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Applying quantitative structure–activity relationship approaches to nanotoxicology: Current status and future potential

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ABSTRACT

The potential (eco)toxicological hazard posed by engineered nanoparticles is a major scientific and societal concern since several industrial sectors (*e.g.* electronics, biomedicine, and cosmetics) are exploiting the innovative properties of nanostructures resulting in their large-scale production. Many consumer products contain nanomaterials and, given their complex life-cycle, it is essential to anticipate their (eco)toxicological properties in a fast and inexpensive way in order to mitigate adverse effects on human health and the environment. In this context, the application of the structure–toxicity paradigm to nanomaterials represents a promising approach. Indeed, according to this paradigm, it is possible to predict toxicological effects induced by chemicals on the basis of their structural similarity with chemicals for which toxicological endpoints have been previously measured. These structure–toxicity relationships can be quantitative or qualitative in nature and they can predict toxicological effects directly from the physicochemical properties of the entities (*e.g.* nanoparticles) of interest. Therefore, this approach can aid in prioritizing resources in toxicological investigations while reducing the ethical and monetary costs that are related to animal testing. The purpose of this review is to provide a summary of recent key advances in the field of QSAR modelling of nanomaterial toxicity, to identify the major gaps in research required to accelerate the use of quantitative structure–activity relationship (QSAR) methods, and to provide a roadmap for future research needed to achieve QSAR models useful for regulatory purposes.

1. Introduction

Nanomaterials have different and useful properties compared to bulk materials, and their commercial applications are growing rapidly. However, nanomaterials properties may also pose risks to health or the environment, and regulatory agencies are urgently seeking ways of assessing these risks (Hansen et al., 2008; Morris et al., 2011; Pumera, 2011; Cattaneo et al., 2010). Toxicological characterization of the immense number of structural combinations that can be engineered would be extremely demanding (if not impossible) in terms of time, costs and experimental facilities, and so the adoption of *in silico* toxicology methods as a way to complement and reduce animal testing is a high priority (Clark et al., 2011).

In this context, the application of the structure–toxicity paradigm to nanoparticles appears as a logical adaptation of QSAR modelling concepts as applied to chemicals. Indeed, the encoding of existing knowledge into computational models that formalize the relationships between molecular structure and toxicological effects has been successfully applied in the pharmaceutical field and in regulatory toxicology (Mombelli and Ringeissen, 2009; Bassan and Worth, 2007). QSAR or QSTR (quantitative structure–activity (or toxicity) relationship) models can predict continuous (*e.g.* lethal doses) or categorical (*e.g.* genotoxic *vs.* non-genotoxic) endpoints and they can reduce the need for, or extent of, animal testing. Moreover, the rationalization of knowledge provided by these models offers to all the stakeholders affected by toxicological regulations a common conceptual framework upon which informed discussions could take place.

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Despite inherent difficulties related to the toxicological characterization of nanomaterials, toxicological studies are beginning to highlight the role that the structure of nanoparticles has in triggering and modulating adverse effects (Shvedova et al., 2010; Rivera Gil et al., 2010; Maynard et al., 2011). Size has been proven to play an important role not only for biodistribution of nanomaterials (Choi et al., 2010) but also for their toxicity (*e.g.* Pan et al., 2007). Furthermore, as can be intuitively expected, shape has a function in mediating toxicological responses. For instance, carbon nanotubes that remain as isolated long fibres are more inflammogenic in the outer regions of the lung than nanotubes that form tightly bundled aggregates with non-fibrous shapes (Osmond-McLeod et al., 2011). Another structural characteristic that does not have a wholly unexpected role in producing toxicological effects is the high surface area of nanomaterials that dramatically increases the areas of contact between nanoscale entities and biological environments. The impact of this property on toxicity has been analyzed by Monteiller and co-authors (2007) who showed that surface area is a key factor in determining pro-inflammatory effects on epithelial cells *in vitro*. These authors identified a clear and significant relationship between pro-inflammatory effects and surface area for a variety of nanoparticle types and, according to their analysis, as surface area dose increases above 1 cm^2/cm^2, dose response relationships collapse almost onto a common trend (Monteiller et al., 2007). Similarly, the functional properties imparted by a high surface area have also been put into use in the pharmaceutical field in order to enable nanoparticles to interact with receptors over a larger cell surface area while improving the nanoparticle binding affinity and selectivity (Wang and Dormidontova, 2010).

The difference in electrostatic potential between the stationary layer of fluid surrounding the nanoparticles and the bulk fluid (zeta potential) is another important physicochemical property that has proven to be critical in modulating cytotoxicity effects of nanoparticles (Sayes and Ivanov, 2010). For instance, it is proposed that nanoparticle cytotoxicity could be modulated by controlling the electrostatic interaction between nanoparticles and cellular targets (Feris et al., 2010; Mura et al., 2011).

Perhaps less intuitive, but experimentally proven, is the finding that oxidized carbon nanotubes have an increased toxicological potential with respect to their pristine counterparts because of an enhanced dispersion in the experimental medium and of a higher negative charge introduced by oxygenated functionalities (Pietroiusti et al., 2011). The effect that structure has on toxicity becomes even more complex if we consider that essential biological functions such as cellular uptake can be influenced by the nature of the surface coating on nanomaterials that may be purposely introduced (such as silica or dextran (Kunzmann et al., 2011)) or modified by the biological environment through adsorption of proteins and other biomolecules (Monopoli et al., 2011).

The few examples reported above highlight the fact that structure–toxicity data are potentially multivariate in nature. Therefore, the extraction of meaningful relationships between nanostructures and toxicological properties to yield predictive QNTR (quantitative nanostructure–toxicity relationship) models requires specific techniques. Firstly, chemical or structural properties of nanomaterials are represented by mathematical objects called descriptors, many of which can be calculated rather than measured. Examples of descriptors suitable for nanomaterials include particle size, shape and surface area, ionization potentials of metals, heats of formation of metal oxide clusters (Puzyn et al., 2011), zeta potentials, and physicochemical properties (*e.g.* lipophilicity, hydrogen bond donor or acceptor strength) of molecules attached to nanoparticles surfaces. Secondly, using additional mathematical techniques, subsets of descriptors are chosen that are most likely to relate to the biological property (*e.g.* cell apoptosis, metabolism, or signalling pathway modulation) being modelled. Statistical modelling (*e.g.* regression models) or machine learning methods, often employing neural networks, generate a mathematical model linking the descriptors to the biological activity (Mombelli and Ringeissen, 2009; Burden and Winkler, 1999; Le et al., 2012).

Finally, the model's robustness and ability to predict properties of new materials is assessed by statistical cross-validation techniques, or by predicting properties of materials in a test set not used to develop the model (Fig. 1 and see Le et al., 2012). Although QSAR approaches have only recently been used to predict biological effects of nanomaterials, they have shown encouraging initial results as reported in Table 1 (see also Burello and Worth, 2011; Fourches et al., 2011; Puzyn et al., 2009; Epa et al., 2012a,b).

These pioneering studies suggested that binary classification models based on size, relaxivities, and zeta potential can be used to predict the effect that nanoparticles, characterized by different core compositions and surface attachments, have on cellular physiology (Fourches et al., 2010). However, subsequent studies have shown that some of these properties, particularly relaxivities, may simply generate correlative rather than causative relationships. Interestingly Fourches et al. (2010) also pointed out that the cellular uptake of nanoparticles possessing the same metal core but different organic molecules on their surface can be predicted by taking into account the chemical structure of the coating molecules.

Therefore, the structural determinants of the biological behaviour of nanoparticles can be found both at the core of nanoparticles and at their surface. Because of all the possible combinations and interdependencies that can exist between core and surface compositions, it appears that the characterization of nanoparticles by means of physicochemical descriptors is a domain of research in itself that can benefit from a wide range of scientific studies ranging from classical molecular dynamics simulation to quantum chemical computations (Barnard, 2009; Liu and Hopfinger, 2008). It has anyway to be pointed out that in some cases, as the work by Burello and Worth (2011) and Puzyn et al. (2011) showed, biological effects of nanoparticles can be explained in a simple way by enthalpies of formation of gaseous cations and orbital energies. These straightforward relationships show that a reductionist analysis of the relationship between the structure of nanoparticles and their biological behaviour is possible and pave the way for systematic studies on the impact that nanostructures have on biological systems (Epa et al., 2012b). Nevertheless, the modelling of the behaviour of nanomaterials presents different challenges compared to drugs and chemicals, as discussed in the following paragraphs where we also point out research needs and present a roadmap for the development of QNTR models as a basis for regulatory decision making.

2. Defining the biologically relevant entity

Unlike chemicals, the surface properties of nanomaterials may change in an environment-specific manner (Fig. 2). When taken up by humans, a nano-bio interface (corona), consisting mainly of proteins in the systemic circulation and of phospholipids in the lung, is generated. Protein or phospholipid binding in biological fluid is not a static process, being characterized by continuous association and dissociation events that reach equilibrium whereupon continued exchange does not affect the corona composition (Dell'Orco et al., 2010; Monopoli et al., 2011). The composition of the protein corona may be considered a fingerprint of a specific nanomaterial in a given compartment. However, if the nanoparticle moves from one compartment to another (*e.g.* from the lung to blood), the corona may be modified. Another dynamic process occurring in biological systems is the exchange between the agglomerated and the dispersed forms

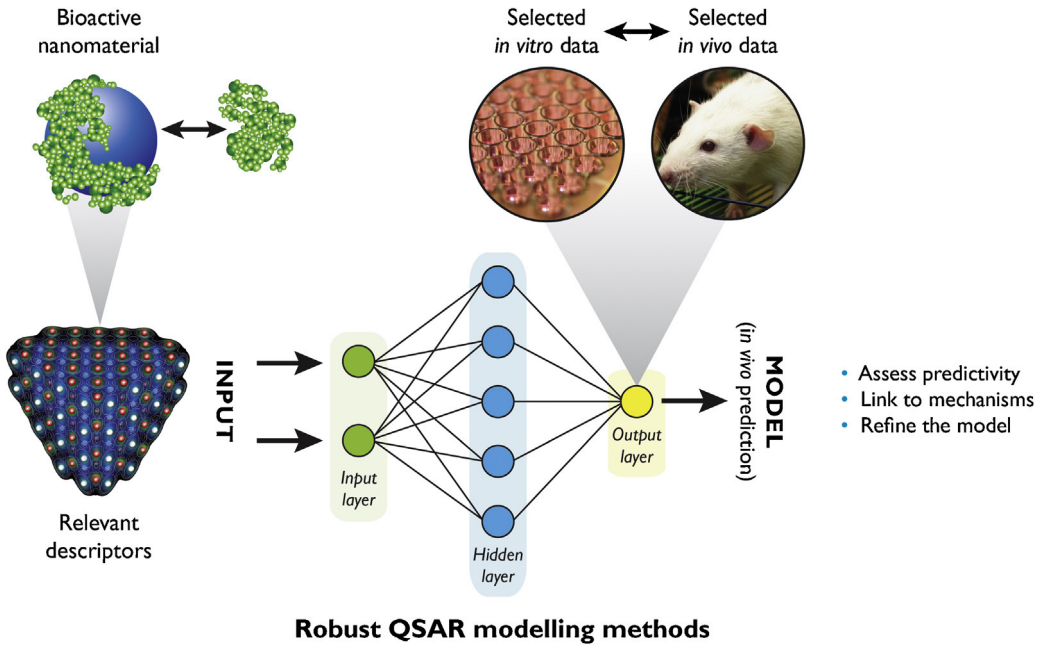

Fig. 1. QSAR method. The bioactive form of nanoparticles for a given environment is converted into suitable molecular descriptors. *In vivo* or relevant *in vitro* data and descriptors are used to train a neural network or to generate a statistical regression model. Once validated, the model can be used to predict properties of new nanomaterials, or to elucidate biological mechanisms and processes.

of nanoparticles, which may change as the environment changes and is currently poorly understood. In addition, potential dissolution of nanoparticles in certain environments may be modified by the composition and surface coverage of its corona. Modelling and prediction of the biological effects of nanomaterials requires a better understanding of these dynamic processes through additional experiments.

High throughput experimental methods can provide information on corona composition through rapid measurements of binding affinities of many potential corona components and nanoparticle types, potentially many thousands of experiments (Damoiseaux et al., 2011). Analysis of this large volume of experimental data will provide a better understanding of the competitive binding behaviour of nanoparticle corona components, and how this leads to specific nanoparticle corona compositions. This will allow the generation of QNTR models linking nanoparticle and adsorbent properties to the resultant nanoparticle corona composition that can be used to predict corona formation more broadly. The data can also parameterize and validate competitive binding models that predict corona properties.

A range of analytical techniques (Table 2) can be used to investigate the interactions of nanoparticles with molecules in their environments as described by Lynch and Dawson (2008). For example, surface plasmon resonance (Tassa et al., 2010) has recently been used to measure the affinity of proteins for nanoparticles. Magnetically responsive nanosensor arrays (up to 100,000 sensors/cm^2) have been employed to quantify protein–binding interactions at sensitivities in the zeptomolar range (Gaster et al., 2011). Fluorescence correlation spectroscopy has been used to follow the kinetics of protein binding to nanoparticle surfaces (Röcker et al., 2009). These types of studies are deepening our quantitative understanding of the protein corona on nanoparticle surfaces.

Recently, a biological surface adsorption index was developed to predict the molecular interactions of nanoparticles with proteins. A set of small molecule probes that mimic amino acid residues were allowed to competitively adsorb onto a set of nanoparticles and the adsorption coefficients for the probes were measured. By assuming the adsorption was governed by five basic molecular forces, the measured adsorption coefficients were used to develop descriptors

Table 1
Early harvest: QNTR (quantitative nanostructure–toxicity relationship) modelling studies.

Reference	Results
Toropov et al. (2010)	Modelled toxicity of nanoscale (metal) oxides towards *E. coli* bacteria using SMILES-based optimal descriptors (available at http://www.caesar-project.eu/posters/SETAC2009/Toropov_nanooxides_setac_1.pdf)
Fourches et al. (2010)	Generated QNTR models predicting the results of *in vitro* cell-based assays for nanoparticles with (i) different metal cores and (ii) similar cores but different surface modifiers. In the first case a QNTR model could be obtained as a function of experimental descriptors (size, relaxivities, and zeta potential) whereas in the second case a model was derived as a function of the structure of the organic molecule attached to the surface of nanoparticles.
Puzyn et al. (2011)	Modelled bacterial toxicity of metal oxide nanoparticles as a linear function of the enthalpy of formation of a gaseous cation having the same oxidation state as in the metal oxide structure.
Burello and Worth (2011)	Demonstrated that the oxidative stress potential of oxide nanoparticles with a diameter larger than 20–30 nm can be predicted by taking into account their band energy.
Fourches et al. (2011)	Using a dataset of carbon nanotubes decorated with a series of congeneric organic molecules, modelled acute toxicity as a function of the structure of the organic molecules in the surface coatings.
Liu et al. (2011)	Using cytotoxicity data, developed a QNTR model based on the atomization energy of the metal oxide, period of the nanoparticle metal, and nanoparticle primary size, in addition to nanoparticle volume fraction (in solution).
Epa et al. (2012b)	Generated quantitative and predictive QNTR models from *in vitro* cell-based assays for nanoparticles with (i) different metal cores and (ii) similar cores but different surface modifiers. They generated robust and predictive quantitative models of smooth muscle apoptosis induced by metal iron oxide nanoparticles (MION), and cellular uptake of surface modified nanoparticles.

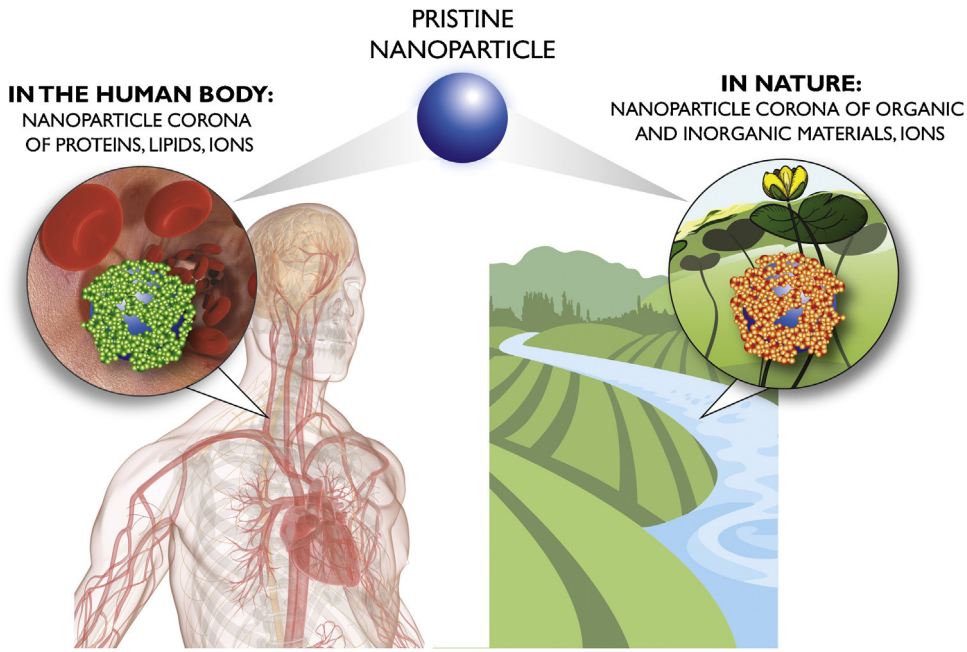

Fig. 2. Effects of different environments on pristine nanoparticles. Pristine nanoparticles become coated with a variety of molecules and ions in the body or in the natural environment to generate the biologically relevant entity.

that represent the relative contributions of each of the forces, which, in turn, could be used in an *in silico* model to predict the adsorption of small molecules to other nanomaterials (Xia et al., 2010).

In a follow-up study, the authors determined the surface adsorption forces of 16 different nanomaterials (Xia et al., 2011). When the five-dimensional information of the nanodescriptors was reduced to two dimensions, the 16 nanomaterials were classified into distinct clusters according their surface adsorption properties. This is the first success in quantitative characterization of the surface adsorption forces of nanomaterials in biological conditions, and could open a quantitative avenue in predictive nanomedicine development, risk assessment, and safety evaluation of nanomaterials.

Another promising computational approach for predicting the formation of protein corona, biodistribution and bioavailability of nanoparticles is provided by the Hansen solubility parameters (Hansen, 2007) that have been applied to the prediction of the solubility and dispersability of carbon nanotubes in order to select the most appropriate solvents to use (Detriche et al., 2008).

Although defining the biologically relevant entity of nanomaterials in the natural environment they occupy is a challenge, the colloidal stability, agglomeration and dissolution properties of metal-containing nanoparticles in simple systems are relatively well understood. Ecotoxicity of chemicals and particles are both affected by abiotic factors like pH, salinity, water hardness, temperature, and naturally occurring organic matter. For example, environmental ions are effective at shielding surface charges of nanoparticles, in turn affecting agglomeration properties. Other factors such as pH and water hardness can influence size, shape, surface properties, solubility, and, ultimately, biological effects of nanoparticle agglomerates and aggregates. However, there have been relatively few (but an increasing number of) studies on how these factors influence the environmental toxicology of engineered nanoparticles (Handy et al., 2008; Klaine et al., 2008; Batley et al., 2012; Peng et al., 2011). There has been also an increasing focus on the ability of nanoparticles to adsorb and desorb toxic metals such as lead and arsenic, largely for environmental remediation purposes, but also providing an additional source of data on

Table 2
Methods suitable for measuring interactions of nanoparticles with environmental molecules (Lynch and Dawson, 2008).

Method	Strengths and weaknesses
Surface plasmon resonance (SPR)	Fast, sensitive but can only measure one protein at a time, suffers from non-specific binding, and is not amenable to high-throughput analyses.
Size exclusion chromatography (gel filtration)	Fast, discriminates different proteins, gives information on kinetics and association rates. Limited ability to identify proteins, results affected by operating conditions.
Magnetic nanosensor arrays	High throughput, very sensitive, measure binding kinetics of proteins with high spatial and temporal resolution but can only measure one protein at a time.
Fluorescence correlation spectroscopy	Fast, sensitive, can follow kinetics of corona build up, hydrodynamic dimensions and photophysical properties can be determined, but limited to fluorescent species.
Shotgun proteomics analysis	Identifies identities and quantities in mixtures of proteins but very slow, cannot do kinetics or monitor dynamic processes.
SDS-PAGE (polyacrylamide gel electrophoresis)	Quick, quantifies relative amounts of proteins. Cannot identify proteins.
LC MS/MS (liquid chromatography mass spectrometry)	Provides very good information on identities and quantities of bound proteins. Limited ability to monitor dynamic processes, not amenable to high throughput.
Isothermal titration calorimetry	Good for thermodynamics of nanoparticle-protein or ion interactions. Slow and not suitable for mixtures of biological samples.

nanoparticle toxicity in the natural environment (Hu and Shipley, 2012).

Nanomaterials may enter the human body by various routes, such as the mouth, nose, skin or eyes. Additional challenges are posed by the fact that nanoparticles move around the body (Péry et al., 2009) and encounter different environments, changing the composition of the corona and the properties of nanoparticles over time. Methods using radioactive tracers and magnetic resonance imaging can track movement of nanoparticles around the body. Other techniques such as electron and confocal microscopy can image nanoparticles in cells. New experimental techniques like scanning near-field ultrasonic holography allow much-improved imaging of the interaction of nanoparticles with cells (Tetard et al., 2008), increasing knowledge of uptake and fate as well as effect on cell function. These methods require further development to allow *in vivo* tracking of nanoparticles at the typical concentrations in the body during likely occupational exposure, to determine their kinetics of transport, and fate. The best methods use some intrinsic property of the nanoparticle, for example the radioactivity of an isotope of one of the nanoparticle components, so that the labelled nanoparticle will behave the same way as the unlabeled (native) nanoparticle as it traverses the body. In the case of metal-containing nanoparticles, intrinsic labelling allows also the simultaneous tracking of dissolved and nanoparticulate metals in the body or other complex environment, thus improving our understanding about the dissolution properties of these types of nanoparticles. In contrast, external tagging (by fluorescent labels, for example) seems less suitable as the label may alter nanomaterials properties, affecting interactions with organs and cells and transport through the body.

Once the nanoparticle characterization, corona composition, and translocation data described above become available in sufficient quality and quantity for a wide range of environments and nanoparticle types, we will be able to predict the bioactive form of nanoparticles in a given environment. To this end, QNTR approaches can play a valuable role. Such approaches can model and predict the *in situ* forms of nanoparticles, and the time- and environment-dependent changes in the nanoparticle composition from the high-throughput experimental data.

3. Choosing the right assays to develop pertinent models

Clearly, the generation of large volumes of *in vivo* data is not possible from ethical or cost perspectives. However, regulators and other risk assessors need to estimate the potential hazard of a given nanomaterial in the workplace, home, or environment. Consequently, it is essential to understand the major mechanisms of toxicity for nanomaterials and define relevant *in vitro* testing procedures (assays) that can measure the toxic effects of the nanomaterials that correlate well with their effects *in vivo*. The selection of the biological properties measured will almost certainly be end-use dependent. As with nanoparticle characterization and corona composition measurement, high throughput and high content (*i.e.* measuring several biological responses in cells simultaneously, Damoiseaux et al., 2011) *in vitro* toxicity assays, such as those developed for the pharmaceutical industry (Service, 2008), can be adapted for nanoparticles (Feliu and Fadeel, 2010; Damoiseaux et al., 2011). This will greatly increase the amount of nanotoxicity data that can be generated for use in modelling and improve our knowledge of mechanisms of toxicity of nanoparticles (Pumera, 2011). As an example, George et al. (2010) recently reported a rapid cytotoxicity screen for metal oxide nanoparticles that exploits high content screening methods. They developed a fluorescence assay that simultaneously measured four different responses in cells to oxidative stress caused by ZnO, CeO_2, and TiO_2 nanoparticles. Using the information, they were able to reduce the cytotoxicity of ZnO nanoparticles by decreasing ZnO dissolution through Fe doping. These authors also reported the use of a multiparametric, automated screening assay for high-throughput analysis of commercial metal/metal oxide nanoparticles and employed zebrafish (*Danio rerio*) embryos in order to compare the *in vitro* with the *in vivo* responses (George et al., 2011). Moreover, Shaw et al. (2008) also reported on the assessment of nanoparticle effects using multiple cell types and multiple *in vitro* assays. Hierarchical clustering of the data identified nanomaterials with similar patterns of biologic activity across a broad sampling of cellular contexts, yielding robust structure–activity relationships for the nanomaterials tested. Furthermore, a subset of nanoparticles were tested in mice, and nanoparticles with similar activity profiles *in vitro* exerted similar effects *in vivo*, using monocyte number as the endpoint. These data suggest a strategy of multi-pronged characterization of nanomaterials *in vitro* that can inform the design of novel nanomaterials and guide studies of *in vivo* activity.

A model for how high throughput methods might be used to assess adverse effects of nanomaterials is ToxCast, a 2007 initiative of the U.S. Environmental Protection Agency (EPA) to accelerate toxicity testing of industrial chemicals (Collins et al., 2008). Researchers examined 320 different chemicals in a wide variety of cell-based assays, which had all undergone extensive conventional toxicological testing, looking for 400 endpoints that correlated with adverse effects. This analysis revealed both known and novel targets that play important roles in toxicity (Knudsen et al., 2011).

Another high-throughput technology that can assist in assessing the adverse effects of nanomaterials is whole genome microarrays (Yang et al., 2010). For instance, Zhang et al. examined cells treated with two dosages of PEG-silane quantum dots and measured both phenotypic changes and altered transcription of the whole genome, providing clues to modes of toxicity (Zhang et al., 2006). Similarly, the ToxCast project uses rapid, high-content assessment of impact on gene regulatory networks by chemicals to elucidate toxicity mechanisms and establish a 'fingerprint' or profile that correlates with *in vivo* adverse effects (Martin et al., 2010; Judson et al., 2010). Roh et al. (2009) investigated the ecotoxicity of silver nanoparticles in *Caenorhabditis elegans* using whole genome microarrays and the integration of gene expression data with organism and population level endpoints. The results suggest that oxidative stress might be an important mechanism in silver nanoparticle-induced toxicity in this model organism.

For organic chemicals, a surrogate assay approach to predict *in vivo* genetic, reproductive and developmental toxicity *in silico* has been utilized (Matthews et al., 2006a,b). Surrogate assays are faster, cheaper experiments that yield results that are strongly indicative of results from a desired, more complex, expensive and time-consuming assay. These authors analyzed *in vitro* genetic toxicity data, reproductive and developmental toxicity studies, and rodent carcinogenicity bioassays to identify surrogate *in vitro* endpoints that best correlated with rodent carcinogenicity observations. In the near future, a similar approach could be adopted for predicting adverse *in vivo* effects of nanomaterials since the development of *in vitro* assays is an active field of research which aims at developing validated protocols (Park et al., 2009).

Choosing relevant assays for predicting adverse effects on the environment is arguably more challenging than for human toxicity. Species in the environment are exposed to many types of naturally occurring nanoparticles such as colloids and volcanic dust, but the concern is that manufactured nanoparticles may have properties that are substantially different to these. The diversity of organisms is very high and susceptibilities to toxic materials vary widely across kingdoms and even between species. Currently, there are relatively few data on the effects of nanomaterials at realistic environmental concentrations on organisms such as fish and

crustaceans, and limited data that enable calculations of "50% of maximal effect" and "no effect" concentrations (Batley et al., 2012). In contrast, the toxic effects of silver nanoparticles on bacteria have been amply demonstrated (Rai et al., 2009).

Handy et al. (2008) reviewed issues involved in the comprehensive assessment of environmental effects of nanomaterials and identified the major data gaps. These gaps include: a better understanding of environmental fate; more studies on uptake and distribution within organisms; measurement of ecotoxicological data across a broader range of terrestrial, marine and plant species; detailed investigations of absorption, distribution, metabolism and excretion on species from the major phyla. Assessment of the environmental impacts of nanomaterials will be accelerated by adoption of high-throughput technologies, if suitable standard reference materials, assays, and procedures can be established internationally. However, as a cautionary note, standard procedures should be adopted with care, as the sequence of steps and time taken to prepare nanomaterial samples for testing can influence observed toxicities (Oberdörster et al., 2005), and so early adoption of standardized procedures may result in not all adverse effects being identified.

4. Modelling of complex nanomaterials–biology interactions

The large volumes of data that may be generated by the high throughput experimental methods referred to above will allow development of QNTR models for properties such as corona composition and cellular toxicity for specific environments and organs, *in vitro*. Such models will allow *in vitro* responses of new nanomaterials to be predicted. This allows experimental work to be focused more effectively, by identifying materials or properties of particular concern. QSAR modelling of large data sets therefore requires cycles of iteration between experiments and modelling that allows predictions to be tested and, subsequently, models to be refined. The refined models will be better predictors of biological responses to new nanomaterials.

Ultimately, *in vivo* effects of nanoparticles are the most important for regulatory purposes, although they are the most expensive and difficult to obtain by experiment. The combination of results from *in vitro* assays (or predictions from QNTR models of *in vitro* assays) and nanomaterials descriptors such as size, shape, composition, zeta potential, elemental and molecular properties and corona composition constitute nanoparticle 'fingerprints' that can be used to derive QNTR models of *in vivo* activity. For example, nanoparticle fingerprints and experimental *in vivo* data could be used to train a neural network that predicts the *in vivo* responses more broadly. A similar approach has been effective in predicting *in vivo* toxicities of industrial chemicals using *in vitro* assay results and molecular descriptors (Lee et al., 2010).

Existing QSAR modelling tools include: statistical methods like multiple linear regression, polynomial and kernel regression; machine learning methods like artificial neural networks (Burden and Winkler, 1999) and support vector machines; and clustering methods like random forest and decision trees (Lee et al., 2010; Fourches et al., 2010; Katritzky et al., 2010; Nantasenamat et al., 2010). They are used to find mathematical relationships that link the microscopic (*e.g.* molecular) or physicochemical properties of nanomaterials to their biological properties. These relationships are often very complex and nonlinear, and QSAR and QNTR methods have the advantage that complete knowledge of intermediate processes and mechanisms is not required to construct useful models. These methods have been shown to predict *in vivo* toxicities of industrial chemicals or drugs in animals and humans (*e.g.* Lessigiarska et al., 2006) and ciliates (*e.g.* Richard et al., 2008) with useful fidelity. The existing QSAR modelling tools appear adequate to model experimental data from nanomaterials.

Recent papers have suggested that QNTR methods can generate useful models of the *in vitro* biological effects of nanomaterials (Puzyn et al., 2011; Fourches et al., 2010; Epa et al., 2012b). Puzyn et al. reported on the cytotoxicity of 17 different types of metal oxide nanoparticles to *Escherichia coli* while Fourches et al. studied the cellular uptake of 109 different nanoparticles with similar core but diverse surface modifiers. These proof-of-concept models are promising, but more studies are needed using larger datasets and emphasis on additional endpoints relevant to the nanosafety assessment for consumers, workers, patients, and for the environment.

5. The relationship between QNTR methods and other computational approaches

Physics-based methods such as quantum chemistry (Barnard, 2009) and molecular dynamics (Liu and Hopfinger, 2008) complement QNTR methods by elucidating mechanisms and generating useful molecular descriptors. Quantum chemical methods are based on approximate solutions of the Schrödinger equation, while molecular dynamics is a computational method for investigating the physical movements of atoms and molecules. Quantum chemistry computations are particularly suited to investigating the geometry and stability of packed carbon nanotubes; the application of this form of computational approach allowed an effective similarity comparison among nanostructures by taking into account energy gaps (*e.g.* energy differences between the highest occupied molecular orbital and the lowest unoccupied molecular orbital), chemical potentials, chemical hardness and Parr electrophilicity (Poater et al., 2010)

Three of the major roadblocks to applying QSAR methods to modelling biological properties of nanoparticles are insufficient experimental data on the composition of the bio-corona on nanoparticle surfaces, the lack of *in vitro* data predictive of *in vivo* effects of nanomaterials, and the paucity of 'nanoparticle-specific' descriptors. Nanomaterials differ substantially in structure from small organic molecules and chemicals for which the existing descriptors were developed. Although existing descriptors work well for modelling of some nanomaterials, it is clear that further research is required to generate nanomaterials-specific mathematical descriptors. In this respect, an interesting perspective is provided by the use of spectral information (*e.g.*, NMR, infrared, UV) as a descriptor of nanostructures. Indeed, spectra represent a unique fingerprint of chemicals and they have been used for designing photoactive nanocatalysts (Yang et al., 2011) and for characterizing nanotubes (Zhou et al., 2008).

6. A roadmap for the future

Policy-makers, regulators and scientists must harmonize efforts and rationalize nanoparticle characterization, bioactive entity, and toxicological data for an effective development of QNTR models for regulatory purposes. Since the discipline of computational nanotoxicology is in its infancy, we are at a critical juncture for organizing a synergy between experimentalists and theoreticians, and policy-makers, industrialists and scientists.

A recent international COST (European Cooperation in Science and Technology) workshop on the use of QSAR methods to model biological effects of nanomaterials (www.cost.esf.org/events/qntr) identified roadblocks to achieving useful models for assessing nanoparticle risks, and methods for overcoming them. A number of tasks that need to be completed in order to create models useful for nanoparticle regulation within the ten-year time frame requested

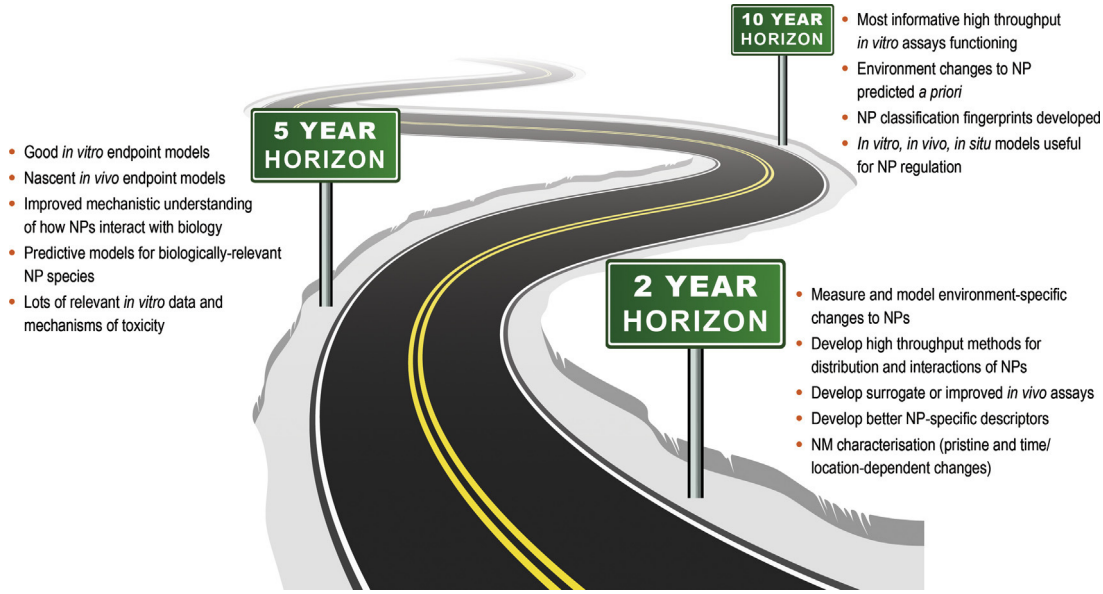

Fig. 3. QNTR (quantitative nanostructure–toxicity relationship) roadmap showing achievable objectives in short, medium, and long term horizons. The outcome will be a set of data and computational tools that can guide regulators in assigning the correct level of risk to nanomaterials.

by regulators, were divided into three time horizons that the expert consensus of COST workshop participants identified as being realistically achievable (Fig. 3).

Within two years, the following were considered achievable: establishment of well characterized materials for experiments; development of specific nanoparticle descriptors; development of high throughput *in vitro* assays for toxicologically relevant endpoints; development of high throughput methods for measuring the interactions of a diverse set of commercially relevant nanoparticles with plasma proteins; refinement of methods for tracking nanoparticles in the body. Commercial samples often contain nanoparticles with a wide range of sizes and shapes, surface properties, and states of aggregation. When studying the biological effects of nanoparticles, it is important that properties of the pristine nanoparticle (*i.e.* as supplied, before exposure to environmental influences), as well as their (altered) properties in a biological system, are well understood so that valid inferences can be made about how these properties affect nanoparticle behaviour in complex systems (Fubini et al., 2010). It is essential to establish an agreed set of reference nanomaterials for experimentation, such as those defined by the Organization for Economic Cooperation and Development (OECD) Working Party on Manufactured Nanomaterials (WPMN; http://www.oecd.org/env/nanosafety). These commercially sourced materials include: fullerenes, carbon nanotubes, silver, gold, iron, titanium dioxide, aluminium oxide, cerium dioxide, zinc oxide, silicon dioxide nanoparticles, dendrimers and nanoclays. An internationally agreed set of nanomaterials characterization methods and organism toxicity measurements also needs to be defined, and the OECD WPMN is also taking the lead on this.

Traditionally, toxicological profiles of drugs or chemicals rely on *in vivo* studies in laboratory animals, which are expensive, low-throughput, and inconsistently predictive of human biology and pathophysiology. Improvements to *in vivo* assays, and particularly the use of *in vitro* assays that correlate with *in vivo* outcomes, will provide reliable information of immediate use by regulators and will allow construction of *in vivo* QNTR models as described previously. To this end, the US National Institutes of Health (NIH) and Environmental Protection Agency (EPA) in the US have developed a suite of cell-based assays to profile the toxicity of chemical compounds in a variety of cell types. The ultimate goal is to identify *in vitro* chemical signatures that could act as predictive surrogates for *in vivo* toxicity (Matthews et al., 2006a,b). A similar approach, coupled with QNTR modelling methods, could yield useful *in vivo* predictions for nanomaterials.

The most important research component of computational nanotoxicology, namely the development of nanoparticle-specific descriptors, will be achieved within this two-year time frame. Also during this period, development of mechanisms for information sharing and close collaboration between experimentalists and computational scientists, with cooperation and input from regulators and industry, is essential. Establishment of tools for data storage and sharing, toxicity mark up languages and informatics tools (like ToxML, a toxicology database language; DSSTox, a distributed toxicity database network; and ACToR, an online warehouse of all publicly available chemical toxicity data), and development of ontologies (formal representations of knowledge as a set of concepts, and the relationships between those concepts) for nanomaterials (Richard et al., 2008) will facilitate this.

Within a five-year timeframe the amount of *in vivo* data on the effects of nanoparticles will be significantly increased; data storage and sharing methods will be developed; reliable *in vitro* models of *in vivo* endpoints will be developed; the first models of nanoparticle corona in different environments will be achieved; and the mechanisms of entry of nanoparticles into cells and mechanisms of toxicity such as free radical production, genotoxicity and apoptosis will be understood. High throughput technologies will provide substantial improvements in the ability to measure *in vitro* responses to nanomaterials, and an improved understanding of mechanisms of toxicity. Based on the limited but promising QNTR models of biological effects of nanoparticles, and the successful track record of this approach to modelling *in vitro* and *in vivo* toxicity data for small molecules, predictive models of the biologically relevant entity and a number of important *in vitro* endpoints will be possible. The larger quantity of data will also allow benchmarking of different QNTR modelling methods to be carried out, identifying those that are most reliable and that generate the most robust models. Concurrent accumulation of *in vivo* data from animal and ecotoxicological experiments and relevant *in vitro* data will see development of

the first models that can be used by regulators to predict *in vivo* responses to new nanoparticles.

The aim of the ten-year plan is to create QNTR models of *in vitro* and *in vivo* effects of nanoparticles and to obtain sufficient experimental and mechanistic data to allow regulators to make decisions on nanoparticle risk. In the final five years, based on achieving the earlier milestones, the following outcomes are considered to be realistic. *In vitro* assays most informative of *in vivo* effects of nanoparticles will be identified, models will be available that can reliably predict corona in diverse environments, nanoparticle 'fingerprints' will be developed that are useful for classification, and QNTR models of *in vivo* effects sufficiently reliable for regulatory purposes (with accuracies similar to models used to regulate industrial chemicals) will be available. We expect that nanomaterials classification fingerprints – physicochemical, genomic, and/or biological profiles of nanomaterials that group materials with similar *in vivo* effects – can also be developed. This would allow regulators to classify nanomaterials into hazard classes in a similar way to that currently adopted for industrial chemicals. This approach is particularly important since it will provide a rationale for forming chemical categories of nanoparticles to be used in regulatory toxicology to fill data-gaps (Diderich, 2010; Hansen et al., 2007).

Achieving the milestones in this roadmap requires a number of things to happen. Firstly, we need to maintain and expand the network of experimental and computational researchers, regulators and policy-makers, such as will be achieved through the COST Action MODENA (http://www.cost.eu/domains_actions/mpns/Actions/TD1204). Secondly, it is essential that the needs of the end-users of the experimental and modelling research outcomes remain a prominent driver for the work. Thirdly, it is important to focus on the high throughput experimentation flagged above as this will provide the essential data required for the QNTR models, will elucidate how environment affects nanoparticles, and will increase our knowledge of how nanoparticles enter, move through, and affect the biology of human and environmental systems. Finally, a funding mechanism needs to be developed to support a strong collaborative network of stakeholders, and to fund the research component of the work to be done. If these four important elements can be achieved, we are confident that the computational models developed, and increased knowledge of nanoparticle behaviour in biological systems, will generate outcomes that will assist regulators to assess nanoparticle risk within a 10-year time frame. This will facilitate finding the best balance between commercial development of these valuable materials and protection of workers, the public, and the environment from adverse effects.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References

Barnard, A.S., 2009. How can *ab initio* simulations address risks in nanotech? Nat. Nanotechnol. 4, 332–335.
Bassan, A., Worth, A.P., 2007. Computational tools for regulatory needs. In: Ekins, S. (Ed.), Computational Toxicology, Risk Assessment for Pharmaceutical and Environmental Chemicals. John Wiley & Sons, Hoboken, NJ, pp. 751–775.
Batley, G.E., Kirby, J.K., McLaughlin, M.J., 2012. Fate and risks of nanomaterials in aquatic and terrestrial environments. Acc. Chem. Res., http://dx.doi.org/10.1021/ar2003368.
Burden, F.R., Winkler, D.A., 1999. Robust QSAR models using Bayesian regularized neural networks. J. Med. Chem. 42, 3183–3187.
Burello, E., Worth, A.P., 2011. A theoretical framework for predicting the oxidative stress potential of oxide nanoparticles. Nanotoxicology 5, 228–235.
Cattaneo, A.G., Gornati, R., Sabbioni, E., Chiriva-Internati, M., Cobos, E., Jenkins, M.R., Bernardini, G., 2010. Nanotechnology and human health: risks and benefits. J. Appl. Toxicol. 30, 730–744.
Choi, H.S., Ashitate, Y., Lee, J.H., Kim, S.H., Matsui, A., Insin, N., Bawendi, M.G., Semmler-Behnke, M., Frangioni, J.V., Tsuda, A., 2010. Rapid translocation of nanoparticles from the lung airspaces to the body. Nat. Biotechnol. 28, 1300–1303.
Clark, K.A., White, R.H., Silbergeld, E.K., 2011. Predictive models for nanotoxicology: current challenges and future opportunities. Regul. Toxicol. Pharmacol. 59, 361–363.
Collins, F.S., Gray, G.M., Bucher, J.R., 2008. Toxicology. Transforming environmental health protection. Science 5, 906–907.
Damoiseaux, R., George, S., Li, M., Pokhrel, S., Ji, Z., France, T., Suarez, E., Rallo, R., Mädler, L., Cohen, Y., Hoek, E.M.V., Nel, A., 2011. No time to lose—high throughput screening to assess nanomaterial safety. Nanoscale 3, 1345–1360.
Dell'Orco, D., Lundqvist, M., Oslakovic, C., Cedervall, T., Linse, S., 2010. Modelling the time evolution of the nanoparticle–protein corona in a body fluid. PLoS ONE 5, e10949.
Detriche, S., Zorzini, G., Colomer, J.F., Fonseca, A., Nagy, J.B., 2008. Application of the Hansen solubility parameters theory to carbon nanotubes. J. Nanosci. Nanotechnol. 8, 6082–6092.
Diderich, R., 2010. Tools for category formation and read-across: overview of the OECD (Q)SAR Toolbox. In: *In Silico* Toxicology: Principles and Applications. The Royal Society of Chemistry, Cambridge, UK, pp. 385–407.
Epa, V.C., Winkler, D.A., Tran, L., 2012a. In: Fadeel, Pietroiusti, Shvedova (Eds.), Adverse Effects of Engineered Nanoparticles. Academic Press, London, ISBN 978-0-12-386940-1.
Epa, V.C., Burden, F.R., Tassa, C., Weissleder, R., Shaw, S., Winkler, D.A., 2012b. Modelling biological activities of nanoparticles. Nano Lett. 12, 5808–5812.
Feliu, N., Fadeel, B., 2010. Nanotoxicology: no small matter. Nanoscale 2, 2514–2520.
Feris, K., Otto, C., Tinker, J., Wingett, D., Punnoose, A., Thurber, A., Kongara, M., Sabetian, M., Quinn, B., Hanna, C., Pink, D., 2010. Electrostatic interactions affect nanoparticle-mediated toxicity to gram-negative bacterium Pseudomonas aeruginosa PAO1. Langmuir 26, 4429–4436.
Fubini, B., Ghiazza, M., Fenoglio, I., 2010. Physico-chemical features of engineered nanoparticles relevant to their toxicity. Nanotoxicology 4, 347–363.
Fourches, D., Pu, D., Tassa, C., Weissleder, R., Shaw, S.Y., Mumper, R.J., Tropsha, A., 2010. Quantitative nanostructure–activity relationship modelling. ACS Nano 4, 5703–5712.
Fourches, D., Pu, D., Tropsha, A., 2011. Exploring quantitative nanostructure–activity relationships (QNAR) modelling as a tool for predicting biological effects of manufactured nanoparticles. Comb. Chem. High Throughput Screen. 14, 217–225.
Gaster, R.S., Xu, L., Han, S., Wilson, R.J., Hall, D.A., Osterfeld, H.Y., Wang, S.X., 2011. Quantification of protein interactions and solution transport using high-density GMR sensor arrays. Nat. Nanotechnol. 6, 314–320.
George, S., Pokhrel, S., Xia, T., Gilbert, B., Ji, Z., Schowalter, M., Rosenauer, R., Damoiseaux, R., Bradley, K.A., Mädler, L., Nel, A.E., 2010. Use of a rapid cytotoxicity screening approach to engineer a safer zinc oxide nanoparticle through iron doping. ACS Nano 4, 15–29.
George, S., Xia, T., Rallo, R., Zhao, Y., Ji, Z., Lin, S., Wang, X., Zhang, H., France, B., Schoenfeld, D., Damoiseaux, R., Liu, R., Lin, S., Bradley, K.A., Cohen, Y., Nel, A.E., 2011. Use of a high-throughput screening approach coupled with in vivo zebrafish embryo screening to develop hazard ranking for engineered nanomaterials. ACS Nano 5, 1805–1817.
Handy, R.D., Owen, R., Valsami-Jones, E., 2008. The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. Ecotoxicology 17, 315–325.
Hansen, C.M., 2007. Hansen Solubility Parameters. A user's Handbook, 2nd ed. Taylor & Francis Group, Boca Raton.

Hansen, S.F., Larsen, B.H., Olsen, A.B., Baun, A., 2007. Categorization framework to aid hazard identification of nanomaterials. Nanotoxicology 1, 243–250.
Hansen, F.S., Maynard, A., Baun, A., Tickner, J.A., 2008. Late lessons from early warnings for nanotechnology. Nat. Nanotechnol. 3, 444–447.
Hu, J.X., Shipley, H.J., 2012. Evaluation of desorption of Pb (II), Cu (II) and Zn (II) from titanium dioxide nanoparticles. Sci. Total Environ. 431, 209–220.
Judson, R.S., Houck, K.A., Kavlock, R.J., Knudsen, T.B., Martin, M.T., Mortensen, H.M., Reif, D.M., Rotroff, D.M., Shah, I., Richard, A.M., Dix, D.J., 2010. In vitro screening of environmental chemicals for targeted testing prioritization: the ToxCast project. Environ. Health Perspect. 118, 485–492.
Katritzky, A.R., Kuanar, M., Slavov, S., Hall, C.D., Karelson, M., Kahn, I., Dobchev, D.A., 2010. Quantitative correlation of physical and chemical properties with chemical structure: utility for prediction. Chem. Rev. 110, 5714–5789.
Klaine, S.J., Alvarez, P.J.J., Batley, G.E., Fernandes, T.F., Handy, R.D., Lyo, D.Y., Mahendra, S., Mclaughlin, M.J., Lead, J.R., 2008. Nanomaterials in the environment: behavior, fate, bioavailability, and effects. Environ. Toxicol. Chem. 27, 1825–1851.
Knudsen, T.B., Houck, K.A., Sipes, N.S., Singh, A.V., Judson, R.S., Martin, M.T., Weissman, A., Kleinstreuer, N.C., Mortensen, H.M., Reif, D.M., Rabinowitz, J.R., Setzer, R.W., Richard, A.M., Dix, D.J., Kavlock, R.J., 2011. Activity profiles of 309 ToxCast™ chemicals evaluated across 292 biochemical targets. Toxicology 282, 1–15.
Kunzmann, A., Andersson, B., Vogt, C., Feliu, N., Ye, F., Gabrielsson, S., Toprak, M.S., Buerki-Thurnherr, T., Laurent, S., Vahter, M., Krug, H., Muhammed, M., Scheynius, A., Fadeel, B., 2011. Efficient internalization of silica-coated iron oxide nanoparticles of different sizes by primary human macrophages and dendritic cells. Toxicol. Appl. Pharmacol. 253, 81–93.
Le, T.C., Epa, V.C., Burden, F.R., Winkler, D.A., 2012. Quantitative structure–property relationship modelling of diverse materials properties. Chem. Rev. 112, 2889–2919.
Lee, S., Park, K., Ahn, H.S., Kim, D., 2010. Importance of structural information in predicting human acute toxicity from in vitro cytotoxicity data. Toxicol. Appl. Pharmacol. 246, 38–48.
Lessigiarska, I., Worth, A.P., Netzeva, T.I., Dearden, J.C., Cronin, M.T., 2006. Quantitative structure–activity–activity and quantitative structure–activity investigations of human and rodent toxicity. Chemosphere 65, 1878–1887.
Liu, J., Hopfinger, A.J., 2008. Identification of possible sources of nanotoxicity from carbon nanotubes inserted into membrane bilayers using membrane interaction quantitative structure–activity relationship analysis. Chem. Res. Toxicol. 21, 459–466.
Liu, R., Rallo, R., George, S., Ji, Z., Nair, S., Nel, A.E., Cohen, Y., 2011. Classification NanoSAR development for cytotoxicity of metal oxide nanoparticles. Small 7, 1118–1126.
Lynch, I., Dawson, K.A., 2008. Protein–nanoparticle interactions. Nano Today 3, 40–47.
Matthews, E.J., Kruhlak, N.L., Cimino, M.C., Benz, R.D., Contrera, J.F., 2006a. An analysis of genetic toxicity, reproductive and developmental toxicity, and carcinogenicity data: I. Identification of carcinogens using surrogate endpoints. Regul. Toxicol. Pharmacol. 44, 83–96.
Matthews, E.J., Kruhlak, N.L., Cimino, M.C., Benz, R.D., Contrera, J.F., 2006b. An analysis of genetic toxicity, reproductive and developmental toxicity, and carcinogenicity data: II. Identification of genotoxicants, reprotoxicants, and carcinogens using in silico methods. Regul. Toxicol. Pharmacol. 44, 97–110.
Mombelli, E., Ringeissen, S., 2009. The computational prediction of toxicological effects in regulatory contexts. Current use and future potential of (Q)SAR tools. Actual. Chim. 335, 52–59.
Monopoli, M.P., Walczyk, D., Campbell, A., Elia, G., Lynch, I., Bombelli, F.B., Dawson, K.A., 2011. Physical–chemical aspects of protein corona: relevance to in vitro and in vivo biological impacts of nanoparticles. J. Am. Chem. Soc. 133, 2525–2534.
Martin, M.T., Dix, D.J., Judson, R.S., Kavlock, R.J., Reif, D.M., Richard, A.M., Rotroff, D.M., Romanov, S., Medvedev, A., Poltoratskaya, N., Gambarian, M., Moeser, M., Makarov, S.S., Houck, K.A., 2010. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. Chem. Res. Toxicol. 23, 578–590.
Maynard, A.D., Warheit, D.B., Philbert, M.A., 2011. The new toxicology of sophisticated materials: nanotoxicology and beyond. Toxicol. Sci. 120, S109–S129.
Monteiller, C., Tran, L., MacNee, W., Faux, S., Jones, A., Miller, B., Donaldson, K., 2007. The pro-inflammatory effects of low-toxicity low-solubility particles, nanoparticles and fine particles, on epithelial cells in vitro: the role of surface area. Occup. Environ. Med. 64, 609–615.
Morris, J., Willis, J., De Martinis, D., Hansen, B., Laursen, H., Sintes, J.R., Kearns, P., Gonzalez, M., 2011. Science policy considerations for responsible nanotechnology decisions. Nat. Nanotechnol. 6, 73–77.
Mura, S., Hillaireau, H., Nicolas, J., Le Droumaguet, B., Gueutin, C., Zanna, S., Tsapis, N., Fattal, E., 2011. Influence of surface charge on the potential toxicity of PLGA nanoparticles towards Calu-3 cells. Int. J. Nanomed. 6, 2591–2605.
Nantasenamat, C., Isarankura-Na-Ayudhya, C., Prachayasittikul, V., 2010. Advances in computational methods to predict the biological activity of compounds. Expert Opin. Drug Discov. 5, 633–654.
Oberdörster, G., Maynard, A., Donaldson, K., Castranova, V., Fitzpatrick, J., Ausman, K., Carter, J., Karn, B., Kreyling, W., Lai, D., Olin, S., Monteiro-Riviere, N., Warheit, D., Yang, H., 2005. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part. Fibre Toxicol. 2, 8.
Osmond-McLeod, M.J., Poland, C.A., Murphy, F., Waddington, L., Morris, H., Hawkins, S.C., Clark, S., Aitken, R., McCall, M.J., Donaldson, K., 2011. Durability and inflammogenic impact of carbon nanotubes compared with asbestos fibres. Part. Fibre Toxicol. 13, 8–15.
Pan, Y., Neuss, S., Leifert, A., Fischler, M., Wen, F., Simon, U., Schmid, G., Brandau, W., Jahnen-Dechent, W., 2007. Size-dependent cytotoxicity of gold nanoparticles. Small 3, 1941–1949.
Park, M.V., Lankveld, D.P., van Loveren, H., de Jong, W.H., 2009. The status of in vitro toxicity studies in the risk assessment of nanomaterials. Nanomedicine 4, 669–685.
Peng, X., Palma, S., Fisher, N.S., Wong, S.S., 2011. Effect of morphology of ZnO nanostructures on their toxicity to marine algae. Aquat. Toxicol. 102, 186–196.
Péry, A.R.R., Brochot, C., Hoet, P.H.M., Nemmar, A., Bois, F.Y., 2009. Development of a physiologically based kinetic model for 99m-Technetium-labelled carbon nanoparticles inhaled by humans. Inhal. Toxicol. 21, 1099–1107.
Pietroiusti, A., Massimiani, M., Fenoglio, I., Colonna, M., Valentini, F., Palleschi, G., Camaioni, A., Magrini, A., Siracusa, G., Bergamaschi, A., Sgambato, A., Campagnolo, L., 2011. Low doses of pristine and oxidized single-wall carbon nanotubes affect mammalian embryonic development. ACS Nano 5, 4624–4633.
Poater, A., Gallegos Saliner, A., Solà, M., Cavallo, L., Worth, A.P., 2010. Computational methods to predict the reactivity of nanoparticles through structure–property relationships. Expert Opin. Drug Deliv. 7, 295–305.
Pumera, M., 2011. Nanotoxicology: the molecular science point of view. Chem. Asian J. 6, 340–348.
Puzyn, T., Leszczynska, D., Leszczynski, J., 2009. Toward the development of "nano-QSARs": advances and challenges. Small 5, 2494–2509.
Puzyn, T., Rasulev, B., Gajewicz, A., Hu, X., Dasari, T.P., Michalkova, A., Hwang, H.M., Toropov, A., Leszczynska, D., Leszczynski, J., 2011. Using nano-QSAR to predict the cytotoxicity of metal oxide nanoparticles. Nat. Nanotechnol. 6, 175–178.
Rai, M., Yadav, A., Gade, A., 2009. Silver nanoparticles as a new generation of antimicrobials. Biotechnol. Adv. 27, 76–83.
Richard, A.M., Yang, C., Judson, R.S., 2008. Toxicity data informatics: supporting a new paradigm for toxicity prediction. Toxicol. Mech. Methods 18, 103–118.
Rivera Gil, P., Oberdörster, G., Elder, A., Puntes, V., Parak, W.J., 2010. Correlating physico-chemical with toxicological properties of nanoparticles: the present and the future. ACS Nano 26, 5527–5531.
Röcker, R., Pötzl, M., Zhang, F., Parak, W.J., Nienhaus, G.U., 2009. A quantitative fluorescence study of protein monolayer formation on colloidal nanoparticles. Nat. Nanotechnol. 4, 577–580.
Roh, J.Y., Sim, S.J., Yi, J., Park, K., Chung, K.H., Ryu, D.Y., Choi, J., 2009. Ecotoxicity of silver nanoparticles on the soil nematode *Caenorhabditis elegans* using functional ecotoxicogenomics. Environ. Sci. Technol. 43, 3933–3940.
Sayes, C., Ivanov, I., 2010. Comparative study of predictive computational models for nanoparticle-induced cytotoxicity. Risk Anal. 30, 1723–1734.
Service, R.F., 2008. Can high-speed tests sort out which nanomaterials are safe? Science 321, 1036–1037.
Shaw, S.Y., Westly, E.C., Pittet, M.J., Subramanian, A., Schreiber, S.L., Weissleder, R., 2008. Perturbational profiling of nanomaterial biologic activity. Proc. Natl. Acad. Sci. U.S.A. 105, 7387–7392.
Shvedova, A.A., Kagan, V.E., Fadeel, B., 2010. Close encounters of the small kind: adverse effects of man-made materials interfacing with the nano-cosmos of biological systems. Annu. Rev. Pharmacol. Toxicol. 50, 63–88.
Tassa, C., Duffner, J.L., Lewis, T.A., Weissleder, R., Schreiber, S.L., Koehler, A.N., Shaw, S.Y., 2010. Binding affinity and kinetic analysis of targeted small molecule-modified nanoparticles. Bioconjugate Chem. 21, 14–19.
Tetard, L., Passian, A., Venmar, K.T., Lynch, R.M., Voy, B.H., Shekhawat, G., Dravid, V.P., Thundat, T., 2008. Imaging nanoparticles in cells by nanomechanical holography. Nat. Nanotechnol. 3, 501–505.
Toropov, A.A., Toropova, A.P., Benfenati, E., Leszczynska, D., Leszczynski, J., 2010. SMILES-based optimal descriptors: QSAR analysis of fullerene-based HIV-1 PR inhibitors by means of balance of correlations. J. Comput. Chem. 31, 381–392.
Wang, S., Dormidontova, E.E., 2010. Nanoparticle design optimization for enhanced targeting: Monte Carlo simulations. Biomacromolecules 11, 1785–1795.
Xia, X.R., Monteiro-Riviere, N.A., Mathur, S., Song, X., Xiao, L., Oldenberg, S.J., Fadeel, B., Riviere, J.E., 2011. Mapping the surface adsorption forces of nanomaterials in biological systems. ACS Nano 5, 9074–9081.
Xia, X.R., Monteiro-Riviere, N.A., Riviere, J.E., 2010. An index for characterization of nanomaterials in biological systems. Nat. Nanotechnol. 5, 671–675.
Yang, Y., Qu, Y., Lü, X., 2010. Global gene expression analysis of the effects of gold nanoparticles on human dermal fibroblasts. J. Biomed. Nanotechnol. 6, 234–246.
Yang, Y., Lin, T., Weng, X.L., Darr, J.A., Wang, X.Z., 2011. Data flow modeling, data mining and QSAR in high throughput discovery of functional nanomaterials. Comput. Chem. Eng. 35, 671–678.
Zhang, T., Stilwell, J.L., Gerion, D., Ding, L., Elboudwarej, O., Cooke, P.A., Gray, J.W., Alivisatos, A.P., Chen, F.F., 2006. Cellular effect of high doses of silica-coated quantum dot profiled with high throughput gene expression analysis and high content cellomics measurements. Nano Lett. 6, 800–808.
Zhou, H., Mu, Q., Gao, N., Liu, A., Xing, Y., Gao, S., Zhang, Q., Qu, G., Chen, Y., Liu, G., Zhang, B., Yan, B., 2008. A nanocombinatorial library strategy for the discovery of nanotubes with reduced protein binding, cytotoxicity, and immune response. Nano Lett. 8, 859–865.