 10^{-1} day is calculated and hence, CFw of $2.8 \text{ PAF day m}^3 \text{ kg}^{-1}$ is obtained. And more, considering scenario 4 ($\alpha = 0.001$, particle concentration of $SPM 1.10 \cdot 10^{11} \text{ m}^{-3}$ $\rho = 2.0 \text{ g/cm}^3$ and a radius of 8 nm a FFW,w,i of 1.15 day is calculated (Table S.8) and a CFw of $32.1 \text{ PAF day m}^3 \text{ kg}^{-1}$ is obtained.

5. Conclusions and Outlook

The understanding of the underlying mechanisms relating to the potentially adverse effects of ENPs on aquatic organisms is a prerequisite for determining appropriate hazard assessment strategies. Unfortunately, this understanding is hampered by lack of knowledge concerning behaviour in the environment, parameters determining bioavailability, mechanisms of toxicity, and dependency of size (e.g. aggregates or small particles or their mixture). These challenges carry over to the assessment of freshwater ecotoxic impacts of ENPs, in the field of LCA. One specific challenge is that the CFs for toxicity associated with release of ENPs as nano-TiO2 into the environment have not yet been properly calculated in LCIA methods and LCA studies are unable to assess the ecotoxic impact related to emission of ENPs in the environment. The aim of this study was to calculate the CF of nano-TiO2 for freshwater ecotoxicity. The main conclusions of the study are highlighted hereunder.

- i)The conceptual framework adopted by the LCIA characterisation USEtox™ for the evaluation of the fate and effect factors can be applied for this new class of contaminants.
- ii)The literature survey on aquatic ecotoxicology showed a high variability of the toxic data for nano-TiO2, which may lead to a scarce robustness of the EF. This study proposes a set of criteria to select the EC50 values of ENPs as nano-TiO2 in order to increase the reliability of the ecotoxicity dataset and, consequently, of the EF estimated.
- iii)The HC50/EC50 chronic values obtained adopting GM species-level and GM trophic-level approach confirms the choice of the averaging approach as a relevant issue to the calculation of the EF. Here an

approach based on GM-trophic level is adopted in order to put equal weight on the three trophic levels which can differ in the number of data available.

iv) Conservatively, due to the lack of knowledge on bioavailability of poor soluble ENPs as nano-TiO₂, the exposure factor has been assumed to be equal to 1; thus all nano-TiO₂ dispersed in fresh- water is assumed bioavailable.

v) On the basis on the well-established multimedia box model, the colloidal nature of nano-TiO₂ has been taken into account to calculate the FF. Thus, specific nano-fate processes were considered. An environmental realistic approach has been followed considering that the nano-TiO₂ are dispersed in freshwater following a size class distribution, and hence, the aquatic organisms are exposed to a size distribution of aggregates.

vi) A CF_w of 0.28 PAF day m³ kg⁻¹ for the impact category of freshwater ecotoxicity for nano-TiO₂ is proposed.

The prediction of environmental fate of ENPs is best described when site-specific local conditions are considered. The study of Praetorius et al., 2012 showed how the properties of SPM, such as the density, may influence the fate and transport of nano-TiO₂ in freshwater (Rhine River). Therefore, further investigations are needed in which also size distribution of SPM and site-specific conditions should be included into the FF calculation. More efforts towards the introduction of spatial differentiation in regional/local impact categories such as ecotoxicity are relevant too (Zamagni et al., 2008). In ordinary LCA the location of the processes which release toxicants to the environment is usually not precisely known and, therefore, site-specific models cannot easily be used. Most often large average landscape data and environment conditions are assumed. To date, the site-independent approach seems a limitation of the assessment the potential toxic impact of ENPs in LCA. In fact kinetic modelling is conceptually difficult for global or continental model, as colloidal behaviour is strongly depended on the local condition. Furthermore, the emission of a toxicant listed in a life-cycle inventory is regarded as a single pulse without time duration and, therefore, time and space are integrated in the assessment posing further restrictions to the modelling. This is in contrast with the environmental behaviour of the ENPs for which the environmental fate and behaviour (stability and persistence) have been observed as concentration-dependent. In addition, over time, the physical form and intrinsic properties of ENPs (e.g. size distribution of aggregates, surface charge) may be subject to transformations, therefore leading to a different bioavailability and route of exposure.

References

- Alvarez PJJ, Colvin V, Lead JR, Stone V. Research Priorities to Advance Eco- Responsible Nanotechnology. ACS Nano 2009, 7: 1616-1619.
- Aruoja V, Dubourguier HC, Kasemets K, Kahru A. Toxicity of nanoparticles of CuO, ZnO, and TiO₂ to microalgae *Pseudokirchneriella subcapitata*. Sci Total Environ 2009; 407:1461-1468.
- Arvidsson R, Molander S, Sandén BA, Hassellöv M. Challenges in exposure modelling of nanoparticles in aquatic environments, human and ecological risk assessment. An Int J 2011; 17: 245-262.
- Bhatt I, Tripathi BN. Interaction of engineered nanoparticles with various components of the environment and possible strategies for their risk assessment. Chemosphere 2011; 82: 308-317
- Blaise C, Gagne F, Ferrard JF, Eullaffroy P. Ecotoxicity of selected nano-materials to aquatic organism. Environ. Toxicol. 2008; 23 (5): 591-598.
- Clement L, Hurel C, Marmier N. Toxicity of TiO₂ nanoparticles to cladocerans, algae, rotifers and plants-Effects of size and crystalline structure. Chemosphere 2013; 90 (3): 1083-90.
- Crane M, Handy RD, Garrod J, Owen R. Ecotoxicity test methods and environmental hazard assessment for engineered nanoparticles. Ecotoxicology 2008; 17: 421-437.
- Dabrunz A, Duester L, Prasse C, Seitz F, Rosenfeldt R, et al. Biological surface coating and motility inhibition as mechanism of TiO₂ nanoparticle Toxicity in *Daphnia magna*. PLoS ONE 2011; 6 (5): 1-7.
- Dalai S, Pakrashi S, Chandrasekaran N, Mukherjee A. Acute toxicity of TiO₂ nanoparticles to *ceriodaphnia dubia* under visible light and dark conditions in freshwater system. PLoS ONE 2013; 8 (4): 1-11.
- Das P, Xenopoulos MA, Metcalfe CD. Toxicity of silver and titanium dioxide nanoparticle suspensions to aquatic invertebrate, *Daphnia magna*. Bull Environ Contam Toxicol 2013;91:76-82.
- Eckelman MI, Mauter MS, Isaacs JA, Elimelech M. New perspectives on nanomaterial aquatic ecotoxicity : Production Impacts exceed direct exposure impacts for carbon nanotubes. Environ Sci Technol 2012; 46 : 2902-2910.
- Farré M, Gajda-Schranz K, Kantianu L, Barcelò D. Ecotoxicity and analysis of nanomaterials in the aquatic environment. Anal. Bioanal. Chem. 2009; 393: 81-95.
- Garcia A, Espinosa R, Delgado L, Casals E, Gonzalez E, Puentes V, Barata C, Font X, Sanchez A. Acute toxicity of cerium oxide, titanium oxide and iron oxide nanoparticles using standardized tests. Desalination 2010; 269 (1): 136-141.
- Gavankar S, Suh S, Keller AF. Life Cycle assessment at nanoscale: review and recommendations. Int J LCA 2012; 17(3): 295-303.
- Gottschalk F, Scholz RW, Nowack B. Probabilistic material flow modeling for assessing the environmental exposure to compounds: methodology and an application to engineered nano-TiO₂ particles. Environ Modell Softw 2010; 25: 320-332.

Gottschalk F, Sun TY, Nowack B. Environmental concentrations of engineered nanomaterials: Review of modeling and analytical studies. *Environmental Pollution* 2013; 181: 287-300.

Griffitt RJ, Luo J, Gao J, Bonzongo JC, Barber DS. Effect of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. *Environ Tox Chem* 2008;27:1972-1978

Guinée J, Heijungs ., Van Oers I, Wegener SA, Van de Meent D, Vermeire T, Rikken M. USES-Uniform System for the evaluation of substances. Inclusion of fate in LCA characterisation of toxic releases applying USES 1.0. *Int J LCA* 1 (1996): 133:138.

Hall S, Bradley T, Moore JT, Kuykindalla T, Minella L. Acute and chronic toxicity of nano-scale TiO₂ particles to freshwater fish, cladocerans, and green algae, and effects of organic and inorganic substrate on TiO₂ toxicity. *Nanotoxicology* 2009; 3 (2): 91-97.

Handy RD, Von der Kammer F, Lead JR, Hasselov M, Owen R, Crane M. The ecotoxicology and chemistry of manufactured nanoparticles. *Ecotoxicology* 2008; 17: 287-314.

Hartmann NB, Engelbrekt C, Zhang J, Ulstrup J, Kusk KO, Buam A. The challenges of testing metal and metal oxide nanoparticles in algal bioassays: titanium dioxide and gold nanoparticles as case studies. *Nanotoxicology* 2013; 7 (6): 1082-1094.

Hartmann NB, von der Kammer F, Hofmann T, Baalousha M, Ottofuelling S, Baun A. Algal testing of titanium dioxide nanoparticles-Testing considerations, inhibitory effect and modification of cadmium bioavailability. *Toxicology* 2010; 269: 190-197.

Heinlaan M, Ivask A, Blinova I, Dubourguier HC, Kahru A. Toxicity of nanosized and bulk ZnO, CuO and TiO₂ to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. *Chemosphere* 2008; 71: 1308-1316.

Henderson AD, Hauschild MZ, Van de Meent D, Huijbregts MAJ, Larsen HF, Margni M, McKone TE, Payet J, Rosenbaum RK, Jolliet O. USEtox fate and ecotoxicity factors for comparative assessment of toxic emission in life cycle analysis: sensitivity to key chemical properties. *Int J LCA* 2011; 16: 701-709.

Hischier R, Walser T. Life Cycle assessment of engineered nanomaterials: state of art and strategies overcome existing gaps. *Sci Total Environ* 2012; 425 : 271-282.

Hischier R. Life cycle assessment of manufactured nanomaterials: inventory modelling rules and application example. *Int J LCA* 2014; 19: 941-943.

Hotze EM, Phenrat T, Lowry GV. Nanoparticle aggregation: challenges to understanding transport and reactivity in the environment. *J Environ Qual* 2010; 39: 1909-1924.

Hund-Rinke K, Schlich K, Wenzel A. TiO₂ nanoparticles- relationship between dispersion preparation method and ecotoxicity in the algal growth test. *Umweltwiss. Schadst. Forsch.* 2010; 22: 517-528.

Hund-Rinke K, Simon M. Ecotoxic effect on photocatalytic active nanoparticles (TiO₂) on Algae and Daphnids. *Environ. Sc.i & Pollut. Res.* 2006: 1-8.

ISO (International Organization for Standardization). Technical specification UNI EN14040: Environmental management-life cycle assessment (LCA)-Principles and Framework. Switzerland: Geneve 2006a.

ISO (International Organization for Standardization). Technical specification UNI EN14044: Environmental management- LCA-Requirement and guidelines. Switzerland: Geneve 2006b

ISO, (International Organization for Standardization) Technical specification ISO/TS 27687:2008(E): Nanotechnologies -- Terminology and definitions for nano-objects -- Nanoparticle, nanofibre and nanoplate. 2008.

Ji J, Long Z, Lin D. Toxicity of oxide nanoparticles to the green algae *Chlorella* sp. *Chem. Eng. J.* 2010; 170: 535-530.

JRC-IES (European Commission Joint Research Centre and Institute for Environment and sustainability).ILCD handbook: recommendations for life cycle impact assessment in the European context. 1018-5593 Luxemburg; Publication Office of the European Union 2011 978-92-79-17451-3:2011.

Keller A, Wang H, Zhou D, Lenihan HS, Cherr G, Cardinale BJ, Miller R, Ji Z. Stability and aggregation of metal oxide nanoparticles in natural aqueous matrices. *Environ. Sci. Technol.* 2010; 44 (6): 1962-1967.

Kim KT, Klaine SJ, Cho J, Kim SH, Kim SD. Oxidative stress responses of *Daphnia magna* exposed to TiO₂ nanoparticles according to size fraction. *Sci Total Environ* 2010; 408: 2268-2272.

Klaine SJ, Koelmans AA, Horne N, Carley S, Handy RD, Kapustka, L, Nowack B, von der Kammer F. Paradigms to assess the environmental impact of manufactured nanomaterials. *Environ Toxicol Chem* 2012; 31 (1): 3-14.

Klepper O, Bakker J, Traas TP, Van de Meent D. Mapping the potentially affected fraction (PAF) of species as a basis for comparison of ecotoxicological risk between substances and region. *J Hazard Mat* 1998; 61: 337-344.

Larsen HF, Hauschild M. Evaluation of ecotoxicity effect indicators for use in LCIA. *Int J LCA* 2007(a); 1 (2): 24-33.

Larsen HF, Hauschild M. GM-Troph A Low Data Demand Ecotoxicity Effect Indicator for Use in LCIA. *Int J LCA* 2007(b): 12 (2): 79-91.

Lead JR. Manufactured nanoparticles in the environment. *Environ Chem* 2010; 7:1-2.

Lee SW, Kim SM, Choi J. Genotoxicity and ecotoxicity assay using the freshwater crustacean *Daphnia magna* and the larva of the aquatic midge *Chironomus riparius* to screen the ecological risks of nanoparticle exposure. *Environ Toxicol. Phar* 2009; 28: 86-91.

Lee WM, An YJ. Effect of zinc oxide and titanium dioxide nanoparticles on green algae under visible , UVA and UVB irradiations: no evidence of enhanced algal toxicity under UV pre-irradiation. *Chemosphere* 2013; 91: 536-544.

Li S, Wallis LK, Ma H, Diamond SA. Phototoxicity of TiO₂ nanoparticles to a freshwater benthic amphipod: are benthic system at risk?. *Sci Total Environ* 2014; 466-467: 800-808

Lin D, Tian X, Wu F, Xing B. Fate and transport of engineered nanomaterials in the environment. *J Environ Qual* 2010; 39: 1896-1908.

Liu HH, Cohen Y. Multimedia Environmental distribution of engineered nanomaterials. *Environ Sci Technol* 2014; 48: 3281-3292.

Lovern SB, Kapler R. *Daphnia magna* mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles. *Environ Toxicol Chem* 2006; 25 (4): 1132-1137.

Ma H, Brennan A, Diamond SA. Phototoxicity of TiO₂ nanoparticles under solar radiation to two aquatic species: *Daphnia magna* and Japanese Medaka. *Environ Toxicol Chem* 2012; 31 (7):1621-1629.

Mackay CE, Johns M, Salatas JH, Bessinger B, Perri M. Stochastic probability modeling to predict the environmental stability of nanoparticles in aqueous suspension. *Integr Environ Assess. Manag* 2006; 2 (3): 293-298.

MacLeod M, Scheringer M, McKone TE, Hungerbuhler K. The state of multimedia mass balance modeling in environmental science and decision making. *Environ Sci* 2010; 44 (22): 8360-8364.

Meesters JA J, Koelmans AA, Quik JTK, Hendriks AJ, van de Meent D. Multimedia Modeling of Engineered Nanoparticles with SimpleBox4nano: Model Definition and Evaluation. *Environ. Sci. Technol.* 2014; 48: 5726-5736.

Miseljic M, Olsen SI. Life-cycle assessment of engineered nanomaterials: a literature review of assessment status.» *J Nanopart Res* 2014; 16: 2427.

Navarro E, Baun A, Renata B, Hartmann NB, Filser J, Miao AJ, Quigg A, Santschi PH, Laura S. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. *Ecotoxicology* 2008; 17: 372-386.

Oberdörster G, Stone V, Donaldson K. Toxicology of nanoparticles: a historical perspective. *Nanotoxicology* 2007;1: 2-25.

Petosa AR, Jaisi DP, Quevedo IR, Elimelech M, Tufenkji N. Aggregation and deposition of engineered nanomaterials in aquatic environments role of physicochemical interactions. *Environ Sci Technol* 2010; 44 (17): 6532-6549.

Piccinno F.; Gottschalk, F.; Seeger, S.; Nowack, B., Industrial Production Quantities and Uses of Ten Engineered Nanomaterials for Europe and the World. *J. Nanopart. Res.* 2012, 14, 1109.

Praetorius A, Scheringer M, Hungerbuhler K. Development of environmental fate models for engineered nanoparticle-a case study of TiO₂ nanoparticle in the Rhine river. *Environ Sci Technol* 2012; 46 (12): 6705–6713.

Praetorius A, Tufenkji N, Goss KU, Scheringer M, von der Kammer F, Elimelech M. The road to nowhere: equilibrium partition coefficients for nanoparticles. *Environmental Science: Nano* 2014; 1 (4): 317-323.

Quik JTK, Vonk JA, Hansen SF, Baun A, Van de Meent D. How to assess exposure of aquatic organism to manufactured nanoparticles?. *Environ. Intern.* 2011; 37: 1068-1077.

Rosenbaum RK, Bachman TM, Gold LS, Huijbregts M-A.J., Jolliet O, Juraske R, Koehler A, Larsen HF, MacLeod M., Margni M. USEtox-the UNEP-SETAC toxicity model: recommended characterisation factors for human toxicity and freshwater ecotoxicity in Life Cycle Impact Assessment. *Int. J.LCA* 2008, 13 (7): 532-546.

Rosenbaum RK, Marhni M, Jolliet O. A flexible matrix algebra framework for the multimedia multipathway modeling of emission to impacts. *Environ Int* 2007; 33: 624-634.

Sadiq IM, Dlai S, Chandrasekaran N, Mukherje A. Ecotoxicity study of titania (TiO₂) NPs on two microalgae species: *Scenedesmus* sp. and *Chlorella* sp. *Ecotox Environ Safe* 2011; 74: 1180-1187.

Salieri B, Bauman J, Pasteris A., Koser J, D'amato R, Righi S., Filser J. The influence of exposure mode in the hazard identification of metallic nanoparticles: the case of TiO₂ and *Daphnia magna*. *SETAC World Congress* 2012,

Securing a sustainable future: Integrating science, policy and people. Berlin: SCIENTIFIC COMMITTEE OF SETAC EUROPE, 2012 pp.435.

Seitz F, Bundschuh M, Rosenfeldt RR, Schulz R. Nanoparticle toxicity in *Daphnia magna* reproduction studies: the importance of test design. *Aquatic toxicology* 2013; 163-168.

Seitz F, Rosenfeldt RR, Schneider S, Schulz R, Bundschuh M. Size, surface and crystalline structure composition-related effects of titanium dioxide nanoparticles during their aquatic life cycle. *Sci Total Environ* 2014; 493: 891-897

Sillanpää M, Paunu TM, Sainio P. Aggregation and deposition of engineered TiO₂ nanoparticles in natural fresh and brackish waters. *Journal of Physics: Conference Series* 2011; 304: 1-8.

Som. C, Berges M, Chaundhry Q, Dusinka M, Fernandes TF, Olsen SI, Nowack B. The importance of life cycle concepts for the development of safe nanoproducts. *Toxicology* 269 (2010): 160-169.

Strigul N, Vaccari L, Galdun C, Wazne M., Liu X, Christodoulatos C, Jasinkiewicz K. Acute toxicity of boron, titanium dioxide, and aluminium nanoparticles to *Daphnia magna* and *Vibrio fischeri*. *Desalination* 2009; 248: 771-782.

UNEP (United Nations Environment Programme). Global guidance principles for life cycle assessment database. A basis for greener processes and products; 2011 [Report available at/lcinitiative.unep.fr]

Walser T, Demon E, Lang DJ, Hellweg S. Prospective environmental life cycle assessment of nanosilver T-Shirt. *Environ Sci Technol* 2011; 45: 4570-4578.

Warheit DB, Hoke RA, Finlay C, Donner EM, Reed KL, Sayes CM. Development of a base set of toxicity tests using ultrafine TiO₂ particles as a component of nanoparticle risk management. *Toxicol Lett* 2007; 171 (3): 99-110.

Wiench K, Wohlleben W, Hisgen V, Radke K, Salinas ZS, Landsiedel R. Acute and chronic effects of nano- and non-nano-scale TiO₂ and ZnO particles on mobility and reproduction of freshwater invertebrate *Daphnia magna*. *Chemosphere* 2009; 76: 1356-1365.

Xiong D, Fang T, Yu L, Sima X, Zhu W. Effects of nano-scale TiO₂, ZnO and their bulk counterparts on zebrafish: acute toxicity, oxidative stress and oxidative damage. *Sci Total Environ* 2011; 409: 1444-1452.

Zamagni A, Buttol P, Porta PL, Buonamici R, Masoni P, Guinée J, Heijungs R, Ekvall T, Bresani R, Bienkowska A, Pretato U. Critical review of current research needs and limitations related to ISO-LCA practice. Roma: ENEA Italian National Agency for New Technologies Energy and the Environment, 2008.

Zhu X, Chang Y, Chen Y. Toxicity and bioaccumulation of TiO₂ nanoparticle aggregates in *Daphnia magna*. *Chemosphere* 2010; 78: 209-215

Zhu X, Zhu L, Chen Y, Tian S. Acute toxicities of six manufactured nanomaterial suspensions to *Daphnia magna*. *J Nanopart Res*, 2009: 67-75.