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(Q)SAR Modelling of Nanomaterial Toxicity - A Critical Review

Ceyda Oksel[1], Cai Y. Ma[1], Jing J. Liu[2,1], Terry Wilkins[1] and Xue Z. Wang[1,2*]

[1]Institute of Particle Science and Engineering, School of Chemical and Process Engineering, University of Leeds, Leeds, LS2 9JT, UK

[2] School of Chemistry and Chemical Engineering, South China University of Technology, Guangzhou, CHINA 510641

* Correspondence author, Professor Xue Z. Wang:

Tel.: +44 (0) 113 3432427, or +86(20)87114000

E-mail: x.z.wang@leeds.ac.uk, or xuezhongwang@scut.edu.cn

Abstract

There is an increasing recognition that nanomaterials pose a risk to human health, and that the novel engineered nanomaterials (ENMs) in the nanotechnology industry and their increasing industrial usage poses the most immediate problem for hazard assessment, as many of them remain untested. The large number of materials and their variants (different sizes and coatings for instance) that require testing and ethical pressure towards non-animal testing means that expensive animal bioassay is precluded, and the use of (quantitative) structure activity relationships ((Q)SAR) models as an alternative source of hazard information should be explored. (Q)SAR modelling can be applied to fill the critical knowledge gaps by making the best use of existing data, prioritize physicochemical parameters driving toxicity, and provide practical solutions to the risk assessment problems caused by the diversity of ENMs. This paper covers the core components required for successful application of (Q)SAR technologies to ENMs toxicity prediction, and summarizes the published nano-(Q)SAR studies and outlines the challenges ahead for nano-(Q)SAR modelling. It provides a critical review of (1) the present status of the availability of ENMs characterization/toxicity data, (2) the characterization of nanostructures that meets the need of (Q)SAR analysis, (3) the summary of published nano-(Q)SAR studies and their limitations, (4) the in silico tools for (Q)SAR screening of nanotoxicity and (5) the prospective directions for the development of nano-(Q)SAR models.

Keywords: nanomaterial toxicity, nanotoxicology, QSAR, nanoSAR, in silico prediction of toxicity

1. Introduction

The potential human exposure to engineered nanomaterials (ENMs) and the release of into the environment have become more likely with the increasing use of ENMs for commercial purposes. Moreover, recent studies have revealed that the distinctive characteristics of ENMs not only make them superior to traditional bulk materials but also affect their potential toxicity (Arora, Rajwade, & Paknikar, 2012) and present a challenge for the existing regulatory system (Falkner & Jaspers, 2012). There is now a growing body of literature on the potential undesirable effects caused by the exposure to different types of ENMs (Horie & Fujita, 2011; Jeng & Swanson, 2006; Karlsson, Gustafsson, Cronholm, & Möller, 2009; Magrez, et al., 2006). Although the awareness of the potential adverse effects of ENMs is increasing, there are still numerous unanswered questions which complicate the appropriate evaluation of toxicity at the nano-scale dimension.

The toxicological evaluation of ENMs is complicated by many factors (e.g. the presence of a large number and variety of ENMs, the difficulties in categorising nanomaterials (NMs) for toxicological considerations and the fact that even a slight variation in characteristics of nanostructures may also be reflected in their biological response) which dramatically increase the effort required to evaluate the adverse effects of ENMs. It seems that the only reasonable approach to obtain toxicity information for the numerous ENMs without testing every single one is to relate the biological activities of ENMs to their structural and compositional features.

The need to use in silico methods, such as the (quantitative) structure-activity relationship ((Q)SAR) approach, for toxicity prediction of ENMs has been apparent since the EU's REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) Regulation promoted the use of alternative toxicity assessment methods (T. Puzyn, Leszczynska, & Leszczynski, 2010). As the name suggests, (Q)SAR is a computational technique which attempts to predict the biological activity of a compound by relating it to a set of structural and compositional properties such as particle size, size distribution, particle shape, surface area, zeta potential and crystal structure. The basic idea behind this approach is that different types of toxic effects (i.e. cytotoxic, genotoxic and inflammatory effects) can be related to measurable or calculable physicochemical descriptors. A schematic representation of nano-(Q)SAR workflow is given in Figure 1. .

This data-driven approach brings many advantages in terms of cost, time-effectiveness and ethical concerns. Although it has been satisfactorily used to predict the physicochemical properties of NMs, such as solubility (Gajewicz, 2012; Sivaraman, Srinivasan, Vasudeva Rao, & Natarajan, 2001; Toropov, Leszczynska, & Leszczynski, 2007; Toropov, Toropova, Benfenati,

Leszczynska, & Leszczynski, 2009) and elasticity (Mohammadpour, Awang, & Abdullah, 2011; Toropov & Leszczynski, 2006), development of reliable (Q)SAR models becomes more complicated when the actual processes and the endpoints of interest are biologically complex.

Despite all the challenges and open questions, there are some pioneering studies investigating the use of (Q)SAR models to predict the toxicity of ENMs (Epa, et al., 2012; Fourches, et al., 2010; R. Liu, Rallo, et al., 2013; R. Liu, Zhang, et al., 2013; T. Puzyn, et al., 2011; Sayes & Ivanov, 2010; Xue Zhong Wang, et al., 2014; Zhang, et al., 2012). We are now at the stage of getting the results of initial nano-(Q)SAR modelling attempts. Although the initial findings are encouraging, there is also a strong need to ensure the reliability of these models for gaining the acceptance of regulatory bodies and the confidence of potential end-users. We believe that once the main challenges related to the extension of the conventional (Q)SAR approach to nanotoxicology have been overcome, nano-(Q)SAR models will be able to reach their full performance potential and their outcome will be more valuable for predicting the toxicity of ENMs.

This review will focus on (Q)SAR analysis of ENMs for the purpose of toxicity modelling. The main aim of this paper is to give the reader a detailed understanding and critical analysis of the nano-(Q)SAR process, the concepts behind it, the appropriate tools to be used and the remaining knowledge gaps in this area. To that end, it covers major components that play an important role in both the development of (Q)SAR models and the practical use of these models for nanotoxicity prediction purposes.

2. Nanomaterial Toxicity

Nanotechnology is not entirely a new phenomenon since several natural ENMs like clays have been in existence in the environment for centuries. Several studies of nanoscale dimension have been conducted for many years in polymer science, prior to the birth of nanotechnology (Paul & Robeson, 2008). However, the living organisms have now adapted to the natural NPs while the manufactured ones are completely new and unprecedented (Sadik, 2013). The safety of ENMs falls into a very new field called nanotoxicology. These newly fabricated NMs have the ability to easily enter body, accumulate in tissues and cause harm (Oberdorster, et al., 2005). In recent years, some types of ENMs have been shown as hazardous to human health. It has been demonstrated in literature that carbon nanotubes (CNTs) are capable of inducing reactive oxygen species (ROS) (C. S. Sharma, et al., 2007) and pulmonary effects (Shvedova, et al., 2005). It has also been shown in toxicological studies that nano-sized titanium dioxide (TiO2) particles have the potential to induce cytotoxic (Saquib, et al., 2012; Setyawati, et al., 2012), genotoxic (Shukla, et al., 2011; Trouiller, Reliene, Westbrook, Solaimani, & Schiestl, 2009) and inflammatory

effects (Grassian, O'Shaughnessy, Adamcakova-Dodd, Pettibone, & Thorne, 2007; S. G. Han, Newsome, & Hennig, 2013). Another important example of the ENM which raises toxicological concerns due to its widespread use in consumer products is nano-silver. Although nano-silver was known to be harmless, recent studies (Asare, et al., 2012; Foldbjerg, Dang, & Autrup, 2011; Hussain, et al., 2006; Kim, et al., 2009) have provided convincing evidence of toxicity associated with the exposure to nano-silver. More detailed information about the potential adverse effects of various NMs has been provided by several researchers (Arora, et al., 2012; Holgate, 2010; Horie & Fujita, 2011; Jeng & Swanson, 2006; Magrez, et al., 2006; Saquib, et al., 2012; Sharifi, et al., 2012; Wani, Hashim, Nabi, & Malik, 2011).

Toxicological endpoint is the measure of toxic effect of a substance which determines how hazardous a substance is. In (Q)SAR analysis, the endpoint of interest is a measure of a specific type of activity, such as viability and cytotoxicity, which is going to be modelled and predicted. The toxicity of compounds can be evaluated by conducting in vivo, in vitro and in silico studies. Although in vitro assays are commonly preferred to in vivo tests due to their time and cost effectiveness, there is also a well-recognised need in the nano-science community to compare and validate the in vitro findings with in vivo observations. (Q)SAR models can be built and used for the prediction of all toxicological endpoints as long as sufficient toxicity data is provided as input (T Puzyn, Leszczyński, & Cronin, 2010). Ideally, biological effects of various compounds of different size, structure and complexity under relevant exposure conditions should be evaluated with standardized methods for the successful development of nano-(Q)SAR models.

3. Physicochemical Descriptors of ENMs

In traditional (Q)SAR analysis, molecular descriptors are used to characterize and quantify the physicochemical properties of chemicals which are potentially related to the endpoints of interest. Theoretical descriptors provide a great variety of physico-chemical information sources and valuable insights into the understanding of the potential relationship between molecular characteristics and biological activities. They can be derived from either different theories/semi-empirical methods or commercial software packages. Although more than 5000 descriptors (T Puzyn, et al., 2010) have been proposed and calculated to represent the structure of molecules, most of them are either inapplicable to ENMs or need at least some level of adaptation to be used at the nanoscale. The main problems in the computation of theoretical descriptors for nano-systems are the complexity and non-uniformity of ENMs which make the appropriate transformation of the nanostructures into a language for computer representation challenging and extremely time-consuming. Alternatively, the key variables, such as size, shape and surface charge can be measured by various experimental techniques and used as descriptors for

developing (Q)SAR models. Although the procedure of traditional (Q)SAR analysis is almost standardized, nano-(Q)SAR is still under development as there is still no clear consensus on measurement and modelling standards. The lack of deeper knowledge and clarification regarding how to characterize ENMs prior to or during the toxicity tests is widely recognised as one of the major challenges that must be addressed for successful application of (Q)SAR modelling approach for ENMs. To that end, this section identifies characteristics that may potentially influence the toxicity of ENMs and presents techniques for measuring these toxicity-related parameters.

3.1 Possible factors affecting the toxicity of ENMs and their measurements

The first step in the modelling of ENM toxicity is the identification of toxicity-related properties which can be used as potential determinants of adverse effects of ENMs. Since a complete and exact list of parameters influencing the toxicity of ENMs has not been established yet, a detailed material characterization prior to toxicity testing is essential to determine the factors contributing to the biological activities of ENMs and their potential hazards. Although there is still no scientific consensus on the minimum set of relevant nano-characteristics for toxicological evaluation, some particular physicochemical features are repeatedly emphasized in the majority of recommendations (Kevin W Powers, Carpinone, & Siebein, 2012). The size of ENMs is one of the most prominent key characteristics which is held responsible for the changing properties and behaviour of ENMs and hence included in the recommendation list of almost all nanotoxicologists. However, as stated by (Oberdorster, et al., 2005), the size of particle is not the only factor which causes the changes in biological activities of materials at the nano-scale dimension. The following characteristics may also be linked to nanotoxicity: size distribution, agglomeration state, shape, crystal structure, chemical composition, surface area, surface chemistry, surface charge and porosity. (Kevin W Powers, et al., 2012) have investigated the key elements of NM characterization and expanded the list provided by (Oberdorster, et al., 2005) to include purity, solubility and hydrophobicity. In the recent review on the minimum set of physicochemical properties needed to characterize NMs, (Pettitt & Lead, 2013) have suggested that in addition to the parameters that are most likely to have an effect on NM behaviour such as size, surface properties, solubility and aggregation characteristics, information about the production process and history of ENMs should also be provided to avoid incorrect interpretation of toxicity data. Although it is a reasonable suggestion, the quantification of historical properties is the prerequisite for their use as descriptors in (Q)SAR studies. One of the most comprehensive lists of the important physico-chemical characteristics for toxicological studies has been provided by the OECD's Working Group on NMs (OECD, 2010). The research results have described the

physico-chemical properties of NMs that need to be addressed for characterization as they may be relevant to (eco)toxicity. The relevant properties mentioned in this guidance are listed in Table 1. The term composition in Table 1 covers chemical identity, molecular structure as well as degree of purity, impurities and additives. Another term in this list which is often broadly defined is the surface chemistry. It is meant here to identify various modifications of the surface (i.e. coating) and composition of outer layer of NMs. In OECD's list, there are also many properties such as dustiness and n-octanol-water partition coefficient that have not been specified as pre-requisites for NM characterization by other researchers. (Kevin W Powers, et al., 2012) have taken the dustiness as an example and argued that such a measurement for dry NM applications should be standardized first since the presence of well-established analytical techniques for the measurement of intended properties is essential to express the results in comparable terms. For the detailed description of potential toxicity-related physico-chemical properties as shown in Table 1, please refer to OECD's guidance on testing ENMs (OECD, 2010).

3.1.1 Particle size and size distribution

The size of ENM is regarded as one of the most critical properties determining the toxicity potential of ENMs. The surface area to volume ratio increases with decreasing particle size. The change in surface-to-volume ratio also affects the surface energy and hence reactivity of the material. In addition to surface reactivity, the interaction of ENMs with living systems, the uptake and deposition of ENMs within the human body are also affected by particle size (Powers et al., 2007). It is generally believed that the risk posed by materials containing nano-sized particles increases with decreasing particle size (Monteiro-Riviere & Tran, 2007). Indeed, (Gurr, Wang, Chen, & Jan, 2005) have shown that the oxidative damage induced by TiO_2 particles is size-specific; the smaller the particle size, the greater the oxidative damage induced. Another ENM showing a size-dependent toxicity is nano-silver. (M. V. Park, et al., 2011) have compared the cytotoxicity, inflammation, genotoxicity and developmental toxicity induced by different-sized silver ENMs (20, 80 and 113nm) and stated that nano-silver particles with the smallest size have exhibited higher toxicity than the larger ones in the assays studied. All such findings suggest that the size of particles is one of the possible factors which may contribute to the toxicity of chemicals; however, in some cases no relationship has been observed between the toxicity of particles and their sizes (Karlsson, et al., 2009; Lin, et al., 2009).

There are several techniques that can be used to measure the size of ENMs. Although not a comprehensive list, the most common particle size measurement techniques applicable to ENMs are given in Table 2.

The results of different particle sizing techniques are usually not in compliance with each other as the measurement principles behind each sizing method are different. In general, it is possible to classify particle sizing methods applicable to NMs into three categories: microscopy-based, light scattering-based and separation techniques (Savolainen, et al., 2013). Electron microscopy techniques, based on scattered electrons (SEM) or transmitted electrons (TEM), provide very accurate information and give a clear view of individual and aggregated particles. Therefore, these methods can also be used for poly-disperse particle samples. The scanning electron microscopy (SEM) technique provides information about size, size distribution, particle shape and morphology but there is a risk of influencing particle properties during sample drying and contrasting (Bootz, Vogel, Schubert, & Kreuter, 2004). Unlike electron microscopy techniques, a vacuum environment is not needed to obtain atomic force microscopy (AFM) images which allow the measurement of particle size under ambient conditions (Gwaze, Annegarn, Huth, & Helas, 2007).

Dynamic light scattering (DLS) is based on the Brownian motion of suspended particles in solution. The main advantages of DLS techniques are their simplicity and rapidity while their main weaknesses are the high sensitivity to sample concentration and inability to differentiate between large individual particles and aggregates (Monteiro-Riviere & Tran, 2007). Dynamic centrifugal sedimentation (DCS) and analytical ultracentrifugation use the difference in sedimentation rates of different sized particles to separate a sample. (Tantra, et al., 2012) have emphasized that one of the main disadvantages of DCS is the requirement to know the exact density of the particle including coatings and adsorbed analytes on the surface. Another dry sizing method is the BET surface area analysis which calculates the mean particle diameter from surface area measurement based on the assumption that the particles are non-porous and spherical. Additionally, there are several other size measurement methods including laser diffraction, mobility analysis, acoustic methods, field flow fractionation (FIFFF) and fluorescence correlation spectroscopy (FCS), each of which has its own pros and cons. (Domingos, et al., 2009) have provided a good example of size measurement by multiple analysis methods including TEM, AFM, DLS, FCS and NPMTA and FIFFF. They have confirmed that particle size measured by DLS is typically higher than those obtained using other sizing methods. It has been concluded in this study that there is no ideal nano-sizing technique which is suitable for all sample types. Various factors such as the nature of the substance to be measured, the constraints of cost and time, the type of information needed play a decisive role in the choice of sizing method to be used. Additionally, structural properties of NMs, sample preparation and polydispersity have significant impact on the measurement results of different NM sizing techniques.

There are three important criteria that should be met for accurate measurement of particle size: a well-dispersed system, selection of representative sample and appropriate selection of sizing method considering the nature of ENM and its intended use (Kevin W. Powers, Palazuelos, Moudgil, & Roberts, 2007). It should also be kept in mind that some methods such as DLS, NPTA and DSC require dispersion. The aggregation/agglomeration of particles in dispersion leads to an increase in the measured particle size. Although it may lead to inaccuracy in the measurements, it can also be seen as advantageous in nano-toxicity studies since NMs will actually no longer be in a dry form when they are in contact with human cells/organs.

It is our view that the combination of microscopic technique (i.e. TEM or AFM) and the ensemble technique (i.e. DLS) seems appropriate for monodisperse systems, since they can provide a complete picture of size characteristics in dry form and suspension. For poly-disperse systems, the DLS technique has serious problems; hence, it should be replaced or complemented with an alternative sizing approach. To sum up, it is usually useful to combine a single particle sizing technique with an ensemble method in order to have a rich dataset of particle size and size distribution, especially when the compound is unknown. The results of seven studies undertaken by different researchers with the aim of comparing different ENM-sizing techniques are given in Table 3. It should also be pointed out that, compared to the average value of the particle size, the size distribution measures provide more reasonable representation of particle size information, which is a critical attribute in nanotoxicology. However, measurement of particle size distributions usually provides a large amount of data (e.g. hundreds of size distribution components) which may cause problems in the (Q)SAR analysis (e.g. increased chance correlations). Therefore, it is important to find a reasonable way of representing all components of the size distributions with a few variables which still retain all the information present in the input data. (Xue Zhong Wang, et al., 2014) carried out principal component analysis on size distribution data, which consists of a large number of particle size distribution measurements, in order to reduce the number of descriptors to a manageable size. This study is a good example of how to handle large size distribution datasets prior to nano-(Q)SAR analysis. Instead of reporting mean particle size values or statistical variations, the researchers should also take into account the variations in the size distribution as a whole since the ENM samples consist of a range of particle sizes, not only a single type of particles.

3.1.2 Particle Shape

The shape of ENM is another important feature influencing the biological activities of the particulate matter. The hydrodynamic diameters of spherical and rectangular particles with the same mass, and hence their mobility in solution, vary due to shape effects. Moreover, shape

characteristics greatly affect the deposition and absorption kinetics of NPs in a biological environment (Monteiro-Riviere & Tran, 2007). The importance of shape to toxicity has been proven for carbon nanotubes (CNTs). (Poland, et al., 2008) have showed that long MWCNTs are more toxic than short/tangled MWCNTs. The study undertaken by (Kevin W. Powers, et al., 2007) has revealed that the antibacterial activity of silver NPs is shape-dependant. In another study, (Gratton, et al., 2008) have demonstrated that rod-like (high aspect ratio) NPs are drawn or internalized more efficiently into the cell than cylinder NPs. Although there are several studies investigating and confirming the potential impacts of NP shape on toxicity, it is still not possible to draw certain conclusions or define any particular shape inherently 'toxic' with current knowledge. To date, most of the research in this field has focused on shape assessment of spherical NPs while very few have looked at non-spherical NPs or aggregates (Albanese, Tang, & Chan, 2012). Further research is needed to explore the role of NP shape in toxicity with an emphasis on NPs with similar composition but different shape.

There are several non-dimensional shape indexes such as sphericity/circularity, aspect ratio/elongation, convexity and fractal dimensions that can be used to quantify shape characteristics of particles. The shape index of NPs is usually determined using microscopic tools such as SEM and TEM which provide the ability to determine both particle size and shape at the same time. Additionally, the ratio of two particle sizes measured by different techniques such as DLS and TEM/SEM can be used as a simple expression of particle shape (Hosokawa, Nogi, Naito, & Yokoyama, 2007). Since shape characteristics and distribution of NPs may vary when they are in contact with organisms, shape measurement should also be made "as-exposed" form, as well as "as-received" form. (Xue Z. Wang & Ma, 2009) defined the shape of a crystal according to the normal distance between each surface of the particle and its geometrical centre. They carried out the principal component analysis (PCA) approach on the shape description dataset for data compression. The calculated surface-centre distances or the resultant PC values may be directly used as shape indexes of NPs, especially non-spherical ones, in nano-(Q)SAR. Moreover, these values can also be employed as dynamic shape factors to study the time and size dependence of shape once this modelling methodology is applied to model the aggregation/agglomeration behaviour of NPs. If aggregation/agglomeration occurs, some normal distances for some faces may disappear with some new ones being generated. If breakage happens, some new normal distances will be identified to represent the new faces. Such alternative approaches would be useful for nano-(Q)SAR applications as they take into account the dynamic nature of NP shape.

3.1.3 Crystal structure (crystallinity)

NMs with the same chemical composition may have different toxicological properties due to their unlike atomic arrangements and crystal structure. (Jiang, et al., 2008) has investigated the effect of crystallinity on NP activity by comparing ROS generating capacity of TiO_2 NPs of similar size but different crystal phases (amorphous, anatase, rutile and anatase/rutile mixtures). The study has demonstrated that amorphous samples showed the highest level of ROS activity followed by pure anatase and anatase/rutile mixtures while pure rutile produced the lowest level of ROS. Nano-silica which occurs in multiple forms is another nanomaterial whose toxicity may vary depending on the nature of its crystal structure (Napierska, Thomassen, Lison, Martens, & Hoet, 2010).

A widely used technique to obtain information about crystalline phases, purity, crystal structure, crystallite size, lattice constants and defects of NPs is X-Ray Diffraction (XRD). It is a primary tool to characterize nanostructures since it provides non-destructive evaluation of structural characteristics with no need for exhaustive sample preparation (Edelstein & Cammaratra, 1998). Its non-contact and non-destructive features make XRD ideal for in-situ measurements (R. Sharma, Bisen, Shukla, & Sharma, 2012). Measurement in the desired atmosphere is allowed in XRD. This makes this technique advantageous for toxicological characterization in which collection of crystal structure data in a biologically relevant media becomes an important issue.

Additionally, high resolution transmission electron microscopy (HR-TEM) and selected-area electron diffraction (SAED) can be used to obtain information about crystal structure, especially when the data acquisition from individual nanocrystals is needed. We believe that, due to sample-damaging and the user-dependant nature of TEM, conventional XRD should be preferred for crystallographic investigation of nanostructures.

3.1.4 Surface Characteristics

3.1.4.1 Surface functionalization (e.g. coating or modification)

Surface chemistry is another factor that needs to be considered for the complete characterization of NPs since it plays an important role in the surface interactions and aggregation behaviour of NPs in liquid media. Therefore, if the surface of NM is intentionally functionalized with diverse modifications, the chemical species on the surface and functional groups should be identified. The influence of coating on the toxicity of Ag-NPs has been investigated by many researchers (Caballero-Díaz, et al., 2013; Nguyen, et al., 2013; Silva, 2011; X. Yang, et al., 2011; Zhao & Wang, 2012). The results from Nguyen et al. (2013) have showed that uncoated Ag-NPs are more toxic than coated Ag-NPs. However, most probably coating is not the only factor that reduces the

toxicity of Ag-NPs; the changes in aggregation state and particle size as a result of coating may also be important.

Information about the NM surface affecting the interactions of NPs in a biological environment can be obtained from different techniques such as electron spectroscopy (i.e. X-ray photoelectron spectroscopy (XPS) or Auger electron spectroscopy (AES)), scanning probe microscopy (AFM and STM), ion-based methods (i.e. secondary ion mass spectrometry and low energy ion scattering) and other spectroscopic techniques (i.e. IR, NMR, Raman) (Baer, Gaspar, Nachimuthu, Techane, & Castner, 2010). The most important advantage of electron spectroscopy is its high surface sensitivity. XPS is one of the most commonly used techniques for surface analysis (Tougaard, 2005). Both XPS and AES can be used to get information about the presence, relative surface enrichment, composition and thickness of coatings.

3.1.4.2 Surface charge

Surface charge is another important characteristic that may affect the toxicity of NPs. The biological interactions of NPs, and hence their biological activities, are highly surface-charge dependant. (Y.-H. Park, et al., 2013) have analysed the effect of surface charge on the toxicity using negatively and weakly-negatively charged silica-NPs. They have observed that negatively charged silica-NPs have shown a higher level of cytotoxicity than weakly-negatively charged silica-NPs. In another study, the core of silicon-NPs has been covered with different organic mono-layers to obtain different surface charges: positive, negative and neutral (Bhattacharjee, et al., 2010). The study has demonstrated that positively charged silicon-NPs is more toxic than neutral ones while negatively charged silicon-NPs have induced almost no cytoxicity.

As it is challenging to directly measure the charge at the surface of particles, zeta potential measurement utilizing dynamic or electrophoretic light scattering is usually used to quantify surface charge. According to (Xu, 2008), among three techniques that can be used for the determination of zeta potential, namely electrophoretic light scattering (ELS), acoustic and electroacoustic, ELS is preferred for various applications due to its certainty, sensitivity and versatility. However, classic ELS cannot successfully determine the zeta potential of turbid samples because the light cannot penetrate the sample. Preferably, the sample should be optically clean and non-turbid for accurate measurements. It has been also noted in the same study that the accuracy of zeta potential measurement is greatly affected by environmental conditions, e.g. pH and ionic strength. The pH-dependence of zeta potential should also be taken into account since changing the pH in a solution may greatly alter the dispersion of surface charge.

The current level of knowledge regarding the relationship between surface charge and toxicity is severely limited, mainly because of the incapability of existing in-situ measurement techniques

and environment-dependence of zeta potential measurements (Jiang, Oberdörster and Biswas 2009). Since the value of zeta potential measurement obtained may change between different techniques and experiments (Glawdel & Ren, 2008), multiple tests should be conducted for the best possible accuracy in determination.

3.1.5 Aggregation State

Some NPs have a tendency to approach each other and form large agglomerates both in the dry form and in suspension. If NPs form clusters, they may behave like larger particles due to their increased hydrodynamic size (Buzea, Pacheco, & Robbie, 2007). Since agglomeration could affect critical physico-chemical features such as particle size and size distribution, the biological effects of these changes should be identified to avoid incorrect estimation of toxic potential of ENMs (Dhawan & Sharma, 2010; Jiang, Oberdörster, & Biswas, 2009).

The state of aggregation is often quantified by measuring the size distribution of existing agglomerates. It can be monitored and quantified by microscopic techniques such as TEM, SEM and AFM. Additionally, DLS can also be used for the investigation of NP aggregation. However, the characterization of the agglomerate size of NPs in suspensions is very challenging since the degree of aggregation can be influenced by external conditions (e.g. pH, temperature, humidity). Ideally, in-situ instruments which are capable of measuring the size, shape and number of all aggregates in the relevant medium are required to characterize the state of aggregation. The particle size information used in earlier nanotoxicological studies usually refers to the primary size of individual NPs and disregards the effect of aggregation. Although accurate characterization of the aggregation state prior to nanotoxicity testing is seen as a pre-requisite by several researchers (Boverhof & David, 2010; Jiang, et al., 2009; Von der Kammer, et al., 2012), there is still no clear consensus on how to characterize aggregation, but the possibility of characterizing aggregation shape using fractal dimensions, which provide an index of complexity by measuring the space filling capacity of an object, may be the way forward (Schaeublin, et al., 2012).

3.2. NP-specific descriptors

As the properties of nanoscale materials are remarkably different from conventional ones, it is very likely that the toxicity of ENMs is also associated with different features (Burello & Worth, 2011). Therefore, the development of nano-specific descriptors with the capability to describe the distinctive properties of NPs is one of the major research needs in the area of computational

nanotoxicology. In this section, different approaches for developing novel NP-descriptors will be presented.

(Glotzer & Solomon, 2007) proposed an approach to characterize NPs based on microscopic images. They defined eight orthogonal dimensions, including surface coverage, aspect ratio, faceting, pattern quantization branching, chemical ordering, shape gradient and variation in roughness, each of which can be used as an NP-descriptor to compare the structural similarity of different NPs (Figure 2). Although the development of new descriptors based on microscopic images is a promising idea, the numerical expression of these eight dimensions is still an unresolved problem.

The idea suggested by (Glotzer & Solomon, 2007) has inspired other researchers such as (T. Puzyn, Leszczynska, & Leszczynski, 2009) to use microscopic images of NPs for the extraction of structural information. They proposed to quantify each pixel in SEM, TEM and AFM images using RGB colour codes or grayscale representation and then produce a rectangular array of numbers (Figure 3). They also emphasized that these numerical values of image pixels can be employed as new descriptors for encoding the structural properties of NPs.

In another study, (X.-R. Xia, Monteiro-Riviere, & Riviere, 2010) developed a multi-dimensional biological surface adsorption index (BSAI), which consisted of five quantitative nano-descriptors, namely lonepair electrons, polarity/polarizability, hydrogen-bond donor, hydrogen-bond acceptor and London dispersion. These five nano-descriptors represent the fundamental forces governing the adsorption process of NPs in a biological environment. In their follow up study, (X. R. Xia, et al., 2011) performed PCA on five-dimensional nano-descriptor dataset for reducing dimensionality, obtaining two-dimensional representation of molecular interaction forces in biological systems and hence facilitating the characterization of surface properties of ENMs (Figure 4). After obtaining two dimensional nano-descriptors via PCA, they managed to classify 16 different NMs into separate clusters based on their surface adsorption properties.

(Burello & Worth, 2011) proposed that different types of spectra (e.g. NMR, IR, Raman, UV-Vis) can be used as nano-descriptors since they contain fingerprint-like information (Fig. 5). The first step is spectral measurement followed by conversion of spectrums into numerical matrix. This data matrix can be seen as spectra-derived descriptors and used for (Q)SAR analysis. It is not entirely a new perspective since spectral information has already been used in a number of studies in the literature. The use of IR information for (Q)SAR analysis has been shown to be promising in the study carried out by (Benigni, Passerini, Livingstone, Johnson, & Giuliani, 1999). They compared the InfaRed (IR) spectra with several descriptors commonly used in

(Q)SAR studies and found that IR spectra carries unique information which cannot be obtained from molecular descriptors. (Zhou, et al., 2008) used the spectra of MWNTs for characterization purposes while (Y. Yang, Guo, Hu, Wang, & Wang, 2004) attempted to link XRD data to photocatalytic performance tested by dye decolourisation rate. We strongly believe that the use of spectra-derived descriptors in (Q)SAR modelling of NMs is an interesting approach and deserves further investigation.

The final properties of materials are related to not only chemical composition and structure of materials but also preparation, synthesis and processing methods. To this end, (Le, Epa, Burden, & Winkler, 2012) suggested to combine molecular descriptors characterising physicochemical properties of compounds with historical descriptors describing the sample preparation and synthesis techniques of materials in order to develop reliable and predictive models. Although historical descriptors can be useful for modelling traditional materials, their implementation to nano-(Q)SAR models can be very difficult since they would probably have no ability to distinguish between ordinary and nano-sized particles. The determination of 3D descriptors suitable for nanostructures and NP representation is another promising approach and undoubtedly will be put into practice in the near future. In addition, the development of more sophisticated image analysis approaches (e.g. texture analysis-based methods) would facilitate the rapid extraction of morphological information (e.g. particle size, shape, surface area and aggregation state) from microscopic images of NPs.

4. Nano-(Q)SAR and Modelling Techniques

A QSAR is a mathematical model that attempts to relate the biological activities or properties of a series of chemicals to their physico-chemical characteristics in a quantitative manner (T Puzyn, et al., 2010). Although the first use of QSAR models is attributed to (Hansch, 1969), who has brought the physical organic chemistry and the study of chemical biological interactions together to propose the first QSAR approach, the relationship between the chemical structure and biological activity has also been reported in several earlier studies (Brown & Fraser, 1868; Overton, 1901; Richet & Seances, 1893.). Hansch's QSAR approach has found applications in many disciplines such as drug design, chemical and biological science. Moreover, numerous modification of Hansch's approach to QSAR modelling have been developed by many other researchers (Kubinyi, 2008).

It is assumed in QSAR models that the observable biological activity is correlated to the structure of compounds and this correlation can be expressed in a mathematical equation. In QSAR, the presumed relationship between the activity and structure is expressed with the following form of

mathematical equation:

$$y = f(x_i) \quad (1)$$

where y is the biological activity of the chemical (i.e. toxicity) and $f(x_i)$ is a function of structural properties. A set of well-characterized compounds with known biological effects is required to obtain this mathematical algorithm. Structural features of compounds with known biological activities are represented by measured or calculated molecular descriptors. Then, a mathematical model relating the measured activity to the descriptor sets is obtained through regression analysis. The last step is the evaluation of the reliability of the model and its applicability to other compounds. One of the most critical steps, which is often skipped, is to define the model's boundaries and limitations to demonstrate how well it performs when applied to substances that are not used in model building.

4.1. Nano-(Q)SAR research

The opinion papers focusing on in silico modelling of ENM toxicity are listed in Table 4 while solid attempts to model and predict the toxicity of ENMs with (Q)SAR analysis are given in Table 5. The majority of existing nano-(Q)SAR studies focused on the metal oxide (MO) NPs due to their common commercial use and high production volume. One of the first attempts to demonstrate how computational (Q)SAR can give valuable information to nanotoxicity has been reported by (Jianzhong Liu & Hopfinger, 2008). They used molecular dynamic simulation to investigate the effect of CNT insertion on the cellular membrane structure. Four potential toxicity sources were examined through membrane interaction-(Q)SAR analysis. Although the result of this study was very informative and encouraging, a proven (Q)SAR model was not established due to the absence of experimental data.

(Sayes & Ivanov, 2010) assessed the presence of ENM-induced cell damage based on the release of lactate dehydrogenase (LDH) from cells. Six different physical characteristics including primary particle size, size in water and buffered solutions, concentration and zeta potential were measured for each of the two selected metal oxide ENMs, TiO_2 and ZnO. First of all, they performed principal component and correlation analysis on the pre-processed dataset to reveal possible correlations between the physical properties and LDH release measurements. Although strong correlation between some of the physical features, such as particle size and concentration in water, were observed, no correlation was found between the measured physical properties and cellular cell damage in the principal component analysis. Their initial intention was to use the same dataset for developing a regression and classification model. However, they were unable to develop statistically significant regression model using the TiO_2 and ZnO dataset. The results of

classification analysis were better since they managed to produce a classifier with zero resubstitution error. A clear description of experimental design, NM preparation, cell culture conditions and methodology were given in the paper. The inclusion of such knowledge in toxicological research is very important since it greatly improves the interpretability of collected data and enhances its comparability with other studies. The downside of the study is undoubtedly the small number of NMs and physical descriptors used. It is unrealistic to build a (Q)SAR model with a few NMs since it does not allow the sub-setting of original datasets into training, validation and test sets. The number of final descriptors used to develop a (Q)SAR model can be six or less but it is desirable to have a much larger number of initial descriptors, especially in the absence of certain knowledge regarding the relevance of particular properties to nanotoxicity.

In another study, two different experimental nano-toxicity datasets were employed to derive a mathematical relationship between the toxicity of NPs and their physicochemical properties (Fourches, et al., 2010). The advantage of the data used in this study was the concurrent testing of ENMs under the same circumstances. In the first case study, three distinct clusters of ENMs were identified based on their biological activity and support vector machine (SVM) models with high accuracies were developed. In the second case study, it was observed that a descriptor quantifying lipophilicity was the most significant predictor of biological activity since it accurately discriminated between ENMs with low and high values of $PaCa_2$ cellular uptake. Overall, it has been demonstrated in this study that the (Q)SAR approach can provide useful information for toxicity prediction of new ENMs. The methodology used in this work fulfilled all the principles of OECD for the validation of (Q)SAR models.

(T. Puzyn, et al., 2011) were one of the first researchers who managed to derive a mathematical equation based on the dataset of cytotoxicity and molecular descriptors. Initially a set of 12 structural descriptors were quantum-chemically calculated using the semi-empirical PM6 method. Among the pool of descriptors, only one structural descriptor, ΔH_{Me+}, representing the enthalpy of formation of a gaseous cation having the same oxidation state as that in the metal oxide (MO) structure was utilized to establish the following nano-(Q)SAR model:

$$\log\left(\frac{1}{EC_{50}}\right) = 2.59 - 0.50\Delta H_{Me+} \quad (2)$$

A set of 17 MO-NPs can be considered as small from the modelling perspective, but the development of such predictive nano-(Q)SAR models is helpful to encourage new investigations.

Another simple, but statistically powerful nano-(Q)SAR model was developed by (Epa, et al., 2012) based on the results of in vitro cell-based assays for nanoparticles. The dataset used by (Fourches, et al., 2010) was also employed here with minor changes. The difference was that new descriptors encoding the presence or absence of some particular features, such as coating, were

added and used as descriptors by (Epa, et al., 2012). They managed to build the following nano-(Q)SAR equation based on these dummy variables:

$$Smooth\ Muscle\ Apoptosis = 2.26(\pm 0.72) - 10.73(\pm 1.05)I_{Fe2O3} - 5.57(\pm 0.98)I_{dextran} - 3.53(\pm 0.54)I_{surface\ charge} \quad (3)$$

where $I_{Fe2O3}, I_{dextran}$ and $I_{surface\ charge}$ stands for indicators (taking 1 or 0) for the core material, surface coating and surface charge, respectively. This was the second quantitative model developed to predict the toxicity of nanostructures. Compared to the equation (2), this mathematical expression was developed from more diverse set of data.

Recently, the hypothesis that NP toxicity is a function of some physicochemical properties has been tested by (Xue Zhong Wang, et al., 2014). A panel of 18 NPs including carbon-based materials and metal oxides were selected and used in this study. Different types of cytotoxicity assays such as LDH, apoptosis, necrosis, haemolytic and MTT, were performed and several structural and compositional properties were measured. Initially, they applied PCA to the cytotoxicity data in order to combine the toxicity values measured at different doses into a single value that describes all the data points on the dose-response curve. It should be mentioned here that, as the toxicity is highly dose-dependent, the toxicological effects are usually evaluated at multiple concentrations in a series of tests the results of which are represented with a dose-response curve. Figure 6 shows an example of dose-response curves obtained for 18 NPs (Xue Zhong Wang, et al., 2014). As can be seen from this graph, the cell viability is lower in the cells treated with N3 (nanotubes), N14 (zinc oxide) and N6 (aminated beads). There are different methods to analyse and compare dose response curves such as area under the curve, slope of the curve, threshold values, min/max response and benchmark dose approach. In this study, (Xue Zhong Wang, et al., 2014) performed PCA in order to integrate the entire curve and used the resulting principal components as an overall measure of cumulative response. They concluded that, compared to other approaches, PCA-based representation of the dose-response curves provides more reasonable results when ranking the ENMs according to their hazard potential. Due to the high toxicity level of four particular ENMs, i.e. zinc oxide, polystyrene latex amine, Japanese nanotubes and nickel oxide, nano-(Q)SAR analysis has focused on these four ENMs to examine the potential factors behind their observed toxicity. It was concluded in this study that physicochemical characteristics leading to the toxicity of ENMs were different and it was not possible to draw a general conclusion which was valid for all toxic ENMs screened in this study. However, the nano-(Q)SAR method was found useful to reveal that some of the measured properties such as metal content, high aspect ratio and particle charge were correlated to the toxicity of different nano-sized materials.

(R. Liu, Rallo, et al., 2013) developed a classification-based (Q)SAR model based on multiple toxicity assays, 44 iron oxide core NPs, and 4 simple descriptors (size, zeta poteantial and relaxivities). They argued that existing nano-(Q)SAR models did not take into acount the acceptance level of false negative to false positive predictions. Unlike previously constructed nano-(Q)SAR models, they also explored the decision boundaries of the nano-(Q)SARs subject to different acceptance levels of false negative/false positive predictions.

In another study, (R. Liu, Zhang, et al., 2013) attempted to relate the physicochemical properties of MO- NPs to their toxicity by developing a structure-activity relationship. A number of classification nano-(Q)SAR models were developed on a large toxicity dataset of 24 MO-NPs. A set of 30 molecular descriptors were calculated for each NPs and only two of them, the conduction band energy and ionic index, were identified as the key molecular descriptors on which the best performing nano-(Q)SAR model was built. Their conclusion was in a good agreement with the results of previous researchers (Burello & Worth, 2011) who stated that the conduction band energy of oxide NPs is related to their toxicity. Similar findings have also been reported by (Zhang, et al., 2012) who indicated that the oxidative stress induced by MO-NPs could be linked to their conduction and valance band energies.

More recently, (Singh & Gupta, 2014) attempted to build classification and regression nano-(Q)SAR models using ensemble methods such as decision tree forest (DTF) and decision tree boost (DTB). Five different datasets were used to demonstrate and confirm the suitability of these techniques for the (Q)SAR modelling process by comparing the accuracy of the developed nano-(Q)SARs with past studies. It was concluded by the authors that the nano-(Q)SAR models constructed had high performance and statistical significance together with superior predictive ability.

From our point of view, the common problem that exists in the majority of published (Q)SAR studies is that it is not possible to generalize their results in the absence of explanatory information regarding underlying reasons for system behaviour. It limits the use of their findings for external compounds. When the result of (Q)SAR analysis is only valid for tested compounds, (Q)SAR becomes a data analysis tool with no predictive ability. In order to ensure the reliable use of the established nano-(Q)SARs, the modellers should also address the model uncertainty arising from experimental error and lack of knowledge. Moreover, most of the existing nano-(Q)SAR studies use small datasets to establish a link between nanostructure and toxicity. Although the small datasets can be useful to describe or explain relationship between NP structure and activity, they may not be very useful for predictive purposes.

Table 5 summarises the previously reported nano-(Q)SAR studies by comparing their methodology in respect to OECD principles (e.g. (1) a defined endpoint, (2) an unambiguous algorithm, (3) applicability domain and (4) model validation for stability and predictivity).

4.2. Nano-(Q)SAR modelling techniques

In principle, a variety of methods that have proven to be effective in classic (Q)SAR modelling, such as statistical methods, neural networks and decision trees, can all be applied to nano-(Q)SAR. In practice, however, their direct use in ENM toxicity modelling has difficulties. The major obstacle comes from the availability of data since some (Q)SAR algorithms require large datasets which are currently not available for ENMs. Considering the current scarcity of nanotoxicity data, it would be reasonable to use modelling tools which can make effective use of smaller datasets. In addition, there is still insufficient knowledge about physicochemical descriptors that can influence the toxicity of ENMs. Therefore, current nano-(Q)SAR studies should focus on identifying toxicity-related physicochemical characteristics as well as predicting potential toxicity values. The ease of use (i.e. the ease of model building and of interpretation of the results) is another critical consideration, particularly in nano-(Q)SAR world where the ability to interpret the resulting models is the key to understand the correlation between different forms of biological activity and descriptors. Overall, the following factors have to be considered when selecting nano-(Q)SAR modelling techniques:

- Minimal data requirements: Should be able to make effective use of limited data, without relying on the availability of large datasets.
- Transparency: Models should be transparent rather than black-box, intuitive, and able to help identify the physicochemical descriptors that contribute to the toxicity of ENMs
- Ease of model construction: Should be easy to use and easy to implement.
- Non-linearity: Should be able to reveal non-linear relationships/patterns in the dataset
- Low over-fitting risk: Should have the low risk of over-fitting, which may reduce the generalization performance of the model.
- Descriptor selection function: Should have the capability of feature selection in order to exclude redundant descriptors before model building.
- Ease of interpretation: Should be able to produce meaningful and interpretable outcomes and explain how the outcomes are produced.

- Low modeller dependency: Should have the low sensitivity to the changes in model parameters.

Below, some (Q)SAR modelling methods including decision trees, statistical methods, support vector machines, neural networks, multi-dimensional visualisation and knowledge-based expert systems are examined. The focus is on examining their suitability for nano-(Q)SAR modelling, rather than on introducing the individual algorithms. Additionally, feature selection and model validation methods will also be briefly discussed.

Decision Trees (DTs). Automatic generation of decision trees from data is a powerful machine learning technique that can be used as a classification or regression tool for categorical and numerical predictions of biological activity in (Q)SAR studies (Chao Y Ma, Buontempo, & Wang, 2008). DTs can be constructed with small, large or noisy datasets and used to detect non-linear relationships. They have a tree-like structure that splits data points into different classes based on the decision rules in order to categorise and model input data. Various decision tree generation algorithms are available, and can be broadly classified as shown in Figure 7. The most significant advantages of DT methods are their capabilities to automatically select the input variables (i.e. the physicochemical descriptors that contribute to the observed toxicity) and to remove the descriptors that are not related to the endpoint of interest. In a previous study, (Buontempo, et al., 2005) demonstrated the use of a genetic programming-based decision tree generation technique for in silico toxicity prediction. They developed a decision tree model, involving five descriptors selected from a pool of more than a thousand descriptors, that has good predictive performance for both training and test datasets. This 'knowledge discovery' capability is no doubt very valuable at present to identify the physicochemical descriptors that contribute to the toxic effects of ENMs. Such knowledge has even further benefits for eliminating or minimizing the risk of ENMs through engineering approaches (i.e. modification of physicochemical properties that influence the toxicological response through the active engineering of ENMs). Another benefit of using DT analysis is its capability to avoid the (Q)SAR model being over biased towards data cases in the dense areas - a problem with some other techniques such as linear regression and neural networks. Small data cases, i.e. data outside the dense data area, can also be modelled as branches of a decision tree.. An additional merit of DT is the ease of its interpretability (Apté & Weiss, 1997) and transparency (Chao Y Ma & Wang, 2009). Study on DTs for the purpose of modelling ENM toxicity requires more research, since in addition to the above mentioned many advantages, there are researchers who have voiced concerns about the generalization ability and predictive power of DTs (Bengio, Delalleau, & Simard, 2010). To date, DTs (and their extension known as “random forest”) have been

investigated for (Q)SAR modelling in a number of studies (Andres & Hutter, 2006; Arena, Sussman, Mazumdar, Yu, & Macina, 2004; L. Han, Wang, & Bryant, 2008; Chao Y Ma, et al., 2008; Sussman, Arena, Yu, Mazumdar, & Thampatty, 2003). Further research on DT should focus on maximizing its advantages and overcoming its limitations. An interesting such example is random decision forest. Several studies have shown its improved generalisation ability (Díaz-Uriarte & De Andres, 2006; Genuer, Poggi, & Tuleau-Malot, 2010; Chao Y Ma & Wang, 2009; Teixeira, Leal, & Falcao, 2013).

Statistical Methods and Feature Selection. Several different statistical methods, such as Multiple Linear Regression (MLR), Principal Component Regression (PCR) and Partial Least Squares (PLS) Regression, have been extensively studied in (Q)SAR analysis due to their ease of use and interpretation (Yee & Wei, 2012). PLS is a linear regression method that handles data cases where the number of predictors is higher than the number of compounds. The PLS method works well when there are several noisy and inter-correlated descriptors, and also allows multiple responses to be modelled simultaneously (Eriksson & Johansson, 1996). The usefulness of PLS in (Q)SAR studies, especially when the descriptors are highly correlated and numerous, has been proven by several researchers (Cramer, Bunce, Patterson, & Frank, 1988; Dunn, Wold, Edlund, Hellberg, & Gasteiger, 1984; Eriksson, Gottfries, Johansson, & Wold, 2004; Gu, et al., 2012; Luco, 1999; Luco & Ferretti, 1997). However, this method can only be used for the solution of linear regression problems. To overcome this problem, non-linear versions of the PLS method have been developed based on different algorithms, such as kernel-based PLS (Rosipal & Trejo, 2002), neural network PLS (Qin & McAvoy, 1992) and genetic algorithm based PLS (Hasegawa, Miyashita, & Funatsu, 1997). These extensions allow non-linear relationships to be modelled in (Q)SAR studies, which is not otherwise possible with the simple PLS technique. Although MLR is one of the most common modelling techniques used to develop regression-based QSAR models, there are three main factors limiting the use of MLR in nanotoxicity modelling: the linearity assumption, i.e. it cannot detect non-linear causal relationship, the restriction on the ratio of compounds to predictors in the data, i.e. the lowest ratio of the number of NMs to the number of descriptors should be 5 to 1, and the dependence of its performance on redundant variables, i.e. the presence of correlated input variables, as well as input variables that are irrelevant to the output, may lead to poor model performance (Shahlaei, 2013). Dimension reduction methods such as PCA can be useful for eliminating correlations between input variables (i.e. physicochemical descriptors) without removing information about the irrelevant variables which may still affect the model performance. Overall, the main advantage of linear models (such as MLR and PLS) over the non-linear ones is their transparency since one can directly get some information of the relative importance of the physicochemical descriptors from a linear model via

examining the weights, but some non-linear models such as neural networks cannot give such direct information.

The feature selection process is different from the above mentioned dimension reduction technique, i.e. PCA, in that it selects only the inputs that have an impact on the outputs. The input variables that have no or little impact on the outputs are removed during the model building process. Among the various methods for automatic input feature selection, genetic algorithm (GA) has demonstrated excellent performance. The GA feature selection approach can be applied together with almost all (Q)SAR model building algorithms. GA starts from a population of possible solutions (called individuals of chromosomes) which can be randomly generated. In here, each gene in the first generation of solutions consists of randomly selected descriptors. Using the randomly selected descriptors in each chromosome, a (Q)SAR model can be built. (Q)SAR models built based on the individuals in the initial population of solutions in this first generation are evaluated using a defined fitness function. Based on Darwin's theory of 'survival of the fittest', the individuals are selected to undergo some operations such as mutation and crossover to generate the population of individuals in the next generation. In summary, a GA algorithm has the following essential steps:

(1）Generate a set of solutions randomly (the number of solutions can be set by the user) and code into vector group with fixed length.

(2) A new generation is produced by the method below, or is generated to substitute the individuals in the current population.

（2a） Selection of parent individuals based on the value of fitness function.

（2b）Crossover takes place to generate one or several sub-individuals.

（2c）Mutation operation is applied to some individuals.

(3) Repeat step 2 and the algorithm stops when one of the stopping criteria is met, either having reached the maximum number of generations or time limit, or having satisfied the stop criterion for the fitness function. For more details, the interested reader is referred to (Goodarzi, Saeys, Deeb, Pieters, & Vander Heyden, 2013; R. F. Li, Wang, & Abebe, 2008; J Liu & Zhou, 2007; Chao Y. Ma & Wang, 2011; Reddy, Kumar, & Garg, 2010).

Support Vector Machines (SVM). There is an increasing interest in the use of SVM, which can handle both regression and classification problems, as an alternative to linear modelling methods such as MLR and PLS in (Q)SAR studies (Czermiński, Yasri, & Hartsough, 2001; Mei, Zhou, Liang, & Li, 2005). SVM can handle many issues such as non-linear relations, collinear descriptors, small datasets and model over-fitting that usually affect the performance of other (Q)SAR modelling techniques (Mei, et al., 2005). It appears to have good potentials for (Q)SAR

analysis due to its accuracy and high generalization capability. On the other hand, the main disadvantages of SVM are the high sensitivity of model performance to the selection of design parameters (e.g. Kernel functions) and the complexity of direct interpretation of SVM decision. To date, it has been utilized in numerous studies for the construction of classification (Czermiński, et al., 2001; Niu, 2007; Xiaojun Yao, et al., 2005) and regression (Darnag, Minaoui, & Fakir, 2012; Mei, et al., 2005; Niu, Su, Yuan, Lu, & Ding, 2012; XJ Yao, et al., 2004) based (Q)SAR models. As mentioned earlier, GA-based feature selection can be integrated with SVM in (Q)SAR modelling, as proved in near infrared chemometrics (Chao Y. Ma & Wang, 2011).

Artificial Neural Networks (ANNs). ANNs are the computing systems that are created by imitating how the human brain works and simulating the human brain activity on the computer. Although, in some cases, the poorly understood structure of this technique affects its practical reliability, the successful applications of ANNs in the (Q)SAR world (Habibi-Yangjeh, Danandeh-Jenagharad, & Nooshyar, 2006; M Jalali-Heravi, Asadollahi-Baboli, & Shahbazikhah, 2008; Mehdi Jalali-Heravi & Parastar, 2000; Ventura, Latino, & Martins, 2013) keep the interest in this method alive. ANNs offer several advantages to (Q)SAR developers which include the ability to deal with the non-linear nature of structure-activity relationships and the large descriptor datasets including unnecessary variables. However, it also has several disadvantages such as the difficulties in interpreting the outcome and selecting the optimum complexity, risk of over-fitting and high sensitivity of its generalization power to the changes in the parameters and network topology. In some applications, ANN models are treated as a black box due to its inability to give a deep insight into the encoded relationship between the predictors and predicted outcomes (Guyon & Elisseeff, 2003). There are also others highlighting that ANN systems should not be seen as inexplicable models any more (I. I. Baskin, Palyulin, & Zefirov, 2009; Sussillo & Barak, 2013) since a number of methodologies facilitating the interpretation of model outcomes have been developed (I. Baskin, Ait, Halberstam, Palyulin, & Zefirov, 2002; Burden & Winkler, 1999; Guha, Stanton, & Jurs, 2005). Also, it has to be pointed out that, just like other modelling techniques, ANN can be used together with GA-based feature selection algorithm in order to remove redundant variables during the model building process. In addition, some researchers have investigated the use of sensitivity analysis method for minimization of input data dimension and extraction of information about the relative importance of inputs to an output (Zurada, Malinowski, & Cloete, 1994).

Multidimensional Visualization. Multidimensional visualisation techniques, such as the parallel coordinates (Albazzaz & Wang, 2006; Brooks & Wilson, 2011; Inselberg, 2009; X. Z. Wang, Medasani, Marhoon, & Albazzaz, 2004) and heat maps, are also very effective tools for (Q)SAR

analysis of toxicity data. It can visually display the causal relationships between nanomaterials' physicochemical descriptors and the toxicity endpoints, handle limited datasets, and allow investigators to interactively make analysis with the help of the interactive functions and multiple colours built in software tools. To provide an example, the data generated by (Shaw, et al., 2008) are scaled, displayed and coloured (Fig. 8) using a parallel coordinates graph produced by C Visual Explorer (CVE) tool.

Knowledge Based Expert Systems. (Q)SAR often refers to data driven modelling. But one should not underestimate the usefulness of knowledge based expert systems, as evidenced by the success of the expert system DEREK of Lhasa Ltd, for toxicity predictions (LHASA, 1983). This expert system which draws its knowledge from both literature and databases has been considered as one of the most powerful tools for toxicity predictions of molecules. As a matter of fact, considering the gaps and variations in the available NM toxicity data (i.e. incomplete characterisation of physicochemical descriptors and different measures of toxicity), it is our belief that knowledge based expert systems, ideally with some kind of 'text data mining' capability that can continuously capture new knowledge appearing in literature, might be one of the most effective approaches for nano-(Q)SAR.

Model Validation. Regardless of the method used for constructing the (Q)SAR models, the validity of the outcome of the predictive models should be evaluated both internally and externally. Internal validation is the process of evaluating the prediction accuracy of (Q)SAR models based on the dataset used in the modelling process. The most common internal validation techniques used in (Q)SAR studies are least squares fit (R2), chi-squared ($\chi 2$), root-mean squared error (RMSE), leave-one-out or leave-many-out cross validation, bootstrapping and Y-randomization (Veerasamy, et al.). The use of external validation techniques, not in place of but alongside internal validation methods, is increasingly being recommended by researchers (Gramatica, 2007; Tropsha, 2010; Veerasamy, et al., 2011) and authorities (OECD, 2007) for the assessment of (Q)SAR model reliability in the best possible and trustworthy way. Moreover, it is always beneficial to use more than one validation metrics to quantitatively measure the accuracy of the model prediction.

Definition of the applicability domain of the constructed and statistically validated model is the last, but one of the most important steps, in the (Q)SAR model building process. There are several approaches (e.g. geometry, range, distance or probability density function based approaches) proposing to define the applicability domain region of statistical models based on different algorithms. For more detailed information about the available approaches for defining the (Q)SAR model applicability domain, interested readers are encouraged to refer to the review

papers by others (Jaworska, Aldenberg, & Nikolova, 2005; Sahigara, et al., 2012).

4.4. Input data for nano-(Q)SAR and its current availability

In nano-(Q)SAR models, the importance of high-quality and well-described dataset is even more pronounced since the novel properties of ENMs are mostly associated with particular size and conditions (Gajewicz, 2012). Ideally, the input data required to build a reliable (Q)SAR model should be (1) obtained from a preferably single and standardized protocol, (2) examined in terms of accuracy and suitability for (Q)SAR analysis and (3) large enough to allow rational division of the data into training and test sets. Since nano-(Q)SAR is a data-based method, the accuracy of the data determines the quality of the final model. Therefore, it is very important to create a comprehensive nanotoxicity database and make it broadly accessible.

In a recent study, (Lubinski, et al., 2013) developed a framework to help modellers evaluate the quality of existing data for modelling (e.g. nano-(Q)SAR) purposes. In the first part of their study, they provided a set of criteria which are mostly related to the source and quantity of the data, experimental procedures and international standards followed during the characterization process and documentation. In the second part, they assessed the quality of a collection of nanotoxicity data by scoring them according to the proposed criteria. The majority (201 out of 342 data points) of the dataset being collected and scored were evaluated as useful with restrictions for developing (Q)SAR-like models. It seems that the authors were a little over-optimistic.

In fact, there is now a great amount of data on nanotoxicity. However, the majority of the available data on NP toxicity come from studies focusing on a few ENMs and hence are not useful for modelling purposes. It is often the case that the physicochemical properties measured are not directly related to the toxicity of NPs since characterization has been carried out in the absence of test medium. Moreover, the data obtained by different research groups are often incomparable due to the differences in experimental procedures and ENMs being used.

The pre-defined data formats are necessary to facilitate storage, maintenance and exchange of ENM data between different researchers. There are a large number of freely available toxicity databases most of which are more general in scope and not customized for particular purposes. Commercially available NP-specific databases are still at the research stage and limited to a few applications. ISA-TAB-NANO introduced by (Thomas, et al., 2013) is a standard NM data sharing format that facilitates the import/export of NM data and enables data exchange between different nanotechnology laboratories and researchers. The ISA-TAB-NANO uses four different

spreadsheet-based file formats: investigation, study, assay and material file format. The main features of each file format are given in Table 6.

The OECD Database on Research into Safety of Manufactured Nanomaterials was launched in 2009 with the aim of collecting research projects which are focused on human health effects and environmental risks of ENMs. It aims to identify knowledge gaps in the literature and to enhance co-operation between researchers. It contains information about project (i.e. title, status and summary), total funding, investigator, outcomes and categorisation (i.e. relevance to NM safety, research themes). However, this is not a database that provides direct access to data since the overall outcomes and outputs section is usually filled as "publications".

NANOhub is a database for managing information about ENMs. Currently, it hosts several projects but the access to data is usually restricted to projects participants only. Another data sharing portal which provides access to NP characterization and in vitro toxicity data is caNanoLab. The main aim of this data repository is to facilitate the sharing of knowledge on nanomedicine.

An alternative approach that can be taken for collecting nanotoxicity data is the use of text mining techniques to develop a customized knowledge repository system. The NHECD database is an initiative text mining tool which allows the automated extraction of information on the effects of ENMs on human health and the environment from scientific papers. However, the current performance of such NM databases employing text mining algorithms is not very pleasing due to the non-standardized recording of ENM information. At this stage, it is critical to ensure that all data is recorded in a universally agreed format which facilitates the extraction of NM information from the literature. The existence of specifications for NM information sharing is also very important from the point of view of (Q)SAR modelling since the establishment of predictive (Q)SAR models requires close collaboration between different disciplines and research groups. The development of an agreed ontology for ENMs and nano-safety research (i.e. a formal representation of nanostructures, biological properties, experimental model systems, conditions and protocols) will facilitate not only collection of nanotoxicity data but also data mining and resource integration efforts.

5. Final Remarks

(Q)SAR models have been successfully used by engineers, physical and medicinal chemists to predict hazardous properties of molecules for over 50 years (T Puzyn, et al., 2010). Although the adaptation of the (Q)SAR approach to nano-toxicology has been encouraged by many investigators (Burello & Worth, 2011; T. Puzyn & Leszczynski, 2012) and supported by the EU's

REACH regulation, there are still several barriers that need to be overcome in order to establish predictive, reliable and legally acceptable nano-(Q)SAR models. Unfortunately, the current toxicity measurement methods used for bulk materials are not very adequate to examine ENMS. The absence of standardized methods and procedures for nanotoxicity testing gives rise to conflicting and incomparable findings which may hinder the development of risk reduction strategies for ENMs.

One of the main issues that complicates the adaptation of computational toxicity approaches to nanotoxicology is the scarcity of consistent and high-quality experimental data without which the development of robust and predictive nano-(Q)SAR models is obstructed. The scarcity of such data is mainly caused by difficulties in standardizing nanotoxicity testing procedures and characterization conditions for physicochemical properties. The establishment of standard protocols is essential to enable accurate measurements of physicochemical and biological properties of ENMs. The choice of realistic characterization medium/conditions and also appropriate toxicity endpoints for the ENMs makes the accurate measurement of physicochemical and biological properties possible.

The lack of knowledge about the interactions of ENMss with biological systems brings into question the effects of several factors such as aggregation and coating on the toxicity of ENMs. If the particle size is the critical factor that directly affects the biological activity of ENMs, then the size of aggregates in biological systems should also be considered in the context of nanotoxicity modelling. However, there is still no clear consensus about how to characterize ENMs aggregation in relevant media. The remaining problems in the characterization of NPs for toxicity testing are directly related to the establishment of the relationship between physicochemical characteristics and toxicological response. Therefore, the development of reliable nano-(Q)SAR models requires in situ and careful characterization of ENMs in a relevant biological environment by taking into account the possible impacts of nano-bio interactions on the basic properties (i.e. particle size, aggregation state and coating) of ENMs (Powers et al., 2007). In order to be able to draw certain conclusions about the properties influencing the toxicity of ENMs, it is critical to adequately define time and media dependent nano-characteristics. However, the inclusion of some kind of interactions and aggregation mechanisms in the nano-(Q)SAR modelling process is still unclear.

Another issue that make the accurate measurement of physicochemical properties of NPs difficult is the high polydispersity of NPs. In order to advance the quality of experimental characterization data, it is needed to have new analytical methods/instruments that can deal with polydispersity and heterogeneity of NP samples. The complex and dynamic nature of NP-media interactions should be taken into account very carefully when characterizing NP samples in order to ensure

that the measured properties are directly associated with the toxicological response. For more detailed information regarding the factors influencing NP-biomolecule interactions, please refer to the recent review by (Mu, et al., 2014). Although the characterization of ENMs is the key issue without which it is not possible to identify the relationship between nano-characteristics and biological activity, it is also equally important to speed up the safety assessment of ENMs to keep the pace with the rapid growth of nanotechnology. Therefore, the use of practical and rapid assessment platforms, such as high throughput screening method, for toxicity screening of ENMs would provide several benefits in terms of time and cost reductions. High throughput screening systems, which are capable of rapidly assessing multiple toxicants in multiple cell lines (at multiple doses), have already been used for assessing hazard potential of ENMs (George, et al., 2011; Harris, et al., 2013; Rallo, et al., 2011; Shaw, et al., 2008). We believe that HTS data will be extremely useful in near future for establishing nano-(Q)SAR models and identifying the parameters that are responsible for the toxicity of ENMs, as they include comprehensive toxicological information.

In addition to the guidance on what, how and where to measure, it is also important to have standardized data reporting formats in nanotoxicology in order to facilitate consistent reporting of the outcomes of nanotoxicity studies. The development of such an agreed ontology for nano-safety research will greatly facilitate data collection, database development, data mining and resource integration efforts in the field of nanotoxicology.

The size dependent properties of ENMs also greatly affect the data collection strategy in (Q)SAR model building. Data used in classic (Q)SAR analysis includes different chemicals and measured/calculated descriptors. However, nano-(Q)SAR studies require a larger set of data which should include not only different chemicals but also a different-sized form of the same chemicals due to the size-dependent toxicity of ENMs. Furthermore, it is important to realise that a NP cannot be simply considered as an equivalent of a molecule. Since NP sample can have variations in size distribution, shape, size, surface area etc., it might be that a NP sample at given values of its physicochemical descriptors is an equivalent of a molecule. A different sample of the same material that has different values of its physicochemical descriptors should be considered as a new molecule.

As the available nanotoxicity data is far from ideal for modelling purposes, the choice of nano-(Q)SAR tool should be made by considering the nature of existing data. It is our viewpoint that the nano-(Q)SAR tools at present should be able to make use of limited data, identify physicochemical descriptors that influence biological responses, reveal non-linear relations in the dataset and produce interpretable outcomes.

Finally, the existing challenges are not all scientific. The self-concentrated disciplines and the lack of communication, motivation and integration lead to repetition and confusing literature in nanotoxicology. More focused research, integrated processes and more dialogue are needed. In fact, there are now a growing number of European projects and international efforts focusing on various areas of ENM toxicity. However, despite these endeavours, there are still numerous well-recognized but still unfilled knowledge gaps in the area of nanotoxicology. Once the key issues, such as systematic and consistent toxicological data and proper characterization of ENMs are solved, we believe that it will be possible to predict the toxicity of ENMs and to interpret their mode of toxic action through the established nano-(Q)SAR models. In addition to (Q)SAR analysis, there are also other computational modelling techniques, such as physiologically based pharmaco-kinetic (PBPK) models, which can provide useful outputs for estimating and prioritising health risks posed by ENMs. PBPK models can describe the movement of particles throughout the body after exposure. The involvement of PBPK models in toxicological evaluation of ENMs can enhance understanding of ENM kinetics and distributions as these models are capable of proposing a realistic representation of ENM distribution (M. Li & Reineke, 2012). We believe that the integration and harmonization of such in silico models with nano-(Q)SAR models would greatly contribute to the development of risk assessment strategies for ENMs.

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Table 1: Physicochemical Properties and Material Characterization (WR: where relevant, IA: if applicable, WA: where available, AA: as appropriate)

Characterization (as on the shelf)		**Characterization (in respective media)**
Appearance (IA)	Dissociation constant (IA)	Composition/purity
Melting point (IA)	pH (IA)	Size, size distribution
Density (IA)	Agglomeration or aggregation	Agglomeration/aggregation
Size, size distribution	Crystalline phase	Zeta-Potential
N-octanol-water partition coefficient (WR)	Crystallite and grain size	Biophysical properties (AA) (protein binding/corona characterization, residence times, adsorption enthalpy, conformation changes on binding)
Water solubility/dispersibility, hydrophilicity	Aspect ratio, shape	
Solubility/dispersability in organic solvents, oleophilicity	Specific surface area	
Auto flammability (IA)	Zeta potential	Test item preparation protocol, conditioning, homogeneity and short term stability
Flammability (IA)	Surface chemistry (WA)	
Stability in solvents and identity of relevant degradation products	Stability and homogeneity (on the shelf, in water and organic solvents)	
Oxidising properties (IA)	Dustiness	
Oxidation reduction potential	Porosity, pore and pour density	
Explosiveness (IA)	Photocatalytic activity	
Storage stability and reactivity towards container material	Catalytic activity	
Stability; thermal, sunlight, metals	Radical formation potential	

Table 2: Particle size measurement techniques

Method	Parameters Measured	Sample Required	Particle Size Range	Additional Information
Electron Microscopy	Particle size Size distribution Particle shape Agglomeration	Dry	0.3nm- µm	(+) High resolution (-) Expensive and complex (-) Vacuum is needed (Dhawan and Sharma, 2010)
Atomic Force Microscopy	Particle size Size distribution Morphology Surface structure Agglomeration	Wet/Dry	1nm- µm	(+) 3D images, (+) Works well in ambient air (-) Particles should be on the surface (Powers et al., 2006)
Dynamic Light Scattering (DLS)	Particle size Size distribution Agglomeration Zeta Potential	Wet	1nm-6µm	(+) Cheap and fast (-) Sample polydispersity may distort the results (Tomaszewska et al., 2013)
NP Tracking Analysis (NPTA)	Particle size Size distribution Agglomeration	Wet	10nm-2µm	(+) Particle-by-particle basis (-) Dependence on the settings (Hassellöv and Kaegi, 2009)
Centrifugal Sedimentation	Particle size Size distribution	Wet	5nm-10µm	(+) Accurate and repeatable results (-) Takes long time for small particles to sediment (Laidlaw and Steinmetz, 2005)
BET Surface Area Analysis	Particle size Surface area	Dry	5nm- µm	(-) Size distribution is not provided (Dhawan and Sharma, 2010)
Laser Diffraction	Particle size Size distribution	Wet/Dry	40nm-3mm	(+) Fast and flexible (-) Dependent on optical parameters (Kübart and Keck, 2013)
Mobility Analysis	Particle size Size distribution	Dry	2nm-2µm	(+) Commonly used for aerosols (-) Interpretation of results may require additional information (Oberdorster et al., 2005)
Acoustic Methods	Particle size Size distribution Zeta potential	Wet	20nm-10µm	(+)Effective in concentrated suspensions (-) Difficult to interpret the data (Powers et al., 2006)

Table 3: Nanoparticle mean size measurement results obtained from different sizing methods

Particle Size (nm)							
Ref.	Thiele et al. (2010)				Lee et al. (2013)	Akbari et al. (2011)	Borchert et al. (2005)
Method	**Ta**	**$TiSi_2$**	**Ni**	**C**	**SiO_2-7nm**	**Al_2O_3**	**$CoPt_3$**
BET	8	19	35	45	18	27	
TEM	7	13	24	31	19	24	4.86
DLS	316	157	1300		13		
Others						XRD:20; PCS:96	XRD:5; SAXS:4.97
Ref.	Hoo et al. (2008)				Supaka (2012)		Boyd et al. (2011)
Method	**PS-100**	**PS-20**	**PS-20&100**	**PS-20&101**	**CRM-60**	**CRM-100**	**Latex**
DLS	114	23	109	245	73	105	110
AFM	99	16	15-95	16-98	58	58	98
Others					SEM:79	SEM:79	NTA:99

Table 4: Review and opinion papers focusing on in silico modelling of ENM toxicity

Author	Description
Gallegos et al. (2009)	computational modelling, a few NP descriptors and nano-QSPR studies
Puzyn et al. (2009)	use of (Q)SAR approach for risk assessment of NMs.
Burello, Worth (2011a)	(Q)SAR models for nano-toxicity predictions (single example study), challenges
Burello,Worth (2011b)	(Q)SAR modelling of NMs, NP descriptors for nano-bio interactions.
Fourches et al. (2011)	chemoinformatic approachesto estimate the biological effects of ENMs.
Cohen et al. (2012)	the use of in silico models for hazard assessment of ENMs.
Gajewicz (2012)	computational methods/tools to support risk assessment of ENMs
Nel et al. (2012)	development of predictive toxicological paradigms for ENMs.
Winkler et al. (2012)	summary of the current status and known gaps of nano-QSAR modelling

Table 5: Previously reported nano-(Q)SAR studies

Authors	NPs	Descriptors	Endpoints	(Q)SAR tool	Criteria met
Sayes and Ivanov (2010)	24 NP susp., 2 MOs	Size measures, conc., zeta pot.	LDH	MLR, LDA	1,2,4
Fourches et al. (2010b)	44NPs, diverse core	Size, relaxivities, zeta potential	ATP, Red, Apop., Mito	SVM-classification	1,2,3,4
	109NPs, diverse modifier	105 MOE descriptors	Cellular uptake	KNN-regression	1,2,3,4
Puzyn et al. (2011)	17 MO-NPs	12 theoretical descriptors	EC_{50}	MLR-GA	1,2,3,4
Chau and Yap (2012)	105NPs, diverse modifier	679 theoretical descriptors	Cellular uptake	NB, LR,KNN,SVM	1,2,3,4
Zhang et al. (2012)	24 MO-NPs	Size, crystallinity, band gap energy, conduction/valance band, dissolution, zeta pot.	MTS, ATP, LDH, DCF, MitoSox, Fluo4, JC1, PI	Regression tree	1,2,4
Epa et al. (2012)	31NPs, diverse core	Indicator variables, size, relaxivities, zeta potential	ATP, Red, Apop., Mito	MLR, SLR, feature selection, ANN	1,2,4
	109NPs,diverse modifier	691 theoretical descriptors	Cellular uptake		
Wang et al. (2014)	18NPs, MOs and C-based	size, shape, area, porosity, free radicals, reactivity, metal conc. and charge	LDH, Apop., Nec., Proinflammatory, Hemolysis, MTT, DiOC6, morphology	PCA	1,2,4
Liu et al. (2013a)	44 iron oxide core NPs	Size, relaxivities, zeta potential	ATP, Red, Apop., Mito	NBC,LGR,LDA,NN	1,2,3,4
Liu et al. (2013b)	24 MO-NPs	30 molecular descriptors	MTS, ATP, LDH, DCF, MitoSox, Fluo4, JC1, PI	NBC, LR, LGR, LDA, SVM	1,2,3,4
Singh and Gupta (2014)	44 iron oxide core NPs	Size, relaxivities, zeta potential	ATP, Red, Apop., Mito		1,2,3,4
	109NPs,diverse modifier	691 theoretical descriptors	Cellular uptake	Ensemble learning (EL)-based techniques	
	17 MO-NPs	Oxygen percent, molar refractivity, polar surface area	Cytotoxicity (EC_{50})		
	80 MWCNTs	6 topo. and geo. descriptors	Cell viability		
	48 fullerene derivatives	10 descriptors	The binding affinity		
Kar et al. (2014)	109 NPs, diverse modifier	307 theoretical descriptors	Cellular uptake	GFA, MLR, PLS	1,2,3,4

Table 6: ISA-TAB-NANO file types (Thomas et al., 2013)

ISA-TAB-NANO file types	Types of information entered in each ISA-TAB-Nano file
1. Investigation file	Reference information about each investigation, study, assay, protocol, Study file, and Assay file.
2. Study file	Names and attributes of protocols used for preparing samples for analysis; source and characteristics of bio-specimens.
3. Assay file	Values of measured endpoint variables and references to external data files for each analysed sample.
4. Material file	Descriptions of the material sample, its structural parts and chemical components; linkage descriptions between chemical components; reference information about external material data files.

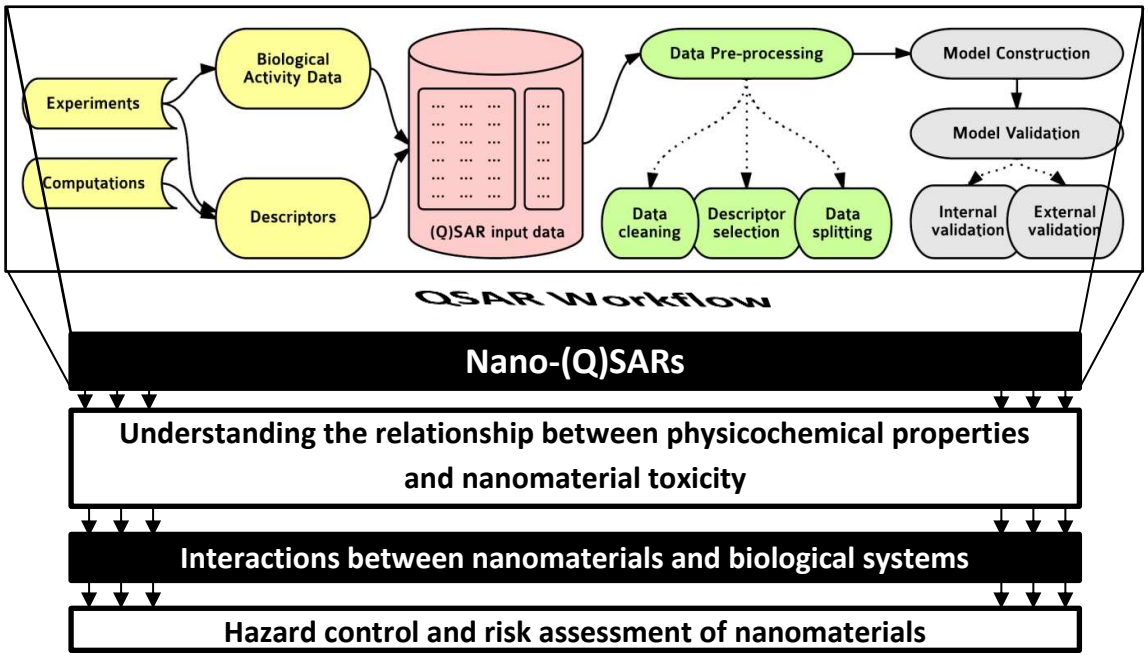

Figure 1: (Q)SAR modelling of nanomaterial toxicity

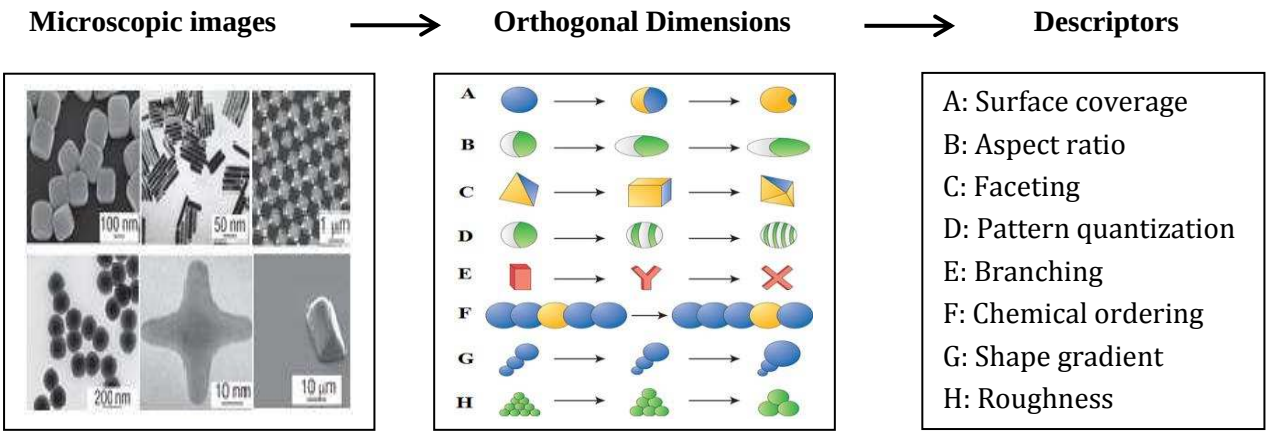

Figure 2: Derivation of eight qualitative descriptors based on microscopic images, proposed by Glotzer and Solomon (2007)

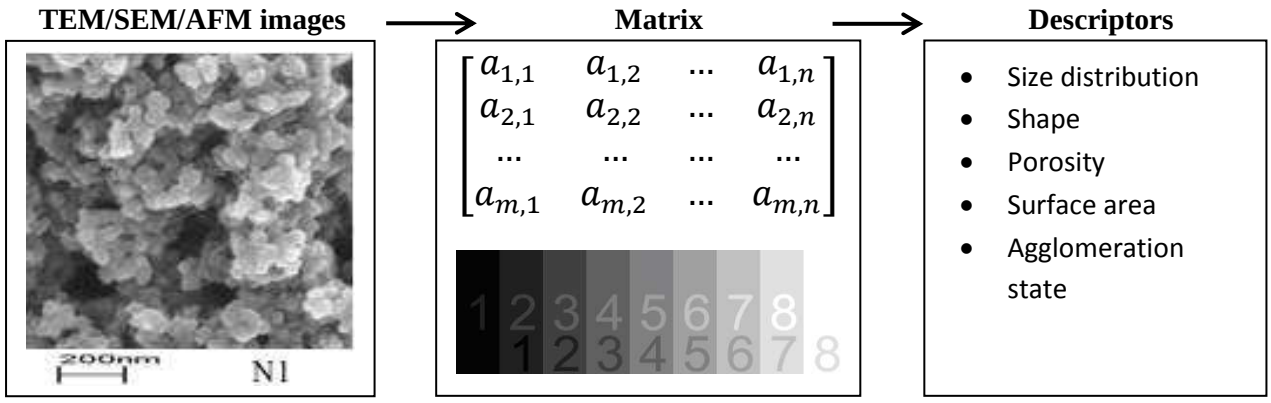

Figure 3: Derivation of structural descriptors based on microscopic images, proposed by Puzyn, Leszczynska, and Leszczynski (2009)

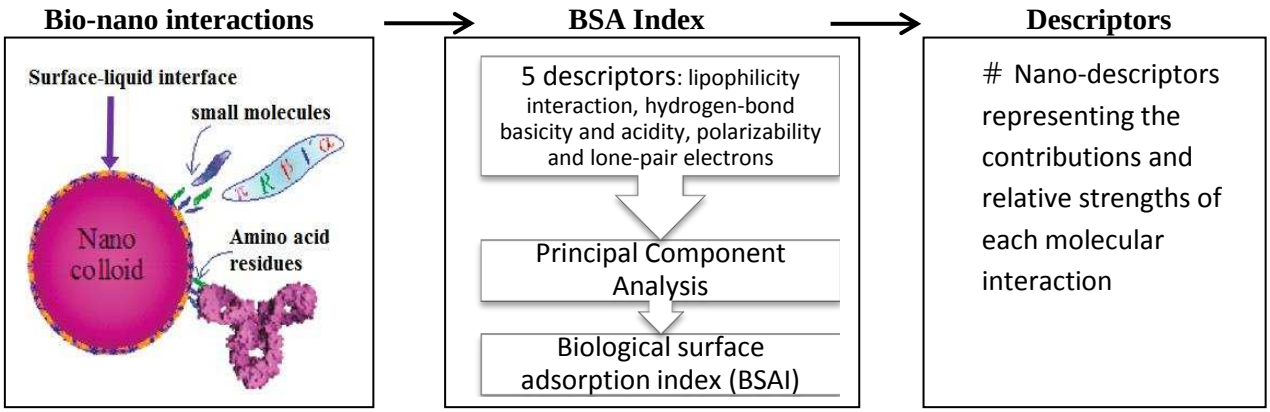

Figure 4: Derivation of descriptors that represent the fundamental forces governing the adsorption process of NPs, proposed by Xia, Monteiro-Riviere, and Riviere (2010)

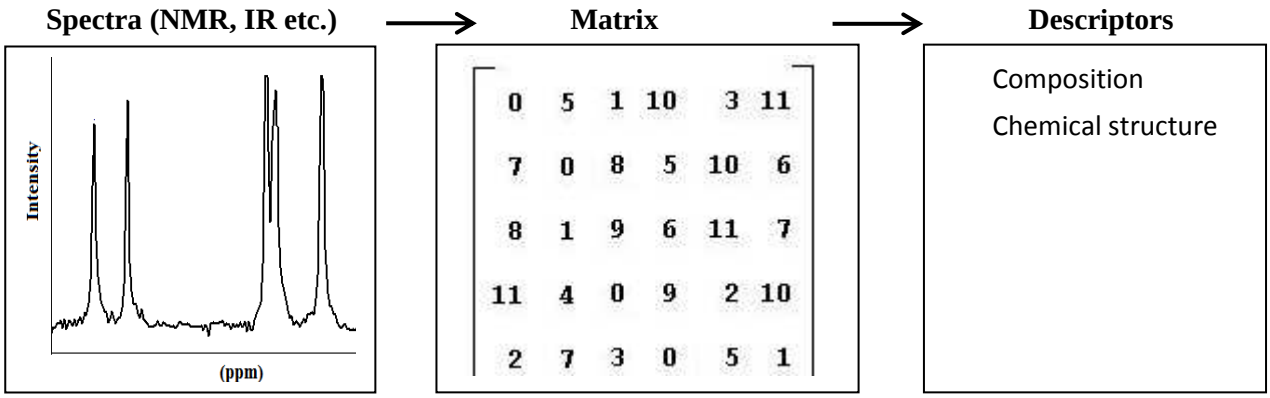

Figure 5: Derivation of NP-descriptors based on spectra of ENMs, proposed by Burello and Worth (2011)

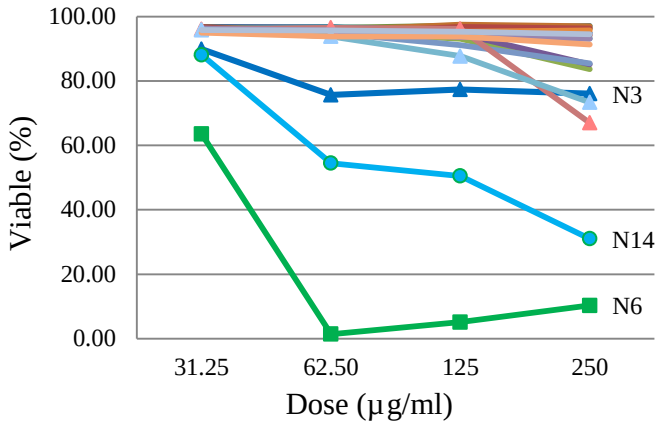

Figure 6: Viability results for 18 NMs (Wang, et al., 2014)

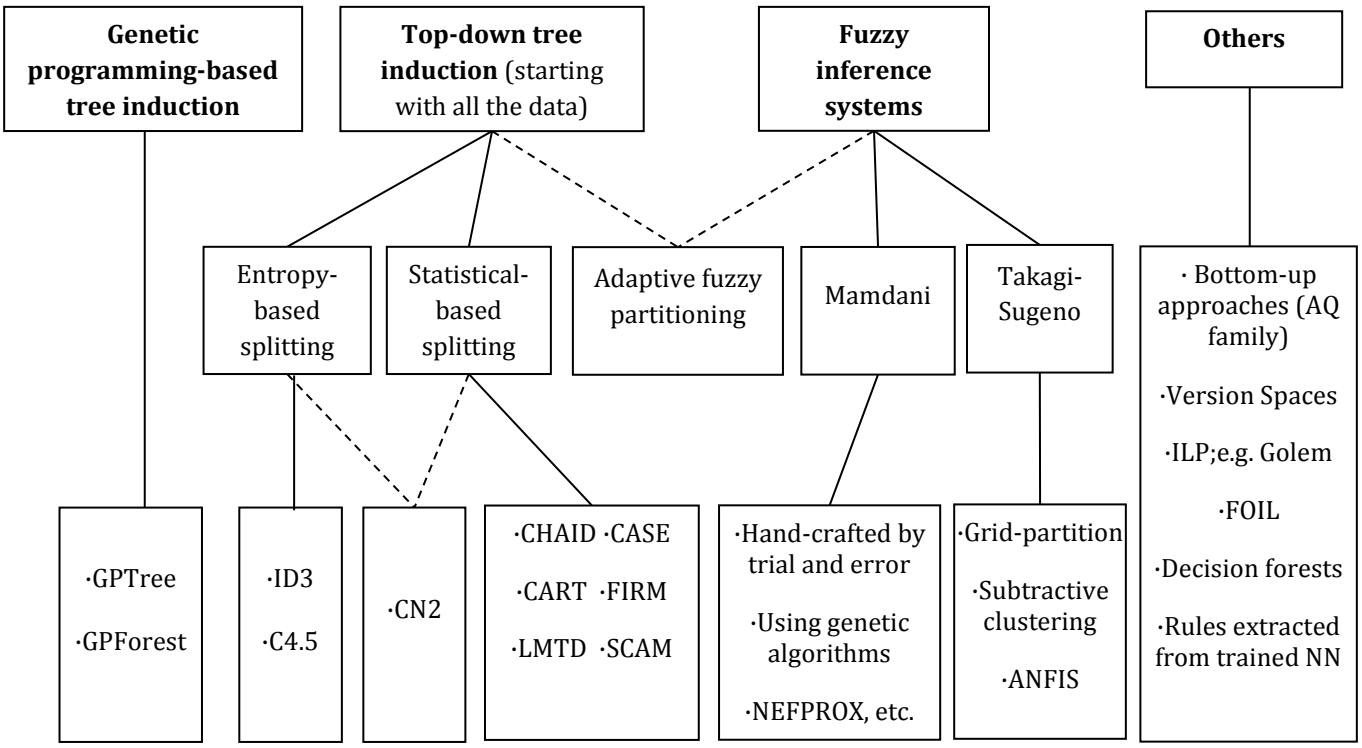

Figure 7: A family tree of proposed inductive learning techniques, showing a selection of specific implementations of each type.

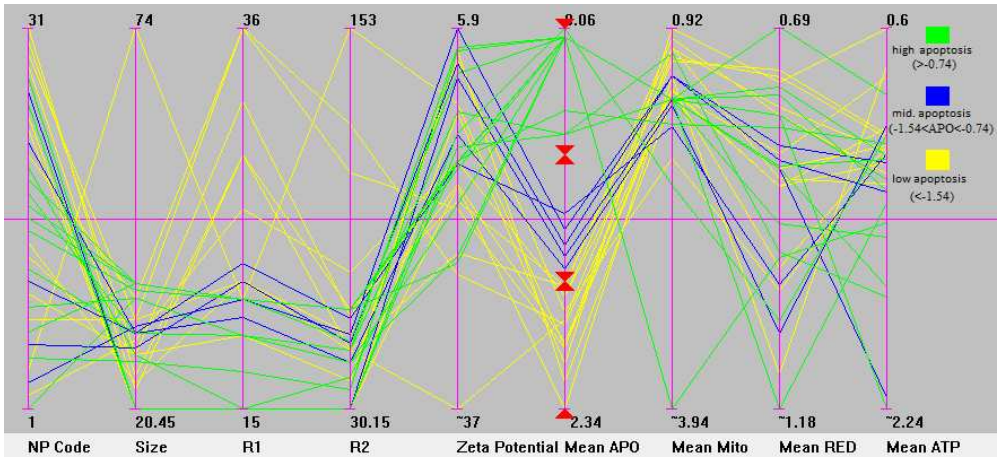

Figure 8: CVE plot of the data collected by Shaw, et al. (2008) (Descriptors: Size, Relaxivities (R1 and R2) and Zeta potential; Toxicity Endpoints: apoptosis (APO), mitochondrial potential (Mito), reducing equivalents (RED), ATP content (ATP)). The mean apoptosis data is divided into three categories; low (<-1.54), medium (-1.54<APO<-0.74) and high (>-0.74) and each category is highlighted in different colors.