Risk assessment strategies, toxicity testing and policy aspects of nanomaterials

Blagica Jovanova*[1], Renata Slaveska Raicki[2], Tatjana Kadifkova Panovska[3]

[1,3] *Department of Toxicology, Institute of Applied Biochemistry, Faculty of Pharmacy, Ss. Cyril and Methodius University, Majka Tereza br. 47, 1000, Skopje, R. Macedonia*

[2] *Department of Pharmaceutical Technology, Institute of Pharmaceutical Technology, Faculty of Pharmacy, Ss. Cyril and Methodius University, Majka Tereza br. 47, 1000, Skopje, R. Macedonia*

blagicajovanova@hotmail.com[1], rera@ff.ukim.edu.mk[2], taka@ff.ukim.edu.mk[3]

Abstract

Background/Objectives: Nanomaterials possess unique properties, but the way they were designed may cause adverse effects. These adverse effects are issues for the establishment of regulatory frameworks for their safe use.
Materials and Methods: Nanostructures able to interact with biological molecules via several mechanisms. However, the dramatic benefits of their applications lately create a potential safety concern of their possible effects on human healthand environment. These potential harms that may follow, lead to the need of a hazard assessment procedure to determine the safety of nanomaterials. Therefore, two main approaches have been designed: Life Cycle Assessment (LCA) and chemical Risk Assessment (RA).
Findings: The Risk assessment/Risk management framework developed by the U.S. National Research Council consists of three components: research, risk assessment and risk management. The aim of this framework is to collect data in order to determine whether an agent has a potential to cause adverse effect, to define the dose-response relationship, exposure status and the probability of an exposure event in a population at current exposure levels. However, current regulatory frameworks are not designed to address nanotechnology research and the progress of risk assessment research of nanomaterials, because new nanoproducts are arriving on the market rapidly. Therefore, the current knowledge about the nanomaterials on the market is insufficient, leading to lack of risk assessment data that can be used to develop any kind of regulation.
Application/Improvements: Toxicity testing is insufficient to reduce the adverse effects of nanomaterials. Tiered risk management strategy is one of the key steps in establishing a control limit for these unwanted effects.

Keywords: risk assessment, risk management, life cycle assessment, nanomaterials, toxicity testing

1. Introduction

The small size of nanomaterials changes their physical behavior compared to their bulk form, affecting their solubility, melting point and catalytic properties, which contributes to their greater toxicity [1]. This explains why nanostructures such as nanoparticles with a large surface area are able to form free radicals through several mechanisms and lead to disruption of human biological tissues and cells. Moreover, due to their small size, nanoparticles can easily interact with DNA, RNA and other genetic material, altering genetic information of the cell. They can also interact with range of proteins altering their function. Their small size also allows them to accumulate in different tissues, preventing the immune system to recognize the accumulation.

All of the above mentioned interactions increase the risk of potential toxicity of nanomaterials which are already on the market. Therefore, it is essential to include risk assessment in the procedure for the determination of the safety of nanomaterials. There are two main approaches for testing the health and environmental impacts of nanomaterials: Life cycle Assessment (LCA) and chemical Risk Assessment (RA). Several regulatory bodies have used either one of these approaches or a combination of both, depending on the type of analysis needed [2]. These findings are based on the available data gained by introducing toxicity testing obtained by *in vivo*, *in vitro* and *in silico* testing methods.

Most of the entities and companies which use or produce nanomaterial products seemed not to attribute enough for the assessment of potential risks of nanomaterials. A survey conducted in 40 companies using nanomaterials in Germany and Switzerland showed that most of the companies did not give high priority to risk assessment of nanomaterials and there is a lack of general industrial framework for evaluating the potential toxicity of these nanomaterials. This leads to increasing of the concern and awareness about the implications on workers, end users and environment [3].

All of these adverse effects that nanomaterials are capable of inducing are issues for the establishment of regulatory frameworks for their safe use.

2. Materials and methods

2.1. Approaches for the assessment of potential risks of nanomaterials

2.1.1. Lifecycle assessment (LCA) of NM

LCA is a comprehensive, systematic methodology for testing the potential environmental impacts of a nanomaterial, attributable to the entire life cycle of a product. This approach enables the assessment of the potential hazardous effects of the NM throughout all life cycle stages, from raw material extraction and acquisition, through both energy and material production and manufacturing process, uses and final disposal of the defined product. The LCA methodology usually covers a broad range of environmental impacts, such as climate change, resource depletion, toxicological stress on both human health and ecosystems and many others [2]. According to ISO standards, LCA assessment is conducted in four main phases: (i) Definition of the goal and scope of the study, (ii) Establishment of an LCA inventory, (iii) Performing Life cycle impact assessment (LCIA) which evaluates the potential impacts of the system on the environment, (iv) Interpretation of the results from the assessment which would provide the support for the critical decision-making in relation to the goal and scope of the study [4]. The LCA approach is adequate to make a comparison between the potential environmental and human health impacts of different nanomaterials as long as their designed products have the same function due to the fact that LCA as an assessment method is based on a functional unit of the examined nanomaterials.

Government agencies could also benefit from the LCA assessment in a manner of providing information which could help in the design of regulations and legislation in regard to occupational health and safety, consumer protection and environmental protection. Also, LCA results can be used to inform the public of the potential benefits and risks of nanoproducts. In industry sectors, LCA analysis might help bringing better decisions in regard of the product's design, marketing, development and manufacturing. LCA could also provide a sound solid basis for marketing nanoproducts as environmentally friendly products, which is another benefit for industry stakeholders [2,4].

This method cannot offer a detailed description of nanomaterials effects, especially in regards to the dose-response information. However, LCA is a standardized methodology that mostly contributes to giving an overview of the impacts of nanomaterials in each of their life cycle phases.

2.2. Risk assessment of NM

Risk assessment (RA) is another methodology used for the evaluation of the toxic potential of nanomaterials. This approach brings the correlation between the specific properties of the nanomaterials with the risks related to human or environmental exposure and the possible hazardous consequences. A risk assessment includes four general components:

(A) Exposure assessment that refers to the identification and characterization of the populations exposed;
(B) Hazard assessment, which involves:
(1) Hazard characterization;
(2) Hazard identification;
(C) Final risk assessment based on exposure and hazard assessments.

Unlike LCA, RS is a tool for the hazard evaluation of several nanoproducts in one part of their life cycle at a time [5].

A variation of this testing method is the Intelligent testing strategy (ITS) which was upgraded in a way that allows a more accurate and efficient risk analysis. This was accomplished by introducing a more thorough physicochemical

characterization of nanomaterials in the ITS method, yet the same basic principles as in the traditional risk assessment approach were retained. Another upgrade to the system was cross-cutting grouping, ranking and modelling of nanomaterials by common characteristics and ranking them by different parameters such as potential of exposure or intrinsic toxicity [6].

A new classification of the nanomaterials according their potential toxicity was proposed as an algorithm which included series of questions about the potential risk and hazard assessment of nanoparticles concerning their solubility, size distribution, if there is any evidence confirming the toxic, biological and oxidation effects and/or disruption of genetic and endocrine function from nanoparticles, any evidence confirming the possiblity of accumulation of nanoparticles in surrounding environment, human/animal organisms and their possible transfer down the food chains. The RA methodology has also introduced the control banding method that leans upon the OHB (Occupational Hazard Band) toxicology scale that consists of five levels. According to this scale, nanomaterials could be rated at OHB level 4 and OHB level 5 that applies for any insufficiently examined chemical [7,8].

Risk assessment strategy could be a quite intricate way to understand all the effects that nanomaterials could possibly lead to, but on the other hand it also gives a more specific explanation of the potential harms that may follow.

2.3. Exposure assessment of NM

Physical (i.e. size, shape, surface area, agglomeration state), chemical (i.e. chemical composition, charge, chemical reactivity), biological (i.e. route of administration, metabolism, excretion, adduction to biological molecules) and environmental (i.e. presence of microbes, temperature, pH, salinity, acidity, viscosity) factors play a very important role in the exposure assessment studies of nanomaterials [9].

Exposure in experimental studies is usually expressed as dose on mass/body weight basis, or as concentration. However, the dose or concentration may not be the best metric to predict the effect of engineered nanomaterials (ENM). Surface area is considered a more relevant factor [10]. Although occupational exposure may occur via several routes (such as dermal contact, ingestion, inhalation), the most common exposure studies are those which consider the inhalation route of exposure, since this is a frequent threat to workers in the occupational settings where nanomaterials are manipulate and used [11]. In line with this, several models have been suggested for inhalation studies, but only few of them are optimized as nano-specific models for inhalation in the occupational settings. Nano-specific models used in exposure studies lack a correlation between the mass concentration of the nanomaterial as a model output and the actual particle number concentration in the settings of exposure. Examples of nano-specific refined model systems are Stoffenmanager Nano® (NL) and NanoSafer® (DK). These models provide more comprehensive exposure assessment compared to other models where only the potential for emission of nanoparticulate matter is used as a factor in exposure studies. However, in order to develop a quantitative nano-specific model which will provide exact correlation between the exposure degree and nanoparticle number concentration, further exposure examinations are needed [9,11,12].

2.4. Hazard assessment of NM

2.4.1. Hazard characterization of NM

First step in a hazard assessment process is a hazard characterization defined by a physical and chemical examination of the nanomaterial. Physical and chemical properties of the bulk form (if exists) may vary from those of the nanomaterial, bringing out the indispensability of the physicochemical characterization in the hazard assessment process [11].

Prior the risk assessment takes place, nanomaterials are being examined and their native physical and chemical properties, as well as their concentration in the medium are initially being determined using various techniques such as Energy Dispersive X-ray Analysis (EDS), Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma Spectroscopy (ICPS), Brunauer, Emmett and Teller (BET) technique and Inverse Gas Chromatography (IGC) which can be used to estimate size and surface area, while Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM) are routinely used for evaluation of size and morphology. Dynamic Light Scattering (DLS), Zeta Potential and UV-Visible Spectroscopy are routinely performed in solution to provide size, charge and composition characteristics [1,13].

However, a physical and chemical characterization of the nanomaterial is insufficient concerning its potential for toxic effects [11].

Hence, even if it is possible to attain a well-defined physicochemical description of the examined nanomaterial, it may be insufficient for the following steps in the hazard assessment.

2. 5. Hazard identification of NM

2.5.1. Nanoparticle properties and their cell uptake in hazard identification

Part of the hazard identification step is to examine how the physicochemical properties of the ENMs influence their uptake via different routes of exposure. Nanoparticles possess a greater surface area per mass than larger particles of the same compound, making them easier for uptake by cells, leading to their greater activity in the biological systems, therefore more prone to cause adverse effects. Other ENM characteristics which may influence their toxicity include size, shape, surface functionalization, surface reactivity (ability for reactive oxygen species production), solubility, association with biological proteins, binding to receptors, their strong tendency for agglomeration (mainly by Van der Waals forces, electrostatic forces and simple physical entanglement). One study showed a positive correlation between the concentration and smaller ENM size with the speed of agglomeration [10,12].

The nano size of nanoparticles is probably one of the most influential factors for the manifestation of the predetermined as well as the adverse effects of nanomaterials.

2.5.2. Mechanisms of toxicity of NM

Depending on the biological molecules they encounter in the body fluids or at the body surfaces, nanomaterials may undergo series of modifications which may alter several native physical and chemical properties through the processes of degradation/dissolution and aggregation. Therefore, it is essential that these modifications are taken in consideration when nanotoxicological studies are ongoing [14].

The toxicity of nanomaterials can occur via several mechanisms based on their physicochemical characteristics, steric orientation, possibility of releasing chemical constituents from the nanomaterial, surface properties, and possibility to act as vectors for the transport of other toxic chemicals [9]. There are approximately ten mechanisms of toxicity which include generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), oxidative stress, frustrated phagocytosis, changes in protein structure and function, protein unfolding response, immune response activation, fibrogenesis and tissue remodeling, vascular injury, neurotoxicity, genotoxicity and other possible mechanisms of toxicity given the wide range of novel NM physicochemical properties [15,16,17,18].

A nanomaterial could also act through more than one mechanism of toxicity, inducing several types of changes in sensitive tissues. Once the nanoparticle enters the cell, toxicity can occur via one or a combination of several mechanisms [9].

Four mechanisms have been identified as most common and explained in detail. The **first** mechanism involves the release of chemical constituents which lead to toxicity through the release of toxic molecular fragments. The **second** mechanism is based on the steric orientation of the nanoparticle and depends on its shape and size. If there is a possibility for steric interferences, the nanoparticles can interfere with important binding sites for macromolecules. The **third** mechanism of toxicity is related to the surface properties of the material, such as photochemical properties, charge densities and local electric fields. The **fourth** mechanism of toxicity is based on the capacity of nanomaterials for transport of other toxic chemicals to sensitive tissues [19].

As above mentioned the most common route of exposure in occupational settings is via inhalation [1].

Most important mechanisms of toxicity via inhalation are:

1) generation of ROS and RNS, causing lipid peroxidation of cellular membranes, DNA and enzyme damage;
2) oxidative stress;
3) ROS-stimulated carcinogenic changes; and
4) inflammation-driven processes which may result in lung mutagenesis and carcinogenesis.

Engineered nanoparticles (ENPs) have the ability to increase the formation of ROS and RNS which may lead to initiation and promotion of carcinogenic mechanisms or/and genotoxicity. However, this is a long process of neoplastic changes, and rarely comes to the final stage of carcinogenic development. This is also confirmed with the little evidence found for the carcinogenic effects of engineered nanoparticles in humans [20].

To sum up, the mechanisms of toxicity of nanomaterials are in a very tight correlation with their fundamental characteristics; this means that by manipulating some of their characteristics, it is possible to reduce their toxicity if the change has been made to the right segment of their structure.

2.5.3. Challenges in risk assessment of NM

There are many challenges regarding the risk assessment of nanomaterials: method of preparation of the NM, their interaction with the environment where it is found during its life cycle i.e. biological systems, food chain, or ecosystems where it may be released after use (soil, air, water). Another challenge is the definition of a special protocol for the identification of specific properties and the design of the appropriate dose metric correlation in toxicological studies with the sole purpose – risk assessment approaches for nanomaterials [11].

Theoreticians and practitioners of LCA agree that Life Cycle Assessment is the best option at the beginning of a new product development to identify the main areas of concern. Moreover, it has been recommended a combination of LCA and RA for the completion of the evaluation process for the potential health and environmental risks of new nanoproducts.

Although both LCA and RA are successful in giving information about the potential toxicity of nanoproducts, there are several gaps in the whole examination process using both methods. In the case of LCA, lack of robust data regarding emissions and exposures that are related to the production process, use and disposal of NM and limited data regarding the environmental fate, behavior and toxicological impacts of NM are of great significance. Other oversights are the unclear metrics which were applied in this methodology, the inconsistencies in data sources, the variability in NM characterization and their purity, as well as the probable inadequacy of the gained results for application in decision-making processes.

Risk assessment methodology may provide insufficient measured exposure data for NM, lack of validated exposure estimation models, unclear NM characterization and identification, inappropriate metric(s) for expressing exposure and hazards, and also unclear applicability of test guidelines and (eco)toxicological studies [2].

2.6. Combination of LCA and RA for an overall risk assessment

The combination of both methodologies could offer a way to overcome the flaws of each approach when applied separately.

The LICARA project combines Life cycle assessment, Risk assessment and Multi criteria decision analysis to assess the environmental benefits and risks of new nanoproducts. LCA contributes by observing the full life cycle in comparison to a reference product, while the RA is used to assess the risks of the hazard and exposure of the examined nanoproduct, in each processes of its life cycle separately. In some cases, gained benefits and risks of a nanoproduct are not directly comparable and the uncertainty of the decision making for the safety of that nanoproduct is overwhelming. Therefore, the LICARA concept includes the MCDA as well, in order to weigh-up the specific factors that result in certain benefits and risks of the new nanoproduct [21].

Another reason for the combination of LCA and RA approaches is the higher risk of exposure at the workplace compared to the exposure via the environment. LCA evaluate the toxicity hazards for the environment and the general population providing very little information available on the risks of workers exposed to NM. Therefore, the RA approach broadens the scope and includes the safety and social issues in the sustainability assessment and the impact of nanomaterial risks for both workers and general population [22].

However, full integration of both approaches cannot answer all the questions because of their different scopes and end results. Therefore, a complementary concept is more desirable, regarding involvement of RA approach inside every stage of a product's life cycle, prioritization of data by LCA needed for the RA approach and setting a hypothetical basis by LCA followed by a more detailed RA analysis [23].

Both methods require technical data and strong expert knowledge, therefore the combination of LCA and RA together may result in filling in several gaps, but it wouldn't solve all questions completely. Even though both strategies have points in favor of and against their application, the Risk Assessment strategy appears as a better choice as long as the main aim of the study is to elucidate the hazardous effects of a single agent.

2.6.1. Risk management model for nanotechnology

The objective of risk management is the identification and management of significant risks. It consists of several key steps, with feedback through a control andmonitoring process [5]. By wider definition, risk management involves evaluating the extent of risks and deciding on the most appropriate exposure control measures. In general, it involves research in several areas of interest such as societal and occupational levels. The societal level is relevant for the rights of workers to have safe and healthy workplace and a right to know about potential hazards. Another aspect is the responsibility of the employers to provide a healthy and safe workplace for their employees. Therefore, risk management is an essential part of the clause for the safety of workers at the occupational settings, and includes hazard, exposure and risk information [24].

Goudarzi has suggested a 10-step qualitative risk management model for nanotechnology (NT) risk managers which could enable to detect significant risks in a systematic approach [5], as the following:

1) Basic knowledge of the work and adequate consultation between stakeholders, managers and employees
2) Organization of the work into sections, subsections and tasks or process-units according to Work Breakdown Structure (WBS)
3) Identification of all nanoparticles that are, or will be, used or produced in every work unit and process
4) Identification of the type of nanoparticle (e.g., engineered insoluble nanoparticles in a matrix, or more hazardous forms such as free nanoparticles)
5) Providing information about the nanomaterials in the Material Safety Data Sheets (MSDS) by the supplier, but if not available, it is necessary to obtain adequate information from other sources (textbooks, provided standards, technical reference sources, scientific papers, reports, trade journals, electronic online databases) or experience from a previous use of similar substances or processes
6) How hazardous are nanoparticles released into the working area? Are persons exposed to hazardous nanoparticles through respiration, skin, ingestion or eye contact, or is there a possibility of accidental injection into the body?
7) Determining the nature and severity of the hazard and the degree of exposure of people involved in the process
8) In case the assessment shows that there are significant risks to health, further actions should be acquired if needed
9) The record should be concise and should include: a description of the work unit, name of the assessment team personnel, date, time and a list of hazardous nanomaterials used or produced in the project unit, a summary of the process containing a description of normal operations in the project unit (with a note of any changes observed or anticipated which might affect accuracy of assessment); risk identification, including possible routes of exposure; procedure for assessment of exposure; the degree of exposure and existing control procedures
10) Review and regulation of the assessment (if needed).

Risk management overlaps with other management processes in most projects, which is usually undertaken as part of a normal project management [5].

Alongside the physicochemical profiling of the nanomaterials as an essential fragment of an excellent risk management plan, a structurally managed communication between workers, managers and stakeholders is also one of the key notes for a successful risk management operation. A step-by-step sequence of initiatives is the most convenient approach in resolving the hazard issues with nanomaterials that are exploited in many areas.

2.7. Toxicity testing of nanomaterials (NM)

2.7.1. In vivo or in vitro toxicity assays – is one of them a better choice, or both?

Many national and international regulatory agencies are making efforts to develop a tiered strategy for toxicity tests. These strategies include two stages of testing: first stage refers to *in vitro* assessment (preliminary screening of nanoparticles (NP), followed by their benchmarking and selection of those which potentially cause harm) and the second stage involves *in vivo* assessment using laboratory animals (toxicity testing of the selected potentially toxic nanoparticles). According to this concept, *in vivo* studies contribute to clarifying the toxicity mechanisms and targets of action at different levels of NM life cycle, in order to improve the overall understanding of the possible adverse outcomes resulting from the exposure of nanoproducts [25].

A different approach of the above mentioned, is to include *in vivo* studies as a fundamental tool being essential for setting the basis of a toxicological experiment. *In vivo* studies investigate which organ systems are susceptible to

the potential toxic effects of the nanomaterials, which somehow gives a direction for the *in vitro* studies that follow. Therefore, *in vitro* studies cannot be assumed to capture the same behavior of NM as in *in vivo* settings, since the nanoparticle may experience transformations or interact with biological components (such as proteins) after absorption. According to the second theory, *in vitro* studies are useful for more specific examinations, such as identifying the mechanisms of toxicity and targets of toxicity action on a molecular level [26,27].

Although *in vitro* data are not a substitute for *in vivo* studies, their use may contribute to reduce the number of laboratory animals needed, reveal a general mechanism of toxicity which can give a direction of further assessment of the potential risk of exposure to nanomaterials using *in vivo* testing [1,25]. This is highly supported by the 3Rs' principle, which was described by Russell and Burch in their book *Principles of Humane Experimental Technique*. The 3Rs' principle has been endorsed by many government agencies, scientific associations and funding bodies and refers to the practice to *reduce, refine,* and *replace* animal experimentation whenever possible [26].

Therefore, *in vivo* and *in vitro* assays cannot independently give concrete results as definite unchangeable facts. In addition, *in vitro* experiments must have *in vivo* validation in order to be useful [27].

Both assays are a useful approach for completing a general picture based on several aspects of the examined nanomaterial of whether the nanoproduct is toxic, needs to be further examined, or did not show significant toxic effects and therefore its use is considered relatively safe. The decision of which of these assays may give more relevant results could not be made, mainly because both assays explain the same dilemma from different aspects (e.g., *in vivo* assays could show which organ is affected, while *in vitro* assays could explain the mechanism of toxic action).

2.7.2. Limitations of in vivo and in vitro models

Animal models have several limitations which can be grouped in three categories: ethical issues, economic issues and lack of prediction. Some of the *in vivo* limitations include the following:

1.No animal model is perfect. This means that part of the variables in animal models are neglected on purpose in order to achieve a simplified animal model. However, many factors such as differences between species, differences in the pharmaco- and toxicokinetics of the examined substance, age, sex and group size variations, consecutive stress in animals and other factors reflect the final results, which sometimes might lead to wrong conclusions;

2. Lethality in acute toxicity may be a side-effect;

3. Economic factors impact on animal use.

In vitro testing includes the following limitations:

1. Transformation or immortalization of the cell lines which may alter the properties of the cells,

2. Selective toxicity in which some cells are more sensitive than others,

3. Difficulties in the isolation of the cells from their natural environment,

4. Difficulties in studying integrated groups of cells or organ systems.

5. Lack of specificity in clarification of the toxicological pathway because multiple stimuli might result in the same outcome in the same assay, which results in a poor correlation between the biological outcome and specific nanomaterial properties.

Another disadvantage is that *in vitro* assays cannot give information regarding the sublethal toxicity doses [1,15,26,28,29].

2.7.3. Possible alternatives for Ecotoxicity and Nanotoxicity studies

An interesting alternative for the toxicity assessment of engineered nanomaterials are bacteria which are sensitive to the effects of nanomaterials in their environment. Bacteria residing in water, soils and sediments may be exposed to engineered nanomaterials, but this effect is actually mutual: As long as ENMs can influence their life cycles, bacteria could also change ENMs fates. Bacteria are important receptors in Ecological nanotoxicology, but they are also convenient as test subjects in toxicological screening for rapid hazard identification. However, experiments about ENMs toxicity using bacteria are very limited and partial, which makes these testing methods only applicable in ecotoxicological studies for the assessment of ENMs degradation [30].

A more approachable and cost-effective alternative for the nanotoxicity studies are the *in silico* methods. Their integration with *in vivo* and *in vitro* approaches is critical, since their development often depends on the input of either *in vitro* or *in vivo* data [31].

2.7.4. In silico methods as additional method for toxicity testing

Biological testing should be based on robust scientific paradigms which can further be used for screening of multiple toxicants. This represents a predictive toxicological paradigm, defining the *in vivo* toxic potential by applying *in vitro* and *in silico* methods, instead of costly animal experiments that examine one toxicant at a time. This paradigm is based on four major requirements:

1. Designing compositional and combinatorial ENM libraries aimed for collecting knowledge about the material properties and the potential for biological harm.
2. Development of *in vitro* cellular screening assays
3. Development of HTS (high content throughput screening) and RTS (rapid throughput screening) platforms that are aimed to assess the material composition and properties
4. Application of *in vitro* data in the *in silico* modeling for establishment of QSARs (Quantitative Structure-activity relationships) and optimization of the hazard ranking for the prioritization of animal experiments [15].

The idea of using *in silico* methods as a predictive tool for the biological behavior of nanoparticles is associated with the need of developing an alternative approach which would reduce animal testing, secure early screening, provide details of toxicity mechanisms and allow more time effective planning of experiments. These methods are based on the relationship between biological effects of nanoparticles in the organism and their physicochemical properties. *In silico* prediction models are able to translate the *in vitro* outcome into the outcome expected for *in vivo* tests [31]. Existing *in silico* methods include QSAR (Quantitative structure-activity relationship) and QSTR (Quantitative structure-toxicity relationship) models, expert systems, CADD (computer-aided drug design) models and ADME models [29,31]. QSAR and QSTR models aim to describe the relationship between the biological activity (and toxicity) and one or several molecular descriptors associated with the physicochemical properties of the tested nanomaterial. The most relevant molecular descriptors for the model development are the ones associated with the mechanism of activity and/or toxicity of the selected nanoparticles [31,32,33].

Although *in silico* methods have a significant applicability in nanotoxicological researches, they also have several limitations such as insufficient experimental data about the physicochemical properties, often are based entirely on correlations even when a molecular descriptor is of no biological significance and often these predictions can be better than their input if there are random errors [31].

Although *in vitro* techniques partially replaced *in vivo* testing strategies, *in silico* studies are still the stems to every new toxicity study, since they are based on predictive tools which allow safer, cheaper and faster obtaining of preliminary results. They are essential for every new toxicity study, yet insufficient when it comes to bringing the decision about new nanomaterial usage.

3. Results and Discussion

3.1. Regulation and policy platform

World and European organizations, such as: (i) Organization for Economic Co-operation and Development (OECD), (ii) European Commission (Registration, Evaluation, Authorisation and Restriction of Chemicals legislation — REACH, NANO Safety Cluster), (iii) European Food Safety Authority (EFSA), (iv) Scientific Committee on Consumer Products (SCCP), and (v) Environmental Protection Agency (EPA) are taking steps to regulate and describe the necessary end points that contain information useful in risk assessment of nanomaterials. The largest number of described experimental and theoretical research protocols can be found in OECD documents. In these documents, there are few groups of experimental parameters: (1) Nanomaterial Information/Identification; (2) Physical–Chemical properties and Material Characterization; (3) Environmental Fate; (4)Environmental/Mammalian Toxicology; (5) Material safety [9].

The Risk assessment/Risk management framework developed by the U.S. National Research Council collects data to determine whether an agent has a potential to cause adverse effect, to define the dose-response relationship, exposure status and the probability of an exposure event in a population at current exposure levels. This framework

consists of three components: research, risk assessment and risk management. The task of the risk assessment compartment is to evaluate the above mentioned parameters, while the risk management compartment uses the gained results to regulate the exposure to the examined agent. These results serve to estimate the probability of occurrence of an adverse effect when a population or part of a population is exposed to the examined agent [10,30].

Regulators and policy-makers have responded to these regulatory issues in different ways. At one end, regulatory authorities have adopted the "wait-and-see" approach and delayed regulatory action until sufficient knowledge about risks has become available. At the other end, authorities promoted further research of the potential risks of new technologies and limit or prevent potential harm from uncertain risks. Which approach is a better choice is still a question to be answered by regulatory agencies, as it involves a wide range of factors, from political policies to economic interests [34].

Current regulatory frameworks are not designed to address nanotechnology research and the progress of risk assessment research of nanomaterials, because new nanoproducts are arriving on the market rapidly. Therefore, the knowledge we have about nanomaterials is lagging behind the development of new products, leading to lack of risk assessment data that can be used to develop any kind of regulation at this stage [35].

However, policy-makers and regulatory agencies have started to make progress by developing short-term regulatory frameworks which contain basic regulatory schemes for the safety of the exposed population. An additional issue is how to regulate a technology that we know relatively little about in terms of potential risks, regarding the fact there is a significant knowledge gap between the health and environmental effects on nanotechnology [35,36].

Many regulatory bodies appear to make efforts for the establishment of a policy platform, while interim this process of resolving initiation they keep their distance from the real issue. Whether it is a political reason, or plain economic matter, remains to be clarified by the in time progress achieved in this area of expertise.

4. Conclusion

On the basis of current knowledge about the potential risks and toxicity of nanomaterials, it is obvious that risk management and toxicity studies are a vital part of the regulative measures for a safer use of nanomaterial products on the market. Life Cycle Assessment methodology usually provides sufficient data about the environmental impacts of the examined nanomaterial, while Risk Assessment Methodology is mainly oriented towards the clarification of the health hazards which conceivably follow after handling nanomaterial products. The results gained from *in vivo, in vitro* and *in silico* assays are highly relevant for the design of a regulatory framework, especially because these regulatory challenges are best solved by people who already are in contact with the examined nanomaterials, either as consumers or as researchers. Nanoscientists are a crucial element in the big design of a regulatory network which will contain relevant and up-to-date information on the safety of nanomaterials. Creating an international NM database with information on the chemical properties, toxicity and consumer use, would assist in the categorization of nanomaterials. Both consumers and producers of nanomaterials could provide useful information on that matter. A legally enforced information duty of the NM producers might be the most effective way to ensure both quality and safety.

5. Acknowledgement

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

6. References

1. A.M.Schrand, L.Dai, J.J.Schlager, S.M.Hussain. Toxicity testing of Nanomaterials. In: New Technologies for Toxicity Testing. *Springer Link: Advances inExperimental Medicine and Biology.*2012*;* 745, 58-75.
2. Khara D. Grieger, Alexis Laurent, Mirko Miseljic, Frans Christensen, Anders Baun, Stig I. Olsen. Analysis of current research addressing complementary use of life-cycle assessment and risk assessment for engineered nanomaterials: have lessons been learned from previousexperience with chemicals. *Journal of Nanoparticle Research.* 2012; 14(7), 2-9.
3. Aasgeir Helland, Martin Scheringer, Michael Siegrist, Hans G. Kastenholz, ArnimWiek, Roland W. Scholz.Risk assessment of engineered nanomaterials: a survey of industrial approaches. *Environmental Science and Technology*. 2007; 42(2), 640-646.
4. J.Jahnel, T.Fleischer, S.B.Seitz.Risk assessment of nanomaterials and nanoproducts - adaptation of traditional approaches. *Journal of Physics: Conference Series.* 2013;429(1), 5-6.
5. SaeidGoudarzi, AbdolrezaBabamahmoodi, Mohammad Farhadi, Seyed Kamran Kamrava, FariborzMobarrez. Nano technology risks: A 10-step risk management model in nanotechnology projects. *Hypothesis*. 2013; 11(1), 1-4.
6. Vicki Stone, Stefano Pozzi-Mucelli, Lang Tran, Karin Aschberger, Stefania Sabella, Ulla Vogel, Craig Poland, Dominique Balharry, Teresa Fernandes, Stefania Gottardo, Steven Hankin, Mark G.J. Hartl, Nanna Hartmann, DanialHristozov, Kerstin Hund-Rinke, Helinor, Johnston, Antonio Marcomini, Oliver Panzer, DavideRoncato, Anne T. Saber, Håkan Wallin, Janeck J. Scott-Fordsmand. ITS-NANO-Prioritising nanosafety research to develop a stakeholder driven intelligent testing strategy. *Particle and Fibre Toxicology.*2014; 11(9), 2-7.
7. Rasoul Yarahmadi, Reza Abbaszadeh Dizaji, Ali Asgar Farshad, Fatemeh Teimuri, Mohammad Soleimani. Occupational Risk Assessment of EngineeredNanomaterials by Control Banding Method in Chemistry Laboratories. *Journal of American Science.* 2013; 9(6s), 42-46.
8. L.Gridelet, P.Delbecq, L.Hervé, G.Fayet, D.Fleury, S.Kowal, P.Boissolle. Risk assessment method for the implementation of materialsdivided up to the nanometric scale. *Journal of Physics: Conference Series.* 2013; 429, 3-4.
9. Agnieszka Gajewicz, Bakhtiyor Rasulev, C. Dinadayalane Tandabany, Piotr Urbaszek, Tomasz Puzyn, Danuta Leszczynska, Jerzy Leszczynski. Advancing risk assessment of engineerednanomaterials: Application of computational approaches. *Advanced Drug DeliveryReviews.* 2012; 64(15), 1664-1666.
10. Robert A. Yokel, Robert C.MacPhail. Engineered nanomaterials: exposures, hazards, and risk prevention. *Journal of Occupational Medicine and Toxicology.* 2011; 6(7), 5-21.
11. M.J.D.Clift, I.Gosens, N. Boschung. An example of the advantages and disadvantages related to producing aprotocol database for nanoscience. *Summary of the Joint JRC Nano event and 2nd ENPRA Stakeholders Workshop.* 2011; 29-39.
12. Igor Linkov, F. Kyle Satterstrom, Lisa M.Corey. Nanotoxicology and nanomedicine: making hard decisions. *Nanomedicine: Nanotechnology, Biology, and Medicine*. 2008; 4(2), 167-171.
13. David B. Warheit, Paul J.A. Borm,Christa Hennes, Jürgen Lademann. Testing strategies to establish the safety of nanomaterials: Conclusions of an ECETOC workshop*. Inhalation Toxicology.*2007; 19(8), 634-642.
14. Steve Hankin, Diana Boraschi, Albert Duschl, Claus-Michael Lehr, Hera Lichtenbeld. Towards nanotechnology regulation-Publish the unpublishable. *Nano Today.* 2011, 6(3), 228-231.
15. Tian Xia, Huan Meng, Saji George, Haiyuan Zhang, Xiang Wang, Zhaoxia Ji, Jeffrey I. Zink, Andre E. Nel. Strategy for Toxicity Screening of Nanomaterials. *Material Matters.* 2010, 5(3), 82-84.
16. Ramakrishna Devaki, Pragna Rao. Nanoparticle - Is toxicity a concern? *The Journal of the International Federation of Clinical Chemistry.* 2011, 22(4), 3-7.
17. Tin-Tin Win-Shwe, Hidekazu Fujimaki. Nanoparticles and neurotoxicity. *International Journal of Molecular Sciences.* 2011, 12(9), 6268–6273.
18. Arnaud Magrez, Sandor Kasas, ValérieSalicio, Nathalie Pasquier, Jin Won Seo, Marco Celio, Stefan Catsicas, Beat Schwaller, László Forró. Cellular toxicity of carbon-based nanomaterials. *Nano Letters.* 2006, 6(6), 1122-1124.
19. Igor Linkov, Jeffery Steevens, Gitanjali Adlakha-Hutcheon, Erin Bennett, Mark Chappell, Vicki Colvin, J. Michael Davis, Thomas Davis, Alison Elder, Steffen Foss Hansen, Pertti Bert Hakkinen, Saber M. Hussain, Delara Karkan, Rafi Korenstein, Iseult Lynch, Chris Metcalfe, Abou Bakr Ramadan, F. Kyle. Satterstrom. Emerging methods and

tools for environmental risk assessment, decision-making, and policy for nanomaterials: summary of NATO Advanced Research Workshop. *Journal of Nanoparticle Research.* 2009; 11(3), 519-527.
20. Thomais Vlachogianni, Konstantinos Fiotakis, Spyridon Loridas, Stamatis Perdicaris, Athanasios Valavanidis. Potential toxicity and safety evaluation of nanomaterials for the respiratory system and lung cancer. *Lung Cancer: Targets and Therapy.* 2013; 4, 72-77.
21. C.Som, E.Zondervan-van den Beuken, T.Van Harmelen, J.Güttinger, M.Bodmer, D.Brouwer, H.E.Buist, R.Carroll, C.Coll, W.Fransman, A.Hartmanis, I.Hincapie, R.Hischier, T.Karachalios, K.Kouravelou, E.Kuijpers, T.Ligthart, D.Notter, B.Nowack, U.Seibold, G.Schneider. LICARA Guidelines for the sustainable competitivenessof nanoproducts. St. Gallen: Dübendorf,Zeist. 2014.
22. C.Hidalgo, D.González-Gálvez, G.Janer, M.Escamilla, S.Vázquez-Campos. Life cycle assessment of nanomaterials: a comprehensive approach. *The 6th International Conference on Life Cycle Management in Gothenburg* 2013. Spain, 2013, 1-4
23. J.Jahnel, T.Fleischer, S.B.Seitz. Risk assessment of nanomaterials and nanoproducts-adaptation of traditional approaches. *Journal of Physics: Conference Series*. 2013; 429(1), 2063.
24. Paul A. Schulte, Charles L. Geraci, Laura Hodson. Overview of risk management for engineered nanomaterials. *Journal of Physics: Conference Series.* 2013; 429, 3-4.
25. Teresa Coccini, Luigi Manzo, Elisa Roda. Safety evaluation of engineered nanomaterials for health risk assessment: an experimental tiered testing approach using pristine and functionalized carbon nanotubes. *International Scholarly Research Notices: Toxicology.* 2013; 2013, 1-11.
26. Thomas Hartung, Enrico Sabbioni. Alternative in vitro assays in nanomaterial toxicology. *WIREs Nanomedicine and Nanobiotechnology.* 2011; 3(6), 2-4.
27. Hans C. Fischer, Warren C.W.Chan. Nanotoxicity: The growing need for in vivo study. *Current Opinion in Biotechnology.* 2007; 18(6), 568-570.
28. Thomas Hartung. Food for thought on animal tests. *Altex.* 2008; 25, (1), 3-9.
29. Thomas Hartung, George Daston. Are *in vitro* tests suitable for regulatory use. *Toxicological Sciences.* 2009; 111, (2), 234-235.
30. Patricia A. Holden, Joshua P. Schimel, Hilary A. Godwin. Five reasons to use bacteria when assessing manufactured nanomaterial environmental hazards and fates. *Current Opinion in Biotechnology.* 2014; 27, 73-78.
31. Thomas Hartung, Sebastian Hoffmann. Food for thought on *in silico* methods in toxicology. *Altex.*2009; 26(3), 155-166.
32. David A. Winkler, Enrico Mombelli, Antonio Pietroiusti, Lang Tran, Andrew Worth, Bengt Fadeel, Maxine J. McCall. Applying quantitative structure–activity relationship approaches to nanotoxicology: current status and future potential. *Toxicology.* 2013; 313(1), 15-20.
33. M. Balls, R.D. Combes, N. Bhogal. *In silico* methods for toxicity prediction. In: New Technologies for Toxicity Testing. *Springer series: Advances in Experimental Medicine and Biology.* 2012; 745, 98-110.
34. Robert Falkner, Nico Jaspers. Regulating nanotechnologies: Risk, Uncertainty and the global governance gap. *Global Environmental Politics.* 2012; 12(1), 30-55.
35. Elizabeth A. Corley, Dietram A. Scheufele, Qian Hu. Of risks and regulations: how leading U.S. nanoscientists form policystances about nanotechnology. *Journal of Nanoparticle Research.*2009; 11(7), 1574-1576.
36. Gerald Rebitzer, Tomas Ekvall, Rolf Frischknecht, David Hunkeler, Gregory A. Norris, Tomas Rydberg. Life cycle assessment Part 1: Framework, goal and scope definition, inventory analysis and applications. *Environment International.* 2004; 30(5), 701-720.

The Publication fee is defrayed by Indian Society for Education and Environment (iSee). www.iseeadyar.org

Citation:

Blagica Jovanova, Renata Slaveska Raicki, Tatjana Kadifkova Panovska. Risk assessment strategies, toxicity testing and policy aspects of nanomaterials. *Indian Journal of Nanoscience.* 2015; 3 (1), 19-29