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Computational ecotoxicology: Simultaneous prediction of ecotoxic effects of nanoparticles under different experimental conditions

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ABSTRACT

Nanotechnology has brought great advances to many fields of modern science. A manifold of applications of nanoparticles have been found due to their interesting optical, electrical, and biological/chemical properties. However, the potential toxic effects of nanoparticles to different ecosystems are of special concern nowadays. Despite the efforts of the scientific community, the mechanisms of toxicity of nanoparticles are still poorly understood. Quantitative-structure activity/toxicity relationships (QSAR/QSTR) models have just started being useful computational tools for the assessment of toxic effects of nanomaterials. But most QSAR/QSTR models have been applied so far to predict ecotoxicity against only one organism/bio-indicator such as *Daphnia magna*. This prevents having a deeper knowledge about the real ecotoxic effects of nanoparticles, and consequently, there is no possibility to establish an efficient risk assessment of nanomaterials in the environment. In this work, a perturbation model for nano-QSAR problems is introduced with the aim of simultaneously predicting the ecotoxicity of different nanoparticles against several assay organisms (bio-indicators), by considering also multiple measures of ecotoxicity, as well as the chemical compositions, sizes, conditions under which the sizes were measured, shapes, and the time during which the diverse assay organisms were exposed to nanoparticles. The QSAR-perturbation model was derived from a database containing 5520 cases (nanoparticle–nanoparticle pairs), and it was shown to exhibit accuracies of *ca.* 99% in both training and prediction sets. In order to demonstrate the practical applicability of our model, three different nickel-based nanoparticles (Ni) with experimental values reported in the literature were predicted. The predictions were found to be in very good agreement with the experimental evidences, confirming that Ni-nanoparticles are not ecotoxic when compared with other nanoparticles. The results of this study thus provide a single valuable tool toward an efficient prediction of the ecotoxicity of nanoparticles under multiple experimental conditions.

1. Introduction

Modern science has witnessed lately a great and promising development of nanotechnology. In fact, the possibility of manipulating matter at such a small scale has provided the way to create many nanoentities that have become part of the daily human life as e.g. consumer products in optics, electronic devices, sensors or the textile industry (Luque and Varma, 2013). In this context, nanoparticles, considered as materials with nanoscale dimensions (1–100 nm), have played a very important role due to their unique electronic (Chen et al., 2012; Kim et al., 2013; Liz-Marzán and Kamat, 2004), optical/photonic (Chan et al., 2013; Schoen et al., 2013; Zhang et al., 2013), magnetic (Corchero and Villaverde, 2009), and catalytic properties (Biffis and Králik, 2001; Chan et al., 2013; Lu et al., 2013b; Yang et al., 2013). For this reason, several areas like those related with biomedical research have been highly impacted by the use of nanoparticles (Choi and Wang, 2011), which have been applied as agents for optical imaging in anticancer chemotherapy (Li et al., 2006; Zhang et al., 2013), and as valuable elements to implement hyperthermia treatment (Corchero and Villaverde, 2009). Also, some investigations have been performed to study the antimicrobial profiles of nanoparticles (He et al., 2012; Zeyons et al., 2009). Nanomaterials have been very important for gene delivery (He et al., 2013), as well as essential components of drug-delivery systems (Lu et al., 2013a).

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However, one of the most alarming problems associated to the use, disposal and manipulation of nanoparticles has been the appearance of serious toxic effects on ecosystems, and consequently, the occurrence of damages to the environment (Monteiro-Riviere and Tran, 2007). Several works have been carried out with the aim of assessing the ecotoxicity of nanoparticles on different bio-indicators such as *Danio rerio* (zebrafish), *Pseudokirchneriella subcapitata* (microalgae), *Daphnia magna* (planktonic crustacean) and many others (Bar-Ilan et al., 2009; García et al., 2011; Gong et al., 2011; Griffitt et al., 2008; Heinlaan et al., 2008; Hund-Rinke and Simon, 2006; Kasemets et al., 2009; Lin and Xing, 2007; Ma et al., 2010, 2011; Marsalek et al., 2012; Sadiq et al., 2011; Zhu et al., 2008, 2009, 2010, 2012). However, many questions regarding the mechanism of toxicity of nanoparticles remain yet unanswered. Moreover, experimental data pertaining to ecotoxicological profiles is dispersed, and sometimes, several inconsistencies are observed in the conclusions reached from different research works (Lanone et al., 2009). Despite the existence of powerful experimental methodologies such as high throughput/content screening (HTS/HCS) (Holden et al., 2013) to test large samples of nanoparticles, the possibility of covering the huge chemical "nanospace" is very reduced if we consider all possible combinations of nanoparticles exhibiting different compositions, sizes, shapes, and many other physicochemical properties. As a result, the need of large batteries of toxicity assays instigates a remarkable expenditure of financial resources and time. In this sense, alternative computational approaches may help to rationalize the assessment of toxic effects of new and modified nanomaterials.

Quantitative structure-activity/toxicity relationships (QSAR/QSTR) methods have long been particularly useful for predictions of toxic effects of chemicals on different biological systems (Castillo-Garit et al., 2008; Estrada, 1998; Estrada and Uriarte, 2001; Estrada et al., 2001, 2003; Fernandez et al., 2012; Kar and Roy, 2010, 2012; Roy and Ghosh, 2007, 2009), including the modeling of ecotoxicological profiles of chemicals (Speck-Planche et al., 2012). In nanotoxicology, only a few works have employed QSAR/QSTR models (Epa et al., 2012; Fourches et al., 2010; Puzyn et al., 2011; Shao et al., 2013), with several reviews reported about risk assessment of nanomaterials (Burello and Worth, 2011; Cattaneo et al., 2010; Puzyn et al., 2009). However, until now, all the QSAR/QSTR models have been based on classical approaches and derived by resorting to small datasets of nanoparticles, in which the toxicity tests have been carried out against only one biological entity (cell line, bio-indicator, etc.). Thus, and despite their wide applications, classical QSAR/QSTR models preclude having a deeper knowledge about the real ecotoxic effects of nanoparticles. For this reason, the setup of a computational model able to predict the ecotoxic effect of nanoparticles in different assay organisms/bio-indicators and diverse experimental conditions is a task of major importance, because it would contribute to the establishment of efficient rules for risk assessment of nanomaterials to the environment. In a very recent work, a general-purpose perturbation theory for multiple-boundary QSAR problems has been formulated (Gonzalez-Diaz et al., 2013). In that work, several powerful and evolved QSAR-perturbation models were developed and validated, with the aim of performing fast virtual screening of large datasets of chemical species in different research areas, ranging from predictions of chemical reactions to the modeling of ADME (absorption, distribution, metabolism, elimination) properties, including the assessment of different physicochemical properties associated to the process of self-aggregation of drugs into micellar nanoparticles. There are still no reports of nano-QSAR models to simultaneously predict ecotoxicity parameters of nanoparticles targeting multiple structural parameters and/or experimental conditions. In an attempt to overcome this problem, we develop here a QSAR-perturbation model aimed at predicting different ecotoxicological profiles of nanoparticles by considering changes in chemical compositions, sizes and conditions under which sizes were measured, shapes, measures of ecotoxicity, assay organisms/bio-indicator species (aquatic and terrestrial), and time during which those assay organisms were exposed to nanoparticles.

2. Materials and methods

Our raw dataset consist of nanoparticles containing 18 different chemical compositions, namely: titanium(IV) oxide (TiO_2), zinc oxide (ZnO), copper(II) oxide (CuO), aluminum oxide (Al_2O_3), nickel(II) oxide (NiO), aluminum (Al), copper (Cu), nickel (Ni), silver (Ag), iron(II, III) oxide (Fe_3O_4), cerium(IV) oxide (CeO_2), zinc (Zn), lanthanum(III) oxide (La_2O_3), ytterbium(III) oxide (Yb_2O_3), gadolinium(III) oxide (Gd_2O_3), iron(III) oxide (Fe_2O_3), cobalt (Co), and iron (Fe). Given that nanoparticles belonging to these chemicals/materials were assayed against more than one experimental condition (different time, organism/bio-indicator, etc.), and contained also different sizes and shapes, the original dataset comprised 85 cases of nanoparticles (All the details of this dataset are presented in the Supplementary Material; file SM1). The whole dataset was retrieved from the literature (Bar-Ilan et al., 2009; García et al., 2011; Gong et al., 2011; Griffitt et al., 2008; Heinlaan et al., 2008; Hund-Rinke and Simon, 2006; Kasemets et al., 2009; Lin and Xing, 2007; Ma et al., 2010, 2011; Marsalek et al., 2012; Sadiq et al., 2011; Zhu et al., 2008, 2009, 2010, 2012). All the cases mentioned above were tested by using at least 1 of 4 measures of ecotoxicity, against at least 1 of 20 assays organisms/bio-indicators, and the nanoparticles had at least 1 of 7 labels associated to their shape. Additionally, at least 1 of 5 conditions to measure the size of the nanoparticles was taken into account, and the ecotoxicity of a given nanoparticle was measured in at least 1 of 9 intervals of time. In order to carry out a rigorous modeling design, each of the 85 cases was assigned to 1 of 2 possible groups related with their ecotoxicities in a specific experimental condition c_j. Thus, the nanoparticles/cases were considered as non-ecotoxic [$Tox_i(c_j) = 1$] when they exhibited high values of measures of ecotoxicity; otherwise, they were selected as ecotoxic [$Tox_i(c_j) = -1$]. Notice that $Tox_i(c_j)$ is a categorical variable that is used to classify nanoparticles as non-ecotoxic or ecotoxic, and the assignments for all the cases were realized by taking into account certain arbitrary (but rigorous) cutoff values of ecotoxicity (see Table 1). It is necessary to point out that c_j is an ontology of the form $c_j = >(m_t, a_o, p_s, c_p, t_e)$. In this context, m_t is used to define the measures of ecotoxicity (EC_{50}, IC_{50}, LC_{50}, or TC_{50}). The different assay organisms (*D. magna*, *P. subcapitata*, *D. rerio*, etc) are described by the element a_o. On the other hand, p_s is focused on the nanoparticle shape, while c_p defines the condition under which the size of each nanoparticle was measured, i.e., if the nanoparticle size was measured for dry powders or for suspensions of nanoparticles. Finally, t_e is referred to the time during which the assay was performed. Then, it is intuitive to see that the combination of the five elements just mentioned defines a unique

Table 1
Cutoff values for diverse measures of biological effects.

Measure of ecotoxic effect (units)	Concept	Cutoff value[c]
EC_{50} (μM)[a]	Effective concentration of the nanoparticle which inhibits at 50% the growth of the assay organism.	≥168.45
IC_{50} (μM)p	Concentration of the nanoparticle which inhibits the root elongation of the assay organism (plants) at 50%.	≥177.62
TC_{50} (μM)[b]	Concentration which causes toxic effects in 50% of the assay organisms.	≥225.81
LC_{50} (μM)[b]	Lethal concentration which causes mortality in 50% of the assay organisms/bio-indicators.	≥334.08

[a] Based on assays with bacteria and algae.
[b] Based on assays with crustaceans and fish. References use the symbol EC_{50}, but we employed TC_{50} for crustaceans and fish in order to differentiate it from that used for bacteria and algae, because they have different toxicological meanings.
[c] Condition under which a nanoparticle/case was considered as non-ecotoxic.