



## **Review of computational approaches for predicting the physicochemical and biological properties of nanoparticles**

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## **ABSTRACT**

In the growing field of nanotechnology there is a need to determine the physicochemical and potential toxicological properties of nanomaterials since many industrial, medical and consumer applications are based on an understanding of these properties and on a controlled exposure to the materials. This document provides a literature review on the current status of computational studies aimed at predicting the physicochemical properties and biological effects (including toxicity) of nanomaterials, with an emphasis on medical applications. Although a number of models have been published for physicochemical property prediction, very few models have been published for predicting biological effects, toxicity or the underlying mechanisms of action. This is due to two main reasons: a) nanomaterials form a colloidal phase when in contact with biological systems making the definition and calculation of properties (descriptors) suitable for the prediction of toxicity a new and challenging task, and b) nanomaterials form a very heterogeneous class of materials, not only in terms of their chemical composition, but also in terms of size, shape, agglomeration state, and surface reactivity. There is thus an urgent need to extend the traditional structure-activity paradigm to develop methods for predicting the toxicity of nanomaterials, and to make the resulting models readily available. This document concludes by proposing some lines of research to fill the gap in knowledge and predictive methodology.

## LIST OF ABBREVIATIONS

CAS	Chemical Abstract Service
CDNP	Combustion-Derived Nanoparticle
CNN	Computational Neural Networks
CNT	Carbon Nanotube
CPMD	Car-Parrinello Molecular Dynamics
DFT	Density Functional Theory
EINECS	European INventory of Existing commercial Chemical Substances
EU	European Union
JRC	Joint Research Centre
LSER	Linear Solvation Energy Relationship
LSSVM	Least-Squares Support Vector Machine
MC	Monte Carlo
MCDM	Multi-Criteria Decision Making
MD	Molecular Dynamics
MI-QSAR	Membrane-Interaction Quantitative Structure-Activity Relationship
MOT	Mobile Order Theory
MLR	Multilinear Regression
MNP	Manufactured Nanoparticle
NM	Nanomaterial
NP	Nanoparticle
OECD	Organisation for Economic Co-operation and Development
PAH	Polycyclic Aromatic Hydrocarbon
PE	Polyethylene
PM	Particulate Matter
QD	Quantum Dot
QSAR	Quantitative Structure-Activity Relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
ROS	Reactive Oxygen Species
SAR	Structure-Activity Relationship
SMILES	Simplified Molecular Input Line Entry System
SVF	Synthetic Vitreous Fibres
SWNT	Single Wall Nanotube

## CONTENTS

1. INTRODUCTION.....	1
1.1 Definitions .....	1
1.2 Classification of nanoparticles .....	2
1.3 Harmful and beneficial properties of nanoparticles .....	2
1.4 Outline of this review .....	3
2. BACKGROUD ON COMPUTATIONAL METHODS .....	4
2.1 Quantitative Structure Activity Relationship analysis .....	4
2.2 Read-across and grouping .....	4
2.3 Multicriteria decision analysis.....	6
3. REGULATORY CONTEXT.....	8
3.1 General.....	8
3.2 REACH.....	9
4. DESCRIPTORS OF NANOPARTICLE PROPERTIES AND ACTIVITY .....	11
4.1 Shape .....	11
4.2 Surface area and surface reactivity .....	12
4.3 Particle size .....	12
5. COMPUTATIONAL STUDIES OF NANOPARTICLES.....	13
5.1 Molecular modelling studies .....	13
5.2 Modelling of solubility .....	13
5.3 Modelling of Young modulus .....	17
5.4 Modelling of thermal conductivity .....	18
5.5 Modelling of spreading properties.....	18
5.6 Modelling of melting point .....	18
5.7 Modelling of permeability.....	19
5.8 Modelling of cellular toxicity.....	19
5.9 Modelling of enzyme inhibition .....	20
5.10 Modelling of surface reactivity.....	20
6. SOME APPLICATIONS OF CARBON-BASED NANOPARTICLES.....	22
6.1 Antioxidant and neuroprotective properties .....	23
6.2 Antiapoptotic activity .....	23
6.3 DNA photocleavage .....	23
6.4 Anti-HIV activity .....	23
6.5 Antimicrobial activity.....	24
6.6 Enzyme inhibition .....	24
6.7 Drug transport and delivery.....	25
6.8 Electron transfer .....	25
7. SOFTWARE TOOLS FOR NANOPARTICLE ASSESSMENT.....	26
7.1 Development of software tools.....	26
7.2 Grain-Cutter .....	26
8. CONCLUSIONS AND RECOMMENDATIONS.....	27
9. REFERENCES .....	28

# 1. INTRODUCTION

## 1.1 Definitions

Nanomaterials (NMs) are materials with one or more dimensions at the nanoscale, which can exhibit novel characteristics compared to the same material without nanoscale features. Nanoparticles (NPs) can be considered as a subset of nanomaterials, being particles with one or more dimensions at the nanoscale level, with at least one dimension below 100 nm.

Manufactured NPs usually have unique magnetic, electrical, optical, thermal and chemical properties which make them very useful for commercial, technological and therapeutic applications. NMs are also increasingly being used for commercial purposes such as fillers, opacifiers, catalysts, semiconductors, cosmetics, microelectronics, and drug carriers. Engineered NMs are also being used in consumer products such as sporting goods, tires, stain-resistant clothing, self-cleaning surfaces, sunscreens, cosmetics, and electronics and will also be increasingly utilised in medicine for purposes of diagnosis, imaging, and drug delivery (1).

There are two main types of nanostructures which exhibit differentiated intrinsic properties and health risks:

- a) Nanostructures in the form of free particles. The individual particles may be incorporated into another substance, which could be a gas, a liquid or a solid, typically to produce a paste, a gel or a coating.
- b) Nanostructures as integral features of a larger object such as nanocrystalline solids, objects with nanoscale-type surface topography, agglomerates or aggregates.

Agglomerates and aggregates of nanoparticles can have a diameter larger than 100 nm, but they can still pose some specific health and environmental risks since they may break down and release nanoparticles. More specifically, an agglomerate is a group of particles held together by Van der Waals like weak forces, requiring only a physical means for separation; it can also be a group of strongly associated particles that cannot easily be redispersed by mechanical means and may require some chemical means as well. An aggregate is an assemblage of primary particles exhibiting an identifiable collective behaviour.

Nanotoxicology aims to establish relationships between the structural and physicochemical properties of nanoparticles and their fate and toxicity. However, nanoparticles have unique physicochemical properties and functionalities that are different from their bulk counterparts. It is thus crucial to determine the appropriate way to compare physicochemical properties with fate and toxicity. For example, when comparing effects of different sized particles, the effect of size of the nanoparticle on biological activity should be based on the total surface area, rather than on the mass of the sample, as is done in conventional toxicological studies.

## 1.2 Classification of nanoparticles

The structural diversity of nanomaterials has also been classified according to the nanoparticle geometry (2) in zero-dimensional (points), one dimensional (linear), fractal, two-dimensional, and three dimensional structures. Nanowires, nanotubes, very thin fibres, capillaries and pores can be treated as one-dimensional nanoparticles. Carbon nanotubes formed by rolled graphene layers, cylinders and fullerenes can be qualified as two-dimensional structures, while larger crystals form three-dimensional nanostructures.

Nanoparticles have also been classified according to their origin of formation (3). Ultrafine particulate matter (PM<sub>10</sub>), which defines particles less than about 10µm, can be composed by combustion-derived nanoparticles (CDNP) that are particles containing metals and organic volatiles derived from combustion (e.g. vehicle exhaust particles); sodium magnesium compounds derived from sea spray; sulphates and nitrates; calcium and potassium compounds and insoluble materials derived from the earth's crust (e.g. clay); and biologically-derived materials (e.g. endotoxin), among others.

## 1.3 Harmful and beneficial properties of nanoparticles

The increasing use of nanoparticles in biomedicine for probing processes and in drug delivery has opened many possibilities for rational drug design and therapeutics (2-5). However, the potential toxicity and environmental impacts of nanoparticles, which may be related to particle size and biodegradability (2) also have to be considered in environmental risk management. *In silico* modelling of nanoparticle behaviour and interactions in biological systems can be of great value in predicting drug interactions and harmful side-effects (5,6,10).

Whereas some NPs can be used for therapeutic purposes, NPs inhaled from environmental air imply adverse health effects (3,10). The so-called 'nanoparticles paradox' (11) makes reference to the potential harmful effects of NPs, intensified in the last decades due to large scale human and environmental exposure and to increased manufacture for industrial use. There are considerable differences in physical and chemical properties between medicinal nanoparticles and the industrial and combustion-derived nanoparticles studied by particle toxicologists. Medical nanoparticles tend to be composed of materials that are similar to biological molecules and they are generally biodegradable. By contrast, combustion-derived nanoparticles are carbon-centred and contaminated with metals and organics, with a biopersistent core.

To fully address the paradox that nanoparticles can be both beneficial and harmful, there is a need to advance our understanding of the characteristics that determine acute and chronic toxicity, translocation, biodegradation and elimination of all types of nanoparticles likely to gain access to the human body. For instance, occupational lung disease at workplace and in the environment reveal that some particles like asbestos, quartz (crystalline silica), and coalmine dust are associated with lung disease (12). On the other hand, combustion-derived nanoparticles (CDNP) and in special ultrafine particulate matter (PM<sub>10</sub>) also cause adverse health effects, such as hospitalisation or mortality from cardiovascular and respiratory causes, exacerbations of asthma and chronic obstructive pulmonary diseases, lung function decrease and lung cancer, among others (13).

A review on the toxicity of quantum dots (QDs) that relates the physicochemical characteristics and environmental factors to the toxic potency of these nanomaterials has been recently published (14). As a conclusion, not all QDs are alike; engineered QDs cannot be considered a uniform group of substances. QD absorption, distribution, metabolism, excretion, and toxicity depend on multiple factors derived from both inherent physicochemical properties and environmental conditions; QD size, charge, concentration, outer coating bioactivity (capping material and functional groups), and oxidative, photolytic, and mechanical stability have each been implicated as determining factors in QD toxicity. Although they offer potentially invaluable societal benefits such as drug targeting and in vivo biomedical imaging, QDs may also pose risks to human health and the environment under certain conditions.

On the other hand, carbon nanotubes (CNTs) have been investigated as multipurpose innovative carriers for drug delivery and diagnostic applications (15-16). Their versatile physicochemical features enable the covalent and noncovalent introduction of several pharmaceutically relevant entities and allow for rational design of novel candidate nanoscale constructs for drug development. CNTs can be functionalized with different functional groups to carry simultaneously several moieties for targeting, imaging, and therapy (17). What makes CNTs quite unique is their ability, to passively cross membranes of many different types of cells following a translocation mechanism that has been termed the nanoneedle mechanism (18). In this way, CNTs open possibilities for future drug discovery based on intracellular targets that have been hard to reach until today. Moreover, adequately functionalized CNTs can be rapidly eliminated from the body following systemic administration offering further encouragement for their development. CNT excretion rates and accumulation in organs and any reactivity will determine the CNT safety profile and, consequently, any further pharmaceutical development. CNTs are playing an increasingly important role in the emerging field of nanomedicine; however, there is a need to ensure that the opportunities they offer will be translated into feasible and safe constructs to be included in drug discovery and development pipelines.

#### **1.4 Outline of this review**

This manuscript reviews and identifies in the current literature available modelling and material characterization techniques which could be useful for the understanding and the prediction of nanoparticle's toxicity. First QSAR, read-across and grouping methods will be introduced in the context of REACH requirements. Then a survey on nanomaterial properties/descriptors and modelling studies will be outlined. Finally we will describe some of the most promising nanomaterial applications and software tools for nanomaterial safety and toxicity assessment.

## 2. BACKGROUD ON COMPUTATIONAL METHODS

Computational studies aimed at predicting the toxicity of nanoparticles and materials are almost non-existent (19). Theoretical studies of nanostructured materials have been based on different techniques such as quantum mechanical calculations, force field based methods, and classical and *ab initio* molecular dynamics simulations (20, 21). Theoretical modelling of these systems can help to obtain structural information as well as to predict optical, mechanical, chemical, and electronic properties. By contrast, there have been relatively few studies on the modelling of NP fate and toxicity.

### 2.1 Quantitative Structure Activity Relationship analysis

Structure-Activity Relationships (SARs) and Quantitative Structure Activity Relationships (QSARs), collectively referred to as (Q)SARs, are theoretical models that relate the structure of chemicals to their biologic activities. (Q)SARs are used to predict the physicochemical, biological (e.g., toxicological) and fate properties of molecules from knowledge of chemical structure.

More specifically, a SAR is a qualitative relationship between a molecular (sub)structure and the presence or absence of a given biological activity, or the capacity to modulate a biological activity imparted by another substructure. The term substructure refers to an atom, or group of adjacently connected atoms, in a molecule. A substructure associated with the presence of a biological activity is also called a structural alert. A SAR can also be based on the ensemble of steric and electronic features considered necessary to ensure the intermolecular interaction with a specific biological target molecule, which results in the manifestation of a specific biological effect. In this case, the SAR is sometimes called a 3D SAR or pharmacophore.

A QSAR is a quantitative relationship between a biological activity (e.g., toxicity) and one or more molecular descriptors that are used to predict the activity. A molecular descriptor is a structural or physicochemical property of a molecule, or part of a molecule, which specifies a particular characteristic of the molecule and is used as an independent variable in a QSAR (23).

### 2.2 Read-across and grouping

Read-across is a non-formalised approach in which endpoint information for one chemical (called a “source chemical”) is used to make a prediction of the endpoint for another chemical (called a “target chemical”), which is considered to be similar in some way (usually on the basis of structural similarity). In this way it is assumed that similar compounds will exhibit similar biological activity. In principle, read-across can be applied to characterise physicochemical properties, fate, human health effects and ecotoxicity, and it may be performed in a qualitative or quantitative manner. Read-across can either be qualitative or quantitative, depending on the whether the data being used is categorical or numerical in nature. To estimate the properties of a given substance, read-across can be performed in a one-to-one manner (one analogue used to make an estimation) or in a many-to-one manner (two or more analogues used).

A chemical category is a group of chemicals whose physicochemical and human health and/or environmental toxicological and/or environmental fate properties are likely to be similar or follow a regular pattern as a result of structural similarity (or other similarity characteristic). Accordingly, a chemical category is selected based on the hypothesis that the properties of a series of chemicals with common (structural) features will show coherent trends in their properties. The presence of common behaviour or coherent trends is generally associated with a common underlying mechanism of action.

The use of the category approach means that it is possible to identify properties which are common to at least some members of the category. The approach also provides a basis on which to identify possible trends in properties across the category. As a result, it is possible to extend the use of measured data to similar untested chemicals, and generate estimates that may be adequate for regulatory purposes (e.g. classification and labelling and/or risk assessment) without further testing. In addition, knowledge of the expected effects of the category together with information on use and exposure helps to decide not only whether additional testing is needed, but also the nature and scope of any testing that needs to be carried out.

The trend analysis may involve the development of an “internal” model, i.e. a computational model such as a QSAR that is based entirely on the data in the category. This term is used in distinction to “external” model, which refers to a computational model developed using a different or more extensive dataset.

Within a chemical category, data gaps can therefore be filled by using several approaches, namely: a) read-across; b) trend analysis and use of computational methods based on internal models; and c) use of computational methods based on external models. In this context, the term “model” refers to any formalised method for estimating the properties of chemicals, such as a (Q)SAR.

As part of the OECD activities to increase the regulatory acceptance of (Q)SAR methods when data are lacking, the OECD has started the development of a (Q)SAR Application Toolbox (24) as a mean of making QSAR technology readily accessible, transparent, and less demanding in terms of infrastructure costs. The Toolbox is a software application intended to be used by governments, chemical industry and other stakeholders in filling gaps in (eco)toxicity data needed for assessing the hazards of chemicals. The Toolbox incorporates information and tools from various sources into a logical workflow and allows to:

- identify analogues for a chemical, retrieve experimental results available for those analogues and fill data gaps by read-across or trend analysis.
- categorise large inventories of chemical according to mechanism or modes of action.
- fill data gaps for any chemical by using the library of QSAR models.
- evaluate the robustness of a potential analogue for read-across.
- evaluate the appropriateness of a (Q)SAR model for filling a data gap for a particular target chemical.
- build QSAR models.

The JRC commissioned to Ideacon Ltd the development of an open source software, Toxmatch,(25,26,27,28) that encodes several chemical similarity indices, including a variety of structural and descriptor based indices, to facilitate the grouping of chemicals, thereby supporting the development of chemical categories and the application of read-across between

analogues for chemical toxicity assessment. The core functionalities include the ability to compare datasets based on various similarity indices as well as the means to calculate pair wise similarity between compounds or aggregated similarity of a compound to a set.

### 2.3 Multicriteria decision analysis

Multicriteria decision making (MCDM) techniques are used to help people make decisions according to their preferences, in cases where there is more than one conflicting criterion, finding the optimal choice among the alternatives. This group of techniques provides a means of quantifying or prioritising personal or group judgments that are typically intangible and subjective. Decision making analysis compares different kinds of alternatives by decomposing the preferences into the many properties that the alternatives have, determining their importance, comparing and obtaining the relative preference of alternatives with respect to each property, and synthesising the results to obtain the overall preference. Therefore, the strategy consists in breaking down a complex problem down into its smaller components, and establishing importance or priority to rank the alternatives in a comprehensive and general way to look at the problem mathematically.

The starting point of MCDM lies in the attempt to represent often intangible goals in terms of number of individual criteria, i.e. a set of tools that allow comparison of alternatives according to a particular axis or point of view. Each criterion can be represented by a surrogate measure of performance, represented by some measurable attribute of the consequences arising from the achievement of any particular decision alternative.

Over the years, several MCDM methods, also named ranking methods, have been proposed (29) in different areas, with different theoretical backgrounds and facing different kinds of questions and providing different kinds of results. Ranking methods can be roughly divided into total and partial ranking methods: both are based on elementary methods of discrete mathematics. The main difference is the introduction of the 'not comparable' relationship between objects in the partial order techniques, while in the total order techniques the result is always a ranking value for each object.

To provide a research tool for investigating the application of such methods, the JRC commissioned the development of DART (Decision Analysis by Ranking Techniques; 30) This software tool is designed to support the ranking of chemicals according to their environmental and toxicological concern and is based on the most recent ranking theories. Different kinds of order ranking methods, roughly classified as total and partial-order ranking methods are implemented. DART encodes several techniques for MCDM analysis, which can be used to facilitate and make more transparent the cost-benefit analyses that underlie decision-making (such as the decision not to test but to rely on non-animal data, such as QSARs or *in vitro* tests).

DART was developed by Talete srl (Milan, Italy) under the terms of a JRC contract. It is made available as a free download. It implements several total ranking methods and a partial ranking method (the Hasse Diagram Technique). Besides applying ranking methods, DART also allows performing several pre-processing analysis, which can be fundamental to allow the processing of big datasets, characterised by huge numbers of substances and described by several criteria. Cluster analysis by k-Means, and Principal Component Analysis are the best

known pre-processing methods implemented in DART, together with less known methods, like the bins partition and the reduction of significant digit.

Seven total ranking techniques, named Desirability, Utility, Dominance, Concordance, SAR (Simple Additive Ranking), HAR (Hasse Average Ranking) and Absolute Reference Ranking, are implemented in DART, together with several charts and statistics that help the user to better understand the results obtained.

The Hasse diagram partial ranking technique is also implemented in DART. Several indices are provided to evaluate the analysis performed. The theory of these indices is described by Pavan and Todeschini.(31)

## 3. REGULATORY CONTEXT

### 3.1 General

In the regulatory context, there are several regulations that can be applied to nanoparticles according to their uses (22): Safety at Workplace Directives (32), Directive on the Integrated Pollution Prevention and Control (33), Waste Management Directives (34), and the new European Regulation on the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) (35).

However, the application of the regulations to NPs encompasses several issues. On one hand, there are many uncertainties associated to NP behaviour and a general lack of scientific knowledge; on the other hand, NP characterisation and detection instruments are not yet harmonised; finally, although specific surface area is considered a relevant parameter, the optimal parameter to determine the toxicity of nanoparticles is still undefined.

In the regulatory arena, the identification of new risks demands the application of the precautionary principle. Currently, the possibility of proposing a European specific regulation on nanomaterials is considered unfeasible, due to the difficulty of establishing links between strikingly different pieces of legislation and the need to negotiate a sensible specific, in-depth regulatory process (European Commission, Health and Consumer Protection Directorate General, 2004). Instead, the European Commission has adopted the so-called “incremental approach” (22), to adapt existing environmental laws to the regulation of nanotechnologies.

Three commercially available nanoproducts were examined to investigate the effectiveness of this approach. The current regulations were mapped along the life cycle of each product in order to analyse their applicability to nanomaterials, identify their gaps, and suggest solutions.

It was concluded that the applicability of environmental laws is limited due to difficulties in generating sufficient data on the nanomaterials residing in the products according to their life cycles. Further, metrology tools are unavailable; thresholds are not tailored to the nanoscale; and toxicological data and occupational exposure limits cannot be established with existing methodologies.

Initiatives at the international level are being taken to review existing pieces of legislation and to assess the need for their adaptation. In the EU, the “Nanosciences and nanotechnologies: an action plan for Europe 2005 – 2009” specifies that all applications and use of nanosciences and nanotechnologies must comply with the high level of public health, safety, consumer and worker protection, and environmental protection. The Commission has undertaken a regulatory review of EU legislation in relevant manufactured (or engineered) nano-sized and nanostructured nanomaterials (36), which do not occur naturally or are unintentionally produced, e.g. in combustion.

The current legislation covers to a large extent risks in relation to nanomaterials; however, it may have to be modified in the light of new information becoming available, for example as regards the thresholds used for information requirements and hazard classification.

Recently, toxicity and exposure data, combined with therapeutic and other related literature, have been studied to shape human health risk assessments that should be used to regulate the use of nanomaterials in consumer products. In a symposium at the 2005 annual meeting of the Society of Toxicology (38) it was found that characterization of airborne particles indicates that exposures will depend on particle behaviour (e.g., disperse or aggregate) and that accurate, portable, and cost effective measurement techniques are essential for understanding exposure. Under many conditions, dermal penetration of nanoparticles may be limited for consumer products such as sunscreens, although additional studies are needed on potential photo oxidation products, experimental methods, and the effect of skin condition on penetration. Carbon nanotubes apparently have greater pulmonary toxicity (inflammation, granuloma) in mice than fine-scale carbon graphite, and their metal content may affect toxicity. Studies on TiO<sub>2</sub> and quartz illustrate the complex relationship between toxicity and particle characteristics, including surface coatings, which make generalisations (e.g., smaller particles are always more toxic) incorrect for some substances.

### 3.2 REACH

The entry into force of REACH reinforces the use of computational predictive approaches for the reduction, refinement, and replacement of animal testing by non-testing approaches in the interest of cost saving and animal welfare.

REACH considers a substance a “chemical element obtained by any manufacturing process, including any impurity deriving from the process used”. A substance with a different degree of purity and composition can be classified as the same substance provided hazardous properties do not differ significantly. Although REACH obliges producers or importers to provide toxicological data and assess environmental exposure, information requirements are based on mass thresholds. Furthermore, the usually low concentration of nanoparticles in the final article is likely to exclude many nanoengineered articles from the REACH legislation, since no registration is required when the concentrations of a substance is lower than 0.1% w/w.

Therefore, in REACH, there are no provisions referring explicitly to nanomaterials (36, 37). However, nanomaterials are covered by the “substance” definition. When an existing chemical substance, already placed on the market as bulk substance, is introduced on the market in a nanomaterial form (nanoform), the registration dossier will have to be updated to include specific properties of the nanoform of that substance. In order to address the specific properties, hazards and risks associated with nanomaterials, additional testing or information may be required and as a result current test guidelines may need to be modified.

The European Commission is funding research projects to assess the health and environment impacts of nanoparticles under the 7<sup>th</sup> Research Framework Programme. It will also be necessary to carefully monitor over the next few years whether the 1 tonne per year threshold for the registration and the information requirements under REACH are adequate to address potential risks from particles on a nano-scale.

On October the 8th 2008, the EC published a regulation (39) amending the REACH Annex IV to remove carbon and graphite. Although the substances were originally listed in Annex IV, meaning they were exempt from REACH requirements because they were considered to be of minimum risk because of their intrinsic properties, a EU expert committee delisted the substances in June 2008. According to the regulation, there is insufficient information for

carbon and graphite to be listed in Annex IV, “in particular due to the fact that the concerned EINECS and/or CAS numbers are used to identify forms of carbon or graphite at the nano-scale, which do not meet the criteria for inclusion in this Annex”.

Annex XI of REACH states that “Results obtained from valid qualitative or quantitative structure-activity relationship models ((Q)SARs) may indicate the presence or absence of a certain dangerous property.” Results of (Q)SARs may be used instead of testing when the results are derived from scientifically valid (Q)SAR models and they are adequate for the purpose of classification and labelling and/or risk assessment, when the substance falls within the applicability domain of the (Q)SAR model, and when the method is adequately and reliably documented.

The development of QSAR models for the prediction of nanoparticle properties and toxicity is highly desirable, but it requires the availability of quantitative toxicity studies which are still sparse.

## 4. DESCRIPTORS OF NANOPARTICLE PROPERTIES AND ACTIVITY

To identify the physicochemical and structural descriptors suitable for modelling nanoparticles, the relevant properties for the toxicity/activity of NPs should be identified first. At the nanoscale, the features considered to be responsible for the toxicity of nanoparticles differ to the traditional ones. Some of the properties relevant to the activity are solubility; shape; nanoparticle size, measured as surface area and size distribution; chemical composition (purity, crystal structure and porosity); surface structure and properties, i.e. surface chemistry, charge, inorganic or organic coatings, roughness and surface reactivity; electronic properties; and aggregation and agglomeration state.

In particular, there are some studies reporting the relationship between properties such as shape, surface area and surface reactivity, particle size, solubility and other features (such as spreading properties) with toxicity / activity of NPs.

A preliminary ranking of important physicochemical factors that affect nanotoxicity would be: nanoparticles size and size distribution, surface area, shape dimension, surface chemistry and surface charge, redox potential, zeta potential, oxidative stress potential, solubility, agglomeration state, porosity, functionalisation or chemical composition, crystal structure and structure-dependent electronic configuration, method of synthesis and/or preparation and purity of the sample.

Material characterization for toxicity screening studies is most appropriately considered in the context of the studies being undertaken.(40) Characterizing delivered nanomaterial in a test system or a model (e.g. in a wet phase) provides the highest quality of data on dose and material properties that are related to observed responses., but this is limited by current methodological capabilities. For example, the characterization of nanomaterials after administration or injection in the cell culture media is particularly advantageous where a change in the physicochemical characteristics of the material is likely to occur (e.g. when changing from dry to wet phase). Examples of potential changes include agglomeration state, physisorption, or chemisorption of biomolecules and biochemically-induced changes in surface chemistry. In all these cases, the dry-state characterization of materials has limited relevance for understanding the nanoparticle-cell interactions under cell culture conditions.

### 4.1 Shape

Studies on asbestos and synthetic vitreous fibres (SVF) have demonstrated that shape, in the form of fibre length, and biopersistence control pathogenicity (41). Fibres that cannot be internalised by alveolar macrophages ( $> \sim 20 \mu\text{m}$ ) stimulate macrophages to release inflammatory mediators and are slowly cleared. In addition, chemical structure controls biopersistence by controlling whether the fibres dissolve and break; long fibres that break can be readily cleared as short fibres. Nanoparticles in various media form aggregates, chains and dendritic structures, all of which may influence activity.

## 4.2 Surface area and surface reactivity

In the case of insoluble nanoparticles, which have a much larger surface area per unit mass compared with larger particles, the only contact with the biological system is through the surface. Therefore, the surface area and its reactivity are related to the inflammatory potential (42, 43), which is much higher than in larger particles. Even low-reactive surfaces may have a surface area large enough to elicit effects. Particle surface characteristics and chemical composition are considered to be key factors in the generation of free radicals and reactive oxygen species (ROS) formation.

Nanoparticles can be also made of mixtures of compounds and can form core-shell or other complex structures. The reactivity of manufactured nanoparticles (MNP) can depend on the particle surface chemistry and reactive groups bounded to particles surface. MNP are often stabilized with surfactants to maintain the desired properties with coatings or have their surfaces derivatised to prevent agglomeration. These treatments affect the reactivity and can potentially vary the pathogenicity.

Pulmonary bioassay studies with nanoscale and fine-quartz particles in rats demonstrate that toxicity is not dependent upon particle size or surface area but on surface characteristics (44).

## 4.3 Particle size

Due to their small size, nanoparticles may penetrate cells by passing directly through the cell membrane or infiltrate between cells and translocate to new sites and to the blood/lymph and thereby to target organs away from their portal of entry (45). The size of nanoparticles alone may be the critical factor determining their translocation potential, whereas the total surface area is likely to be the predominant metric driving events at the cellular level.

The relationship between the physicochemical properties of nanoparticles (e.g., size, surface area and crystal phase) and their capacity to generate reactive oxygen species (ROS) has been studied for TiO<sub>2</sub> nanoparticles (46), by using a well-defined dose metrics. TiO<sub>2</sub> nanoparticles were prepared by using gas phase synthesis methods that allow for strict control of size and crystal phase. While toxicological effects are usually evaluated by *in vitro* and *in vivo* studies, a rapid cell-free prescreening assay was used to determine the intrinsic potential of particles to generate reactive oxygen species (ROS). Both *in vitro* and *in vivo* tests of engineered nanoparticles (e.g., carbon nanotubes, TiO<sub>2</sub> and quantum dots) indicate that ROS production is related to their toxicity. (47,48,49)

The dependence of ROS activity on size was established using TiO<sub>2</sub> nanoparticles of nine different sizes but the same crystal phase. For a fixed total surface area, an S-shaped curve for ROS generation per unit surface area was observed as a function of particle size. The highest ROS activity per unit area was observed for 30 nm particles, and observed to be constant above 30 nm. There was a decrease in activity per unit area as size decreased from 30-10 nm; and again constant for particles smaller than 10 nm.

The correlation between crystal phase and oxidant capacity was established using TiO<sub>2</sub> nanoparticles of 11 different crystal phase combinations but similar size. The ability of different crystal phases of TiO<sub>2</sub> nanoparticles to generate ROS was highest for amorphous, followed by anatase, and then anatase/rutile mixtures, and lowest for rutile samples. Based on evaluation of the entire dataset, important dose metrics for ROS generation were established.

## 5. COMPUTATIONAL STUDIES OF NANOPARTICLES

### 5.1 Molecular modelling studies

There have been few studies on the modelling of nanoparticles.(50) Computational studies of nanostructured materials are based on different techniques such as quantum mechanical calculations, force field based methods, and classical and *ab initio* molecular dynamics simulations.

An extensive computational modelling review of thermo-mechanical and transport properties of carbon nanotubes has been reported (51). Some of the computational simulation tools that are appropriate for modelling nanomaterials include the molecular dynamics (MD), the Monte Carlo (MC), and the *ab initio* MD simulation methods.

The computational methods used to model nanomaterials are different depending on the number of atoms to be modelled and thus the computational cost. For nanosystems composed from ten to several hundred atoms, *ab initio* simplified quantum mechanical-based methods, such as the density functional theory (DFT), are used. For nano-scale structures composed of several thousand to several million atoms, classical statistical mechanics-based methods, such as the molecular dynamics (MD) simulation method, are applied.

1) Molecular dynamics (MD) simulation method. In an MD simulation, the motion of individual atoms within an assembly of N atoms or molecules is modelled on the basis of either a Newtonian deterministic dynamics or a Langevin-type stochastic dynamics.

2) Monte Carlo (MC) simulation methods. The MC simulation method is based on the use of probabilistic concepts. In this method, the evolution of the initial configuration of the system is generated by successive random displacements of the atoms.

3) *Ab initio* molecular dynamics simulation methods. *Ab initio* molecular dynamics simulation methods are quantum-mechanical based, potential-free, methods in which the forces experienced by the atoms are computed, not from inter-atomic potentials fixed in advance, but from electronic-structure calculations. However, generally it is not possible to obtain an exact solution and different approximations can be adopted. The density functional theory (DFT) approximation, based on the Hohenberg–Kohn theorem, and the Car–Parrinello molecular dynamics (CPMD) are some examples of approximations.

### 5.2 Modelling of solubility

Solubility is an important property from technical applications to bioavailability of compounds, toxicity, and environment. Indeed, aqueous solubility of nanoparticles has many potential uses in medical applications. The following examples use the term solubility for fullerene compounds, which can be considered borderline nanoparticles; however for other NPs the term and concept of dispersion is more appropriate.

The structures of both solute and solvent determine the interactions relevant to the solubility process. The solubility can be modelled quantitatively for a series of solutes in a single solvent or for a series of solvents for a single solute. In the first case, the solute molecules are different in each case but the solvent remains the same and the model expresses the solubility

as a function of the solute descriptors. In this case, the solvent descriptors can be combined into the corresponding coefficients, and solubility is then determined only by the structural characteristics of the solutes. In the second case, where the solute is the same but the structure of the solvent is varied, the solubility is determined by the solvent descriptors. In this case, the solute descriptors can be combined into the corresponding coefficients, thus the solubility relationship depends solely on the structural features of the solvent.

Solubility can be modelled by using a variety of methods: linear solvation energy relationship (LSER) (52,53), mobile order theory (MOT) (54), and QSPR (55).

The LSER method is based on multilinear regression (MLR) analysis of the solubilities of solutes in different solvents. The method was originally developed by Kamlet and Taft (52, 53) and further refined and applied by Abraham and co-workers (56) who have applied it to numerous solutes. The LSER MLR model utilises several characteristics that account for the solvent/solute polarisability, dipolarity, volume, hydrogen bond acidity, and hydrogen bond basicity.

The strength of the LSER approach relies on combining all these characteristics into a single model, thus providing a solid basis to describe the solute-solvent interactions and also the ability to rank each type of interaction for each solute-solvent pair. A limitation is that the characteristics (descriptors) used in the LSER model originate from experimental measurements; these are often unavailable or incomplete when working with diverse compounds within large databases.

The MOT approach (54) has been used extensively to predict mole fraction solubilities of various solutes in non-electrolyte solvents. MOT is based on a thermodynamic treatment of the liquid state that includes terms for describing the effects that solute-solvent, solvent-solvent, and solute-solute interactions have on the chemical potential of the dissolved solute. MOT assumes that hydrogen bonded aggregates are formed temporarily without a distinguishable thermodynamic identity. Partners of hydrogen bonds are not preserved with time but rather change continuously. Such treatment leads to an equilibrium consideration involving the fractions of time during which an amphiphilic proton belongs respectively to a bonded and non bonded state. This differs from the more conventional thermodynamic approaches that treat equilibrium in terms of discrete chemical species.

An example of the study of the solubility of a series of solutes in a single solvent was published by Toropov et al (57). The components of the chiral vector of carbon nanotubes (58), which contain information about rolling up graphite layer, were used as structural descriptors. Two-variable models of water solubility and octanol water partition coefficient calculated with the (x,y) components of the chiral vectors were obtained for a training test (n=8) and a test set (n=8).

The simplified molecular input line entry system (SMILES) notation was used in a study by the same authors as an alternative to molecular graph theory to model the solubility of fullerene C<sub>60</sub> in various organic solvents (benzene derivatives) (59). The QSPR analysis predicted fullerene C<sub>60</sub> solubility with one variable correlations by using a descriptor of correlation weights (DCW) calculated from rules of the SMILES notation calculation. The model was validated by random separation of the dataset into training and test sets.

Large differences between the experimental and calculated values of the C60 fullerene solubility were unexpectedly found for relative simple structures. The possible differences between experimental and predicted values of solubility for simple solvents were possibly due to the formation of specific associations of solvent clusters near the C60 molecules. This shows the limitations in representing molecules by the 'classical' molecular graph approach (i.e. 3D molecular features which might become important for spatial arrangements such as clusters or cages around C60 are not taken into account by 2D descriptors).

The solubility of C60 in a series of 47 solvents was determined by Ruoff et al (60). To obtain a quantitative prediction of the solubility of C60, solubility data were plotted against the following solvent parameters: polarizability, polarity, molecular size, and Hildebrand solubility. The plots obtained served to emphasize that solvents with the parameters close to those of C60 were relatively stronger solvents. However, the clear scatter in measured solubilities plotted against any single solvent parameter indicated that, although obvious trends were apparent, no single solvent parameter could uniformly predict the solubility of C60. Instead, a composite picture of solvents with high solubility for C60 emerged: large index of refraction, dielectric constant around 4, large molecular volume, Hildebrand solubility parameter equal to 10 and tendency to act as a moderate strength nucleophile.

Heymann correlated the solubility of C60 (and C70) in seven alcohols with the Hildebrand solubility parameter of the solvent (61). He extrapolated results to water as solvent, and estimated the solubility of C60 and C70 with an uncertainty of one order of magnitude.

Flunt et al (62) used literature data to correlate the solubility of C60 in organic solvents with various solvent properties. They found that solvent dipolarity, hydrogen-bond acidity and hydrogen-bond basicity were not statistically significant, and that the only descriptors required were the solvent refractive index function, and the solvent cohesive energy density. In a subsequent study (63), they found that the solubility of C60 increases with increasing solvent polarisability and decreases with increasing solvent polarity and cohesive energy density. Various types of multiparameter equations proposed in the literature were also compared.

Sivaraman et al (64) reported a systematic study on the correlation of C60 solubility and solvent properties by using conceptually simple, fundamental, and easily computable properties, such as polarisability parameter, solubility parameter, and molar volume. Solubility of fullerene C60 in 75 organic solvents was examined to develop quantitative structure-solubility relationships. These models are useful for the prediction of the solubility of C60 in solvents for which experimental data are not available. The descriptors used to form the regression models were the polarisability parameter calculated from the refractive index, and the connectivity indices, such as Randic connectivity indices, Hall and Kier valance connectivity indices, and other graph theoretical indices that encode the structural details of compounds. Connectivity indices account for structural features affecting the solubility of fullerene in organic solvents. In addition, an indicator parameter IP which is a combination of atom contributions, group contribution, and contribution due to the position of an atom or a group in aromatic compounds was used. The models suggested for individual data sets such as alkanes, alkyl halides, alcohols, cycloalkanes, alkylbenzenes, and aryl halides had good predictive ability and were better than the models for the combined data sets. Though polarisability parameter and topological indices explained the major amount of data variations, the inclusion of an indicator parameter as a combination of atom contributions and contributions of substituents' position in benzenes improved the predictive ability

significantly. However, although the predictions for the relatively small subsets were good, their treatment utilizes valence connectivity indices of different orders which are highly intercorrelated and an indicator variable containing hidden information.

Models predicting fullerene solubility in 96 diverse solvents were developed using multiple linear regression and feed-forward computational neural networks (CNN) by Danauskas et al. (65). A pool of the best linear models, as determined by rms error, was developed, and a CNN model was developed for each of the linear models. The best CNN model was chosen based on the lowest value of a specified cost function.

For each compound, four types of descriptors were calculated: topological, electronic, geometric, and geometric/electronic hybrids. The most predictive descriptors turned out to be topological, electronic, and hybrid descriptors.

The solubility data of C60 fullerene in various solvents has been treated by multivariate stepwise linear regression applied as the linear solvation energy approach (LSER) in studies by Marcus et al. (66, 67). According to this approach, the solvation energy, i.e., the interaction Gibbs energy of the solute with the solvent depends in a linear manner on a small number of independently determinable physicochemical effects.

Data for several solvents were considered at 298 K (113 solvents) and at 303 K (32 solvents). Solvent properties include molar volume, surface tension, solubility parameter, relative permittivity, dipole moment, the square of the refractive index, the Kamlet-Taft hydrogen bond donicity, electron pair donicity, and polarity/polarizability, and the Dimroth-Reichardt "general polarity" parameter. Other derived quantities such as the molar refraction, the molar polarization, and a function of the volume, were included. Increasing molar volume and solvent polarity diminished the solubility of C60, whereas electron pair donation ability and polarisability enhanced solubility.

Abraham et al. (68) transformed the solubilities of C60 in 20 organic solvents into water-solvent partition coefficients and the latter, as log P values. The dependent variable log P, the partition coefficient of a series of solutes between water and a given solvent, were analysed by the Abraham solvation equation. The independent variables were solute descriptors: the solute excess molar refraction, the solute dipolarity/polarisability, the overall or summation hydrogen-bond acidity and basicity, and the McGowan characteristic volume.

A least-squares support vector machine (LSSVM) method was used as a machine-learning technique for the prediction of the solubility of C60 in a large number of diverse solvents in a study by Liu et al. (69). The molecular descriptors (molecular indices, constitution and quantum-mechanical descriptors, mainly) were calculated with CODESSA software package. Non linear models using LSSVM produced slightly better models with good predictive ability than did the linear regression.

Quantitative relationships between solvent structures and the solvation free energies of individual solutes were studied by Katritzky et al. (55), Solvation free energies of 80 diverse organic solutes were modelled in a range from 15 to 82 solvents using CODESSA software package (70). CODESSA includes a variable selection procedure and a diverse pool of theoretical molecular descriptors. Significant correlations (in terms of squared correlation coefficient) were found for all the 80 solutes.

Torrens et al (71) investigated the solubility of SWNTs in a variety of solvents, finding a class of non-hydrogen-bonding Lewis bases that provided good solubility. The (10, 10) single-wall carbon nanotube (SWNT) presents consistency between relatively small solubility, and large partition coefficients and kinetic stability. QSPR predictive models were reported by Martin et al (72) to model the solubility of series of 22 monocyclic and polycyclic aromatic hydrocarbons (PAH), including a fullerene carbon nanostructure (C60). Due to their extended • electronic system and the structure which consist of fused pentagons and hexagons, PAH and fullerenes have similar properties. Extended knowledge about PAH solubility in different solvents could give insight into the mechanism of solubility of different carbon nanostructures.

Two different condensed media were used: a nonpolar solvent (n-heptane) and an average polarity solvent with an –OH group (1-octanol). Statistically good QSPR models were obtained by using forward selection techniques from a large space of theoretical molecular descriptors.

For the modelling purposes the data was used in logarithmic scale. Three-dimensional CODESSA descriptors including constitutional, topological and geometrical descriptors were calculated by using optimised molecular structures at the AM1 semiempirical level. Quantum chemical descriptors were calculated using information extracted from MOPAC. The QSPR models were derived with Heuristic and best multilinear regression (BMLR) descriptor forward selection approaches as implemented in CODESSA software package.

In both cases, the best models obtained were three-parameter correlations involving topological, charge related and quantum chemical descriptors. The authors obtained two statistically significant models (separately for octanol and heptane) based on 15 training compounds, including 14 PAHs and fullerene C60. The QSPR models constructed for n-heptane and 1-octanol indicate that the solubility of PAH and C60 in nonpolar solvents like n-heptane and in average polar solvents like 1-octanol depends on the compound spatial structure, on electron distribution, reflected by electrostatic molecular descriptors and on interaction energy between solute and solvent molecules. As a general trend, the solubility in these two solvents decreases with increasing the size of the molecule (with the number of cycles in PAH). However there are several exceptions from this trend. Significantly, the solubility of C60 is predicted correctly by the QSPR models for both n-heptane and 1-octanol, even when left out from the model. However, they did not validate the applicability domain of the models. The structural difference between 14 PAHs and the fullerene is probably too large to make reliable predictions for C60 (all PAHs are planar, but the fullerene is spherical).

### 5.3 Modelling of Young modulus

Young modulus (E), which is a measure of the stiffness of a given material and the resistance of an elastic body to deflection or deformation by an applied force, was predicted by using descriptor correlation weights (DCW). The DCW descriptor was a product of the correlation weights calculated for each element of the mentioned special notation by the Monte Carlo method. Young modulus of 29 nanomaterials (different atom composition and conditions of syntheses) was predicted by using a SMILES-like description (73).

The SMILES-like nomenclature for a given nanomaterial contains data on atom composition and the technological conditions of its synthesis and is used as basis for calculating optimal descriptors. The nomenclature used was not analogical to the SMILES, since its function was

restricted to encoding the available information on the genesis of the nanomaterials as commercial products. The information on nano substances included the following characteristics: atom composition, type of substances (bulk or not; ceramic form), and temperature of synthesis. The applied approach allowed the prediction of Young's modulus of eight randomly selected nanomaterials for a test set from a training set of 21 nanomaterials.

#### **5.4 Modelling of thermal conductivity**

The features of nanomaterials relevant for the prediction of thermal conductivity were examined by Toropov et al (74). A number of characteristics that include atom compositions, conditions of synthesis and the features of nanomaterials related to their commercial manufacturing (ceramic, single crystal, glass, bulk and film) were examined as possible descriptors of a given nanostructure. Using an optimization procedure linked to the Monte Carlo method the special correlation weights were calculated for each descriptor and a predictive model for thermal conductivity of nanomaterials was developed.

#### **5.5 Modelling of spreading properties**

Molecular modelling and QSPR methods were used to characterise the molecular properties of novel surfactant systems for soil removal on micro- and nano-structured surfaces (75), and predict new surfactants with good spreading properties. Although rough surfaces are common for floor covering because of their non-slip properties, they are difficult to clean. Spreading and drying abilities, lime soap dispersing properties and emulsification properties were enhanced with the help of an ethoxylated quat additive.

#### **5.6 Modelling of melting point**

Molecular dynamics calculations were applied for calculations of the melting point and glass transition temperature of polymeric nanoparticles (76). These studies were conducted to gain insight into some thermodynamic properties of ultra fine polymer powders. The results of such simulations predicted an interesting reduction of the melting point of nanoparticles in comparison with the bulk polymer systems.

In a first study, molecular dynamics simulations were conducted for polyethylene (PE) particles generated with up to 12000 atoms. By computing molecular volume and total energy as a function of temperature, melting point, glass transition temperature, and heat capacity were obtained. In a second study, nano-sized particles were generated up to 60000 atoms using an efficient MD method, structure and a variety of structural and physical characteristics and were computed by averaging over sets of microstates at particular temperatures. The melting point, glass transition temperature, and heat capacity of the particles as a function of polymer chain length and particle size were obtained by monitoring the molecular volume and total energy. The results of the simulations predicted a reduction of the melting point and significantly smaller compressive modulus in comparison with the bulk system.

## 5.7 Modelling of permeability

A structure-permeability relationship (77) of an ultrathin nanoporous membrane based on nanocrystalline silicon was conducted. The silicon nanomembrane resembles a hypothetical model of the nuclear envelope, a nanoporous membranous organelle that separates the cytoplasm and nucleus of eukaryotic cells. The relationship was applied to assess ion-selective transport dynamics at the silicon nanomembrane and also the diameter of the nuclear pore complex channel. The results showed that the permeability of the ultrathin membrane is determined by the average effective radius rather than its porosity.

Wong-Ekkabut et al. (78) studied the thermodynamics and kinetics of fullerene translocation into lipid bilayers using molecular dynamics simulations. The study suggests that fullerene molecules rapidly aggregate in water but disaggregate after entering inside the membrane. High concentrations of fullerene induce changes in the structural and elastic properties of the lipid bilayer, but these are not large enough to mechanically damage the membrane. The authors conclude that mechanical damage is an unlikely mechanism for membrane disruption and fullerene toxicity.

## 5.8 Modelling of cellular toxicity

The cellular toxicity induced by the insertion of a carbon nanotube into a membrane bilayer was explored by using the membrane interaction quantitative structure-activity relationship (MI-QSAR) methodology (79). The structural organization, dynamical features and transport of small polar molecules across the membrane bilayer with, and without, the inserted carbon nanotube were compared. Additional calculations to determine how the transport of solvated ions through the inserted nanotube might alter the structure of the membrane bilayer were also performed. It was found that the insertion of the carbon nanotube causes two large changes in the bilayer: an alteration in the packing of the molecules, and a change in structural organization that becomes much more rigid than when the nanotube is not inserted. Solvated calcium ions are predicted to transport preferentially through the inserted nanotube as compared to hydrated sodium ions, but the solvated calcium ion also produces an alteration in the local bilayer structure as it passes through the nanotube.

A series of bis-functionalized and water-soluble fullerene C60 derivatives bearing polar chains at different positions on the spheroid has been investigated for their cytotoxic and haemolytic properties, with the aim to correlate structure with toxicity (80). Cationic chains were suspected to induce significant toxicity while the presence of neutral or anionic moieties did not produce any response in the model produced. A tentative explanation of the experimental observations was performed by theoretical studies in which hydrophilic and hydrophobic surface areas were correlated quantitatively with haemolytic properties. The total hydrophobic surface area (ASA\_H) and the total hydrophilic surface area (ASA\_P) were calculated. The ratio between ASA\_H and ASA\_P was plotted against the haemolytic activity measured at two different concentrations. Interestingly, linear correlation plots have been obtained ( $r=0.78$  and  $r=0.94$ ). Even if the coefficients did not indicate a really strong correlation between hydrophilic/hydrophobic area ratio and haemolytic action, these preliminary unexpected indications are a very promising starting point for further detailed studies.

The ability to screen nanomaterials with high-throughput instrumentation can be very helpful given the large number of possible nanomaterial/cell culture compositions and enable robust

structure-activity relationships under controlled conditions. More specifically, one could in principle identify novel nanomaterials that are likely to have favourable in vivo safety based on in vitro activity profiles that are similar to a non-toxic nanomaterial. Shaw et al. (81) evaluated the toxicity of 50 nanoparticles in a high-throughput fashion, testing multiple cell types and multiple assays that reflect different aspects of cellular physiology, and finally collected almost 24,000 data points. By applying hierarchical clustering to the dataset the authors identified nanomaterials with similar patterns of biologic activity across a broad sampling of cellular contexts and were able to infer whether activity profiles were dominated by either core composition or surface modifications. The authors also conclude that given the 'heterogeneity within each cluster, the biological activity of NPs arises from the combined effects of many aspects of their composition and is therefore difficult to predict a priori'. However, no further studies were conducted to verify how the particles behaved in vitro and the materials were only characterised before entering the assays. The analysis indicated that similarity or differences in biological activity is not significantly enhanced by evaluating NPs in multiple different cellular assays and cell types. These findings disagree with Monteiro-Riviere et al., who showed that due to NM interaction with some assay markers, more than one assay might be required for determining NP toxicity (82).

### **5.9 Modelling of enzyme inhibition**

The group of Papadopoulos (83,84) analysed the binding interactions between fullerene based inhibitors and HIV-1 protease employing a combination of docking, 3D QSAR and MD simulations. The latter were applied to both the ligand-free and the inhibitor-bound protease forms revealing a different orientation of the  $\alpha$ -hairpin flaps at these two systems. When the inhibitor is present, the flaps of the enzyme are pulled in toward the bottom of the active site, while in the free enzyme the flaps are shifted away from the catalytic site. Both MD simulations have shown that flap, catalytic and termino-lateral regions of the protease show more flexibility during the simulations. A series of fullerene derivatives was then analyzed using docking and 3D QSAR/CoMSIA methods. The high contributions of the steric fields indicated the importance of the van der Waals interactions between the non polar surface of the enzyme and the fullerene-based inhibitors. The information obtained from the combination of various computational techniques is useful to both rationalise the molecular basis of fullerene HIV-1 protease inhibition and design new and more potent drugs.

### **5.10 Modelling of surface reactivity**

Surface reactivity is another important parameter to explain the behaviour and toxicity of nanomaterials. A nanoparticle can be represented by a periodic array of atoms (i.e. the core) and a surrounding layer of more or less disordered atoms having weakened and dangling bonds as well as irregularities such as steps, kinks and terraces. Therefore nanoparticles are characterised by a short range ordering, an enhanced interaction with environments and a great variety of the valence bond electronic structure. All these features make nanoparticles an interesting material for applications such as catalysis or chemisorption.

A few studies focusing on the characterization of the surface and reactivity of nanomaterials combine theoretical calculations (i.e. DFT) and experimental measurements. Liu et al. (85) synthesised nanoferrhydride nanoparticles using a biomimetic process, which employs ferritin, to form well-dispersed supported monolayers of ferrhydride. This techniques enables the production of nanoparticles with nominal size of 3 and 6 nm. The surface features and

reactivity of the material were studied by combining attenuated total reflection Fourier transform infrared spectroscopy with molecular orbital and DFT frequency calculations. Adsorption and reaction of SO<sub>2</sub> on the nanoparticles, leading to the formation of SO<sub>3</sub><sup>-</sup> species, showed that SO<sub>2</sub> sorption can be a sensitive function of the structural properties and size of nanoparticles.

Vittadini et al. (86) reviewed recent theoretical studies concerning the chemistry of and on TiO<sub>2</sub> anatase surfaces by DFT calculations. Due to its widespread exploitation, numerous studies are focusing on revealing the toxicity of titanium dioxide. The most stable polymorph of this oxide is rutile, but anatase and brookite are also common, especially in nanoscale and synthetic samples. Concern has focussed on nanoparticles of the anatase form because the photocatalytic activity on the surface of these nanoparticles can produce ROS which can eventually lead to oxidative stress and cell damaging. The (001) surface of anatase has been shown to be particularly reactive and the stronger reactivity is confirmed by investigations on the adsorption of formic acid. Calculations show that on the most common surface type (101) the acid remains undissociated, whereas on the (001) surface formic acid adsorbs dissociatively. Similar results are obtained with water which is weakly bound to the (101) surface but adsorbs dissociatively to the (001) surface, the reason being the tensile stress of the latter material due to the unnatural geometrical configuration of the bridging oxygens.

Other recent examples include the characterization of the surface reactivity of gold nanoparticles using extended Hückel theory combined with DFT calculations (87) and a DFT study on the nanotoxicological implications of oxygen adsorption at silver surfaces (88).

## 6. SOME APPLICATIONS OF CARBON-BASED NANOPARTICLES

The unique properties of nanoparticles have led to a number of applications, as illustrated in this section with reference to carbon-based nanoparticles.

The fullerene family, and especially C<sub>60</sub>, has potentially useful photo-, electro-chemical and physical properties (89), which can be exploited in many different biological fields. The unusual spheroidal shape, the exclusive presence of carbon atoms in their structures and the big  $\pi$ -electron cloud make fullerenes good candidates for novel approaches to chemotherapy. Fullerene is able to fit inside the hydrophobic cavity of HIV proteases, inhibiting the access of substrates to the catalytic site of the enzyme. It can be used as radical scavenger; in fact some water-soluble derivatives are able to reduce ROS concentrations. At the same time, if exposed to light, fullerene can produce singlet oxygen in high quantum yields. This action, together with the direct electron transfer from excited state of fullerene and DNA bases, can be used to cleave DNA. So far, fullerene derivatives have shown interesting properties in different fields such as apoptosis, neuroprotection, anti-HIV therapy, antibacterial and DNA-photocleavage activities.

Negative aspects of fullerene-like molecules for use in medicinal chemistry are the well-known lack of solubility in polar solvents (biologically relevant media) and the consequent formation of aggregates in aqueous solutions. The solubility problem can be partially solved by modifying chemically fullerenes. Various functionalisations have been utilized both to increase the hydrophilicity (e.g.  $-\text{OH}$ ,  $-\text{COOH}$ ,  $-\text{NH}_2$ ) and to prepare new compounds presenting biological and pharmacological activity. New ways of action can be induced by the presence of addends on the carbon cage but, in general, the new derivatives are related to the fullerene physical and chemical properties. The lipophilicity of the sphere can be helpful for interactions with the active site of various enzymes or can make C<sub>60</sub> able to intercalate into biological membranes, destabilizing them. These actions could have a role in the antibacterial activity found for several derivatives. For its electrochemical features, the fullerene core can react with free radical species behaving as radical sponge in diseases caused by a hyperproduction of reactive oxygen species (ROS). On the other side, C<sub>60</sub> is able to generate singlet oxygen after irradiation and this can be used to cleave nucleic acids and to oxidize lipids.

It is possible to entrap metal atoms into the fullerene cage obtaining endohedral metallofullerenes, which can be useful as radiotracers in magnetic resonance and x-ray imaging. However, the modified water-soluble derivatives may be toxic. The undesired effect could be attributed to the surfactant properties of the fullerene derivatives, generated by a simultaneous presence of hydrophobic and hydrophilic portions, which most probably induce membrane disruption.

Although there are many examples of papers discussing the relationships between the structure and toxicity of nanoparticles, there are no QSAR studies based on toxicological endpoints for this group of materials. Some of the main biological applications of fullerenes are outlined below.

## 6.1 Antioxidant and neuroprotective properties

Many neurodegenerative disorders such as Parkinson's, Alzheimer's and Lou Gehrig's diseases are due to the hyper-production of oxygen and nitric oxide radical species probably due to the over-excitation of glutamic acid receptors. In other words, the formation of reactive oxygen species (ROS) is considered to be one of the processes involved in neuronal injury.

Oxidative stress by oxygen radicals is known to induce cellular instability by a cascade of events, leading to a programmed cell death. In these situations, the use of radical sponges has been demonstrated to decrease, yet not to eliminate, neuronal death. The neuroprotective activity of fullerenes is based on their capability to react with oxygen radical species such as superoxide hydroxyl radicals, which attack lipids, proteins, DNA, and other macromolecules, taking up many radicals on the carbon cage. In particular, poly-hydroxylated fullerenes named fullereneols or fullerols [C<sub>60</sub>(OH)<sub>n</sub>] have been shown to be excellent antioxidants, reducing apoptosis in cortical neurons cultures: with their high solubility and their ability to cross the blood brain barriers, fullerols have been also demonstrated to absorb many oxygen radicals per fullerene molecule and to reduce the toxicity of free radical damage on neuronal tissue.

## 6.2 Antiapoptotic activity

Apoptosis is a scheduled cell death that is mainly due to the transforming growth factor (TGF- $\beta$ ) protein. In this process, ROS species are released and one way to stop the damage, or at least to decrease it, is an antioxidant treatment. Some fullerene derivatives can prevent apoptosis in hepatic tumour cells by neutralization of the TGF- $\beta$  induced reactive oxygen species.

## 6.3 DNA photocleavage

DNA photocleavage number of studies, nucleotidic chain cleavage can be performed in the presence of fullerene derivatives. This phenomenon takes place only in the presence of light and was studied on animal microbial cells lines (Salmonella) and on plasmids. In both cases the fragmentations of the DNA and RNA filaments were observed.

## 6.4 Anti-HIV activity

In spite of the enormous advances achieved in the therapy of AIDS, the quick mutation of human HIV that leads to resistance toward the modern therapies and the high toxicity of currently used drugs strongly support the discovery of new agents.

A series of bis-functionalized fullerene C<sub>60</sub> derivatives bearing two or more solubilising chains have been evaluated for their activity against HIV-1 and HIV-2 strains (90). Some of the compounds showed activity against HIV-1 type in the low micromolar range. The effect of the positions of the addends on the C<sub>60</sub> nucleus was investigated, indicating that only trans-2 isomers possess promising activity. The presence of a quaternary pyrrolidinium nitrogen is essential to increase solubility. The study was performed to find the relevant structural features for improving anti-HIV activity. As a conclusion, positive charges near to the C<sub>60</sub> backbone could increase potency, while longer solubilising chain seems to induce cytotoxicity.

In a subsequent study, the activity of a series of regioisomeric bis-fulleropyrrolidines bearing two ammonium groups were also evaluated against HIV-1 and HIV-2 (91). Two trans isomers were endowed with interesting antiviral properties, confirming the importance of the relative

positions of the substituent on the C60 cage. In addition, reduced amphiphilicity of molecules to other compounds previously reported decreased their cytotoxicity in CEM cell cultures. None of the compounds showed any inhibitory activity against a variety of DNA and RNA viruses other than HIV.

This study allowed the identification of some structural requirements necessary for bearing anti-HIV-1 properties. From this evaluation, it was possible to underline the fact that specific relative positions of the two substituents (trans-2) and positive charges close to the carbon cage are prerequisite for antiviral activity. In contrast, bulky polar chains on a C60 sphere induce cytotoxicity and seem to reduce potency, suggesting a significant steric control. The toxic action could be attributed to the amphiphilic character of this class of compounds, as recently reported.

### **6.5 Antimicrobial activity**

The discovery of the possible intercalation of fullerenes into biological membranes has encouraged many research groups to study the potential antimicrobial effects of C60. Positive results were achieved on bacteria like *Candida albicans*, *Bacillus subtilis*, *Escherichia coli* and *Mycobacterium avium*.

It was found that water-soluble fulleropyrrolidine inhibited the growth of a strain of *Mycobacterium avium*, a mycobacterium peculiar to birds but dangerous for immunodepressed humans that is resistant to most antimicrobial drugs (92). The exact nature of the action of fulleropyrrolidines is not known, but it is likely that the fullerene derivatives fit inside the tidy structure of the mycobacterial cell-wall, causing its disarrangement and death of the microorganism.

The activity of fulleropyrrolidines was tested against human strains of mycobacteria. Singly charged fullerene derivatives seemed very appealing substrates for the interaction with the cell-wall structure. The simultaneous presence of a charged species together with the hydrophobic spheroid offers the potential advantage of driving the compounds toward the charged double layer and then allowing the spheroid to enter the hydrophobic membrane.

Upon contact with water, C60 spontaneously forms a crystalline stable aggregate with nanoscale dimensions. It has been demonstrated that prokaryotic (microbial) exposure to nano-C60 in water is inhibitory at relatively low concentrations (93).

### **6.6 Enzyme inhibition**

Some fullerene derivatives have shown inhibitory activity against various enzymes as cysteine proteases (papain, cathepsin) and serine proteases (trypsin, plasmin, thrombin). The unique characters of hydrophobicity and electrophilicity together with the high reduction potential are the key elements for this activity but the mechanism is still unknown.

Four different regioisomers of cationic bis-N,N-dimethylfulleropyrrolidinium salts were evaluated as inhibitors of the enzymatic activity of acetylcholinesterase (94). These fullerene-based derivatives were found to be non-competitive inhibitors of acetylthiocholine hydrolysis. Molecular modelling was used to describe the possible interactions between the fullerene cage

and the amino acids surrounding the cavity of the enzyme. The cationic C60 derivatives are potentially able to modulate the enzymatic activity of acetylcholinesterase.

### **6.7 Drug transport and delivery**

Ultrathin needles (95) provide a low invasive and highly selective means for molecular delivery and cell manipulation (96). The geometry and the stability of a family of packed carbon nanoneedles (CNNs) formed by units of 4, 6 and 8 carbons has been studied by using quantum chemistry computational modelling methods (97-99). At the limit of infinite-length, these CNNs might act as semiconductors, especially when the number of terminal units is increased. CNNs are also potentially able to stabilise ions around their structure. Therefore, due to the apolar characteristics of CNNs and their ability to carry ionic species, they would be suitable to act as drug carriers through non-polar biologic media. Fullerenes could be also used as drug carriers for selective tissue targeting (19).

### **6.8 Electron transfer**

C60 is an excellent acceptor; in alkali-metal intercalated complexes, it shows superconductivity; and in charge-transfer salts, it exhibits ferromagnetic properties. Furthermore, C60 is a great partner for light-induced electron-transfer processes. It would be very promising to be able to modulate the electronic properties of fullerene derivatives. However, most of C60 derivatives have decreased electronegativity, and attempts to increase the electron affinity of C60 have led only to partial success.

The electron affinity of C60 can be increased by attaching strong electron-withdrawing groups to C60 or through periconjugation mechanisms. The improvement of the electron-accepting properties of C60, coupled with the versatility of the organic chemistry of fullerenes, can lead to more efficient charge transfer processes.

The electrochemical properties of a family of N-methylpyrrolidinium fullerene iodide salts, possessing one (or two) solubilising chains, were studied (100). These species showed enhanced electron-accepting properties with respect to both the parent fulleropyrrolidines and C60.

## 7. SOFTWARE TOOLS FOR NANOPARTICLE ASSESSMENT

### 7.1 Development of software tools

An initiative to integrate a computer-based approach for high throughput kinetic and virtual screening is the informatics toolbox that has been developed for TOPCOMBI, a research project funded by the European Commission for Nanotechnology and Nanoscience (101). It is composed of a collection of modules dealing with laboratory analytics, robotics, data handling, optimization, database processing and visualization. The system is based on a workflow paradigm that enables the capture and re-usage of processes. Two case studies dealing with the kinetic study of glycerol catalytic oxidation using parallel equipments, and integrated QSAR applied in heterogeneous catalysis have been reported.

### 7.2 Grain-Cutter

A new modelling tool, Grain-cutter (GRcut) (102), was developed to generate computational models systematically, for atomistic simulation of isolated nanoparticles. It generates atomic coordinates in crystalline nanoparticles with equivalent crystallographic surfaces. In addition to 32 crystallographic point groups, nine non-crystallographic point groups with five-fold symmetries are implemented for modelling icosahedral and decahedral clusters in a systematic way. Curved surfaces with spherical, ellipsoidal and cylindrical shapes are also available. This software is useful as a pre-processing tool for molecular simulation on metallic clusters, quantum dots, or fullerenes in high symmetries. The GRcut is distributed as a Java applet via the Internet to be used on web browsers.

## 8. CONCLUSIONS AND RECOMMENDATIONS

To date, there have been a number of theoretical studies on the prediction of optical, mechanical, chemical, and electronic properties of NPs but there have been relatively few published studies on the computational modelling of toxicity.

To extend the traditional (Q)SAR paradigm to predict NP toxicity, the main challenge is to identify the relevant properties for modelling NP reactivity/toxicity. However, the development of QSAR models requires the availability of quantitative toxicity studies which are still sparse. In particular, data on the characterisation of NPs, data on toxicological endpoints of interest (oxidative stress/inflammation, immunotoxicity, and genotoxicity), and physicochemical properties and calculated structural descriptors are needed.

There is an urgent need to develop a rational structure-activity based paradigm to screen large numbers of NPs for nanotoxicity and hazard assessment. QSAR has been widely used to predicting toxicity of substances in bulk form but, up to date, QSAR studies for the prediction of nanoparticle toxicity have been rarely reported. Indeed, there have been few systematic efforts to derive structure activity relationships for nanoparticles (3, 103-105).

Some of the main pathobiological processes that are considered important NP effects are translocation, cell damage, cell death, oxidative stress, inflammation and genotoxicity (3).

To date, the only common feature that has been identified as a structural correlate for NP toxicity for non-fibrous pathogenic particles is the potential to cause oxidative stress, which has been suggested as a metric.

Some exceptions to this are the cases of: asbestos and synthetic vitreous fibres (SVF), where a structure activity paradigm has been defined between the length and chemistry and the pathogenicity (106); quartz, whose pathogenicity depends on the surface reactivity and the ability to cause oxidative stress (42); CDNP which are also related to oxidative stress (11).

A promising approach could be to study the forces and interactions which occur at the interface between nanostructures and biological materials. Nanoparticles interacting with proteins, DNA and membranes establish a series of nanoparticle-biological interfaces that depend on colloidal forces as well as dynamic biophysicochemical interactions (107). These interactions lead to the formation of protein coronas, particle wrapping, intracellular uptake and biocatalytic processes that could have biocompatible or bioadverse effects. Probing these various interfaces allows the development of predictive relationships between structure and activity of nanomaterials.

The combined and synergistic use of different computational methodologies is therefore necessary to develop robust approaches for predicting the biological and toxicological properties of NPs.

### **Disclaimer**

Any views and conclusions expressed in this report are those of the authors alone and do not represent an official position of the European Commission.

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### Abstract

In the growing field of nanotechnology there is a need to determine the physicochemical and potential toxicological properties of nanomaterials since many industrial, medical and consumer applications are based on an understanding of these properties and on a controlled exposure to the materials. This document provides a literature review on the current status of computational studies aimed at predicting the physicochemical properties and biological effects (including toxicity) of nanomaterials, with an emphasis on medical applications. Although a number of models have been published for physicochemical property prediction, very few models have been published for predicting biological effects, toxicity or the underlying mechanisms of action. This is due to two main reasons: a) nanomaterials form a colloidal phase when in contact with biological systems making the definition and calculation of properties (descriptors) suitable for the prediction of toxicity a new and challenging task, and b) nanomaterials form a very heterogeneous class of materials, not only in terms of their chemical composition, but also in terms of size, shape, agglomeration state, and surface reactivity. There is thus an urgent need to extend the traditional structure-activity paradigm to develop methods for predicting the toxicity of nanomaterials, and to make the resulting models readily available. This document concludes by proposing some lines of research to fill the gap in knowledge and predictive methodology.

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