EVALUATION OF THE POTENTIAL HAZARD OF MANUFACTURED METAL-BASED NANOMATERIALS TO HEALTH OF AQUATIC ECOSYSTEMS: STATE OF THE ART

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Abstract

Rapidly growing production and use of engineered nanomaterials (NMs) is accompanied by increased emissions into the environment. Existing data gaps in the scientific knowledge on the fate of NMs in the environment and their potential biological adverse effects makes the evaluation of the environmental risks of NMs difficult if not impossible. We propose that the assessment of environmental risks and regulation and control of the release of metal-based NMs should be performed on the basis of total concentration of metals comprising these NMs. This would assure that the safe levels of NMs in the environment were not exceeded until enough environmentally relevant tests have been carried out for more realistic evaluations.

Keywords: *nanomaterials, toxicity, hazard, exposure, risk assessment*

1. INTRODUCTION

Rapidly growing production of metal-based nanomaterials (NMs) used in the different sectors of the world economy is inevitably accompanied by increased emissions of NMs into the environment via different pathways. According to Piccinno et al. [1] annual production volumes of NMs were highest for SiO2 (5500 tons) and TiO2 (3000 tons), followed by ZnO (550 tons) and carbon nanotubes (300 tons) whereas iron, cerium and aluminium oxide NPs as well as silver NPs were produced at about 55 ton level per year. The high production volume of metal-based NMs is the main reason why the current review focuses on metal-based nanomaterials.

The modelling of the potential release of NMs has shown that nearly half of the NMs used in different applications will end their life cycle in the landfills and the rest mostly in the soil and water compartments [2]. NMs may reach the environment at different stages of their life cycle and via different pathways [3,4]. For example, aquatic ecosystems may be polluted via effluent discharges, directly when NMs are applied as pesticides or for remediation purposes [5] and also via secondary pollution, e.g. by swimming people spreading nanoTiO_2-based UV-protection cosmetics into natural aquatic ecosystems. Recent report on the environmental risk assessment of nanomaterial use in Denmark pointed out that in addition to TiO_2 (mostly due to its high production volume and thus high environmental exposure), silver and CuO NMs may also pose a considerable risk to the aquatic environment close to the points of discharge, mostly due to their high toxicity to aquatic organisms [6].

According to the European Commission, the following definition of the term 'nanomaterial' has been recommended: *'Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm*' [7]. The physicochemical properties of NMs distinctly differ from the micro-sized (bulk) particles made from the same material due to the larger surface area per mass (specific surface area) leading to higher reactivity [8]. Due to unique properties of NMs, the hazard related to environmental pollution also may significantly differ from those of bulk (micro-size) materials as a result of biological effects of NMs being dissimilar to their bulk form with the same chemical composition. During past ten years, huge amount of data on potential hazard of this new group of chemicals to biota has been published [9]. However, despite the exponentially increasing amount of nanotoxicological peer-reviewed papers data on ecotoxicity of synthetic NPs is still incomplete and existing information is rather heterogeneous and contradictory. It has been reported that NMs may pose threat to living organisms due to novel properties, but no consensus has been reached concerning realistic potential hazard of NMs and suitable regulation for the NM emission into the environment. In spite of thousands published articles and reports on ecotoxicity of NMs, the situation is still the same as reported nine years ago by EC Scientific Committee on Emerging and Newly Identified Health Risks [10], i.e. there is still lack of high quality data needed for risk assessment [11]. The lessons which can be learned from more than 10 years of studies and the existing gaps in the scientific knowledge on the fate of nanomaterials in the environment and their potential ecological effects will be discussed in the current publication using the information that has been accumulated on ecotoxicity of metal-based NPs.

2. MATERIALS AND METHODS

A critical analysis of available information needed for evaluation of risks related to metal-based nanomaterials entering into the environment has been performed based on the published data and authors' experience in this field.

3. CHALLENGES IN REALISTIC ENVIRONMENTAL RISKS ASSESSMENT OF NMS

Assessing environmental hazard of manufactured NMs is a real challenge for the scientific community. The current approach to environmental risk assessment of chemicals is based on the comparison of toxic effect levels obtained from laboratory testing (*potential hazard to biota*) with predicted environmental concentrations (*environmental exposure*) [12]. However, this approach fails to provide predictive information for adequate risk assessment of NMs. This raises the question whether current risk assessment methodologies, which were developed for the conventional soluble chemical substances, are suitable for testing nano-specific properties.

3.1. Uncertainties in evaluation of potential hazard of NMs to the biota

The main aims of the laboratory toxicity testing of chemical compounds are (i) to evaluate their toxicity to different test species and (ii) to reveal the mechanisms of their toxic effects. The first aim provides the information on the 'safe' concentration, i.e. pollution level concerning the certain chemical which does not lead to negative changes in the ecosystems. There are two main problems which complicate the interpretation of the laboratory test results obtained on NMs: characterisation of the exposure concentration of NMs during the toxicity test and ecological relevance of the data obtained in the laboratory tests.

3.1.1. Exposure concentration of NMs in the toxicity test

In the case of metal-based NMs, the most important problem is the evaluation of the exposure concentration of NMs as their bioavailability (and thus toxicity) in different test media varies due to speciation. The correct extrapolation of laboratory test results to natural ecosystem is possible only if we know what fraction of NMs actually accounts for the toxic effect: nanoparticles or toxic metal species formed during testing (e.g., dissolved metal ions that often are main cause of the toxic effect of metal-based NMs [13]). Specific properties of NMs may result in unexpected fate characteristics and enhanced reactivity compared to the corresponding bulk materials or the dissolved metal species. In the absence of information on NMs speciation in the test medium we may lead to obtaining irrelevant ecotoxicological data for given NMs.

Aggregation, sedimentation and dissolution are the main processes affecting bioavailability of partially soluble metal-based nanomaterials. The behaviour of NMs in the test medium depends largely on the chemical composition of the test medium, but also on the methods used for the preparation of the NM suspensions for the testing (ultrasonication etc). Sonication is needed to avoid rapid aggregation and settling of NMs in the test vessels and provide even distribution of NMs in the dilution series.

Characterisation of the speciation of NMs during the test is a very difficult task. Even quantification of the dissolved fraction of tested NMs is impeded by lack of appropriate methods. During last decades, numerous models have been elaborated and used for the prediction of bioavailability of metals in different test media [14, 15], but these models were designed for soluble metal salts. Thus, the possibilities for modelling the behaviour of nanomaterials in the aquatic compartment are limited as both theoretical description and experimental data are currently insufficient for elaboration of exposure assessment models for NMs [16]. In addition, these processes (aggregation, dissolution) are also time-dependent [17] that even more complicates the elaboration of well-working models which could be used for interpretation of toxicity test results. Due to that, it is practically impossible to predict equilibrium speciation for diverse metal-based NMs in media of different composition. So far, the parallel tests with respective soluble metal salts have been used to separate the biological effects of metal ions and particulate NMs [18]. However, such an approach provides only approximate results, and can be used just for preliminary conclusions.

Practically all scientists working in the field of toxicity evaluation of NMs are unanimous that currently used standardized testing protocols for risk assessment are not suitable for ecotoxicity evaluation of NMs [19-21]. It has been emphasized that the development of prescriptive testing guidelines for NMs, including preparation of NMs suspensions and providing reliable methods for quantification of NMs in complex media, are needed in order to improve their applicability for hazard assessment of MNs [22].

However, it should be mentioned that the scientific 'nano'toxicity testing community has been mainly focusing on quantification of exposure concentrations of NMs in the test media and less on the ecological relevance of laboratory tests.

3.1.2. Ecological realism of the laboratory toxicity test results

The low ecological relevance of standardized ecotoxicity test methods is the biggest problem in environmental hazard assessment of chemicals [23, 24]. It is very difficult to predict the actual hazard of NMs to ecosystem health on the basis of results obtained in standardised tests. The main limitations that hamper extrapolation of laboratory test results to aquatic ecosystems are common for all chemicals [25]:

- too high exposure concentrations (not comparable to real environmental exposures);
- unrealistic exposure conditions (test media, climatic conditions);
- limited number of test species;
- impossibility to predict the interactions and indirect effects that regulate the functioning of biological communities.

In the case of too high exposure concentrations of NMs, observed adverse effects may result not only from the toxicity of investigated compound but also from decreasing viability of test organisms due to mechanical stress caused by the NMs' particles. In addition, the dissolution-aggregation rates noticeably differ in suspensions with high and low concentrations of NMs. Sonication of the NMs suspensions before testing also decreases environmental reliability of laboratory tests as such processes never occur in the environment.

Chemical composition of test media is a crucial parameter in ecotoxicity testing with aquatic organisms. Use of the standardized artificial freshwater significantly increases uncertainties in extrapolation of laboratory result to natural ecosystems. Very simple chemical composition (a mixture of mineral salts) and absence of dissolved organic matter in the test media may lead to erroneous assessment of the real risks related to pollution by metal-based NMs as their bioavailability to aquatic organisms may significantly differ depending on water composition. For example, it has been shown that acute toxicity of CuO nanoparticles to freshwater microcrustaceans in six different natural waters decreased 35-80-fold as compared with artificial test medium, whereas toxicity of soluble copper salt decreased only 2-12-fold in the same test media [26]. So, toxicity mitigating effect of natural waters was more remarkable for CuO NMs than for soluble compounds of the same metal (Cu-ions). The variation (up to 10-fold) of the bioavailability of the same NMs in the different lake waters has been reported also in other publications [27,28]. As it was mentioned above, working models for prediction of NMs speciation in different types of natural water are still absent as currently for elaboration of such models there is not enough experimental data on behaviour of NMs in the test media and their interactions with natural colloids [16].

It has been emphasized that introducing of modifications into the design of ecotoxicological experiments instead of using standardised protocols would improve the ecological realism of data obtained in laboratory toxicity testing [29]. Use of low environmentally relevant exposure concentrations and natural water as test medium in long-term tests are the main conditions that should be applied in ecotoxicological assessment of NMs [20]. The use of multispecies test formats such as micro- and mesocosms, semi-field studies (model ecosystems) yield more environmentally relevant data for evaluation of potential hazard of NMs to aquatic ecosystems. But, unfortunately, these approaches are much more expensive: the testing costs increase in parallel to the increased reliability of the test results (Fig.1). Therefore, application of the test formats such as mesocosms and semi-field studies should be justified and reasonable, i.e. applied only when the predicted emissions of certain type of NMs are predicted to be high enough to consider it as a threat to the environment.

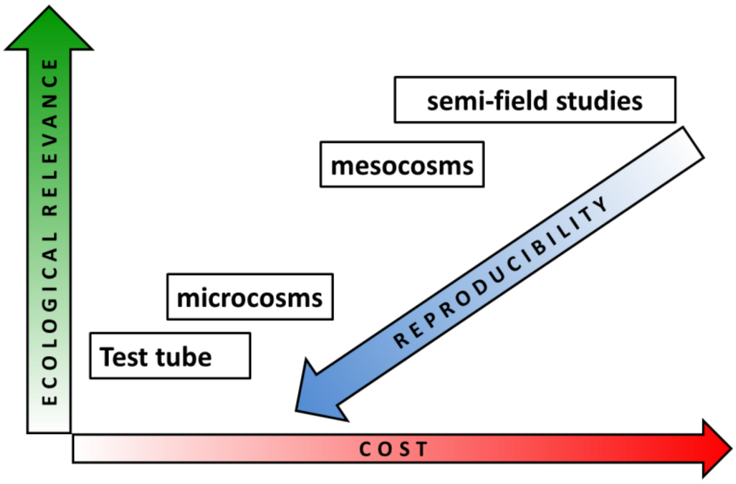

Fig.1. Cost and environmental reliability of test formats

3.2. Challenges in the environmental exposure assessment

In the risk assessment of chemicals, environmental exposure assessment is a key issue along with biological effect assessment. Pollution monitoring and modelling of predicted environmental concentrations or combination of these two approaches are the main tools for assessment of chemicals' environmental exposure. However, due to the fundamentally different physicochemical properties of NMs, compared to 'conventional' water soluble chemicals, applicability of these two approaches to NMs is questionable.

3.2.1. Monitoring

The nano-sized particles play an important role in biogeochemical processes in all environmental compartments [30]. Detection and accurate quantification of manufactured NMs in the environment is currently still a challenge due to low predicted concentrations of NMs in the environment and the presence of background natural nanosized particulate matter and colloids that have often similar composition as the engineered NMs [31]. Existing analytical methods do not allow measuring low NPs concentration in water and especially in the sediments [32]. The measurement and detection of

NMs even in simple media using in the laboratory tests is very complex and needs application of different techniques [33]. Thus, routine monitoring of 'nano'pollution levels in the environment is currently impossible due to the lack of appropriate analytical methods for detection of NMs in soil and natural water. Moreover, measurement of metal-based NMs concentrations in different environmental compartments is also not very informative for evaluation of potential exposure as there is still lack of information concerning bioavailability of NMs species (for example, particles and soluble metal ions) to biota and their toxic potential. Once entered the aquatic ecosystem, manufactured NMs become actively involved in complex biogeochemical processes: precipitation, aggregation, dissolution, complexation with inorganic ligands and organic ligands, accumulation in living organisms and detritus. As a result, most of the NMs entering the ecosystems will dissolve or aggregate. The distribution ratio of insoluble NPs between different ecosystem compartments (water, sediments and biota) is also unstable but most insoluble NMs have been shown to settle rapidly. These processes occur both in natural and artificial (waste water treatment plants, effluents) ecosystems and define the complexity in predicting the fate of NMs in aquatic environment.

3.2.2. Uncertainties in the Modelling

Modelling of the predicted environmental concentrations of NMs consist of two parts: (i) prediction of the pollution load from different sources and (ii) description of environmental fate. The first task is not much more complex for NMs than for other chemicals. Indeed, approximate evaluations of emissions of engineered NMs into environment using different models have been published [2, 34]. The major problems concern the second task. As it was mentioned above, limited knowledge on the behaviour of NMs in the complex environmental matrices hamper modelling of these processes [20]. Uncertainties in the modelling of the behaviour of NMs in the environment depend on the ability to describe numerous abiotic and biotic processes the aquatic ecosystems which affect speciation of the metal-based NMs and which still have not been adequately explored. This makes modelling of the behaviour of NMs in the environmental matrices practically impossible. The numerous types of NMs with various properties (chemical composition, particle size, surface coating, shape and surface functional groups) determining the reactivity and bioactivity of NMs [35] also complicate the application of modelling.

TASK	STATUS	MAIN PROBLEM
Hazard Identification		
Laboratory testing	insufficient	➢ Exposure quantification in terms of speciation ➢ Low ecological relevance
Environmental exposure assessment		
Pollution load	uncertain	➢ Identification of pollution sources ➢ Lack of available data on manufacturing and application of NMs
Modelling of NMs behaviour in the environmental matrices	uncertain	➢ Lack of knowledge and experimental data ➢ Variety of NMs types
Monitoring	impossible	➢ Lack of appropriate analytical methods

Table 1. Uncertainties in environmental risk assessment of manufactured metal-based nanomaterials

4. OPINION

Uncertainties in the environmental risk assessment of manufactured metal-based nanomaterials are summarised in the Table 1. It seems that existing gaps in the scientific knowledge on the fate of this type of nanomaterials in the environment and their potential biological effects make evaluation of the environmental risks of metal-based NMs currently very difficult if not impossible. All above-mentioned problems make it impossible to prepare water quality standards for NMs [36]. The ecotoxicological information published so far on the toxicity of NMs suit more for preliminary hazard evaluation and labelling purposes than for realistic environmental risk assessment. In this context, the question about how to regulate emissions of metal-based NMs into waterbodies is still relevant.

Taking into account the predicted amount of NMs emission into the environment, it is unlikely that current emissions of NMs will cause effects at lethal and even sub-lethal levels in aquatic or soil ecosystems. But we stress that the potential hazard for human exposed during manufacturing and use of NMs must be studied comprehensively and strictly regulated until we do not have enough safety data (precautionary principle). Also, environmental pollution by NMs should be taken under control.

From our point of view, the assessment of environmental risks, regulation and control of the release of metal-based NMs should be performed on the basis of total concentration of metals comprising these NMs. Health and environmental effects of toxic metals are well-known. The cumulative knowledge on ecotoxicity of metal-based NMs collected so far has shown that in case of most metal-based NMs, the toxicity to aquatic organisms was lower than the toxicity of soluble salts of individual metals found in the NMs [37]. The approach based on the total metal concentration in the environment may lead to overestimation of the risk related to NMs entering into aquatic environment, but new data on bioavailability of NMs in aquatic ecosystems obtained with more environmentally relevant test formats will allow to correct the estimations. This would assure that the safe levels of NMs in the environment were not exceeded until enough environmentally relevant tests have been carried out for more realistic evaluations.

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