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Toxicity of nanoparticles

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Introduction

Toxicity of nanoparticles is one of the most attractive and amazing scientific areas of research. In the recent decades, nanomaterials have deeply integrated into our everyday's life. There are numerous examples of already established and possible applications of using nanoparticles such as textile, cosmetics, optical, pharmacy, electronics, etc. According to the recent research [1] over 1000 different manufactured nanoparticles (NPs) were developed and introduced to the market; some of them may cause toxic effects in humans and nature. Although the nanotechnology field is growing rapidly, the potential harmful effects of nanomaterials on human's health or the environment have not yet been identified. Thus, there is a clear need for assessment of such potentially dangerous toxic effects of nanomaterials for human and environment in a short-term period.

The previous review shows that no single particle characteristic can be a hallmark indicator of toxicity, although some particle characteristics show some role in directing the biological fate and toxicity [2]. However, Oberdörster et al. [3] suggested that the particle size is not the only possible factor influencing the toxicity of nanomaterials. The following features should be also considered:

- size distribution,
- agglomeration state,
- shape,
- porosity,
- surface area,
- chemical composition,
- structure-dependent electronic configuration,
- surface chemistry,
- surface charge,
- crystal structure

One of the greatest challenges for assessing the potential risks of manufactured NPs is the lack of rational evidence-based system for understanding the hazard of nanomaterials. Taking into account the increasing number of NPs produced or to be produced in the near future, it is clearly impossible to evaluate the hazard parameters by testing all these NP products on a case-by-case basis. The classical way of assessing

toxicity, e.g. by performing in-vivo experiments, is very expensive and time consuming. Performing such tests for all possible nanoparticle types, sizes and concentrations is practically infeasible. A cheap and efficient alternative to such tests is using predictive computational models, for instance Quantitative Structure-Activity Relationship (QSAR) models.

State-of-the-art of predictive modeling for characterization and evaluation of nanomaterials toxicity

Regarding the NP structure, the class of nanomaterials is not homogenous, combining a range of physico-chemical properties, as well as possible mechanisms of metabolism and toxicity. Thus, it is impossible to assume one common modeling approach for all nanomaterials. Each mode of toxicity and each class of nanomaterials should be studied separately [4].

There are only few Quantitative Nano Structure Activity Relationship models (QNSAR also frequently referred to as nano-QSAR) described in articles [5, 6]. Most of them were developed for carbon-based nanomaterials. Rasulev et al. [7] developed a QNSAR model for the cytotoxicity to the bacterium E. coli of nano-sized metal oxides. They successfully predicted the toxicity of seven compounds (namely, SnO2, CuO, La2O3, Al2O3, Bi2O3, SiO2 and V2O3) from the model trained on the other seven oxides (ZnO, TiO2, Fe2O3, Y2O3, ZrO2, In2O3 and Sb2O3).

Quantitative Structure-Activity Relationship (QSAR) approach allows the possibility of theoretical analyses of a great number of properties in a short time without extra cost and without in vivo experiments. There are five OECD [8] principles for the validation of QSAR models. An ideal QSAR model, which is applicable for regulatory purpose, should be associated with

(i) a well-defined endpoint;
(ii) an unambiguous algorithm;
(iii) a defined domain of applicability;
(iv) appropriate measures of goodness-of-fit, robustness, and predictivity;
(v) a mechanistic interpretation, if possible.

Unfortunately, it is extremely difficult to fulfill all of these principles for QNSARs applicable to nanomaterials. There are two main difficulties related to the development of QNSARs. The first one is a lack of sufficiently numerous and systematic experimental data, while the second one is a very limited knowledge on the mechanisms of the toxic action. There is still no clear notion about a toxic behavior NPs and characteristics that determine this behavior.

Natural and anthropogenic nanoparticles gain access into the human body through the main ports of entry including the lungs, the skin, or the gastrointestinal tract. The unique properties of nanoparticles allow them not only to penetrate physiological barriers but also to travel throughout the body and interact with subcellular structures. Toxicological studies show that nanoparticles can be found in various cells and organellas such as mitochondria, lipid vesicles, nucleus and macrophages [9,10]. Thus, we must consider the following type of interaction between NPs and the organism.

Nanoparticles can induce the formation of **reactive oxygen species (ROS)**, for instance, superoxide radicals, hydroxyl radicals. This results in oxidative stress and inflammation, leading to the impacts on lung and cardiovascular health [11].

Cytotoxicity and Genotoxicity:

It is known that the mechanism of oxidative stress is mainly responsible for the observed genotoxic and cytotoxic effects induced by nanoparticles. Epidemiological studies have shown that nanoparticles might be genotoxic to humans [12]. Irreversible DNA modifications resulting from the activity of ROS may lead to heritable mutations.

Neurotoxicity:

It has been reported that inhaled nanoparticles, depending on their size, may be distributed to organs and surrounding tissues, including the olfactory mucosa or bronchial epithelium and then can be translocated the olfactory nerves to the central nervous system [11].

Immunotoxicity

The effects of nanoparticles on the immune system are still unclear. The toxicological studies have suggested that nanoscale particles interaction with the defense activities of immune cells can change their antigenicity and stimulate and/or suppress immune responses. Direct experiments showed that macrophages uptake of nanoparticle–protein complexes may change the formation of the antigen and initiate an autoimmune response [13]. Some studies have also reported that nanoparticles may induce damage to red blood cells (erythrocytes).

Ecotoxicity

In many cases, lack of data precludes an appropriate implementation of statistical methods, including necessary external validation of the model. The problem of the paucity of data will be solved only when a strict collaboration between the experimentalists and QSAR modelers is established. The role of the modelers in such studies should not be restricted only to rationalization of the data after completing the experimental part, but also they must be involved in the planning of the experimentation. Since the experiments on nanomaterials are usually expensive, a kind of compromise between the highest possible number of compounds for testing and the lowest number of compounds necessary for developing a reliable QSAR model should be reached.

When analyzing the current status of nano-QSAR, the following noteworthy suggestions for further work can be made:

1. There is a strong need to supplement the existing set of molecular descriptors by novel “nanodescriptors” that can represent size-dependent properties of nanomaterials.

2. A stronger than usual collaboration between the experimentalists and nano-QSAR modelers seems to be crucial. On one hand, it is necessary to produce data of higher usefulness for QSAR modelers (more compounds, more systematic experimental studies within groups of structural similarity, etc.). On the other hand, a proper characterization of the nanomaterials structure is not possible only at the theoretical (computational) level. In such situation, experiment-based structural descriptors for nano-QSAR may be required.

Further aspects of modeling studies by using a QSAR approach are described in [11,14]. The paper provides advises on how to develop QSARs in future for nanomaterials given the current experiences with QSAR in ecotoxicology of regular bulk chemicals. One of the recommendations is that separate QSARs need to be developed for individual classes of nanomaterials, and some possibilities for structural descriptors are given.

Results and discussion

During the project published data on nanotoxicity were collected and was uploaded into Online Chemical Database and Modelling Environment [15] (http://ochem.eu/). The main priorities were given to toxicity of metal and metal oxides nanoparticles (Fe, Ag and TiO_2 are our first targets; other metals/metal oxide will be also included). About 500 data points were collected.

Several changes were made to update the OCHEM to be used as a user-friendly data base for the nanotoxicity data collection. To describe the toxic properties of the nanoparticles an abbreviation was used: for each property "Nano" prefix was used to separate the nano-properties from all others.

The basic characteristics of nanoparticles are chemical composition, average particle size (APS) and shape of the nanoparticles.

Chemical composition of NP (Material Nanoparticles of Elements)

Elemental composition describes what elements make up ENMs. Usually there is no correlation between the toxicity of NPs and the toxic properties of bulk materials[16,17]. However, composition of NPs is an important parameter [11].

Average nano-particle size

Nanoscale materials are defined as a set of substances where at least one dimension is less than approximately 100 nanometers. This parameter seems to be at least as important as the chemical composition as far as we move towards the nano-range, and the dependences of the toxicity of diverse inorganic materials versus the particle size can exhibit either a volcano-shape behavior or an exponential curve descending with the increase in the particle size. There are several possibilities to describe the average particle size (APS). On the one hand it is possible to use an

average value. On the other hand one can describe APS using an interval, i.e. as [min_value; max_value].

Shape (morphology) of nanoparticles

The role of this factor is definitely underexplored and underestimated compared to the first two factors. Nanomaterials can be nanoscale in one dimension (eg. surface films), two dimensions (e.g. strands or fibres), or three dimensions (eg. particles). They can exist in single, fused, aggregated or agglomerated forms with spherical, tubular, and irregular shapes. Common types of nanomaterials include nanotubes, dendrimers, quantum dots and fullerenes.

Nanomaterials can be created with various modulation dimensionalities [17]: zero (atomic clusters, filaments and cluster assemblies), one (multilayers), two (ultrafine-grained overlayers or buried layers), and three (nanophase materials consisting of equiaxed nanometer sized grains). Nanomaterials (gold, carbon, metals, metal oxides and alloys) with variety of morphologies (shapes) are depicted in Fig. 1.

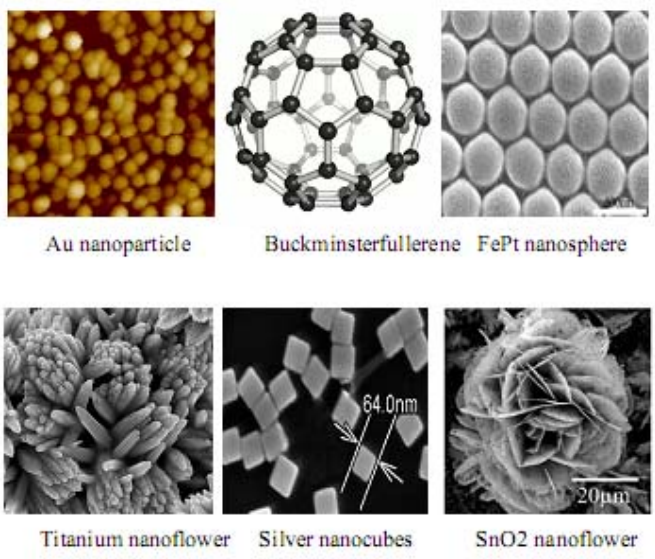
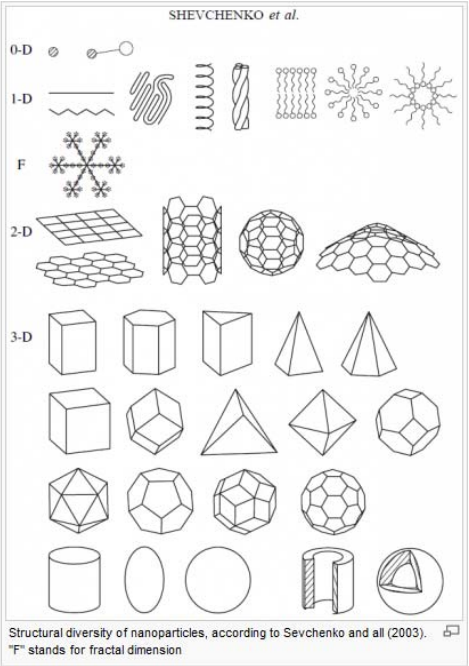


Fig.1 Sample of the forms of the nanoparticles [17].

There are two different classification schemas describing the possible forms of the nanoparticles [18, 19]. Fig. 2 indicates the theoretical shapes of objects. As one can see they are rather different from those observed in experimental measurements (Fig. 1). Shevchenko suggested a classification based on a geometrics apology between shapes of the particles and figures [18]. Most of the figures are well known mathematical objects and can be easily used

to calculate some descriptors for QSAR modelling. Maynard [19] looked at the classification from another point of view. He took as a model some kind of conceptual subjects taking into account reactivity. Nanoparticles belong to nine categories (Fig.2) depending on their structure and properties: i) spherical or compact particles (compositionally homogeneous), ii) highaspect-ratio particles (compositionally homogeneous), iii) complex non-spherical particles (compositionally homogeneous), iv) compositionally heterogeneous particles: core surface compositional variation, v) compositionally heterogeneous particles: distributed compositional variation, vi) homogeneous agglomerates (agglomerates of a single particle class), vii) heterogeneous agglomerates (aggregates of diverse particle types), viii) active particles (particle behaviour and properties depend on external stimuli), and ix) multifunctional particles (particle behaviour and properties depend on functional responses to local environment and stimuli). He also paid an attention to potential mixtures of nanoparticles.

Unfortunately both these descriptions are mainly a nice theoretical approach. When one moves to the practice, then it is not easy to figure out how these classification schemas can be applied. Only few authors mention shape of the particles in their works and even then there is no unity between the authors. For instance if we take titanium or SiO_2 nanoparticles (Fig.1), some authors define this shape like a nanoflower and other call the same form as sea-urchin-like shape.



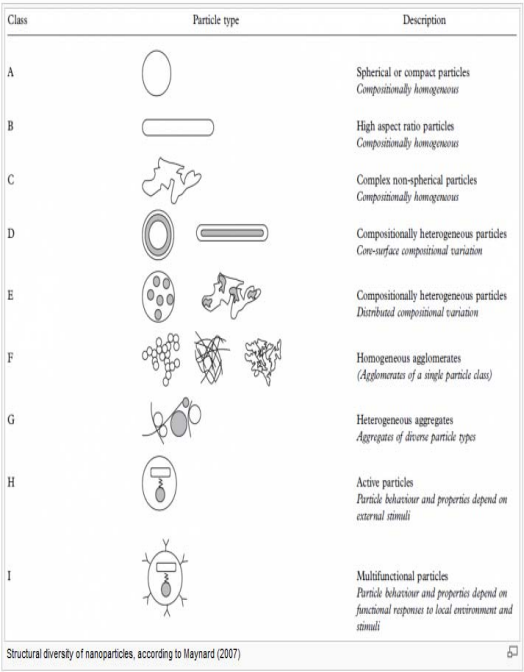


Fig. 2. Sample of the forms of the nanoparticles [18, 19].

Material Nanoparticles of Elements, APS and shape of the nanoparticles were used as obligatory condition for all properties in OCHEM. Thus each record was required to incorporate information about these the most important parameters of nanoparticles. The collected data are summarised in Table 1.

Table 1. Overview of the collected data.

NanoToxicity LC50 aquatic	89 records	13 compounds
NanoToxicity MIC	101 records	7 compounds
NanoToxicity immobilization	25 records	1 compounds
NanoToxicity mortality	75 records	5 compounds
NanoToxicity log(1/EC50)	17 records	17 compounds
NanoToxicity LC20 aquatic	15 records	5 compounds
NanoToxicity LD50	11 records	8 compounds
NanoToxicity EC50	21 records	8 compounds
Nanotoxicity survival	14 records	10 compounds
Nanotoxicity cell viability	32 records	1 compounds
NP aggregation state	3 records	1 compounds
Nanotoxicity [*OH] generation	48 records	2 compounds
NanoToxicity NOEC	20 records	5 compounds

NanoToxicity EC20	6 records	4 compounds

We have developed several test models based on ASsociative Neural Networks (ASNN)[20], linear methods and several others approaches available at OCHEM [15]. We have also reproduced a previously published model [21]. Calculated models were comparable in terms of their statistical parameters with those described in the original article [21]. The originally published and reproduced with Multiple Linear Regression (MLRA) models had R^2=0.85, Q^2 =0.77 and R^2 =0.87, Q^2 =0.83, respectively. R^2 is square of Pearson's correlation coefficient and Q^2 is coefficient of determination (http://en.wikipedia.org/wiki/Coefficient_of_determination) that is another frequently used in QSAR literature statistical parameter. It is also known as cross-validated R^2. The best models were calculated with ASNN and in some cases with MLRA methods (see Table 2).

Table 2. Statistic data of calculated models

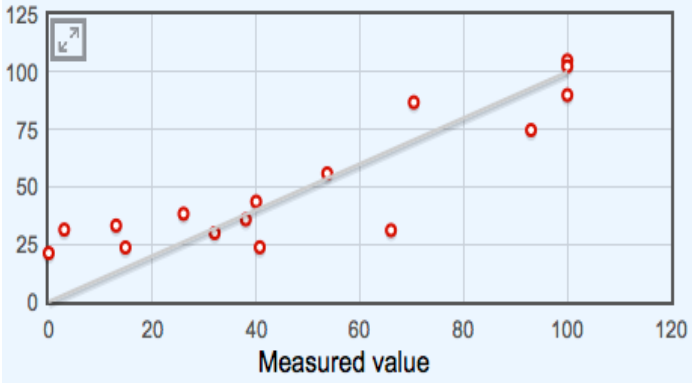
Models	***Properties***	***Descriptors***	***R^2***	***Q^2***
Articles № 16	Log(1/EC50)	ΔH_{Me+}	0,85	0,77
ASNN	Log(1/EC50)	ΔH_{Me+}	0,86	0,78
MLRA	Log(1/EC50)	ΔH_{Me+}	**0,87**	**0,83**
FSMLR	Log(1/EC50)	ΔH_{Me+}	0,87	0,79
KNN	Log(1/EC50)	ΔH_{Me+}	0,77	0,6
ASNN	Immobilization(the same time)	APS; exp.concentration	**0,78**	**0,78**
MLRA	Immobilization(the same time)	APS; exp.concentration	0,47	0,47
FSMLR	Immobilization(the same time)	APS; exp.concentration	0,43	0,4
KNN	Immobilization(the same time)	APS; exp.concentration	0,53	0,51
ASNN	Immobilization(different time)	APS; time; exp.concentration	**0,71**	**0,69**
MLRA	Immobilization(different time)	APS; time; exp.concentration	0,27	0,27
FSMLR	Immobilization(different time)	APS; time; exp.concentration	0,36	0,34
KNN	Immobilization(different time)	APS; time; exp.concentration	0,54	0,49

MLRA (Multiple Linear Regression Analysis); ASNN (ASsociative Neural Networks); FSMLR (Fast Stagewise Multiple Linear Regression) [22]; KNN (K-Nearest Neighbors). All these machine learning methods are available at OCHEM [15]. The red values highlight models with the highest accuracy of predictions, which are shown at Fig. 3.

Several calculated models developed using measured properties of nanoparticles are shown at Fig. 3.

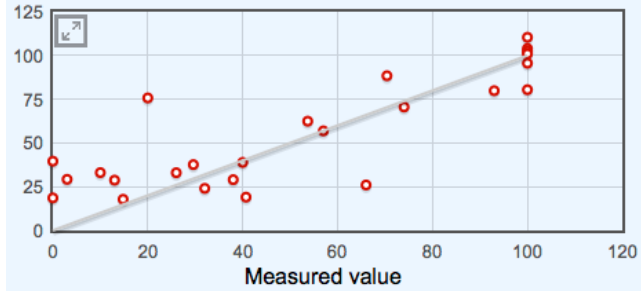
a).

Data Set	#	R2	q2	RMSE	MAE
Training set: Immobil_72h	16 records	0.78	0.78	16.12	12.80



b).

Data Set	#	R2	q2	RMSE	MAE
Training set: Immobilisation_25	25 records	0.71	0.69	19.94	14.47



c).

Data Set	#	R2	q2	RMSE	MAE
Training set: Puzin training	10 records	0.84	0.84	0.20	0.17
Test set: Puzin validation [x]	7 records	0.87	0.83	0.19	0.14

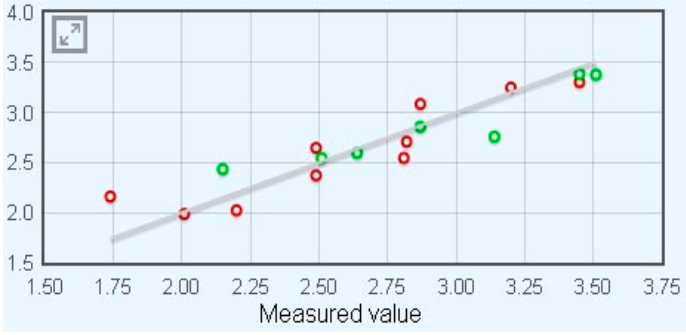


Fig.3 Calculated models **a.** immobilisation without accounting the time dependences (ASNN method [20] was used). Data are taken from the original article [21]. **b.** immobilisation with accounting the time dependence (ASNN) from the same

reference. **c.** EC50 toxicity of the nanomaterials calculated using MLRA (the model was reproduced according to the original publication of Puzyn T [21].

My work was presented as a poster [23] at an international conference Munich Interact 2012 (http://www.munich-interact.org/) and in a nearest future a publication about the main achievement about will be prepared.

Further goals

The main problem with modelling nanoparticles is how possible to describe a structure of the nanomaterials. There are a lot of different types of the nanomaterials with different parameters. It is simply impossible to combine all nano objects in one description. The main idea is to analyse every group separately. During this fellowship the work has been mainly concentrated on the analysis of one class of nanoparticles notably inorganic metal nanoparticles.

At the moment we use a characteristic of the nanoparticles and experiments for designing models. The main question is which combination of properties will be the most appropriate ones for definition of the nanomaterials. To solve this problem we need to collect a reliable dataset with comprehensive description of nano objects and their main important characteristic.

The first steps were made toward QSAR modeling of the nanotoxicity. The OCHEM database (http://ochem.eu) was extended to incorporate nanoscale objects and it will be filled continuously with data on the nanotoxicity of metals and metal oxides.

Further work will include: accumulation and digestion of the available literature and own experimental data (Fe, Ag with algae, daphnia, zebra fishes, planaria, molluscs) on the relationship between the toxicity of inorganic nanomaterials and their chemical composition, size of the nanoparticles, shape (morphology) of nanoparticles and the availability and nature of the grafted groups. The experiments will be carried out in parallel in Moscow, St-Petersburg and Puschino, Russia; and also in Institute of Environmental Sciences (CML) of the Faculty of Science of Leiden University, Leiden, The Netherlands.

In our studies, we plan to use these data to develop predictive QSAR models for nanoparticles toxicity. The further work will include development of new descriptors to characterize nanoparticles according to their chemical composition and size. As a result we would like to have of a user-friendly database containing

experimental physico-chemical and biological properties of nanoparticles and the evaluation of predictability of the toxicity of novel nanomaterials on the basis of newly developed algorithms linking physicochemical substance properties to the observed toxicity profiles of the NPs.

Supported information:

Abbreviations

ASNN − ASsociative neural network
kNN − K-Nearest Neighbors
MLRA − Multiple Linear Regression Analysis
FSMLR − Fast Stagewise Multiple Linear Regression
NP − NanoParticles
QSAR − Quantitative Structure Activity Relationship
ROS − Reactive Oxygen Species
OCHEM – On-line CHEmical database and Modelling environment (http://ochem.eu [15])

Abstract from conference «Munich Interact 2012»

Modeling toxicity of nanoparticles using Online Chemical Modeling Environment

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In the recent decades, nanomaterials have deeply integrated into our everyday's life. There are numerous examples of already established and possible applications of using nanoparticles such as textile, cosmetics, optical, pharmacy, electronics, etc. Although the nanotechnology field is growing rapidly, the potential harmful effects of nanomaterials on human's health or the environment have not yet been identified. Thus, there is a clear need for assessment of such potentially dangerous toxic effects of nanomaterials.

The classical way of assessing toxicity, e.g. by performing in-vivo experiments of hydrobionts, is very expensive and time consuming. Performing such tests for all possible nanoparticle types, sizes and concentrations is practically infeasible. A cheap and efficient alternative to such tests is using predictive computational models, for example Quantitative Structure-Activity Relationship (QSAR) models.

Using QSARs for nanoparticles is a new and still developing area of research. Within our study, we have collected toxicity data for a number of nanoparticles (currently, metals and metal oxides) for different species: daphnids, planaria worms, mussels. Additionally, we have collected the information for different nanoparticles sizes, under different concentrations and exposure intervals. The data has been uploaded to the Online Chemical Modeling Environment (www.ochem.eu) and is publicly accessible by everyone on the Web. In our studies, we plan to use this data to develop predictive QSAR models for nanoparticles toxicity. Several models calculated using measured properties of nanoparticles are presented. The further work will include development of new descriptors to characterize nano-particles according to their chemical composition and size.

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