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Series on the Safety of Manufactured Nanomaterials

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IMPORTANT ISSUES ON RISK ASSESSMENT OF MANUFACTURED NANOMATERIALS

IOMC
INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS
A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

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The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 34 industrialised countries in North and South America, Europe and the Asia and Pacific region, as well as the European Commission, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several countries with special status at the OECD, and from interested international organisations, attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

The Environment, Health and Safety Division publishes free-of-charge documents in ten different series: **Testing and Assessment**; **Good Laboratory Practice and Compliance Monitoring**; **Pesticides and Biocides**; **Risk Management**; **Harmonisation of Regulatory Oversight in Biotechnology**; **Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents;** and **Safety of Manufactured Nanomaterials.** More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (www.oecd.org/ehs/).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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FOREWORD

The OECD Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology (the Joint Meeting) held a Special Session on the Potential Implications of Manufactured Nanomaterials for Human Health and Environmental Safety (June 2005). This was the first opportunity for OECD member countries, together with observers and invited experts, to begin to identify human health and environmental safety related aspects of manufactured nanomaterials. The scope of this session was intended to address the chemicals sector.

As a follow-up, the Joint Meeting decided to hold a Workshop on the Safety of Manufactured Nanomaterials in December 2005, in Washington, D.C. The main objective was to determine the "state of the art" for the safety assessment of manufactured nanomaterials with a particular focus on identifying future needs for risk assessment within a regulatory context.

Based on the conclusions and recommendations of the Workshop [ENV/JM/MONO(2006)19] it was recognised as essential to ensure the efficient assessment of manufactured nanomaterials so as to avoid adverse effects from the use of these materials in the short, medium and longer term. With this in mind, the OECD Council established the OECD Working Party on Manufactured Nanomaterials (WPMN) as a subsidiary body of the OECD Chemicals Committee in September 2006. This programme concentrates on human health and environmental safety implications of manufactured nanomaterials (limited mainly to the chemicals sector), and aims to ensure that the approach to hazard, exposure and risk assessment is of a high, science-based, and internationally harmonised standard. This programme promotes international co-operation on the human health and environmental safety of manufactured nanomaterials, and involves the safety testing and risk assessment of manufactured nanomaterials.

This document is published under the responsibility of the Chemicals Committee of the OECD. It is intended to provide information on the outcomes and developments of the OECD programme on the safety of manufactured nanomaterials.

TABLE OF CONTENTS

ABOUT THE OECD 6
FOREWORD 8
OECD'S PROGRAMME ON THE SAFETY OF MANUFACTURED NANOMATERIALS 10
EXECUTIVE SUMMARY 11
RISK ASSESSMENT OF MANUFACTURED NANOMATERIALS: IMPORTANT ISSUES 12
1. General Introduction 12
2. Background 13
2.a. Health / Environmental Risk Assessment Framework 15
2.b. Approaches to Chemicals' Risk Assessment and Regulation across OECD Countries 17
2.c. Occupational Exposure Assessments 19
2.d. Approaches to Risk Assessment of Particulates 20
2.e. Current Case Studies on Risk Assessment of Manufactured Nanomaterials: Carbon Nanotubes 21
3. Risk Assessment of Nanomaterials: Important Issues 24
3.a. Problem Formulation 24
3.b. Hazard Identification 25
3.c. Hazard Assessment 28
3.d. Issues Relating to Exposure 31
3.e. Risk Characterisation (Assessment) 34
3.f. Relationship of Risk Assessment to Risk Management 35
4. Risk Assessment of Nanomaterials: Strategies and Approaches 37
4.a. Considerations Regarding the Information for Use in a Nanomaterial Risk Assessment 37
4.b. Information for Use in a Risk Assessment 39
4.c. Risk Characterisation 45
4.d. Risk Assessment Strategies 46
5. Research Needs to Address Risk Assessment Issues 49
5.a. Exposure: Public, Occupation and Environment Research Needs 49
5.b. Human Health Effect Research Needs 49
5.c. Ecological Effect Research Needs 50
5.d. Persistence, Bioaccumulation, Fate and Distribution 50
5.e. General Risk Assessment Research Needs 50
References 52

OECD'S PROGRAMME ON THE SAFETY OF MANUFACTURED NANOMATERIALS

The OECD's Programme on the Safety of Manufactured Nanomaterials[1] was established in 2006 to assist member countries to efficiently and effectively address the safety challenges of nanomaterials. OECD has a wealth of experience in developing methods for the safety testing and assessment of chemical products.

The Programme brings together more than 100 experts from governments and other stakeholders from: a) OECD Countries; b) non-member economies such as China, the Russian Federation, Singapore, South Africa, and Thailand; and c) observers and invited experts from UNITAR, FAO, WHO, ISO, BIAC[2], TUAC[3], and environmental NGOs.

Although OECD member countries appreciate the many potential benefits from the use of nanomaterials, they wished to engage, at an early stage, in addressing the possible safety implications at the same time as research on new applications are being undertaken.

The Programme of Work is being implemented through specific projects to further develop appropriate methods and strategies to help ensure human health and environmental safety:

- OECD Database on Manufactured Nanomaterials to Inform and Analyse EHS Research Activities;
- Safety Testing of a Representative Set of Manufactured Nanomaterials;
- Manufactured Nanomaterials and Test Guidelines;
- Co-operation on Voluntary Schemes and Regulatory Programmes;
- Co-operation on Risk Assessment;
- The role of Alternative Methods in Nanotoxicology;
- Exposure Measurement and Exposure Mitigation; and
- Environmentally Sustainable Use of Manufactured Nanomaterials

Each project is being managed by a steering group, which comprises members of the WPMN, with support from the Secretariat. Each steering group implements its respective "operational plans", each with their specific objectives and timelines. The results of each project are then evaluated and endorsed by the WPMN, and subsequently by the OECD Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology.

This document was prepared by steering group six (SG6) of the WPMN, which is leading the project on Co-operation on Risk Assessment and was endorsed at the 9th meeting of the WPMN in December 2011.

More information about the work of the OECD's Programme on the Safety of Manufactured Nanomaterials, as well as OECD's publications regarding safety issues of nanomaterials, is available at www.oecd.org/env/nanosafety.

1 Updated information on the OECD's Programme on the Safety of Manufactured Nanomaterials is available at: www.oecd.org/env/nanosafety

2 The Business and Industry Advisory Committee to the OECD

3 Trade Union Advisory Committee to OECD

EXECUTIVE SUMMARY

Since the document, *Risk Assessment of Manufactured Nanomaterials: Critical Issues,* was initially presented at the 4th meeting of the WPMN in 2008, the WPMN Steering Group Six (SG6) has been developing it taking account of comments/ suggestions from worldwide experts. At the 9th meeting of the WPMN, the title of the document "Critical Issues" was replaced by "Important Issues". The WPMN has recognised that there is important information/ issues in this document which assist when considering the risk assessment of nanomaterials.

This document, *Important Issues on Risk Assessment of Manufactured Nanomaterials*, provides the current practices, challenges and strategies for assessing risk in circumstances where data are limited, and there is a necessity for more research on specific risk assessment issues; however, it is not to be construed to imply scientific and/or policy endorsement of any specific risk assessment methods or models. It should be noted that this document is a living document. It was current at the time of 9th meeting of the WPMN (December 2011) and subject to amendment and refinement as research affords further understanding of how to assess and manage nanomaterials.

RISK ASSESSMENT OF MANUFACTURED NANOMATERIALS: IMPORTANT ISSUES

1. General Introduction

1. The OECD Working Party on Manufactured Nanomaterials (WPMN) has currently eight projects, which are led by steering groups, to address international co-operation with respect to human health and environmental safety related aspects of manufactured nanomaterials. The overall objectives of Steering Group Six (SG6) are to evaluate risk assessment approaches for manufactured nanomaterials through information exchange and to identify opportunities to strengthen and enhance risk assessment capacity.

2. SG6 has agreed to three detailed objectives to: i) Consider risk assessment strategies, methodologies, and supporting tools to carry out risk assessment; ii) Identify and consider any unique issues that manufactured nanomaterials present for risk assessment; and iii) Make recommendations to the WPMN for addressing and filing identified gaps. Also, SG6 will consider the need for provision of guidance on key issues that should be considered when undertaking risk assessments for manufactured nanomaterials as well as the development of empirical evidence to support this guidance.

3. SG6 has developed this document on *Important Issues* to make progress on the mentioned objectives. An initial draft was presented in 2008. It is especially worth noticing that SG6 organised and hosted a *Workshop on Risk Assessment of Manufactured Nanomaterials in a Regulatory Context* in 2009 in collaboration with the Business and Industry Advisory Committee (BIAC) and the Society for Risk Analysis (SRA) in Washington DC, United States. The discussions and outcomes of this workshop have contributed to the content of this document.

4. This document aims at introducing in chapters 2 and 3 the current practices and challenges on risk assessment of manufactured nanomaterials as well as strategies for assessing risk in circumstances where data are limited (chapter 4). Finally, this document makes clear in chapter 5 the necessity of direct research toward specific risk assessment issues in concert with current efforts to develop basic data sets, thus supporting the work on-going in other Steering Groups on the OECD-WPMN in view of further development of methods, models, data, and tools for use by decision makers to develop a systematic and integrated picture of environment, health and safety impacts of nanomaterials production and use.

5. Furthermore, it is important to note that this document should be a living report, subject to amendment and refinement as research affords further understanding of how to assess and manage nanomaterials. SG6 will consider revisions to this document once the risk assessment community has secured further insight into amending risk assessment methodology. Considering the dynamic nature of the field, this document should be regarded as a systematic compilation of current views and recommendations for those performing risk assessments and developing respective methodology. This document should not be construed to imply scientific and/or policy endorsement of any specific risk assessment methods or models. The particular situation or need for the risk assessment and the type and quality of data available will influence the risk assessment approach. Practices and policies may vary depending on applicable rules or regulations in a given area.

2. Background

6. **Core terms.** The *hazard* of a substance is its potential to cause harm whereas *risk* is the likelihood of that harm occurring, taking into account wider considerations of *exposure* and *uncertainty*. Thus, risk assessment requires information on both the potential hazard, the release of the substance into the environments and the likelihood and/or degree of resulting short- and long-term exposure. In cases where the risk of adverse effects at exposures below a safe level is expected to be practically zero, the *Margin of Exposure (MoE)* can be calculated to describe the quotient between expected exposure and the *No-Observed-Adverse-Effect-Level (NOAEL)* or other effect level (e.g., benchmark dose) in the test species. Similarly, the *Margin of Safety (MoS)* reflects, depending on definition, the ratio between exposure and NOAEL or a *Reference Dose (RfD)* derived from the NOAEL (OECD, 2003). The derivation of a RfD or any other exposure limit from effect levels like NOAELs requires knowledge of the appropriate Assessment Factors (AFs) to account for variability and uncertainty in the risk estimates. An initial MoE could be calculated (identified) without knowledge or selection of the appropriate AF and re-evaluated later when more data are available, MoE and MoS methods may be useful in screening risk assessments to evaluate a large number of substances and to prioritize further research.

7. **Risk assessment paradigm**. The classical risk assessment framework includes four main steps: hazard identification, hazard characterisation including dose-response assessment, exposure assessment, and risk characterisation (NRC 1983) (Figure 1). Research studies in various fields provide the data required for the risk characterisation, which provides input to risk management decision-making. At the *SG6 Workshop* it was agreed, that the existing risk assessment paradigm developed for traditional chemicals should also be applied to nanomaterials.

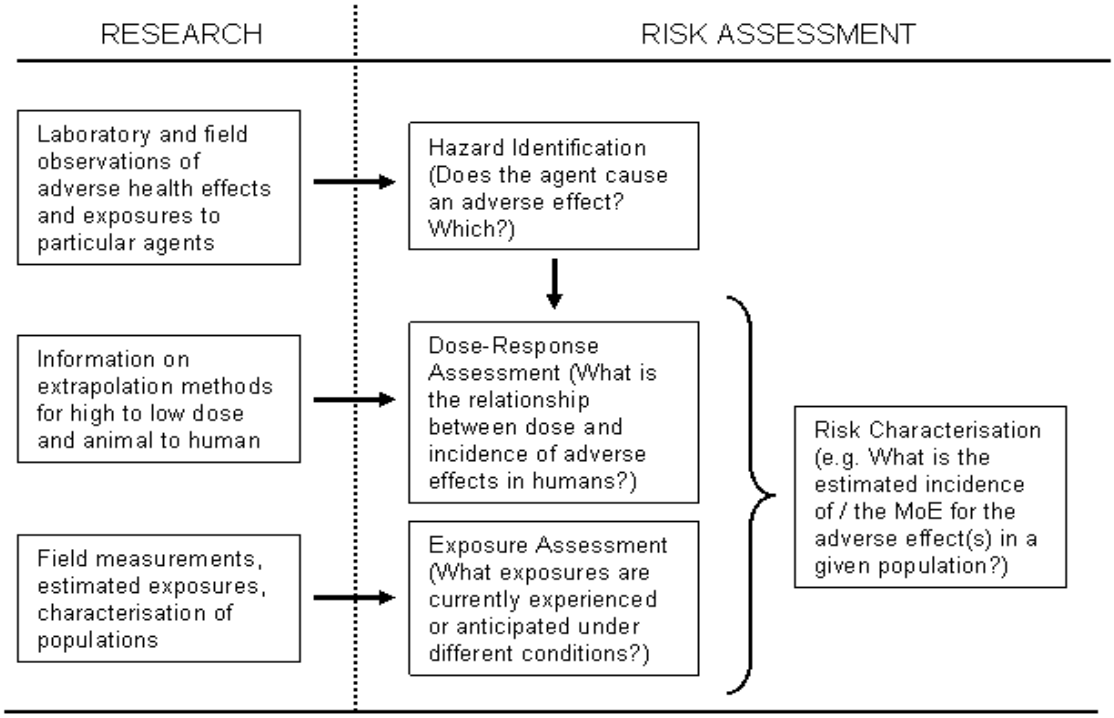


FIGURE 1. The Risk Assessment Paradigm for Human Health Assessment, adapted from NRC (1983).

8. The National Research Council (NRC) recently re-evaluated the 1983 risk assessment framework, in response to a charge from the U.S. Environmental Protection Agency (EPA) to recommend improvements in risk assessment as practiced (NRC 2009). In its report, the NRC recommended retaining the four basic steps of the risk assessment process, and also recommended additional steps to improve both the utility of risk assessment and the technical analyses supporting risk assessment. Among these, the NRC proposed adding an initial step in problem formulation and scoping, as well as revisions to the risk management phase to integrate the risk and non-risk information to systematically evaluate the options (Figure 2). With the goal of improving the utility of risk assessment, this NRC framework explicitly asks the question of what options there are to reduce the hazards or exposures that have been identified, and how can risk assessment be used to evaluate the merits of the various options. This approach can also help to reduce the "paralysis by analysis" problem that has occurred with some risk assessments in practice (NRC 2009). It can be noted that various elements proposed in the framework are currently implemented in regulations such as REACH and other (e.g. iterative risk assessment, formulation of testing proposals, stakeholder involvement).

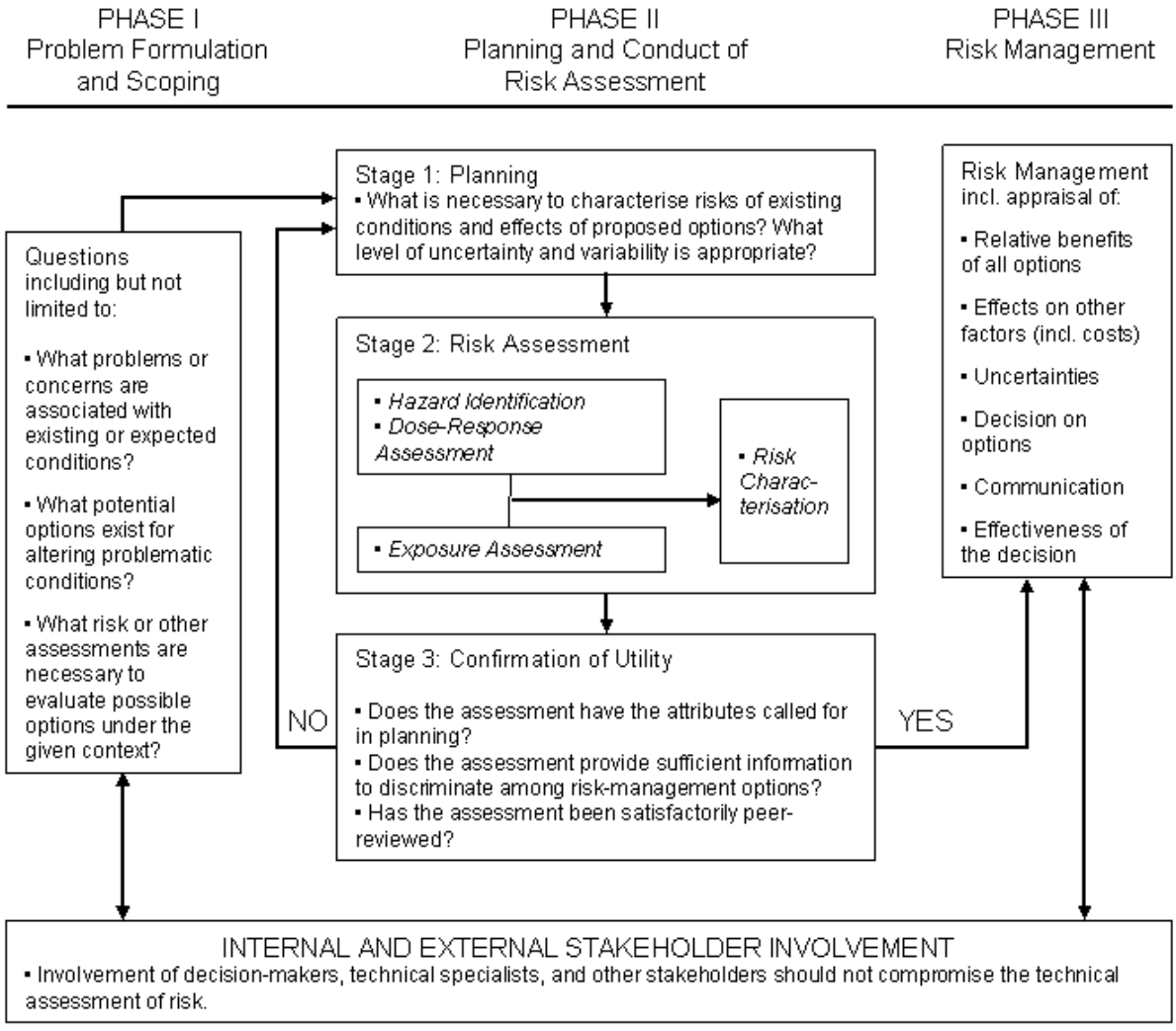


FIGURE 2. A framework for risk-based decision-making that maximizes the utility of risk assessment, adapted with modifications from NRC (2009).

9. Risk Assessment Outputs. The output of a risk assessment varies significantly depending on the availability and the quality of the supporting science, evidence, and analysis, as well as the needs of the end-user. Risk assessment tools and techniques allow for both robust qualitative descriptions of risk significance as well as quantified risk estimates. Although originally risk assessment outputs were usually qualitative descriptions (e.g. 'negligible, 'moderate' or 'severe'), methods have over time been developed to assess risk in quantitative terms and at different levels of sophistication from semi-quantitative and deterministic-quantitative to probabilistic-quantitative (NRC 2009). Such risk estimates will also be required if risk-benefit considerations should be intended. However, it is essential to ensure that risk estimates do not suggest a level of precision that the evidence base, with the uncertainty of mathematical derivations and subjective interpretations, does not support.

10. **Problem formulation.** The development of testable (falsifiable) hypotheses represents the first step in the cycle of formulation – testing – potential falsification – reformulation of scientific theories, which is regarded as an important epistemological (theory of knowledge) foundation for the continuous generation of scientific knowledge. The initial problem formulation step of the risk assessment process may include the formulation of testable risk hypotheses and plans to empirically validate or invalidate these hypotheses. In practice, however, the problem formulation stage remains a problem scoping exercise and a statement of the issue of concern to be addressed. It aims to answer "what/who is at risk?", and "what is it/are they at risk from?" Inadequate problem formulation results in inappropriate risk analysis (Pollard et al. 2004, Owen and Handy 2007), whilst good problem formulation guides the remainder of the assessment on other issues, including the relationship between the risk assessment and other decision components. Nanotechnology may provide an opportunity for upstream assessment of the physical and chemical properties of the materials (e.g. to inform the application of green chemistry approaches or selection of safer substances), which could lead to downstream risk reduction or avoidance. Considering nanomaterial risk assessment in this way during problem formulation may increase the utility of risk assessment to contribute to environmental sustainability.

11. **Bridging and read-across of data.** As an alternative to testing, toxicological properties of one substance may sometimes be inferred from those of a very similar substance or a group of related substances (see also OECD 2007).This relationship between data sets is a consideration at the problem formulation step because the risk assessment should take advantage of existing information where possible, and methods (such as Quantitative Structure-Activity Relationships (QSAR), *in vitro* methods to support bridging to bulk or other NM, etc.; OECD 2007) to "bridge" to existing data would need to be included in planning.

2.a. Health / Environmental Risk Assessment Framework

12. **Initial considerations.** The assessment of the effects of chemical exposure on human health and organisms in any environment involves the consideration of a range of properties and characteristics. Traditionally, the starting point for risk assessments of chemicals is an assessment of the physicochemical properties and possible exposure pathways. This is essential as it determines not only the extent to which various organisms (in environmental ecological risk assessment) or tissues (in human health risk assessment) might be exposed via different exposure routes, and therefore which toxicity data are most relevant, but also whether significant exposure is likely to occur at all.

13. **Human health risk assessment framework.** Based on problem formulation, the risk assessment may include one or more components to assess acute and chronic toxicity with regard to type of effect and targets of toxicity (endpoints) as well as dose-response relationship: an evaluation of acute toxicity, repeated dose toxicity, irritancy, sensitisation potential, genotoxicity, carcinogenicity and reproductive toxicity. Assessment regularly also includes supporting evaluation of toxicokinetic properties as well as mechanistic studies. The specific tests conducted and the routes of exposure used in the testing regime are

governed by the physicochemical properties of the substance, as well as its likely use and human exposure scenarios. Potential exposure routes include oral (delivered in the feed, drinking water or by gavage), dermal, inhalation and injection. The SG6 *Workshop* identified four areas to consider in addressing health risks from nanoparticles (OECD 2010a):

- Focusing testing approaches and the building of databases on enabling and advancing computational tools (e.g., QSARs, Quantitative Property-Property Relationships [QPPRs], physiologically based pharmaco(toxico)kinetic modelling [PBPK]) that facilitate our ability to categorize and otherwise efficiently group materials for decision making. Key to this is linking nanomaterial properties to effects;

- Understanding the particulate nature of nanomaterials, and in particular, particle kinetics which affects the distribution, disposition and the local dose of nanoparticles;

- Identifying whether there are nanoparticle-specific endpoints or nanospecific considerations for currently identified adverse effects of nanomaterials; and

- Advancing epidemiological approaches, including taking advantage of existing data and developing biomonitoring techniques.

14. That said, the overall human health risk assessment concepts for chemicals appear to be applicable to nanomaterials (OECD 2010a); in general the current set of test guidelines is adequate although adaptations may be required for the individual protocol (OECD 2009a); and our existing knowledge gained from the study of chemicals and (macro)particulates provides us with a basis of knowledge from which to investigate the special considerations related to manufactured nano-scale materials.

15. **Environmental risk assessment (ERA) framework.** Environmental risk assessment encompasses an understanding of how the substance behaves in different compartments of the environment, including consideration of its persistence, bioavailability, distribution and bioaccumulation. Studies may include the assessment of (bio)degradation, hydrolysis, bioconcentration, adsorption/desorption screening as well as short term aquatic ecotoxicity, growth inhibition study on algae, long term aquatic ecotoxicity testing, and effects on terrestrial organisms and micro-organisms. Potential exposure compartments to assess include surface water, Sewage treatment plant, soil, sediment, groundwater as well as the assessment of secondary poisoning. The SG6 *Workshop* identified the following components for consideration by risk assessors (OECD 2010a):

- Behaviour of nanomaterials in various media (e.g. dissolution, agglomeration/aggregation, adsorption, etc.): In the absence of empirical data, assessments could assume "worst case" behaviour, (e.g., the nanomaterial does not agglomerate or degrade, but remains dispersed);

- Persistence: Predictive techniques to predict aspects of degradation of certain nanomaterials were reported to exist, and these approaches could be applied when examining the physical persistence[4] of nanomaterials;

4 Persistence in conventional chemical assessment generally refers to the enduring state of a molecular structure, i.e. it is poorly susceptible to chemical change from biotic or abiotic processes. In the case of nanomaterials, persistence is often used to refer to the size and shape of the particles (physical persistence) as well as the more conventional use of the word. However it should be made clear in the use of these terms whether the nanomaterials are still present in another form (e.g., agglomerated).

- Transportation/Distribution: As in the ERA paradigm for traditional chemicals, information on behaviour and persistence should be used to address transport/distribution;

- Predicted Environmental Concentrations (PECs): Metrics of PECs remains a challenge and ERAs should include a justification for why a particular metric was used Furthermore, sufficient and appropriate information on exposure metrics/descriptors during ecotoxicity tests will need to be obtained to allow comparison with environmental exposure concentration information on the same basis or *vice versa*. An understanding should be developed of the forms of the nanomaterial present in the receiving environment (e.g., free primary particles, agglomerates/aggregates, ions etc.);

- Transformation Products and Impurities: Transformations of nanoparticles or their coatings may result in the changes to the particles' properties and can be the result of biotic or abiotic processes.. The importance of these changes to fate, transport, bioaccumulation, and toxicity should be determined. Furthermore, rapid transformations should be taken into consideration in the development of testing strategies particularly in terms of media preparation and dosing technique such that test outcomes reflect the most stable, environmentally relevant transformation product(s). Slow transformation would also be taken into consideration as part of the risk assessment as this may result in a shift in properties which may affect compartmentalisation and uptake. Nanomaterials may also act as carriers for other substances, and the potential for this should be addressed in the assessments;

- Bioaccumulation: No (validated) methods for quantitative prediction of bioaccumulation of nanomaterials exist. In the absence of empirical bioaccumulation data, qualitative judgments could be made based on information on non-nano material or actual data on similar substances. In addition, empirical studies should be further supported addressing the relevance of uptake by an organism in terms of whether the particles may cross cell membranes, whether they will be embedded in tissues and whether they release ions, etc.

- Effects / Predicted No Effect Concentration (PNEC): The basis for effects assessment must be empirical data on nanomaterial or analogue data, given that no predictive capacity currently exists. In addition, the use of acute data to predict chronic toxicity is currently not recommended, as extrapolation factors for nanomaterials are not available. Assessment could consider identifying the margin of exposure (or safety, MoE/MoS) between a (e.g., human-equivalent) NOAEC and exposure rather than employing uncertainty factors to obtain a PNEC.

2.b. Approaches to Chemicals' Risk Assessment and Regulation across OECD Countries

16. **Harmonisation.** Regulatory approaches for chemicals and manufactured nanomaterials differ within OECD countries. However, all are based on the basic risk assessment paradigm (Fig. 1) and the use of similar technical or scientific information to assess risks. With regard to defining, classifying and communicating hazard information, international cooperation has resulted in the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) which now provides common and consistent criteria replacing various different standards (UN 2009).

17. **EU risk assessment approach.** The Regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals **(**REACH)[5] together with the Classification, Labelling and Packaging (CLP) Regulation (1272/2008/EC) provide an excellent example for regulation of risk assessment. REACH includes the requirement for registration of substances (including its forms and

5 Regulation (EC) No 1907/2006; OJ L 396 of 30.12.2006

states) manufactured or imported by a company in quantities of 1 or more metric tonne per year to supply a technical dossier and, especially at volumes of 10 or more metric tonnes per year, a chemical safety assessment to be performed and reported by the registrant. Its provisions are underpinned by the precautionary principle. Although there are no provisions in REACH referring explicitly to nanomaterials, they are covered by the definition of "substance"[6] in REACH, and thus subject to the requirements of the regulation (EC 2008). REACH obliges the registrant to ensure that his registration(s) demonstrate(s) that all forms of the substance in his dossier(s) can be used safely. The focus of attention should therefore be on ensuring that the submitted data are applicable/appropriate for the all form(s) covered in a dossier(s) in question and on ensuring that the registrant has provided all relevant information to allow the safe use of the substance by the downstream users and consumers. Standard information requirements as they are described in the Annexes VII - XI apply equally to nanoforms and bulkforms. The registrant has to make sure that in case tests are performed these must be representative for the form(s) of the registered substance. Alternatively when read-across is used between the forms, the registrant has to make sure that this is scientifically justified. The technical adequacy of the REACH guidance for nanomaterials has been reviewed in REACH Implementation Projects on Nano (RIP-oN1, 2 and 3; Aitken 2011, Hankin 2011, JRC 2011). An assessment of the nanospecific aspects in relation to hazard and risks from nanomaterials on the market presently is about to start in view of implementation of the current legislation by companies. Data submitted by companies to the European Chemicals Agency ECHA due to the passing of the first deadlines of 1 December 2010 and 3 January 2011 for registration and notification should provide useful information regarding this issue.

18. **US risk assessment approach.** Statutory risk assessment controlling the importation and manufacture of new chemical substances in the United States of America is currently controlled under the Toxic Substances Control Act (TSCA)[7]. TSCA requires the US Environmental Protection Agency (EPA) to assess and regulate risks to human health and the environment before a new chemical substance is introduced into the market. Any available data on a new chemical substance (specifically including chemical structure and name) must be submitted as a Pre-Manufacture Notification (PMN) to the EPA. EPA classifies chemical substances as either "new" chemicals or "existing" chemicals, which are listed in the TSCA Chemical Substances Inventory[8]. Occupational risk assessment is conducted by the National Institute for Occupational Safety and Health (NIOSH) as a basis for developing recommended occupational health and safety recommendations. NIOSH transmits its recommendations to the Occupational Safety and Health Administration (OSHA) which is responsible for promulgating and enforcing occupational health and safety regulations in the U.S.

19. **Japan risk assessment approaches.** The recently amended "Chemical Substances Control Law" has introduced a new approach towards the risk of all existing and new chemicals for industrial use. Authorities of the law prioritize chemicals based on available information on hazard and environmental releases estimated from the manufactured amount and usages using a risk prioritisation matrix based on conservative assumption ("Screening" process), and then conduct risk assessment of those prioritized chemicals while collecting further information. This approach is expected to enable efficient risk assessment. A risk assessment approach for chemical substances used in the workplace is also

6 A substance = A chemical element and its compounds, in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used, but excluding any solvent, which may be separated without affecting the stability of the substance or changing its composition.

7 The Toxic Substances Control Act (15 U.S.C. 2601–2692) consists of Public Law 94–469 (Oct. 11, 1976; 90 Stat. 2003) and the amendments made by subsequent enactments.

8 A description of the US EPA approach to determine whether a nanoscale substance is a "new" chemical for the purposes of the TSCA inventory and the PMN requirements is available at: http://www.epa.gov/oppt/nano/nmsp-inventorypaper2008.pdf

implemented under the "Industrial Safety and Health Law". The Law also obliges the employer to endeavour investigation on risks due to chemical substances and taking necessary measures to prevent health impairment to workers. The Authority undertakes the risk assessment for high priority substances (highly hazardous substances) in order to enact rational regulations or measures.

20. **Common information requirements.** A feature of international risk assessment frameworks for chemicals across OECD is that they consider in conjunction the physiochemical characteristics of the chemical, the toxicological and the environmental effects. Although the exact legal requirements differ slightly between countries, all expect a certain degree of hazard identification and assessment. Components may include:

- *Physiochemical properties* – e.g. detailing melting/boiling point, relative density, vapour pressure, water solubility, flammability, partition coefficient (n-octanol/water), physical state;

- *Toxicological information* – evaluation of toxicokinetics, skin irritation/corrosion, eye irritation, skin sensitisation, mutagenicity (bacterial and mammalian cell studies), acute toxicity studies (route dependant on physical state of chemical), short or long term repeated dose toxicity study, reproductive toxicity study, and carcinogenicity; and

- *Ecotoxicological information* – degradation (biodegradability), hydrolysis (as function of pH), bioconcentration (fish), adsorption/desorption screening, short term aquatic toxicity testing (e.g. *Daphnia* and fish), growth inhibition study on algae, long term aquatic toxicity testing (*Daphnia* and fish), effects on terrestrial organisms, micro-organisms and other sediment dwelling organisms.

2.c. Occupational Exposure Assessments

21. **Exposure Measurement.** SG8 on Exposure Measurement and Exposure Mitigation has addressed the generation of exposure data for occupational risk assessments. In addition, the SG6 *Workshop* focused on the following discussion points:

- More exposure data are needed. OECD should develop a database of published exposure information involving all routes of exposure and promote publication of exposure data from companies, etc. The database should be stratified by routes of exposure;

- The detection limit of conventional methods to measure particles in the environment may be limited[9]. Therefore, it may be necessary to develop more sensitive methodologies to measure and characterize nanoparticles; and

- OECD in collaboration with ISO should define standardised exposure measurements for various media and exposure types that could be used to validate exposure metrics and instrumentation.

22. **Exposure Modelling.** Steering Group 8 has noted the availability and use of different simulation approaches and discussed their predictive capacity in relation to possible occupational, human and environmental exposure (OECD, 2009b).

9 It has been noted, that the inaccuracy of a measurement can be of significantly higher practical relevance than an insufficient detection limit for both, common chemicals and nanomaterials (cf. Conference on Workplace Aerosols, Karlsruhe, Germany, 28 June – 2 July 2010, http://www.gaef.de/workplace2010/frames/conference_structure.html).

2.d. Approaches to Risk Assessment of Particulates

23. **General.** Ultrafine particles are considered an aerosol particle in the nanoscale range (e.g. diesel exhaust particulates) and the occupational exposure of workers to ultrafine particles has been a well studied area (e.g. IEH, 1999). Health effects of fine and ultrafine dust originating from various sources including but not limited to combustion also remain an extensively discussed concern in the area of environmental medicine (e.g. WHO 2007). Principles and Critical Issues in their toxicological assessment have been laid-out in, for example, Schlesinger & Cassee (2003).

24. **Generic Occupational Exposure Limits (OELs) for particulate materials.** In the case of particulate materials, OEL settings have not always been scientifically-based. Historically, many particles were regarded as "nuisance" or "low toxicity" dusts, which meant that little attention was given to them although many workers were exposed. Only few dusts/particles have been reported to produce systemic toxicity. In addition, the control of exposure was difficult (e.g. in construction, mines and welding). As a consequence, a generic approach to standard-setting was taken for many particulates resulting in a generic inhalable OEL of 10 mg/m^3 and a respirable OEL of 4 mg/m^3 for many low-toxicity poorly-soluble dusts including aluminium oxides, graphite, platinum, titanium dioxide and others (IEH, 1999). In Germany, the DFG MAK commission recently reduced the OEL (MAK value) for biopersistent granular particles[10] from 3 to 0.3 mg/m^3 (respirable fraction), reflecting concerns about a possible carcinogenic potential (DFG 2011). All these values, however, were not intended for particulate materials with a known inhalation or systemic toxicity (e.g. asbestos and lead, respectively) for which specific OELs were also determined.

25. **Occupational exposure limits.** Currently, there are no specific regulatory OELs established for manufactured nanoparticles. Interim or draft OELs have been developed for certain nanomaterials, including "benchmark exposure levels" based on analogy with OELs for other particles or fibres (BSI 2007), and separate OELs for titanium dioxide based on particle size (NIOSH 2005; Dankovic et al. 2007). In addition, OELs have been proposed by some producers of multi-walled CNTs and an interim OEL for multi-walled CNTs has been issued (NIOSH 2010):

- The BSI[11] approach was intended to provide "pragmatic guidance levels," which were considered to be reasonably cautious levels based on an assumption that the hazard potential of the nanoparticle form is greater than that of the large particle form, for the following groups: Group 1: fibrous (high aspect ratio); Group 2: insoluble; Group 3: carcinogenic, mutagenic, asthmagenic, or reproductive toxicant; and Group 4: soluble nanomaterials.

- U.S. NIOSH developed particle size-based draft OELs for titanium dioxide using quantitative risk assessment methods applied to chronic inhalation data in rats. Benchmark dose estimates were derived from the relationship between particle surface dose and lung tumour response in rats and extrapolated to an equivalent dose in workers using a human lung dosimetry model. In addition, a draft REL (recommended exposure limit) was derived for CNTs (refer to section 2.e for details).

- AIST[12] of Japan proposed interim OELs for multi-walled CNTs, nanoscale titanium dioxide and fullerene (C60) based on pulmonary inflammation response in rats (Nakanishi (ed) a-c).

10 also: poorly soluble, low-toxicity (PSLT) particles

11 British Standards Institution

12 National Institute of Advanced Industrial Science and Technology

- OELs for two types of multi-walled CNTs have also been proposed by their producers using dose-response data from subchronic inhalation studies in rats (Pauluhn 2010a; Ma-Hock et al. 2009; Nanocyl 2009) (see also below).

26. Currently, no epidemiology data are available on adverse health effects of exposures to engineered nanoparticles. Therefore, dose-response data from animal studies are typically used to estimate risk in humans. Experimental studies in animals or *in vitro* are also used to evaluate hazard and understand mechanisms of toxicity.

27. The considerable variety in the types of nanomaterials presents a challenge to the efficient development of OELs for each specific nanomaterial. To improve the efficiency and effectiveness of the risk assessment process, additional strategies and methodologies (beyond a one-at-a-time approach) will be needed to evaluate hazard and risk in a timely manner on the increasing array of nanomaterials being developed. One approach proposed is to develop OELs based on categories of nanomaterials with similar properties and modes of action (Hansen et al. 2007; BSI 2007; Schulte et al. 2010).

2.e. Current Case Studies on Risk Assessment of Manufactured Nanomaterials: Carbon Nanotubes

28. **Risk Assessment for Carbon Nanotubes.** Carbon nanotubes (CNTs) are an example of manufactured nanomaterials that have been the subject of several recent risk assessments. CNTs can have wide variations in structure, size, shape and chemistry (including impurities) affecting their hazard properties, exposure potential and ultimately risk. To facilitate risk assessment of carbon nanotubes through modelling approaches, research is needed to correlate such variations with hazard and exposure potential. For practical purposes, it would be useful to determine the minimum differences that would make the properties of two CNT materials or samples of the same material distinct (i.e., variations from batch-to-batch, process-to-process, plant-to-plant, etc.). The SG6 *Workshop* included presentations on acute and subchronic inhalation studies to form the basis for assessing risk. The issue of dose metric was raised at the SG6 workshop, where data were presented showing dose-response relationships with CNT particle mass or volume (Pauluhn 2010b). Other studies suggest that particle surface area or fibre number may be more relevant to the biological effect (OECD 2010a). Until this issue is resolved, it is often recommended to extend the characterisation of CNT material in hazard and exposure studies in a way that allows for conversions between different metrics if necessary.

29. **Risk Assessment for specific CNTs.** Following the workshop, an approach to derive an OEL was published for a specific multi-walled CNT (produced by Bayer and marketed under the trade name Baytubes) (Pauluhn, 2010a). This type of CNT had been examined in single and repeated (subchronic) rat inhalation studies, also addressing kinetic endpoints, the time course of pulmonary inflammation in response to treatment, as well as reversibility of effects during a 3 and 6 month post-exposure period (Pauluhn 2010b). On this basis, a mechanistic (conceptual) model was developed forming the basis for interspecies extrapolation. When accounting for differences in alveolar deposition, ventilation parameters and particle clearance, the authors derived at an overall extrapolation factor of 2 and a value of 0.05 mg/m^3 was considered to be reasonably protective as an OEL. Uncertainty factors, e.g. to account for intraspecies variability, however, were not applied.

30. Another risk assessment on a multi-walled CNT produced by Nanocyl for BASF was also based on a 90-day inhalation study in rats (following OECD 413 guidelines) (Ma-Hock et al. 2009; Nanocyl 2009). Starting from a LOAEL of 0.1 mg/m^3, an assessment factor of 40 was applied, resulting in an estimated "no effect" concentration in air of 0.0025 mg/m^3 for 8-hr/day exposure (Nanocyl 2009, Lecloux & Luizi 2009).

31. **Derivation of a draft REL for CNTs.** NIOSH in the U.S. recently issued a draft Current Intelligence Bulletin (CIB) on Occupational Exposure to Carbon Nanotubes and Nanofibers which included a risk assessment and a recommended exposure limit (REL) of 7 μg/m^3 (8-hr time-weighted average (TWA) concentration) for public review (NIOSH 2010). The quantitative risk assessment included estimation of benchmark doses using dose-response data from the two subchronic inhalation studies of two types of MWCNTs (Ma-Hock et al. 2009; Pauluhn 2010b), as well as dose-response data from several studies of SWCNTs and other MWCNTs in rats or mice by intratracheal instillation or pharyngeal aspiration. Response endpoints included pulmonary granulomatous inflammation and fibrosis. Risk estimates were derived by assuming either no clearance of the estimated deposited lung dose of CNT or normal clearance based on spherical particle models, which was considered to bound the uncertainty associated with CNT lung dose estimation. There was considerable variability in the risk estimates, although all estimates were associated with low airborne mass concentrations relative to other poorly soluble particles. The variability was due, in part, to the differences across studies including the type of CNT, rodent model, route of exposure, duration, and response endpoint. The data were insufficient to discern a role of the physicochemical properties of the various CNT types and the lung responses. The NIOSH draft REL of 7 μg/m^3 (8-hr TWA concentration) was set at the limit of quantification (LOQ) of the analytical method to measure the airborne CNT in the workplace (NIOSH method 5040 for elemental carbon), The risk estimates indicate a greater than 10% excess risk of early-stage lung effects if exposed at the LOQ over a working lifetime. Based on a study in mice showing similar pulmonary response to carbon nanofibres (CNF), and workplace exposure data showing mixed exposures to CNF and CNT, NIOSH included CNF in the REL for CNT and CNF. NIOSH described areas of uncertainty in the risk assessment and research needs. Among these, the need for data on potential chronic effects, including cancer, was noted.

32. **Conclusions from the SG6 Workshop on Risk Assessment of Manufactured Nanomaterials in a Regulatory Context in Washington DC, 2009.** In addition to CNTs, the Workshop discussed titanium dioxide and silver nanomaterials with regard to the data available, knowledge gaps, and the current risk assessment results. The following conclusions were produced (please refer to the Workshop Report for additional information, OECD 2010a):

1) The risk assessment paradigm for chemicals will continue to guide approaches to the risk assessment of nanomaterials, and no fundamental changes to this paradigm are envisioned. However, because of the limited amount of empirical data on nanomaterials, many of the assumptions and estimations employed in chemical risk assessments (e.g., acute-to-chronic ratios, estimation of bioaccumulation potential, estimation of persistence) need to be evaluated for nanomaterials;

2) As with any risk assessment, extrapolation approaches for nanomaterials should be based on mechanistic data where available and additional research is needed to support the validity of default assumptions. Furthermore, limiting exposures and releases of nanomaterials should be encouraged wherever possible as an interim measure in order to compensate for the current limitations in the science;

3) Although the basic risk assessment paradigm for nanomaterials is essentially the same as for traditional chemicals, research is needed to determine what characteristics of nanomaterials may pose unique hazards;

4) There does not appear to be a scientific rational to justify employing a risk assessment uncertainty factor specifically addressing materials at the nanoscale. In addition, application of standard risk assessment uncertainty factors in nanomaterial risk assessments should undergo validation; justification should also be provided when using invalidated

uncertainty factors in risk assessments. Identification of a "margin of exposure" may be an alternative approach to understanding likelihood of risk; and

5) It is recognised that there is uncertainty concerning the units of measurement (i.e., metrics) used to generate test results employed in risk assessments. It is expected that empirical results will continue to be reported in terms of mass based units; however, risk assessments should include discussion of any limitations this metric may present (e.g., limit of detection, specificity). Characterisation of nanomaterials by various dose metrics (e.g., particle surface area, number concentration, etc) would facilitate evaluation of the metrics most closely associated with mechanism of action and improve risk estimation.

3. Risk Assessment of Nanomaterials: Important Issues

3.a. Problem Formulation

33. **Problem Formulation.** Problem formulation, i.e. formulation of risk hypotheses requires precise definition of relevant sources and targets of suspected harm. Under-utilisation of this tool may lead to poor risk assessment. Furthermore, for nanomaterials, the mostly limited depth of information (qualitative and quantitative) on sources and targets of harm may represent a hurdle in problem-formulation. The SG6 2009 *Workshop* (OECD 2010a) included a discussion on problem formulation needs:

- Consider the "particle nature" of the material, such as the surface properties and interactions, the relation of metrics used, the characteristics of the material;

- Assess and accommodate risk assessment approaches with regard to the effects of test methods and exposure matrix (e.g., dispersion methods) on testing outcomes and on inter-comparability of the data used in the assessment; and

- Include particular attention to the complex nature of the material (e.g., variation in size, surface properties, and composition that create a heterogeneous range of particle types) and its interaction with environmental components and transport mechanisms in exposure and toxicity contexts.

34. **Sources of Potential Harm.** Nanoparticles are known to be unintentionally produced and released into the atmosphere by natural phenomena and many human industrial and domestic endeavours, such as transportation utilizing internal combustion and jet engines,. In recent years a new type of source of nanoparticles has been introduced, within the sphere of intentionally engineered nanoscale components of consumer products and advanced technologies. For these engineered nanoscale components, two separate types of nanostructure may be identified, those where the structure itself is a free particle (or agglomerate or aggregate thereof), and those where the nanostructure is an integral feature of a larger object (e.g. an ultrathin surface coating or semiconducting layer). Particularly the many uncertainties in production, use and fate and behaviour of free insoluble nanoparticles give rise to concerns over possible human health and environmental risks. However, SCENIHR (2009) also concludes that "The hypothesis that smaller means more reactive and thus more toxic cannot be substantiated by the published data. In this respect nanomaterials are similar to normal substances in that some may be toxic and some may not. As there is not yet a generally applicable paradigm for nanomaterial hazard identification, a case by case approach for the risk assessment of nanomaterials is recommended." Nevertheless, information on mode of action and structure-activity relationships may facilitate development of categorical-based hazard and risk characterisation (OECD 2007).

35. **Nanomaterial Identification.** The nanomaterial for which a risk assessment is performed (i.e. the scope) needs proper definition. In the absence of an international definition, OECD WPMN has applied for the practical work a working definition based on ISO and other relevant considerations. The identification of a nanomaterial includes appropriate naming as a key element. Properties to be considered as identifier could include chemical composition, crystallinity, surface coatings, morphology, size (range), etc. (OECD 2009c).

36. **Variability in Composition and Properties.** Unlike discrete chemicals, nanomaterials can be present as substances with a variable composition that goes beyond variations at the level of impurities. Examples include variations in size and size distribution, surface properties or composition of the nanoform itself. Strategies to accommodate for this particular character also during testing should be developed and introduced.

37. **Nanomaterial Characterisation.** In the context of nanomaterial characterisation it is noted that describing the properties of the primary nano-objects of the material (nanoparticles, -fibres, -sheets) themselves is essential but not sufficient. The interactions between the nanoobjects within a given environment or formulation must be considered as well as interactions of the material with components thereof. The physicochemical properties and material characterisation that may be required for testing are described in more detail in OECD (2008). Specialised instrumentation that is not usually available in test facilities may also be needed for characterisation of the material within the vehicle (as prepared for dosing).

38. **Selection of Assessment Endpoints.** To provide direction and boundaries for RA, the specific entity to be protected, such as individuals, a species, a sub-population, a community, an ecosystem, etc. has to be identified. In addition, the concerns or effects to be protected from (e.g. reduced survival and reproductive impairments in ecological RA) generally require definition. For nanomaterials, this may be complicated by an incomplete knowledge about its behaviour throughout the lifecycle and limited experience with toxicity in the target species or population.

39. **Testing Plan**. Generally, good problem formulation allows for clear definition of the minimum data required to show safety. For regulation of conventional chemicals, standard data requirements have been prescribed, based on extensive experience, for substance categories such as pesticides. Such standard requirements may need adaptation for nano-scale materials. The same applies to the practice of use of other existing data and methods to "bridge" to that existing data. Especially the level of generalisation that can and should occur needs to be evaluated and a scientifically sound approach that allows for inclusion of information obtained using dissimilar materials (even non-nanoscale), methods or reporting has to be defined (OECD 2007).

3.b. Hazard Identification

40. **Applicability of Testing Methods.** The direct hazard that specific nanomaterials present to human health and to the environment will depend on the physicochemical (and chemical) characteristics of the surface and core of the nano-objects, and the extent to which the material exhibits interactions with biological systems associated. SCENIHR (2006) noted the insufficiency of scientific information about the physiological responses to nanoparticles, about the mechanisms of interaction at sub-cellular level (see also below), and about the changes in the nanoparticle physicochemical characteristics like agglomeration and aggregation, surface modification, dissociation, degradation, adsorption of different species, etc. Those changes would depend on the size/shape of the particle as well as on the local environmental and cellular conditions (ionic strength, acidity, viscosity, etc.) Therefore, the methods used in the hazard identification and assessment may also need to be augmented to include all of the above considerations.

41. **Endpoints Assessed.** Hazards are commonly identified in standardised acute and chronic (eco) toxicity tests. As concluded by SG4 in its Preliminary Review of OECD Test Guideline for their Applicability to Manufactured Nanomaterials (OECD 2009a,), the OECD guidelines are in general considered applicable to manufactured nanomaterials, particularly with regard to investigating their health effects, with the important proviso that additional consideration needs to be given to the physicochemical characteristics of the material tested, including dosing. In some cases, there may be a need for further modification to the OECD guidelines. Preparation of samples and dose administration are critical considerations for the tests and therefore guidance has been developed on sample preparation and dosimetry for the safety testing of nanomaterials (OECD 2010c). The preliminary review of OECD-WPMN is consequently seen as a "living" document, highlighting the feasibility of various approaches and allowing for continuous updates, given the rapid developments in this area. Nevertheless, there may be remaining uncertainty at the moment in the respect that specific toxicity (mechanisms) related to the size or the particle nature of specific nanomaterial may be overlooked since standardised tests are usually aimed at

a specific endpoint based on experience with, supposedly, non-nanomaterials. As the field of nanotoxicology advances, this uncertainty may be reduced through additional research.

42. **Target Organs.** The current OECD Test Guidelines in principle enable the assessment of the all the possible target organs affected (OECD 2009a). Toxicokinetic studies may provide useful information in this context. More specifically, given the indications that nanoparticles could migrate from the respiratory tract to the blood and on to the brain (or translocate directly via the olfactory nerves), SCENIHR (2006) emphasised the need for the development of quantitative assays that could determine the presence of actual nanoparticles in different tissues of the human or animal body. To date, toxicokinetics usually relies on measurement of the primary matter or bound residues of metal catalysts rather than the nanomaterial as such (e.g. Ti for nano-TiO2 or Co for Baytube CNTs; Chen 2009, Pauluhn 2010a). Taking into account the slow body clearance observed for some nanomaterials (e.g. Chen 2009, Pauluhn 2010a), local accumulation may play an important role.

43. **Effective Dose.** The effective concentration or dose (that results in an adverse biological response) derived for a manufactured nanoparticle from laboratory studies is likely to be influenced by the abiotic (and biotic) composition of the exposure pathway, variations in which may influence nanoparticle structure, form and behaviour.. To give one example, in aquatic systems some relevant abiotic factors are pH, ionic strength and the concentration of humic substances in the aquatic matrix. These are known to influence and modify physicochemical characteristics of the particle, notably agglomeration and aggregation. Notably, the effective dose of a nanomaterial may be smaller on a mass basis than the effective dose of larger particles of the same material if the mode of action relates to the total particle number or surface area (Handy 2008).

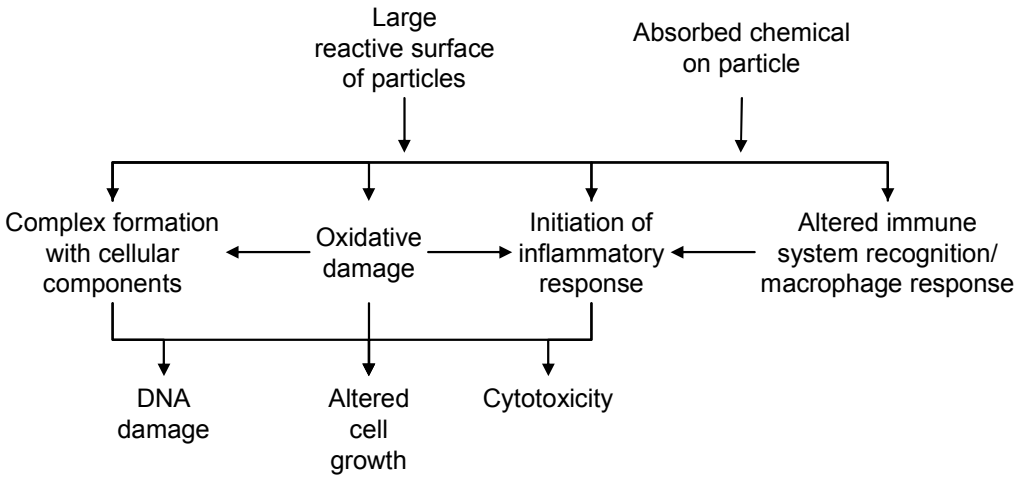
44. **External Factors Influencing Toxicity.** One factor determining particle behaviour is how the particular natural environment will influence important physicochemical characteristics such as surface charge, and/or agglomeration and aggregation. Thus, abiotic factors may play critical roles in the context of bioavailability, distribution, bioaccumulation and, ultimately, toxicity of nanomaterials when exposure occurs in natural settings. In some cases, specific environmental components, esp. biopolymers, absorb stably to a particle surface (Handy 2008). One phenomenon identified in this context is the formation of a "protein corona" (Maiorano 2010).

45. **Variability of External Factors.** A number of abiotic and biotic factors that influence nanoparticle toxicity may be variable themselves as well, depending on the (receiving) environment, which can be highly complex (e.g. estuaries where pH and ionic strength can vary considerably) (Handy 2008). In principle, this is an issue not exclusive to nanomaterials, but the specific factors of relevance, their variability and impact may be different from what is expected.

46. **Definition of Adversity.** OECD Test Guidelines refer to adverse effects and define it in a following manner: "Change in the morphology, physiology, growth, development, reproduction or life span of an organism, system, or (sub) population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences" (OECD 2003). For nanomaterials, there is debate about the definition of adversity for specific effects. One example is, whether the presence of nanoparticles in the brain is an adverse event as such or if there should be (indication of) proof that the brain function or structure is negatively affected by the presence of the nanoparticles before it can be regarded as an adverse effect. This applies to human health hazard assessments as well as to ecotoxicology.

47. **Mechanistic Considerations**. A number of mechanisms by which toxic nanoparticles may exert their effects have been proposed and these are summarised in the figure below (adapted from SCENIHR

2006). However, a full understanding of the various mechanisms involved in nanomaterial toxicity is still lacking.



48. **Non-Nano – to – Nano Extrapolation**. As already pointed out by other organisations and bodies (e.g. SCENIHR 2010), the term "nanomaterial" relates to a categorisation by size, and would not per se imply specific risks or hazard properties. It is noted, however, that reduction in size to the nanoscale may or may not change the characteristics of particles (SCENIHR 2009), and observed differences in biological effects may be due to the increased surface to volume or surface to mass ratio. In addition, size can influence the materials (bio) distribution and the kinetics thereof in an organism or an ecosystem. Finally, the structuring of matter at the nanoscale is characterised by the interplay of classical physics and quantum mechanics which could lead to some novel characteristics vis-à-vis the same material without nanoscale features. It was apparent from discussion during the SG6 workshop that the development of relationships between existing data on nanoscale and non-nanoscale materials may be difficult due to limited or lack of data (OECD 2010a). For some materials (e.g., poorly soluble low toxicity particles), the surface area of the particles has been related to the lung response, such that nanoparticles were more inflammogenic than the same mass of larger particles of the same chemical composition (Bermudez et al. 2002, 2004; Elder et al. 2005). In these cases, hazard/risk grouping strategies may be considered for particles with the same mode of action.

49. **Nano – to – Nano Extrapolation**. The nanomaterial properties determining its toxicokinetics and toxicodynamics are currently not known with confidence. Therefore, it should be realised that different nanoforms/sizes may show differences in effect concentrations and/or effect parameters. Importantly, these uncertainties mean that methodologies to permit extrapolation between different types of nanomaterials and different species are not available, implying that assessments often have to be made *de novo*, on a case-by-case basis (see also "problem formulation"). Additional data are needed to link the biological effects with the physicochemical properties of nanomaterials in order to develop predictive hazard/risk grouping strategies (Rushton et al. 2010, OECD 2010b, OECD 2010c).

50. **Nanomaterials Acting as Carriers**. Chemical risk assessments may consider whether chemicals absorb onto particulate matter as this phenomenon can influence the transport and compartmentalisation of chemicals (SCENIHR 2006). Likewise, the particulate nature of nanomaterials may result in the adsorption of contaminants which could influence the transport of chemicals and metals of concern (Bastian 2009). This is referred to as the "Trojan horse" carrier concept (e.g. arsenic adsorbed to the surface of a nanomaterial travelling across a cell membrane; Shipley 2009).

3.c. Hazard Assessment

51. **Hazard Assessment for Classification and Labelling (C&L)**. It is noted that irrespective of the exposure, a hazard assessment is needed for the purposes of C&L of any substance, including manufactured nanomaterials.

52. **Identification of the Toxic Principle[13].** The SCENIHR 2006 considered the identification of the toxic principle of a given nanomaterial a critical issue to be resolved initially in hazard assessment. The hazard may be due principally to, for example:

- the toxicological properties of the chemical(s) that comprise the core of the nanoparticle, or the influence of functionalisation of the nanoparticle surface;
- the much greater relative surface area of the nanoform and, consequently, the greater potential reactivity;
- the potential, due to the enhanced surface area and possible surface reactivity, for other chemicals of concern to be absorbed onto the nanoparticles; and/or
- contaminants and/or by-products related to the nanoparticle production (e.g., metal catalysts).
- If contaminants or by-products related to the nanoparticle production (e.g., metal catalysts) are responsible for the hazard, this may also affect the results of risk assessment and management (Liu et al. 2008).

53. **Dose Metrics**. For nanomaterials the actual metric that best describes the observed effects in test organisms or environmental fate and distribution may not be mass-based, usually expressed as mg/kg body weight or mg/L (or mg/m^3). There are indications that, for example, the number of nanoparticles, the surface area, or another metric can be in some cases a better metric to relate dose to the observed fate, behaviour, and effects of a specific nanomaterial relative these effects across a range of particle sizes (also rf. to chapter 2 and Aitken 2011, Hankin 2011). Knowledge about the mechanisms underlying the observed effect (but also determining fate) would be required to make a decision on the scientifically most appropriate dose metrics on a case-by-case basis or for defined groups on nanomaterials. Using another metric, however, will need further discussion, and might also have major consequences for the international Mutual Data Acceptance as well as for most legislations. Altering the metrics for hazard would require also using consistent units for exposure and risk estimation. This includes classification and labelling, where most hazards of a substance are related to mass concentration.

54. **Material Heterogeneity and Batch-to-Batch Variation**. Depending on the outcome of the "Nanomaterial Identification" step (see above), there may be substantial variation in properties between samples of the "same" material from producer to producer and/or batch to batch[14]. For carbon nanotubes (CNTs), for example, especially multi-walled CNTs, variation in length, metal content, aggregation and surface chemistry of the produced material is known and expected to influence measured toxicity (Johnston 2010). Heterogeneity in the degree of surface modification and/or aggregation was reported to

13 Toxic Principle: Describes the constituent or the substructure of a given material that is responsible for the toxic effects of that material (e.g. an impurity, an aspect ratio, a surface charge etc.)

14 In principle, this consideration is not limited to nanomaterials and applies to any other form of chemical substances, but the typical spectrum of properties affected would be expected to be different (see also chapter 2).

influence the activity of fullerenes (Chae 2010) and other nanoscale materials. Such variability may cause (quantitative) differences in toxicological effects and thus affect the outcome of hazard characterisation.

55. **Relevant Nanomaterial (Sub) Species**. The specific form(s) or (sub)species of a heterogenous nanomaterial to which humans or the environment may finally be exposed, as well as the specific activities of this particular fraction are largely unknown. This situation is further complicated by the fact that during their life-cycle, nanomaterials can transform from one form to another (e.g. coated – uncoated, oxidised - reduced), agglomerate or aggregate, and dissolve in part or completely. Therefore, information on the state of the nanomaterial in situ and the specific form that causes the observed effect could potentially reduce the degree of uncertainty.

56. **Linking Material Properties to ADME (Absorption, Distribution, Metabolism and Elimination) and Toxic Effects**. In view of the diversity of nanomaterials also within one "material class", there is an urgent need for valid approaches to categorise or otherwise group nanomaterials in order to allow read-across or bridging of data for assessment (and decision making). Development of an understanding how material properties are linked to ADME and toxicity would assist in building of categories and enable QSAR approaches.

57. **Definition of Biologically Relevant Properties**. A well known example of how abiotic and biotic factors influence bioavailability, bioaccumulation and toxicity of nanomaterials is that of asbestos fibres[15]. Here, it is accepted that a combination of the aspect ratio of the fibre (or shape) i.e. length and width, and the durability or biopersistence of the nanofibre, in the context of the physiological response in the airways and the macrophages in the lung (i.e. clearance), are the critical determinants of subsequent toxicity and pathology. This demonstrates the need to acknowledge and understand such complex interactions when predicting toxicity and pathogenicity for nanomaterials when exposure occurs in natural settings. Without such knowledge, the descriptors chosen for substance identification may be unsuitable, leading to inclusion of less or non-hazardous material in a high hazard category and *vice versa*.

58. **Biological Relevance of Testing Conditions**. Another consequence of such complex interactions between nanomaterials and abiotic and biotic factors is that exposure to single nanomaterials following some of the recommended standard test protocols (e.g. a daphnid or fish test using standardised de-ionised water) may have only limited relevance when compared with the natural environment in which exposure occurs. There, abiotic and biotic factors can greatly change the structure, form, behaviour and fate of nanomaterials and may thereby influence their bioavailability and toxicity to a larger extent and through other reactions than known or expected for conventional (non-nano)materials. Relevance is likely to vary with both the method chosen (*in vitro / in vivo*) and exposure route (e.g. water, air).

59. **Assessment of the Quantitative Relevance of Testing Conditions**. The implications of interacting factors such as, for example, dispersion media and protocol and their unknown relevance are that a considerable measure of uncertainty is introduced to the calculation of a Lowest or No Observed Effects Concentration (LOAEC/NOAEC) when using some of the current standard tests employed for chemicals. This is an issue that should be further considered by SG4 in its review of OECD test methods.

60. **Extrapolation from Aquatic to Terrestrial Environment**. With respect to the environment, similar issues of interacting factors and the associated relevance of standard tests will also apply to terrestrial systems although, based on the experience with 'conventional' chemicals, aquatic toxicity data are likely to be the primary information required for assessing risks to both aquatic and terrestrial environments in regulatory contexts (Crane 2008). This is, however, based on the knowledge that for 'conventional' (non-nano) materials aquatic data can be extrapolated based on carbon content to the

15 Asbestos is only given as an example of bioavailability and bioaccumulation on toxicity in this paragraph.

terrestrial environment. At present there is no scientific evidence that this can be done for nanomaterials as well. This issue also applies to the qualitative identification of hazard to the terrestrial systems from aquatic toxicity data.

61. **Relevant Species**. Similarly, the selection of test species for human health but also environmental risk assessment requires consideration. In human health risk assessment, for example, the rat is usually considered as the most sensitive species for inflammatory changes in the lung in repeated dose inhalation toxicity testing of nanomaterials (Becker 2011). This is fuelling an ongoing debate with regard to how (qualitatively and quantitatively) respective findings from inhalation toxicity studies of nanomaterials in rats - but not or to a lesser extent in other species like mouse, hamster or guinea pig - should be extrapolated to man. In addition, it may be assumed that because our current understanding with regard to the physiological and pathological processes involved in adverse reactions towards nanomaterials is in general smaller than for many classes of chemicals, this would also apply for quantitative and qualitative differences of those pathways. In this context, it should be noted that the choice of species may also influence the numbers of animals and dose groups that can be examined with reasonable effort as well as the number of endpoints included and the level of confidence with which these can be assessed.

62. **Nanomaterial Identity and Dose in Historic Data**. Similar to existing chemicals, some nanomaterials have been on the market for over 50 years, e.g. nanoscale forms of silver which have been used for its antibacterial properties, although not necessarily in the same formulations or applications (OECD 2010a). It is generally agreed that the accumulated historic safety information should be taken advantage of. It remains to be resolved on a case-by-case basis, however, how to integrate this data in current hazard assessment. As concluded at the 2009 Workshop of SG6, the relationship is often not clear between current and older data sets on nanoscale materials as different methods may have been used or measurements may have been less precise (OECD 2010a). Today's techniques and equipment for determining nanoparticle properties (BET surface[16], zeta potential, SMPS[17], etc.) and dosage (e.g. ICP-MS[18], particle counting) may not have been available or as sensitive at the time, which could hamper identifying dose equivalence and whether the historical and recent data were determined for the same material. Even if a certain historical product is still on the market today and thus can be analysed with today's techniques, one cannot be certain that the techniques to manufacture the product are still the same. Approaches to provide adequate scientific proof for equivalence may be developed.

63. **Toxicological Endpoints in Historic Data**. A similar uncertainty can be expected with regard to the historic toxicity data collected for such a material. When it is established (or can be reasonably assumed) that the tested material is equivalent to the material under assessment, some validation will be needed to ascertain that historically determined toxicity levels are similar to those that can be established with modern techniques. This issue is clearly not "nano-specific", but it should be taken into account, that measurement endpoints with particular relevance for nanomaterial toxicity (e.g. Bronchoalveolar Lavage (BAL) parameters / parameters indicative of fibrosis or immunotoxic effects[19]) may have been included at a different schedule as other enhancements. Reference is also made to the "Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials" (OECD 2009a).

16 A technique to determine specific surface area using the physical adsorption of gas molecules on a solid surface, first described by S. Brunauer, P. H. Emmett and E. Teller (BET) (J. Am. Chem. Soc., 1938, 60, 309-319).

17 Scanning Mobility Particle Sizing

18 Inductively Coupled Plasma Mass Spectroscopy

19 Included in revised OECD TG 412: Guideline for the Testing of Chemicals: Sub acute Inhalation Toxicity: 28-Day Study, adopted 7 September 2009)

64. **Epidemiological and Medical Data.** Generation of epidemiological data as basis for hazard identification and assessment requires development and follow-up of nanomaterial worker cohorts and exposure registries. Practical experience suggests that case reports need timely validation especially with regard to factual exposure to nanomaterial and identity thereof in order to be useful for risk assessment purposes.

3.d. Issues Relating to Exposure

65. **External Exposure**. Exposure assessment provides an evaluation of the extent to which people and/or the environment are exposed to nanomaterials. Ideally, this assessment is quantitative, but may remain qualitative (yes/no/negligible) in cases. Good problem formulation is needed to consider all relevant sources of potential exposure, including indirect exposure (i.e. exposure to nanoparticles unintentionally transported outside of manufacturing sites and laboratories by workers or unintentionally released into environment during manufacturing and storing). At the same time, initial assessment of variables influencing external exposure to nanomaterials will drive reformulation of risk hypotheses. A set of relevant questions may be developed similar to that proposed by SCENIHR (2006).

66. **Background and Cumulative Exposure**. Nanoparticles of natural origin and those generated unintentionally by human activity involve all individuals to be routinely exposed to nanoparticles throughout life. The increasing use of manufactured nanoparticles adds to this exposure. Hence, the assessment of risks from cumulative and aggregate exposure to nanomaterials requires consideration. In addition, "background noise" may present a challenge to exposure measurements.

67. **Internal Exposure Following Inhalation**. For many applications, the principal route of potential human exposure to nanomaterials is by inhalation in view of their presence in air. The general pathways for the mechanical clearance of insoluble particles deposited in the pulmonary region are thought to be well understood, involving either phagocytosis by alveolar macrophages usually and clearance via the mucociliary escalator into the gastrointestinal tract or passage through/into the respiratory epithelium that may be passive or active (Schlesinger 1995, SCENIHR 2006). Possible ways to use this knowledge e.g. for derivation of refined adjustment factors in risk assessment of particulates have already been suggested, for example by Pauluhn (2010a). However, for improved quantitative hazard assessment, methods and (mathematical) tools similar to PBPK / TK[20] models for "conventional" chemicals to describe not only isolated steps but the pathway(s) as a whole may be required.

68. **Other Routes of Exposure**. The rapidly increasing use of manufactured nanoparticles in consumer products, pharmaceutical preparations and food technology implies that dermal, gastrointestinal, and parenteral routes of exposure are becoming more significant. It may be expected that migration studies and human biomonitoring approaches would improve the current level of knowledge on the factual relevance of these pathways.

69. **Internal Exposure following Ingestion and Dermal Exposure**. Although the present database seems to suggest that exposure of internal tissues to nanomaterials through absorption by the oral and dermal routes is low or undetectable, a mechanistic understanding is required for quantitative assessment (but also qualitative statements). At present, such understanding of the molecular and cellular barriers as well as passages is limited.

70. **Internal Exposure through Distribution**. After deposition of nanomaterial in the respiratory tract, translocation to the lung interstitium, liver, spleen and possibly to the foetus in pregnant females as well as to the brain has been described (MacNee et al 2000, Oberdörster et al 2000, 2002). Nanoparticle

20 Physiologically Based Pharmakokinetik / Toxikokinetic Modelling

translocation to the brain may also occur via the neuronal transport, a "novel" pathway relative to larger particles (Oberdörster et al. 2004). Following oral and parenteral exposure, material was found e.g. in blood, liver, spleen or kidneys (Chen 2009, Wang 2007). There are limited data available on these pathways on both the qualitative and the quantitative aspects. In addition, it may be noted that analytical methods currently available for biodistribution analysis (often ICP-MS or autoradiography) may be limited, for example with regard to discrimination between nano and non-nano (including molecular/ionic) forms of the material.

71. **Applicability of Tools for Exposure Modelling**. For exposure assessment, models are currently in use for chemicals to provide exposure estimates for the environment (e.g. EUSES), consumers (e.g. ConsExpo) and workers (e.g. EASE). Input for environmental exposure models are often based on QSPR[21] calculations using physicochemical properties of the substance, mainly K_{OW} and K_p values. At the moment the applicability of these QSPRs for nanomaterials is being tested (OECD 2011).

72. **Adaptation of Tools for Exposure Modelling**. In addition, the exposure models currently in use were not designed for nanomaterials. Some assumptions that have been incorporated in these models (e.g. the time a particle remains airborne or solubility/insolubility in environmental media) may have to be adjusted for nanomaterials[22]. At the moment, however, there is a lack of reference data in order to incorporate relevant nanospecific parameters into these models. Considering the present uncertainties in exposure to nanomaterials the following information appears to be essential for adaptation of the models:

- information on the life-cycle of the specific nanomaterial. Especially information on the (nano)form to which humans or the environment may be exposed is essential (*e.g.* as manufactured or as used);

- information on the distribution of the (different) nanoform(s) over the environmental compartments (*i.e.* partition coefficients for sediment/water, soil/water and air/water may have to be measured);

- information on the aerodynamic size distribution to estimate the inhalation and deposition fractions, as well as retention or clearance of nanoparticles; and

- information on the fraction of the particles that will pass the barrier of the skin and gastrointestinal tract for a proper estimation of the internal exposure.

73. **Conceptual Exposure Models**. The development and subsequent validation of a conceptual model of exposure for the nanomaterial concerned can help to reduce uncertainty in assessment of exposure and thus risk. Such a conceptual model may be underpinned by a life cycle assessment approach that considers sources and pathways of exposure during production, use and end-of-life (e.g. waste disposal; Köhler et al. 2008). The model(s) can be used to develop emission scenarios and mass flows in the environment (Blaser et al. 2008), but may also suggest areas where a better understanding of behaviour, form and fate in the respective natural systems can reduce associated uncertainties through subsequent model refinement. Such models should, however, be developed with the acknowledgement of complexity as a defining feature of nanomaterials in natural systems, establishing hypotheses for testing through empirical research (validation).

21 Quantitative Structure-Property Relationship.

22 More information may be obtained from the website and publications of the Research Project NANOTRANSPORT (NMP4-CT-2006-033371): http://research.dnv.com/nanotransport/index.htm

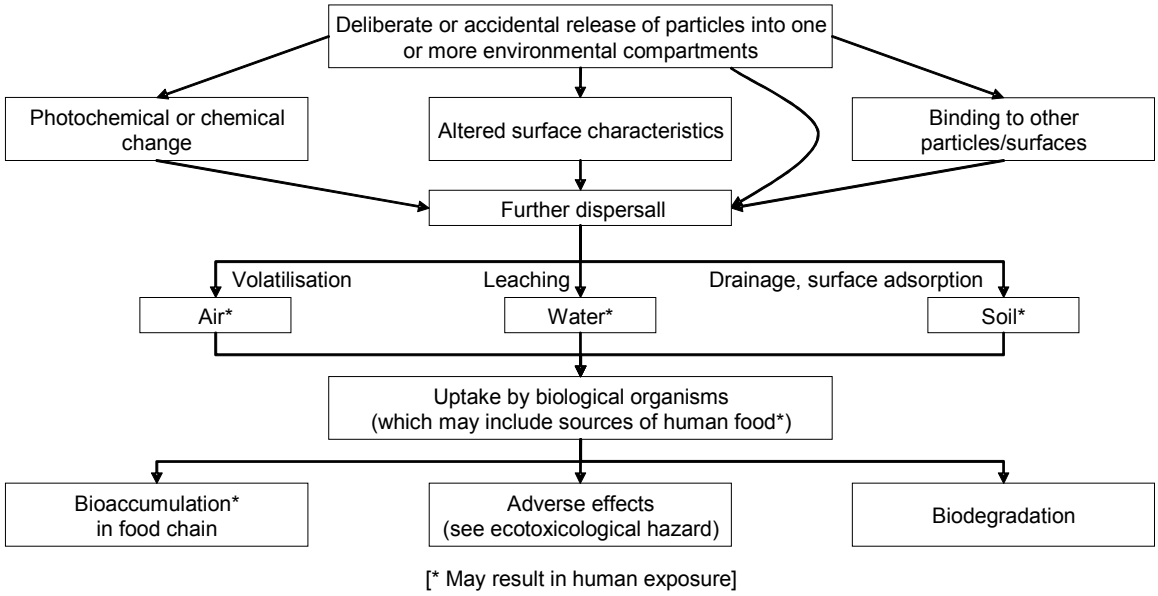


FIGURE 3. The complex fate of a nanomaterial released in the environment is complex and possible stages involved (adapted from SCENIHR 2006).

74. **Parameterisation of Conceptual Exposure Models.** Defining the conceptual exposure model for a nanomaterial is underpinned by understanding its intrinsic physicochemical properties and interactions with the surrounding environment. Such properties include chemical composition, particle size range, surface charge and others as established starting point for understanding the exposure scenario for any given nanomaterial, based on fundamental properties of the material itself. History has shown the benefit of the development of fundamental behaviour and speciation models (e.g. the biotic ligand model for metals in aquatic media) for predicting speciation and bioavailability in complex systems, suggesting that this may also help to reduce uncertainties in nanomaterials risk assessment (Blaser et al. 2008).

75. **Validation of Conceptual Exposure Models**. At present some of the stages/pathways can quite well be predicted for non-nanomaterials (e.g. behaviour, persistence, transport, transformation pathways/products, bioaccumulation, and effects). However, as indicated above for the exposure models, for nanomaterials these models have not (yet) been evaluated and it can be assumed that (some of) the underlying assumptions for these models will have to be adapted for nanospecific characteristics/behaviour (SCENIHR 2009).

76. **Progress in Exposure Modelling.** There is constant and rapid progress in the availability, the use, and the predictive capacity of tools for modelling of human and environmental exposures to nanomaterials. Respective developments are analysed and documented by Steering Group 8 of the WPMN (e.g. OECD 2009b).

77. **Detection Limits**. The sensitivity of conventional methods for detection and characterisation of nanomaterials in the environment may be limited. In this context, the required limits of detection and quantification should not be defined solely with regard to surveillance purposes but also keeping in mind the need for generation of knowledge to feed exposure model development.

78. **Portability of Equipment**. There is inadequate portable (personal) instrumentation for nanoparticles exposure at the workplace and in the environment, including continuous (or "online")

measurements (OECD 2009b). Various activities have been initiated that promise rapid progress in the development of such equipments for routine exposure monitoring[23].

3.e. Risk Characterisation (Assessment)

79. **Interspecies Extrapolation.** In conventional risk assessment (default) factors are used to extrapolate effects in one species (e.g. rats) to other species (e.g. mice, humans). These assessment factors (AF) are established based on extensive historical knowledge on the mechanisms influencing dose and toxicity of non nano-materials. Whether the same factors are appropriate for nanomaterials, and if not how they should be adapted asks for further research considering the potential differences in deposition (pattern), clearance including capacity, and sensitivity.

80. **Intraspecies Extrapolation.** Default (standard) assessment factors between 3 and 10 have been established to account for interindividual differences in workers and the general population (ECHA 2010, EPA 1993). It was noted that, in its recent opinion, the Scientific Committee of EFSA concluded, the current scientific literature would not indicate a need for different assessment factors for nanomaterials (EFSA 2011). However, the uncertainty caused by intraspecies variability is as yet not documented for effects of nanomaterials. A lot of research so far has been focussed on human health and thus on vertebrate toxicity tests, which are often time consuming and costly. The incentive in these tests is to minimize the number of animals involved, which further hampers gaining insight in intraspecies variability.

81. **Time Extrapolation.** Similarly, acute-to-chronic ratios, are often used for time extrapolation from acute (high dose) effects to chronic (lower dose) effects in environmental risk assessment. The same applies to subacute-to-subchronic and subchronic-to-chronic extrapolation in human health risk assessment. The size and use of these factors is justified by the extensive experience and data. Considering that the nanomaterial under assessment may differ fundamentally from the non-nanoscale materials with regard to toxicokinetics, these default values should be evaluated for their usefulness for nanomaterials.

82. **Chemical-Specific Adjustment Factors (CSAFs).** CSAFs are always given preference over default assumptions described above (WHO 2005). However, the definition of CSAFs requires reliable chemical-specific data and good understanding of the fundamentals of the biological response to the material. Limitations in amount and reliability of the specific database as well as the level of specific mechanistic knowledge may cause significant uncertainty in (or may even prohibit) the derivation of CSAFs. Specific approaches were suggested for CNTs (Nakanishi (ed) 2009a, Pauluhn 2010a).

83. **Additional Uncertainties.** A number of options are available to account for additional uncertainties in risk assessment of nanomaterials, drawing from experience with 'conventional' chemicals. One option is to derive safe levels (e.g. derived no effect levels (DNELs), tolerable daily intakes (TDIs) or predicted no effect concentrations (PNECs)) by the application of an increased assessment factors (on a case-by-case basis) depending on the information available (e.g. up to x1000 instead of x100. This is a common (and indeed recommended) approach in chemicals risk assessment when data are scarce or poor in quality (Blaser 2008, ECHA 2010, EPA 1993, WHO 2005) which has been applied in a recent scientific review of the health and environmental safety of different classes of nanomaterials (ENRHES 2010).

84. **Consequences of Large Assessment Factors.** In adopting this approach, the potential management implications (or impacts) must be evaluated. One implication may be that the application of large assessment factors to derive limit values may prove to be over-precautionary. Another implication may be that characterising and managing risk in the context of such low PNECs or Maximum Residue

23 Reference is made to the OECD Database on Research into the Safety of Manufactured Nanomaterials available at http://webnet.oecd.org/NanoMaterials

Levels (MRLs) may require the development and optimisation of highly challenging analytical measurement methods with very low limits of detection (this is also an issue for some 'conventional chemicals', where experience suggests that such analytical challenges can pose significant feasibility concerns).

85. **Validity of Exposure Assessments.** At present there is no information on the validity of the use of current exposure models especially for the environment, although rapid progress is expected (see above). However, the traditional approach of human health and environmental risk assessment depends on the ratio between, for example, NOAEL and exposure or PNEC and PEC. The risk characterisation methodology recommended in the Technical Guidance Documents can thus be followed for nanoparticles if, and only if, both exposure as well as acceptable limit values can be calculated with sufficient confidence. However, risk estimates may be derived for a range of exposure scenarios even if complete exposure data are not available.

86. **Dose Metrics**. For a given particle type and size, any of the possible dose metrics may be sufficient to describe dose-response because they are all correlated within a given material. However, to describe dose-response relationships across a range of particle sizes, the use of mass concentration data alone may be insufficient if size-specific number concentrations and surface area metrics may, in these cases, be more closely related to the biological effect. Under these circumstances, exposure measurements may produce mass related results, while surface area may be the toxicologically relevant dose metric. Thus, consideration must be given to the choice(s) of metric(s) for definition of the limit value, to exposure measurement methods and detection limits, and to reliable methods for conversion if required (OECD 2009b).

3.f. Relationship of Risk Assessment to Risk Management

Role in Problem Formulation

87. The standard risk assessment paradigm of any substance involves comparing potential hazards with expected exposure and determining whether there is a potential for risk. Where risk has been identified, risk management measures shall be undertaken to ensure the substance can continue to be used safely in commerce, but under specific restrictions. However, given that nanomaterial risk assessment may have limited relevant empirical data, introducing risk management measures as part of the problem formulation stage provides opportunity to limit the scope of the risk assessment.

88. In terms of occupational, public health, and environmental exposure risk assessments, restrictions of use and application along with engineering controls can limit exposure, thereby limiting the types of effects that need to be examined in the context of a risk assessment. Fully contained nanomaterials would be generally characterised by the following use and applications:

- The nanomaterial is not directly available to members of the public, or contained within the matrix of an article. However, disposal or aging of that material would need to be considered in the life cycle analysis (and in exposure assessment);

- Environmental exposure is minimised or absent as the application is non-dispersible and releases from facilities are fully controlled. However, the potential hazard of the material would need to be evaluated in the form released as well as changes that may occur during aging or possible transport of the material;

- Exposure to the public is minimised as environmental releases are minimised, and there is no significant exposure to consumers. However, the definition of the level below which release and exposure can be considered insignificant would need to be addressed; and,

- Exposure to workers in production as well as downstream uses is minimised using engineering controls and personal protective equipment. However, the effectiveness of these engineering controls needs to be evaluated in the context of safe exposure levels for nanoparticles.

89. Applications which do not meet all of these standards will require more definitive exposure assessment, as well as development of relevant empirical data to address these exposures. Restricting exposure by means of risk management can be considered while any necessary data is being generated.

Data Deficient Risk Management Action

90. Precedent for taking risk management action in the absence of full scientific certainty has been exercised in the application of the "Precautionary Principle". There are a number of variations on how this principle is defined and may be described as: "where there are threats of serious or irreversible damage or harm, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent this potential damage or harm" (UN 1992). Interpretation of the precautionary principle in terms of "threats of serious or irreversible harm" in the field of nanomaterials, presents a challenge due to the paucity of information linking properties and effects.

91. Undertaking risk management action in the context of data deficiency should consider the available information both on the specific nanomaterial and on other substances with similar chemical and physical properties, as well as identify the key data gaps, in making decisions about the appropriate level of exposure controls. When hazards are uncertain, additional controls may be warranted as a primary prevention measure until further data can be generated to better assess the hazard of the nanomaterial and the risk of exposure to workers or the environment (Schulte and Salamanca-Buentello 2007, HSE 2004). The basis for data deficient risk management action stems from our understanding that nanomaterials exhibit new properties, and given historical experience that new properties have had unexpected (adverse) environmental and human health and safety consequences, it remains in the interest of all stakeholders to apply prudent risk management approaches to nanomaterials in the current environment of scientific uncertainty.

92. In the context of a regulatory agency imposing risk management measures, there has been historically an expectation that such measures are undertaken only when substantial hazard or quantifiable risk has been identified. As to whether regulators can take risk management action based on an absence of information, however, will be a jurisdiction specific consideration.

93. Nevertheless, it is understood that nanomaterials are developed and produced to exhibit specific new, unique properties (for example quantum dots or CNTs). Historically, the risk assessment community has had a range of experiences with materials which exhibit unique properties. In some cases, these unique properties have translated into impacts in unforeseen ways (chlorofluorocarbons (CFCs), polychlorinated biphenyls (PCBs)).In summary, the acceptance that nanomaterials possess unique properties, along with the recognition of current limitations associated with generating meaningful empirical risk assessment data for many nanomaterials, suggests a need for application of specific risk management action based on the best available evidence and commensurate scope for the risk assessment.

4. Risk Assessment of Nanomaterials: Strategies and Approaches

94. While there are no fundamental differences in general risk assessment paradigms for chemicals and nanomaterials (Canady 2010, OECD 2010a), Chapter 3 has identified a range of important issues that should be considered or addressed to enhance nanomaterial risk assessments. This chapter will address how to proceed with risk assessments with limited critical data.

95. However, as research outcomes become available uncertainties associated with undertaking risk assessments for nanomaterials will diminish and data sets will continue to become increasing relevant and tailored to the unique challenges and properties presented by nanomaterials.

96. In terms of a risk assessment strategy, the following issues should be addressed for the particular nanomaterial or class of nanomaterial under investigation:

a. identifying the availability of reliable and relevant data, and in particular physicochemical data, fate and effect data, and exposure information;

b. evaluating the uncertainty associated with drawing conclusions about the fate and distribution of the nanomaterial in the environment, as well as in occupational settings or consumer settings;

c. understanding the limitations in undertaking effects characterisation, as well as how to extrapolate to chronic no-effect or benchmark concentrations;

d. selecting an appropriate method for quantitatively or qualitatively determining whether the nanomaterials will pose a risk; and

e. examining the implications potential of risk management actions which may help limit the scope of the risk assessment (i.e., to focus the risk assessment toward providing the data needed to choose among the available risk management options).

97. It has also been suggested that, in the absence of specific guidelines, it would be critically important to review the problem formulation with stakeholders and decision makers before advancing in the assessment process.

4.a. Considerations Regarding the Information for Use in a Nanomaterial Risk Assessment

98. Information used in the risk assessment of chemicals draws on many decades of research and experience on the type of information necessary to evaluate risk, types of test methods that are appropriate, and even what concerns are inherent to specific subclasses of chemicals. However for nanomaterials, there are limited empirical data, reliability and validity of the data is often not clear, and relevance of the available information to the nanomaterial under assessment may be limited.

99. Nevertheless, the general categories of information used to carry out a risk assessment for nanomaterials are the same for other chemicals and include: i) identity information; ii) physicochemical properties; iii) industrial and consumer uses and environmental releases; iv) environmental fate; and v) ADME[24] and the potential toxicity of the nanomaterial. Likewise, the basic steps in the risk assessment paradigm also apply, including hazard identification, dose-response assessment, exposure assessment, and risk characterisation (NRC 1983, 2009).

24 ADME = Absorption, Distribution, Metabolism and Excretion

Quality, Adequacy and Reliability of Data

100. Experimental data identified for use in a risk assessment should be evaluated for reliability based on whether or not the data has been generated according to an accepted testing or measurement protocol (e.g., OECD Test Guidelines). Test methods which are internationally recognised for chemicals, are being validated for applicability to nanomaterials. For example, SG4 has examined the OECD test methods for applicability to nanomaterials and concluded: i) health effects test methods are generally applicable (with the addition of material characterisation information) ii) ecotoxicity test methods are also adequate, however materials characterisation as well as guidance on preparation, delivery, measurement, and metrology is currently insufficient for testing, and iii) few OECD physical chemical property, fate and degradation methods have direct applicability (OECD, 2009a). Additional guidance is also available from OECD concerning sample preparation and dosimetry (OECD, 2010c) which can assist risk assessors in the review of test data.

101. As mentioned in chapter 3, nanomaterials present particular challenges in terms of behaviour such as agglomeration/aggregation which can impact aspects such as adequate dosing of test organisms and analytical measurements. As a consequence, risk assessors should ensure that the test material being examined: i) has been adequately characterised, ii) represents a realistic worst case form of the material to which an individual or organism has been exposed; iii) has had adequate exposure in the context of effects testing. In addition, multiple dose groups and sufficient spacing are needed to adequately characterize the shape of the dose-response relationship and the study dose levels should include doses that adequately represent a range of potential exposures to the target population (e.g., workers).

Use of Data on Close Analogues

102. When experimental data are not available for the nanomaterial being assessed, or to supplement limited data, experimental data from analogous nanomaterials may be considered (the "read-across" approach). In chemical risk assessments, there are general principles which can be followed when assessing the validity of an analogue (OECD, 2007). General rules do not yet exist for use with nanomaterials, however, as trends in the behaviour of nanomaterials begin to unfold, risk assessors are encouraged to capitalize on this information. Until such time, such read across information should be used with caution and accompanied with scientific rationale to justify its use.

103. Information from non-nanomaterials can also contribute to an assessment in terms of providing a weight of evidence argument, to determine general trends, or as a reference material in comparative potency assays of nanomaterials and non-nanomaterials. Information on the non-nanochemical form typically cannot be used in place of information specific to the nanomaterial. An exception to this would be circumstances where hazard data on the non-nano form indicates a concern. Furthermore, if a relationship is established to describe the dose-response relationship for nanoparticles and larger particles, it may be feasible to convert dose metrics (e.g., from mass to particle surface area or particle number). For those subclasses of nanomaterials for which a relationship between the responses to nanoscale and larger particles has been identified (e.g. particle surface area dose of poorly soluble low toxicity particles and pulmonary inflammation or tumours) (Dankovic et al. 2007; NIOSH 2005), utilizing the available data on that relationship may assist in the risk assessment for that specific subclass.

Model Predictions

104. For certain types of data, particularly physicochemical, mammalian toxicity and ecotoxicity data, there are limited empirical data sets and as a consequence models are not yet been developed. As more refined prediction models become available and trends in the behaviour of nanomaterials begin to unfold, risk assessors are encouraged to capitalize on this information.

Metrics

105. Empirical test results for chemicals are largely communicated in terms of mass-based metrics (e.g. mg/L, mg/m^3, kg body weight). However for nanomaterials, it is recognised that there is uncertainty concerning these units of measurement (OECD 2010a). Although it is expected that empirical results will continue to be reported in terms of mass based units, risk assessments may need to include a discussion of any limitations this metric may present, and consider whether non-mass based metrics (e.g., particle surface area, number of particles) or combinations of them (SCENIHR 2006) would be more effective for communicating results across a range of particle sizes.

106. Research activities are currently examining whether such additional dose metrics would facilitate a more effective presentation of test results. For example, if biological effects of the specific nanomaterial appear to relate to surface area, rather than mass, this should be considered when reporting information (although these various metrics are correlated for a given nanoparticle). That is, although particle surface area may in some cases better describe the relationship between dose and response across various particle sizes, the equivalent mass dose can also be used by taking into account the particle size.

4.b. Information for Use in a Risk Assessment

4.b.i. Nanomaterial Identification

107. Variability in composition or properties of the specific nanomaterial that may occur between batches (or for other reasons) may be accounted for by defining appropriate ranges for all affected identifiers. This would be similar to the approach usually taken for impurities. However, it must be ensured that the hazard (and exposure) data is adequate to cover the defined range. Identifiers to be considered on a case-by-case basis include chemical composition, size (range), crystallinity, surface coatings, morphology, etc. Alternatively, the nanomaterial may be considered and treated as UVCB substance (Substance of Unknown or Variable composition, Complex reactions products or Biological materials).

4.b.ii. Physico-chemical Properties

108. Steering Group 3 of the WPMN has identified a series of physico-chemical, fate and toxicological endpoints for nanomaterials for the testing of selected nanomaterials in the OECD Sponsorship Programme. These properties are anticipated to provide insight into how nanomaterials will behave in biological and environmental compartments. However, these endpoints are described as "exploratory" in nature and will be investigated as to their applicability in risk assessments.

109. In terms of physicochemical properties, the following properties are currently being examined in the SG3 Sponsorship Programme (OECD 2010b):

* Agglomeration/aggregation
* Water solubility
* Crystalline phase
* Dustiness
* Crystallite size
* Representative TEM picture(s)

* Particle size distribution
* Specific surface area
* Zeta potential (surface charge)
* Surface chemistry (where appropriate)
* Photocatalytic activity
* Pour density
* Porosity
* Octanol-water partition coefficient, where relevant
* Redox potential
* Radical formation potential

OECD has developed detailed descriptions of these physical chemical property endpoints and as well as OECD and non-OECD test methods (OECD 2009c).

110. Unlike the OECD Minimum Pre-market Data set (OECD, 1982), these endpoint have not been approved for direct application as regulatory data sets. Nevertheless, where this information is available, risk assessors are encouraged to consider this information as part of a regulatory risk assessment and include adequate justification for how this information contributes to a nanomaterial risk assessment (e.g., determining if the dose-response relationship is influenced by these parameters).

4.b.iii. Characterisation of Entry, Fate, and Exposure

111. During this phase of the assessment, information on how a nanomaterial enters the body or is released into the environment is integrated with information on its fate in order to establish the degree of exposure that is occurring, or may occur, between receptor and the nanomaterial. As with chemical risk assessments, the main steps are:

a. Entry or release characterisation – to understand if, how, and in what quantities a nanomaterial may enter the body or is released into the environment throughout its life cycle (from manufacture or importation through to disposal);

b. Characterisation of fate and distribution – to determine a nanomaterial's fate in different environmental or biological compartments and to understand how an organism comes into contact with a nanomaterial entering a particular medium; and

c. Quantification of exposure – to estimate potential quantities in the human body or the environment and to determine either derived exposure doses or Predicted Environmental Concentrations (PECs) or exposure distributions for relevant human organs (e.g. liver, kidney, brain) or environmental compartments (e.g., air, water, soil, sediment, terrestrial wildlife).

4.b.iv. Entry or Release Characterisation

112. Entry or release characterisation involves identifying where and how a nanomaterial may be released to the environment (e.g., via industrial processes, or in consumer products) and the characterisation of releases from these processes (e.g., quantities, frequency, and duration). This information is critical for determining the relative significance of a source of release and the scale (in terms of both time and space) of potential exposures. Understanding where a nanomaterial enters the environment (e.g., whether it is released to water or to air) is also essential for determining its fate in the environment.

113. Similarly, entry characterisation in humans involves identification of exposure route (e.g. via inhalation, skin, etc.) and characterisation of these exposures (e.g. quantities, frequency, and duration). Understanding how a nanomaterial may enter the human body (e.g. skin, lung epithelium) is essential for determining which body parts will be (primarily) affected.

114. Undertaking entry or release characterisation does not differ from approaches used in standard chemical risk assessments, other than accounting for any changes in nanomaterial behaviours which can impact entry.

4.b.v. Transformation, Degradation and Persistence

115. As with chemicals, nanomaterial transformation can influence distribution within an organism or in the environment. Assessing transformations will need to be considered in terms of assessing the fate of the "core" material, as well as any functionalisation or surface coating, as alternations of either will affect properties and consequently their distribution pattern. In addition, fate of the material may not necessarily be viewed only in terms of degradation; aggregation/agglomeration will impact how materials distribute and whether dis-aggregation is likely upon settling in a tissue or compartment.

116. Transformations can also be viewed in the context of protein corona formation within biological fluids; such coronas can influence the behaviour of nanomaterials and how this effect influences toxicity should be considered (Canady, 2010), e.g. in designing or evaluating experimental conditions in *in vitro* and *in vivo* systems.

117. When generating degradation information, strictly inorganic nanomaterials will not benefit from testing geared toward biotic degradation tests in which test material provides the carbon nutrient source. Biodegradation tests should only be considered in instances where the nanomaterials can serve as an "organic" carbon source

118. Abiotic degradation tests should also be examined. Hydrolysis testing provides meaningful insight where chemical structure of the material or surface coating suggests a potential for such a reaction to occur.

4.b.vi. Distribution and Compartmentalisation

119. As described above, the behaviour of nanomaterials can be evaluated according to more particle-specific physicochemical properties listed in section 4.b.iii. These properties are considered a tool-box of information which will provide necessary evidence to predict where the material is likely to reside. However, to date there is little experience in terms of the roles these properties will play in risk assessment. As this information becomes more and more available, risk assessors are encouraged to use this as part of a weight-of-evidence argument concerning environment distribution. However, in the absence of such data, assessments should assume a reasonable worst case behaviour; for example one can assume the materials are agglomerated during transport, but disagglomerated once established in a compartment or organism.

120. Also given the underlying chemistry of nanomaterials, it is expected that the behaviour of nanomaterials will alter in various media such as ranges of water hardness, pH, presence of humic acids and in biological media. Reasonable worst case scenario of how nanomaterials change according to these media should also be addressed in risk assessments.

4.b.vii. Bioaccumulation

121. For simple organic chemicals, there is an established relationship between octanol water partition coefficient (K_{OW}) and bioaccumulation or bioconcentration factor (BCF). However, there is not a wide body of evidence that this relationship will hold true for many nanomaterials. Consequently, it is not yet recommended that risk assessors make attempts to predict bioaccumulation on the basis of chemical modelling programs. However, empirical BCF tests on the nanomaterial are recommended (understanding the influence the environmental form will have, as well as any corona effect). Empirical studies should be further supported by addressing the relevance of uptake by an organism in terms of whether the nanomaterials may cross cell membranes, whether they will embed in tissues and release ions, whether they are excreted, etc. In the absence of this information, reasonable worst case assumptions based on the size and chemistry will provide insight into potential for bioaccumulation.

4.b.viii. Quantifying Exposure

122. The main objective of quantifying exposure is to determine the concentrations of the nanomaterial in the media in which it is expected to reside following release. Methods for quantifying exposure follow the same general paradigm employed in chemical risk assessments. However, whether using modelled approaches or direct measurement, there must be an understanding of the material's form (e.g., single particle, aggregate or agglomerate, ions, etc.), and whether exposure metrics are compatible with metrics from other parts of a risk assessment (eg. compatible with units from the effects assessment).

4.b.ix Effects Characterisation

123. The overall objective of effects characterisation is to identify the type and severity of adverse effects to human health or the environment, either direct or indirect, following exposure to a nanomaterial or its transformation product(s).

124. Three important issues identified for effects characterisation include i) the appropriateness of test species ii) the appropriateness of test methods, and iii) where there is an adverse effect, the use of uncertainty factors or extrapolation factors to estimate a no-effect level associated with long term exposure.

Test Species

125. Standard species used in chemical toxicity testing have not been examined regarding adequacy to predict (possibly unknown) effects of exposure to nanomaterials. Nevertheless, until such a body of evidence exists to either affirm or refute the use of standard species, and given that test methods are currently geared toward the use of certain species, risk assessors should continue using data from standard species unless research outcomes indicate otherwise. Results from non-standard species may also be considered along with discussion of how these species will better represent toxicity outcomes in the target population.

Appropriateness of Test Methods

126. Employing standard test methods have the advantage of comparability with results from chemical toxicity testing and can serve as an indicator of relative risk. As discussed in paragraph 122, health effects test methods are generally applicable with additional material characterisation information and ecotoxicity

test methods are also adequate assuming material characterisation preparation, delivery, measurement, and metrology issues are addressed.

127. Poor or inadequate material characterisation has been a major barrier to interpreting and comparing studies addressing the human or ecological toxicity of nanomaterials. In response to this a number of international organisations have proposed indicative materials characterisation parameters which should be undertaken when toxicity tests are undertaken (OECD 2009c, ISO/DTR 13014:2011). An ad hoc meeting of researchers and risk assessment scientists developed the Minimum Information for Nanomaterial Characterisation (MINChar) Initiative (MINChar Initiative, 2008). This initiative identifies a series of material characteristics which allow scientists to more effectively interpret the results of health and ecological toxicology studies. By examining these chosen parameters, evaluators can understand which parameters could enhance or eliminate biological responses.

128. The MINCHar Initiative has recommended the following minimum physical and chemical parameters for characterizing nanomaterials on toxicology studies:

What does the material look like?

* Particle size/size distribution

* Agglomeration state/Aggregation

* Shape

What is the material made of?

* Overall composition (including chemical composition and crystal structure)

* Surface composition

* Purity (including levels of impurities)

What factors affect how a material interacts with its surroundings?

* Surface area

* Surface chemistry, including reactivity, hydrophobicity

* Surface charge

129. Also to be considered when characterizing nanomaterials in toxicity studies is:

i. their stability – how material properties change with time (dynamic stability), storage, handling, preparation, delivery, etc., including solubility, and the rate of material release through dissolution; and

ii. context/media – how material properties change in different media; i.e., from the non-nano material to dispersions to material in various biological matrices (characterisation "as administered" is considered to be particularly important).

Use of Uncertainty Factors

130. Uncertainty factors can be used to identify no effect levels by extrapolating: i) temporally (i.e. estimating chronic toxicity based on the results of acute toxicity testing); ii) accounting for intra- and interspecies extrapolation; and iii) accounting for laboratory to human or field conditions. In chemical risk assessments, use of uncertainty factors is widely accepted and although regulatory programs apply these as a matter of policy (Chapman et al, 1998) there nevertheless exists a body of evidence to support their use. For example, studies focussing on organic chemicals have examined acute to chronic toxicity value ratios (ACRs) employed in ecological risk assessments (ECETOC 1993, Persoone and Janssen 1994) show that ACRs typically fall below a factor of 50, although outliners can have factors much higher. Similarly, for human health risk assessment, productivity of the default factor for interspecies variability of 10 for humans was evaluated based on the available experimental data (e.g. Burin and Saunders 1999).

131. Given that empirical studies have yet to examine the scientific basis for the use of variability and uncertainty factors in nanomaterials risk assessment, and considering the unique properties of certain classes of nanomaterials, nanomaterial toxicokinetics (e.g. possible slow clearance), formation of protein coronas, etc, there is a need for additional research on use of these factors. Some of the standard adjustment factors used in risk assessment to account for variability and uncertainty in the data are also considered to be relevant to nanomaterials (e.g., exposure data based on a LOAEL vs. NOAEL; subchronic vs. chronic exposure; animal to human dose extrapolation; and human inter-individual variability). However, substance-specific data should be used when available, and the basis for the adjustment factors used in the risk assessment should be clearly described. Qualitative approaches could also be used until supporting empirical data becomes available for evidence-based adjustment factors for specific classes of nanomaterials. Agency or jurisdiction-specific procedures and practices may also be applicable to risk assessments of nanomaterials.

132. Ideally the use of long(er) term or chronic data is recommended over extrapolation from acute or subchronic to chronic. If these data are not available and default assessment factors are used, this source of additional uncertainty to the RA should be noted. Risk assessors may also consider the use of a margin of exposure/safety rather than employing uncertainty factors, depending on the data available and the information needed for risk management decisions.

133. There has also been some debate concerning the use of nano-specific uncertainty factors to account for unknowns associated with conducting risk assessments in this field. Current consensus suggests there is no need for a separate additional nano-uncertainty factor, as uncertainty factors are best employed for specific area of uncertainty, rather than trying to compensate for a broad unknown. (OECD 2010a, Canady 2010).

Dose descriptor

134. Benchmark dose (BMD) estimation is another standard risk assessment method Crump 1984, 1995; EPA 2005, 2009) to the NOAEL/LOAEL approach. BMD methods have been used in cancer and noncancer risk assessments, including for pulmonary responses to inhaled nano-scale (ultrafine) particles (NIOSH 2005; Kuempel et al. 2006; Dankovic et al. 2007). A BMDLx (benchmark dose lower confidence limit) is defined as "a statistical lower confidence limit for the dose corresponding to a specified small increase [of x %] in level of (adverse) health effect over the background level" (Crump 1984). The BMDL can be used as an alternative to a NOAEL as a point of departure to extrapolate to lower doses to estimate risk (EPA 2005). Advantages of the BMD method are that it takes appropriate statistical account of the sample size and of the shape of the dose-response relationship. In contrast, NOAELs tend to be larger in smaller experiments, and complete information about the dose-response relationship is not used (NRC 2009). BMDL estimates tend not to be dependent on the choice of the dose-response model (since they are computed within the range of the data). Whereas a NOAEL or LOAEL approach assumes a threshold

model regardless of the shape of the dose-response relationship, BMD methods provide an estimate of the dose associated with a specified level of risk based on fitting a statistical model to the dose-response data. BMD methods may provide a more accurate estimate of the true risk given the dose-response data, and also allow (or require) an explicit discussion of acceptable or achievable risk levels for specific responses given the known or estimated exposures. BMD methods may have advantages for risk managers and regulators by providing estimates of significant risk associated with specific exposure scenarios, as well as exposures associated with minimal, acceptable or achievable risk levels (NRC 2009). Consequently, this approach should be considered in jurisdictions where permitted.

Foodchain Considerations

135. Information on persistence and bio-accumulation will inform on the potential for transfer from aquatic species to mammalian wildlife (and further to humans). However, predictive models in turn do not currently exist to describe how to quantify the transfer between species. Empirical trophic transfer experiments may be necessary to measure food chain exposure.

Abiotic Effects

136. These are effects that impact non-living components of the environment (the environment on which life depends). The release of a nanomaterial may, depending on its physical and chemical properties, have impacts that result in a change to the environment, which, in turn, may adversely impact the ability of organisms to inhabit the environment. Adverse abiotic impacts can include altering the chemical make-up of natural waters, e.g. metal content, pH changes, or in soil, chemical-mediated compaction.

137. Characterisation of abiotic effects varies between nanomaterials depending on their physical and chemical properties. This can involve examining the potential reactions and interactions of the nanomaterial in the environment.

4.c. Risk Characterisation

138. Risk characterisation involves a combination of qualitative and quantitative approaches to understand and describe the risk a nanomaterial poses to human health or the environment. The various lines of evidence explored during the assessment are considered in a weight-of-evidence approach to evaluate the potential for harmful effects of a nanomaterial.

139. Chapter 3 has identified the need to resolve a number of important issues. Nevertheless, nanomaterials are actively being marketed and as such there is an immediate need to develop meaningful risk assessment conclusions. Given the limited state of the science, some risk assessments currently undertaken may need to be re-examined as information and methodology improves. However, there are specific circumstances described below where sufficient data are available to use current risk assessment practises combined with risk management tools to provide meaningful risk characterisation outcomes. The best available scientific data should be used in the risk assessment. Depending on the available data, there may be sufficient data to perform only one or more of the four steps in the risk assessment process, which include: hazard assessment, exposure assessment, and dose-response assessment (in a complete risk assessment, information from each of these steps is evaluated in the fourth step of risk characterisation) (NRC 1983, 2009). Information available in any of these risk assessment steps can be used to inform the risk management decision-making, assuming that the uncertainty based on the available data is also taken into account.

4.d. Risk Assessment Strategies

Exposure Minimization

140. Minimizing[25] or eliminating potential exposure is one means of focusing the scope of a risk assessment in cases where there is limited toxicological information. This approach is limited to circumstances where exposure is controllable from the point of import or manufacture until end usage. In particular, this approach focuses on an assessment of the use patterns of the nanomaterial, and the likelihood of human or non-human species interaction. An example of an applicable circumstance may be where the material:

- is manufactured and processed at facilities which do not permit release to the environment, and where exposure to workers is minimized or eliminated through the use of engineering controls (first priority) and personal protective equipment (as needed where exposures are not adequately controlled), recognizing that "no release" and "no exposure" need to be verified with validated sampling and analytical methods; and

- has a use pattern that is limited to products where the nanomaterial is embedded into a matrix minimizing or eliminating exposure to consumers as well as to the environment, after considering possible release if material is modified (e.g., weathering or aging processes or by grinding or sawing the composite material).

141. Minimizing or eliminating exposure may need to be assured using risk management measures. As discussed in Chapter 3, based on the standard risk assessment – risk management paradigm, there are challenges in employing risk management measures when limited data are available for a complete risk characterisation. Risk management practices are traditionally employed in many fields of regulation only when a critical mass of evidence supports the necessity of such action. However, given the limited state-of-science, jurisdictions may consider employing risk management actions in the absence of a standard weight-of-evidence approach – essentially basing risk management on the understanding that the specific nanomaterial may exhibit enhanced or unique properties which may lead to unexpected effects. Such an approach permits placing limits on allowable activity (e.g., use under highly controlled conditions) as a risk management option for dealing with a lack of empirical information, and also thereby focuses the scope of the risk assessment. As more data become available, the scope of the risk assessment could be extended to evaluate potential risk under other conditions of use.

Lack of Bioavailability or Toxicity

142. Another strategy for addressing risk involves developing weight-of-evidence addressing bioavailability. For example, if characterisation data shows substantive and unequivocal evidence that nanomaterials released into relevant media will rapidly and irreversibly form larger aggregates, this may indicate an inability to cross biological membranes. However, the size of the aggregates should be evaluated to determine if they are inhalable, and under what conditions they are stable. This characterisation information in combination with biological effects data showing no evidence of toxicity for the aggregate, may lead to a conclusion that the material will not cause biological effects at those doses. These data can be used to identify NOAELs and develop exposure limits for those materials.

25 The term minimize will need to be considered in the context of individual assessment/regulatory programs. The spirit of this term suggests that any exposure or release is minimal in the context of a particular assessment. However, caution is needed in evaluating the definitions of exposure and release, which may not be acceptable for nanomaterials that are more bioactive on a mass basis than non-nano materials.

143. Alternatively, circumstances may exist where exposure characterisation is not possible or practical but where there is considerable weight-of-evidence showing a lack of toxicity, including in chronic toxicity testing which is supported with ADME evidence indicating no concerns regarding biopersistence and bioaccumulation. This may support a risk characterisation that the material would not pose a risk under reasonably anticipated exposure scenarios, if those exposures are considerably lower than the equivalent exposure at which no toxicity is observed (after application of appropriate uncertainty factors). Furthermore, the lack of toxicity can be identified by an absence of effects at the appropriate "limit" doses as described in the relevant OECD TGs. For example, in OECD TG 407, the Limit test is described as follows:

> "If a test at one dose level of at least 1000 mg/kg body weight/day or, for dietary or drinking water administration, an equivalent percentage in the diet, or drinking water (based upon body weight determinations), using the procedures described for this study, produces no observable toxic effects and if toxicity would not be expected based upon data from structurally related compounds, then a full study using three dose levels may not be considered necessary. The limit test applies except when human exposure indicates the need for a higher dose level to be used."

Quantifying Risk

144. The approaches described above provide for circumstances where clear risk assessment outcomes are possible, even in the absence of comprehensive data sets. However, many applications of nanomaterials have an inherently dispersive use (e.g., paints, fertilizers, waste water treatment) where release/exposure is difficult to be controlled. Furthermore, where such dispersive materials have a potential for biological effects, then quantification of risk would be appropriate.

145. As discussed above, risk quantification may require the use of uncertainty factors. Currently there is policy support for use of existing default UFs for risk assessments of nanomaterials (EFSA 2011), although there, at present, is a lack of empirical evidence supporting the application of these standard uncertainty factors to nanomaterials. Consequently, the use of such standard uncertainty factors should be explained given the data available. Alternatively, a comparison of a valid no-effect-concentration or a specified effect value (adjusted to human-equivalent effect level as appropriate) with the exposure concentration (i.e. determination of a margin of exposure) can provide a point of comparison where there is a high degree of uncertainty in the appropriate adjustment factors.

Iterative Risk Assessments

146. An approach addressing limited data is "adaptive management" based on a plan-do-check-act (PDCA) cycle (Nakanishi 2009d). In this approach the substance is produced and used under a certain set of conditions based on a preliminary assessment, while additional data are collected to periodically evaluate the initial assessment and to modify the conditions as needed to ensure health and safety. There is broad recognition of the use of tiered risk assessment frameworks to inform risk management and identify necessary research (FAO WHO 2009). Incorporating product life cycle considerations into these frameworks prioritizes risk assessment needs for occupational, consumer and environmental receptors (Royal Society 2004; Shatkin 2008; Davis 2007; NNI 2011). This approach could be compatible with a precautionary approach if the initial set of conditions and level of caution based on the preliminary data is related to the degree of uncertainty. That is, extra precaution would be taken when the extent of the hazard is not well known (Schulte and Salamanca-Buentello 2007).

Need for Research

147. Development of risk assessment and risk management decisions in the absence of a comprehensive data-set and scientific understanding is not a scenario unique to nanomaterials. Chapter 5 addresses critical research needs to improve risk assessment and to reduce the uncertainties about the risk of nanomaterials is essential to effective occupational, public health and environmental risk management. Risk assessors are encouraged to stay abreast of on-going developments in this field.

5. Research Needs to Address Risk Assessment Issues

148. Effective resolution of the scientific issues identified as important issues includes cooperation between risk assessors and researchers. The SG6 Risk Assessment Workshop was an opportunity for such a discussion and a summary of identified areas of research is provided (OECD, 2010a). Risk assessment research needs were identified both in terms of broader issues, such as the need to generate high quality information, and in terms of specific aspects of exposure assessment, health human effects and ecological fate and effects. Ultimately, the intent of this research is more than simply the development and generation of information; it represents the systematic development of science and principles which will support future risk assessment of nanomaterials

149. Specific research topics pertaining to Exposure, Human Health Effects and Ecological Fate and Effects, as well as general areas of research identified at the workshop are listed below:

5.a. Exposure: Public, Occupation and Environment Research Needs

- Generating basic data on:
  - exposure of workers at different stages of the materials life cycle releases to the environment from industrial facilities, as well as exposure to consumers.
  - concentrations in and releases from consumer products into environment,
  - translocation and persistence of nanomaterials;
- Developing relationships on how nanomaterials move through different environmental and biological media in relation to morphology, surface chemistry, size, etc, and in addition compare these with those in non-nanomaterials;
- Development of more sensitive and more reliable methodologies to measure and characterize nanoparticles with lower detection limits as the detection limit of currently available conventional methods to measure particles in the environment and workplace may be limited; Decision on logic models for exposure assessment based on particle morphology needs to be developed; and
- Development of new and improvement of existing simulation approaches.

5.b. Human Health Effect Research Needs

- Generation of structure and activity data flanked by the development of databases which will facilitate modelling, QSAR, computational approaches to advance our ability to categorize and group materials for decision making. These tools will allow prediction of toxicity and provide weight-of-evidence to validate other empirical data being generated;
- Understanding the properties of nanomaterials, including particle kinetics in biological systems (i.e., adsorption, distribution, metabolism, and excretion) which influence the internal dose, biopersistence and bioaccumulation. This will also assist risk assessors in interpreting results from toxicology studies;
- Identifying nanoparticle-specific toxicological endpoints or nanospecific considerations for currently identified endpoints. This line of research will ensure that risk assessors are identifying all appropriate biological responses which may lead to adverse outcomes;

- Validation and acceptability of in vitro test methods. Similar to development of models between particle behaviour and toxicity to provide insight into risk, developing relationships between the results of in vitro testing and whole organism testing can be a valuable predictor of health effects;

- Understanding mode of action in mammalian systems, including whether there is variation between species; and

- Advancing epidemiological approaches and developing biomonitoring techniques. Given that it is assumed that substantive human exposures to nanomaterials are in early stages, it is important to identify potential population exposure likelihood to evaluate and validate initial risk estimates, e.g., confirm that estimated no-effect scenarios indeed do not lead to adverse impacts.

5.c. Ecological Effect Research Needs

- Understanding the disposition of nanomaterials (i.e. ADME) within whole organisms in all trophic levels. This information will provide an understanding as to whether standard ecotoxicological studies are an effective indicator of toxicity for nanomaterials, as well as provide insight on mode of toxicity and species sensitivities; and

- Identification of the most sensitive species, including lower trophic species (e.g. mycorrhizal fungi) which are potentially different from the current fish, daphnia algae paradigm. The purpose of this research is to determine which species are the optimal representative test species for use in identifying critical toxicity values for quantifying risk;

5.d. Persistence, Bioaccumulation, Fate and Distribution

- Identify mechanisms of bioaccumulation, as well as developing means for predicting bioaccumulation, as well as potential for food chain transfer. Bioaccumulation and food chain transfer are crucial in conventional chemical risk assessments, however, there is no confidence that approaches employed for chemicals are applicable to nanomaterials;

- Validation of extrapolations and uncertainty factors including acute-to-chronic ecological toxicity for all trophic levels and from pelagic to benthic toxicity. Understanding how and whether these extrapolations will allow more complete risk assessments to be made based on less expensive toxicity testing;

- Generation of foundation data on fate, transport, environmental presence, translocation and persistence;

- Development of trends in behaviour of nanomaterials including: i) comparing how specific properties of nanomaterials relate to biological effects; and ii) how different media impact these properties. This research is important for developing preliminary screening tools for nanomaterials, including possibly establishing thresholds indicative of toxicity or lack of toxicity;

- Development and improvement of new simulation approaches.

5.e. General Risk Assessment Research Needs

- Generation of high quality physicochemical, fate and effects information;

- Identifying the appropriate toxicological endpoints within or if needed outside of normal chemical screening endpoints;
- Validating the use of uncertainty factors used to calculate no-effect concentrations;
- Developing relationship or trends between i) specific properties of nanomaterials, with the potential to elicit biological effects and ii) how different media impact these properties;
- Determination of appropriate metrics for expressing exposure and dose; and
- Development of adequate sample preparation and dosimetry approaches.

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