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Important Issues on Risk Assessment of Manufactured Nanomaterials

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Important Issues on Risk Assessment of Manufactured Nanomaterials

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Foreword

The Working Party on Manufactured Nanomaterials (WPMN) is a subsidiary body of the OECD Chemicals and Biotechnology Committee (CBC). 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. It 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 presents the current state of science on the risk assessment of manufactured nanomaterials and highlight priorities for research toward specific risk assessment issues.

This document is published under the responsibility of the Chemicals and Biotechnology Committee.

Executive Summary

This document, *Important Issues on Risk Assessment of Manufactured Nanomaterials*, provides the current practices, challenges and strategies for assessing risk of manufactured nanomaterials in circumstances where data is limited, and there is a necessity for more research on specific risk assessment issues. As such, the document presents an overview of the chemical risk assessment paradigm and describes how various member-countries have adapted existing regulatory frameworks to the assessment of nanomaterials. It also presents the state of science on nanomaterials risk assessment (as of May 2021), and highlights priorities for research toward specific risk assessment issues.

It should be noted that this document is a living document. As such, it remains subject to refinement as research affords further understanding of how to assess and manage nanomaterials. It is not to be construed to imply scientific and/or policy endorsement of any specific risk assessment methods or models.

Table of Abbreviations

Acronym	Term
ACRs	Acute to Chronic Toxicity Value Ratios
ADME	Absorption, Distribution, Metabolism, and Excretion
AICIS	Australian Industrial Chemicals Introduction Scheme
AOP	Adverse Outcome Pathway
BAF	Bioaccumulation factor
BAL	Bronchoalveolar lavage
BCF	Bioconcentration factor
BMD	Benchmark dose
BMDLx	Benchmark dose lower confidence limit
CEPA	Canadian Environmental Protection Act, 1999
CLP	Classification, Labelling and Packaging, EU
CNT	Carbon NanoTubes
DNELs	Derived no effect levels
DSL	Domestic Substance List, Canada
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency, US
FAIR	Findable, Accessible, Interoperable and Reusable
GD	Guidance document
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
IATA	Integrated Approach to Testing and Assessment
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
ICRP	International Commission on Radiological Protection
ISO	International Organization for Standardization
ISO/TR	ISO Technical Report
ITS	Intelligent Testing Strategy
KE	Key event
K_{OW}	Octanol-water partition coefficient
Kp	Generic partition coefficient between any two phases (e.g. soil-water etc.)
LOAEL	Lowest observed adverse effect level
MIE	Molecular initiating event
MN	Manufactured nanomaterial
NAM	New approach methodology
nano-TiO2	Nano titanium dioxide

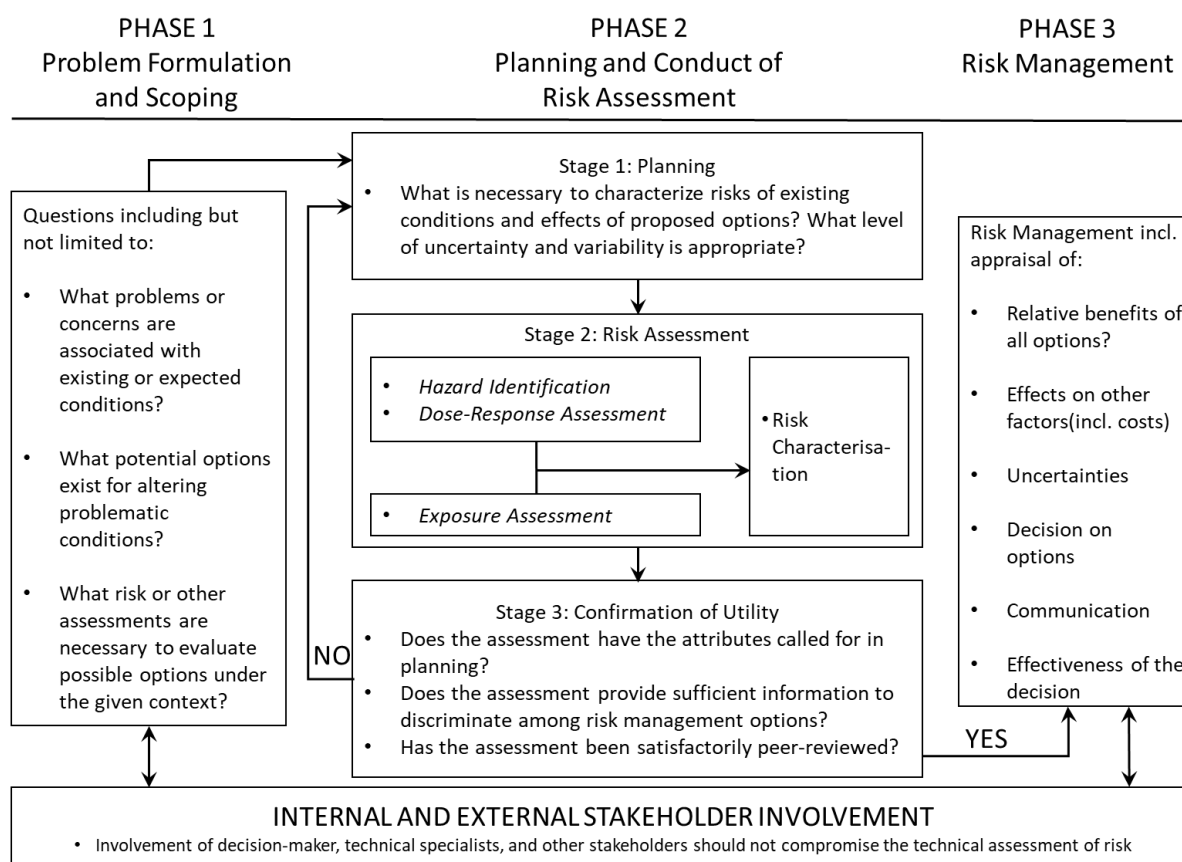| Acronym	Term
NECID	Nano Exposure & Contextual Information Database
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NIOSH	National Institute for Occupational Safety and Health, US
NOAEL	No observed adverse effect level
NRC	National Research Council, US
NRCWE	National Research Centre for the Working Environment, Denmark
NTP	National Toxicology Program, US
OECD	Organisation for Economic Co-operation and Development
OEL	Occupational Exposure Limit
OSHA	Occupational Safety and Health Administration, US
PBPK	Physiologically based pharmacokinetic
PDCA cycle	Plan-do-check-act cycle
PEROSH	Partnership of European Research in Occupational Safety and Health
PMN	Pre-Manufacture Notification
PNEC	Predicted no effect concentration
QSAR	Quantitative Structure-Activity Relationship
QSPR	Quantitative Structure Property Relationship
RAF	Risk Assessment Framework, Canada
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals, EU
REL	Reference Exposure Limit
ROS	Reactive oxygen species
SB4N	SimpleBox4nano
SbD	Safe(r)-by-design
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks, EU
SMPS	Scanning Mobility Particle Sizing
SNAc	Significant New Activity, Canada
TDI	Tolerable daily intake
TG	Test Guidelines
TK	Toxicokinetic
TSCA	Toxic Substances Control Act, US
WPEA	OECD Working Party for Exposure Assessments
WPMN	OECD Working Party on Manufactured Nanomaterials

1 Background

1.   Regulatory decisions are risk-based and must be informed by the best available scientific evidence fulfilling the legislative and regulatory requirements of member countries. The OECD recommendation on manufactured nanomaterials (OECD, 2013b) states that: "to manage the risks of manufactured nanomaterials, [country members should] apply existing international and national regulatory frameworks or other management systems, adapted to take into account the specific properties of manufactured nanomaterials". As such, an overview of the traditional risk assessment paradigm, which is the basis for most regulatory frameworks for chemicals, is provided here, followed by a description of member-country-specific frameworks and their adaptations for the characteristics of nanomaterials.

2.   The classical risk assessment framework includes four main steps: hazard identification, hazard characterisation, exposure assessment, and risk characterisation (NRC, 1983). These steps are informed by research that includes laboratory and field observations of adverse effects and exposures. The research is supported by the use of models and analogues.

3.   Risk assessment outputs can include qualitative descriptions (such as 'negligible, 'moderate' or 'severe'), and quantitative estimates of various levels of sophistication from semi-quantitative and deterministic-quantitative to probabilistic-quantitative (NRC, 2009). Risk estimates should identify a level of precision, uncertainty of mathematical derivations, and subjective interpretations that may underlie the base evidence. Quantitative estimates of risk should always be accompanied by a description of the associated uncertainties. The output of a risk assessment (qualitative vs. quantitative) may depend on the availability and the quality of the supporting science, evidence, and analysis, as well as the needs of the end-user. The evidence base, including uncertainties and assumptions used to estimate risk quantitatively, should be adequate to support the level of precision in those estimates.

4.   In 2009, the National Research Council (NRC) re-evaluated its 1983 risk assessment framework and made recommendations for improvements (NRC, 2009). In its report the NRC recommended retaining the four basic steps of the risk assessment as the central phase of the process, while adding an initial problem formulation and scoping phase and a final risk management phase (Figure 1.1). The updated NRC framework identifies options to reduce hazards or exposures and to evaluate the merits of the various options (NRC, 2009). Various elements proposed in the framework are currently implemented in legislation such as EU's regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH; European Commission, 2006) (e.g. iterative risk assessment, formulation of testing proposals, stakeholder involvement).

Figure 1.1. A framework for risk-based decision-making that maximizes the utility of risk assessment, adapted with modifications from NRC (2009).

PHASE 1	PHASE 2	PHASE 3
Problem Formulation and Scoping	Planning and Conduct of Risk Assessment	Risk Management

Questions including but not limited to:

- What problems or concerns are associated with existing or expected conditions?

- What potential options exist for altering problematic conditions?

- What risk or other assessments are necessary to evaluate possible options under the given context?

Stage 1: Planning
- What is necessary to characterize risks of existing conditions and effects of proposed options? What level of uncertainty and variability is appropriate?

Stage 2: Risk Assessment
- *Hazard Identification*
- *Dose-Response Assessment*
- *Exposure Assessment*
- Risk Characterisation

NO

Stage 3: Confirmation of Utility
- Does the assessment have the attributes called for in planning?
- Does the assessment provide sufficient information to discriminate among risk management options?
- Has the assessment been satisfactorily peer-reviewed?

YES

Risk Management incl. appraisal of:

- Relative benefits of all options?

- Effects on other factors(incl. costs)

- Uncertainties

- Decision on options

- Communication

- Effectiveness of the decision

INTERNAL AND EXTERNAL STAKEHOLDER INVOLVEMENT
- Involvement of decision-maker, technical specialists, and other stakeholders should not compromise the technical assessment of risk

1.1. Health / Environmental Risk Assessment Framework for Chemicals

5.   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 assessment of chemicals is an assessment of the physico-chemical properties and possible exposure pathways. This is essential as it determines not only the extent to which various organisms (in environmental risk assessment) or tissues/(sub)populations (in human health risk assessment) might be exposed, but also indicates the different exposure routes, and therefore which toxicity data are most relevant.

6.   The human health risk assessment may include one or more types of effect, or endpoints. These include potential evaluation of acute toxicity, repeated dose toxicity, irritancy, sensitisation potential, genotoxicity, carcinogenicity and reproductive toxicity. Assessment regularly also includes evaluation of toxicokinetic properties as well as mechanistic studies. The routes of exposure used in the testing regime and the specific tests conducted are governed by the physico-chemical properties of the substance, as well as its likely use and human exposure scenarios. Potential exposure routes used in animal testing include oral (delivered in the feed, drinking water or by gavage), dermal, inhalation and parenteral routes.

7.   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

transformation/(bio)degradation, hydrolysis, bioconcentration, adsorption/desorption, short- and long-term aquatic ecotoxicity, testing algal growth inhibition, secondary poisoning and effects on terrestrial and micro-organisms. Potential environmental compartments include surface water, sewage treatment plants, soil, sediment and groundwater.

1.2. Country-Specific Chemical Risk Assessment Frameworks and Adaptations for the Characteristics of Nanomaterials

1.2.1. Harmonization and Common Information Requirements

8. Regulatory approaches for chemicals and manufactured nanomaterials differ among 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), first published in 2002 and regularly updated, which now provides common and consistent criteria replacing various different standards (UN, 2019). The applicability of the GHS criteria to nanomaterials was reviewed for four nanomaterials and selected health hazard classes (Larsen et al., 2019), concluding that in general the GHS classification criteria are considered applicable for the data on the selected nanomaterials. Nevertheless, the United Nations continues to monitor the applicability of the GHS criteria to nanomaterials.

9. Frameworks require information to identify the chemical, and in the case of nanomaterials this includes physico-chemical properties to enable their physical characterisation. International risk assessment frameworks for chemicals across the OECD also consider in conjunction the physico-chemical characteristics of the chemical and its toxicological and environmental effects. Although the exact legal requirements differ between countries, all require a certain degree of hazard identification and assessment. Adaptations for nanomaterials generally involve inclusion of additional requirements (i.e. added to the general requirements for chemicals). Such nanomaterial-specific requirements may include:

- *Physico-chemical properties* – e.g. dissolution kinetics (in addition to water solubility), particle size distribution, particle size, shape, surface area, surface chemistry (OECD, 2008);
- *Toxicological information* – evaluation of toxicokinetics, mutagenicity in mammalian cells, different exposure routes in toxicity studies (potentially with additional endpoints or organs of interest);
- *Ecotoxicological information* – evaluation of additional environmental fate parameters, e.g. dispersion stability and dissolution rate as well as transformation under environmental conditions.

1.2.2. Australian Risk Assessment Approach

10. The Australian Industrial Chemicals Introduction Scheme (AICIS) replaced the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) on 1 July 2020 as the national regulator of industrial chemicals in Australia (https://industrialchemicals.gov.au). Under AICIS, the decision to assess a chemical introduction pre-market is based on the potential for exposure of workers, the public, and the environment and this is called 'Categorisation' (https://industrialchemicals.gov.au). A chemical importation or manufacture (introduction into Australia) must be categorised into 5 categories:

- Listed (chemicals listed on the Australian Inventory within the terms of listing);
- Exempted (very low risk to human health and the environment);
- Reported (low risk to human health and the environment);
- Assessed (medium to high risk to human health and/or the environment); or
- Commercial evaluation.

11.   Under AICIS, nanomaterials will be categorised as being at the nanoscale based on the proportion of particles present in the nanoscale size range (1-100 nm), the solubility of the chemical, and whether the nanoscale particles are intentionally produced. New nanomaterials assessment will follow the framework for conventional chemicals, using the internationally harmonised risk assessment paradigm. Standard information requirements apply equally to bulk chemicals and nanoforms and generally depend on their introduction volume and hazard characteristics. Substances that are considered to be at the nanoscale may be subject to additional data requirements, determined on a case-by-case basis. Listed chemicals including their nanoforms will be subject to post-market evaluation, based on health and/or environmental concerns.

1.2.3. *Canadian Risk Assessment Approach*

12.   Chemical substances, including nanomaterials, are assessed and managed as part of Canada's Chemicals Management Plan, under the authority of the Canadian Environmental Protection Act, 1999 (CEPA)[1]. Under the CEPA framework, chemical substances are either "new" or "existing". New substances are those that are not listed on the domestic inventory called the Domestic Substances List (DSL). CEPA requires the notification and pre-market assessment of "new" substances prior to their import into or manufacture in Canada. Using available information, including that required of notifiers under the New Substances Notification Regulations (Chemicals and Polymers), Environment and Climate Change Canada and Health Canada jointly conduct pre-market risk assessments and may impose control measures on notified substances if concerns are identified (using instruments such as a Significant New Activity Notice, Ministerial Condition, Ministerial Request for Additional Information, or Prohibition).

13.   Following assessment, a substance may be added to the DSL. When that substance could be manufactured at the nanoscale, a SNAc Order may be published to require nanoforms to be notified and information provided to help fill nanomaterial-specific data gaps for risk assessment. Existing substances are those that are listed on the DSL and available for commerce in Canada. Canada is developing an approach to address existing nanomaterials that entered Canadian commerce without prior assessment, as described in the 2016 consultation document: "Proposed Approach to Address Nanoscale Forms of Substances on the Domestic Substance List". As part of this approach, a mandatory information gathering survey under s.71 of CEPA was published in 2015, which required reporting on 206 nanomaterial substances, of which 53 were identified as being in commerce. Canada has since been identifying data gaps, setting priorities for information gathering and assessment, and developing a risk assessment framework (RAF) for manufactured nanomaterials under CEPA.[2]

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[1] For more information about Canada's risk assessment approach, visit: https://www.canada.ca/en/health-canada/services/chemical-substances/canada-approach-chemicals/risk-assessment.html

[2] Canada's 'Proposed Approach to Address Nanoscale Forms of Substances on the Domestic Substance List' document is available at: https://www.canada.ca/en/environment-climate-change/services/canadian-environmental-protection-act-registry/publications/approach-nanoscale-forms-substances-list.html

1.2.4. Japanese Risk Assessment Approach

14.   The revision in 2009 of the "Chemical Substances Control Law" introduced an approach towards the risk of all existing and new chemicals for industrial use in Japan. 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 prioritization matrix based on conservative assumptions ("Screening" process). A risk assessment is then conducted 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 implemented under the "Industrial Safety and Health Law". The Law obliges employers to investigate risks due to chemical substances and take necessary measures to prevent health impairment of workers. In addition, for chemical substances of high concern, employers have been obliged to investigate the risks since June 2016. The Authority undertakes the risk assessment for high priority substances (highly hazardous substances) in order to enact rational regulations or measures.[3]

1.2.5. Korean Risk Assessment Approach

15.   "Korea's Act on the Registration and Evaluation, etc. of Chemical Substances", enforced in January 2015, is legislation on chemical registration and evaluation, and safety management of chemical products stipulating matters on producing and utilizing hazard and risk dossiers. Any person who intends to manufacture or import a new substance in quantities of more than 100 kilograms per year, or an existing substance in quantities of more than 1 tonne per year, should submit risk dossiers including physico-chemical data, toxicology and scenarios of exposure to the environment based on manufacture/import volumes. The risk/hazard assessment should be conducted based on the technical dossiers. New substances must be registered prior to manufacture or import. However new substances less than 100 kg per year only require notification and do not need to go through hazard evaluation. Chemicals that are considered hazardous in a hazard assessment would be designated as toxic chemicals. And if chemicals are regarded as of risk/concern they would be designated as substances subject to authorization, restriction and prohibition according to risk levels. These substances subject to authorization, restriction, and prohibition are managed by the "Chemicals Control Act". The term 'nanomaterials' has been defined and nano-specific risk assessment approaches are currently being developed.

1.2.6. United States' Risk Assessment Approach

16.   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). 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,

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[3] For more information about Japan's risk assessment approach, visit the following websites:

Regarding risk assessment under the "Chemical Substances Control Law":

- http://www.meti.go.jp/english/policy/safety_security/chemical_management/index.html
- http://www.meti.go.jp/policy/chemical_management/english/index.html
- http://www.meti.go.jp/policy/chemical_management/english/cscl/files/about/02Progres.pdf

Regarding risk assessment under the "Industrial Safety and Health Law":

- https://www.mhlw.go.jp/new-info/kobetu/roudou/gyousei/anzen/dl/180815-01.pdf
- https://www.jisha.or.jp/english/act/index.html

name, and health and safety data) 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 [4]. Occupational risk assessment research is conducted by the National Institute for Occupational Safety and Health (NIOSH) as a basis for developing recommended occupational health and safety measures. 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.

1.2.7. European Union's Risk Assessment Approach

17.   The REACH Regulation (European Commission, 2006) concerning chemicals together with the Classification, Labelling and Packaging (CLP) Regulation (European Commission, 2008) provide an advanced and comprehensive regulatory instrument for the risk assessment of chemicals in Europe. REACH includes the requirement for registration of substances (including their forms and states) manufactured or imported by a company in quantities of 1 or more metric tonne per year. A technical dossier must be submitted and, at volumes of 10 or more metric tonnes per year, a chemical safety assessment is to be performed and reported by the registrant. Its provisions are underpinned by the precautionary principle. While nanomaterials have always been covered by the definition of substance [5] under REACH, with the adopted changes to the REACH Annexes in December 2018, REACH now includes specific provisions for nanomaterials (European Commission, 2018). Provisions are introduced for nanoforms [6], specific forms of a substance that fulfil the conditions in the modified REACH Annex VI, taken ad verbatim from the Commission Recommendation (2011) on the definition of nanomaterial.

18.   The Recommendation was developed for the explicit purpose of ensuring a consistent approach to regulate nanomaterials across different areas of EU legislation. While the substance remains the principal subject of the requirements under REACH, the amended REACH annexes introduce specific requirements when the substance covered by the registration is in nanoform. REACH obliges registrants

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[4] A description of the US EPA approach to controlling the risks of nanoscale materials including whether a nanoscale substance is a "new" chemical for the purposes of the TSCA inventory is available at:
https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/control-nanoscale-materials-under

[5] 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.

[6] Definition of a nanoform and a set of similar nanoforms:

> On the basis of the Commission Recommendation of 18 October 2011 on the definition of nanomaterial (1), a nanoform is a form of a natural or manufactured substance containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm, including also by derogation fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm.

> For this purpose, "particle" means a minute piece of matter with defined physical boundaries; "agglomerate" means a collection of weakly bound particles or aggregates where the resulting external surface area is similar to the sum of the surface areas of the individual components and "aggregate" means a particle comprising of strongly bound or fused particles.

> A nanoform shall be characterised in accordance with section 2.4 below. A substance may have one or more different nanoforms, based on differences in the parameters in points 2.4.2 to 2.4.5.

A "set of similar nanoforms" is a group of nanoforms characterised in accordance with section 2.4 where the clearly defined boundaries in the parameters in the points 2.4.2 to 2.4.5 of the individual nanoforms within the set still allow to conclude that the hazard assessment, exposure assessment and risk assessment of these nanoforms can be performed jointly. A justification shall be provided to demonstrate that a variation within these boundaries does not affect the hazard assessment, exposure assessment and risk assessment of the similar nanoforms in the set. A nanoform can only belong to one set of similar nanoforms

to ensure that their registrations demonstrate that all forms of the substance covered by that registration can be used safely. The focus of attention should therefore be on ensuring that the submitted data are applicable/appropriate for all the 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 bulk forms. The registrant must ensure that test results are representative of the form(s) of the registered substance.

19.   Alternatively, when read-across is used between the forms, the registrant has to make sure that this is scientifically justified. To ensure transparency regarding the coverage of the different nanoforms by the individual registration dossiers, REACH requires registrants to characterise all nanoforms. The dossier must document this in the information on substance composition for each nanoform individually or jointly as part of sets of similar nanoforms[13]. The required characterisation includes information on particle size distribution, shape, area and surface chemistry including functionalization. When a set of nanoforms is used as an assessment entity, the boundary intervals of all characterisers must be clearly provided and the similarity between the included nanoforms regarding hazard, exposure and risk justified in advance.

20.   Further in the dossier, all of the compiled information on use, hazard and safety assessment has to be associated with thus defined entities. Other amendments aim to clarify how to fulfil REACH information requirements including nanomaterial-specific tonnage triggers (e.g. for mutagenicity), emphasis on specific exposure routes (e.g. inhalation for acute toxicity) and endpoints (e.g. toxicokinetics), and the requirement to perform chemical safety assessments of substances in nanoform including downstream uses. The amendments also address, for example, the need for adequate characterisation of test samples, responsible use of specific test methods (e.g. mammalian cell assays such as the comet assay) and adaptation possibilities such as waivers.

21.   The European Chemicals Agency (ECHA) has published guidance on how to fulfil the REACH requirements for nanomaterials in 2012, 2017, 2019[7] and a manual in 2020[8]. The ECHA guidance provides advice, inter alia, on how to distinguish between nanoforms of a substance and how to meet the information requirements set out in Annexes VI-XI to the REACH Regulation; including how to justify the use of hazard data between nanoforms of the same substance.

22.   A risk assessment approach for chemical substances used in the workplace is also implemented under the European Framework Directive on Safety and Health at Work. Since June 2016, the directive requires employers to investigate risks arising from chemical substances in the workplace and to take necessary measures to prevent impairment of the health of workers from these chemicals. The Authority undertakes the risk assessment for high priority substances (highly hazardous substances) in order to enact rational regulations or measures.

23.   In 2018 the European Food Safety Authority (EFSA) updated the Guidance document for assessing the risk assessment for consumers through dietary exposure. The guidance highlights the need for an extensive physico-chemical characterisation of nanomaterials and for adapting the design of the toxicity tests when the rate of dissolution in the gastrointestinal track confirms that particles will reach the intestinal epithelia. In addition, the guidance highlights that from the risk assessment perspective, the consideration of characteristics at the nanoscale is not limited to materials manufactured as nanomaterials and should be also considered when assessing materials that, although not covered by the legal definitions, contains a fraction at the nanoscale.

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[7] https://echa.europa.eu/de/-/updated-guidance-for-registering-substances-in-nanoform

[8] https://echa.europa.eu/manuals

24.   This need has triggered a mandate from the European Commission to EFSA. Following the mandate, EFSA has prepared a guidance with Technical Requirements applicable to conventional materials in the food and feed area that require assessment at the nanoscale. In parallel EFSA has updated again the risk assessment guidance for nanomaterials.

2 Issues in Risk Assessment on Nanomaterials

25.   General risk assessment principles are applicable to both chemicals and nanomaterials. These include: i) substance identity; ii) physico-chemical properties; iii) industrial and consumer uses and environmental releases; iv) environmental fate and behaviour; and v) Absorption, Distribution, Metabolism, and Excretion (ADME) 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; NRC, 2009). However, there are areas of uncertainty that present challenges to nanomaterial risk assessment (Canady, 2010; OECD, 2010). This chapter discusses a range of important issues that should be considered to enhance nanomaterial risk assessments, especially in a context of limited data availability, as well as progress made in the last decade (2010-2020) in addressing key research questions and issues.

26.   As progress is made in collecting relevant data sets, validating methods, and updating regulatory requirements, it is expected that uncertainties associated with risk assessments for nanomaterials may decrease. Additionally, some progress has been made with regard to predictive modelling. For example, a proof-of-concept predictive model used physico-chemical property data to predict relative acute pulmonary inflammation response in rodents (Drew et al., 2017). Others have shown that Quantitative Structure-Activity Relationship (QSAR) modelling can help establish the relationship between a nanomaterial's behaviour in biological systems and its physico-chemical properties (Burello and Worth, 2011).

27.   In terms of a risk assessment strategy, developed during problem formulation, the following pose significant challenges:

- identifying the availability of reliable and relevant data, and in particular quality physico-chemical, fate and effect data, and exposure information;
- lack of information evaluating the uncertainty associated with describing the fate and distribution of the nanomaterial in the environment, as well as in occupational settings or consumer settings;
- understanding the limitations of effects characterisation, and extrapolating to chronic no-effect or benchmark concentrations;
- selecting appropriate methods for quantitatively or qualitatively determining whether the nanomaterials will pose a risk; and
- examining the implications of possible 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).

28.   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.

2.1.   Problem Formulation and Scoping

29.   In the context of risk assessment, problem formulation is a systematic approach that identifies factors critical to risk assessments and formulates risk hypotheses considering the purpose of the assessment, the required scope and depth of analysis, resources and outcomes of the assessment, and the overall risk management goal(s). Problem formulation requires a precise definition of relevant sources and targets of suspected harm and for nanomaterials, the often limited depth of information (qualitative and quantitative) on sources and targets may represent a challenge in the process. Problem formulation provides a clear definition of the minimum data required to demonstrate safety. Problem formulation evaluates the level of generalisation required in the assessment and defines a scientifically sound approach, including appropriate methods and reporting, for use of information from dissimilar materials (OECD, 2014a). The Report of the Workshop on Risk Assessment of Manufactured Nanomaterials in a Regulatory Context (OECD, 2010) included the following recommendations, which remain relevant:

- Consider the "particle nature" of the material, such as the size, surface properties and interactions, the relation of metrics used, and the characteristics of the material (i.e. particle-by-particle characterization);

- Assess and accommodate 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 and biological components as well as transport or translocation mechanisms in exposure and toxicity contexts.

30.   In the scoping of the risk assessment during problem formulation, it should be determined whether exposure to nanomaterials from natural and incidental sources is relevant to the assessment. Nanomaterials are known to be unintentionally produced and released into the atmosphere by natural phenomena and as a by-product of many human industrial and domestic activities such as in the transportation sector from internal combustion and jet engines. The scope should define whether the contribution of these sources (e.g. to aggregate exposure for the nanomaterial being assessed) is of relevance to the assessment. The WPMN is primarily concerned with the safety of manufactured nanomaterials, such as those that are intentionally produced for use as nanoscale components of consumer products and in advanced technologies. So far, the focus of the toxicology community has been on investigating the safety of first-generation MNs and their products, the properties of which have not been subject to change during their use. However, there is an increase in growth of next generation materials and products (Roco 2011). These include second-generation materials and products, the properties and functions of which may be intentionally tailored to change during their use or in response to the environment in which they are used. The third generation of MNs and products involve nanosystems consisting of both first- and second-generation nanomaterials (e.g. synthetic organs, engineered microbes, self-assembling materials) and are expected to change and evolve. Lastly, the fourth generation of nanomaterials and products involve molecular nanosystems with specific functions. In a more recent publication two additional generations were added to capture the latest developments in the field, i.e. immersion of nanotechnology with other emerging and established technologies (Roco, 2017). Thus far, the risk assessment procedures explored for nanomaterials have not considered this changing landscape of the nanomaterial universe.

31.   Another aspect that is considered in the problem formulation stage is the adversity of potential effects. OECD Test Guidelines refer to adverse effects and define them in the 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 MN, there is debate about the definition of adversity for specific effects. One example is, all MN induce tissue inflammation and whether this should be considered as an adverse response or simply a defence mechanism, is not clear. Another 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. It is also important to note that some of the responses observed following exposure to nanomaterials in animal experiments (e.g. tissue inflammation or lung fibrosis) are currently not considered in risk assessment of chemicals. As a result, internationally harmonised methodologies for their assessment do not exist.

32.   The problem formulation should also consider the approach for assessing conventional materials, not covered by the legal definitions for nanomaterials, containing a fraction of particles at the nanoscale. This need follows regulatory decisions such as the consideration of nanomaterials as "nanoforms" of the same substance in REACH (European Commission, 2018) or EFSA's establishment of Technical Specifications for conventional materials containing a "nanofraction".

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

33.   Considering the lack of high quality reliable and relevant empirical data, other methods for filling data gaps may be considered. These may include read-across, grouping, comparative potency, or estimation of Occupational Exposure Bands for use in control banding (Kuempel et al., 2012; OECD, 2012a; Gordon et al., 2014; ISO/TR 18637: 2016; ISO/TR 12901-2: 2014; NIOSH, 2017; Dunn et al., 2018).

2.2.1.   Quality, Adequacy and Reliability of Data

34.   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, have been evaluated for their applicability to nanomaterials. OECD Test Guidelines (TGs) specific to nanomaterials for three endpoints: dispersion stability, subacute inhalation toxicity and subchronic inhalation toxicity were published in 2017 and 2018 (OECD, 2017; OECD, 2018a; OECD, 2018b), accompanied by an updated Guidance Document on inhalation toxicity (OECD, 2018c). For physico-chemical property characterisation two documents were published in 2019 (OECD, 2019a; OECD, 2019b). In 2020 new OECD Guidance Documents (GDs) 317 for Aquatic And Sediment Toxicological Test and 318 for the testing of Dissolution and Dispersion Stability of Nanomaterials were also published (OECD, 2020c; OECD, 2020d). A number of other TGs for physico-chemical property characterisation are in preparation[9]. Rasmussen et al. (2019) presents an overview of OECD TGs under development that are applicable to testing nanomaterials. Nanomaterials present particular challenges in terms of behaviour. For example, agglomeration/aggregation impacts the exposure characterisation (including deposition and distribution of nanomaterials) making it difficult to derive dose-response relationships and interpret analytical measurement results. Most published studies lack adequate physico-chemical

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[9] The OECD is developing a number of TGs addressing physico-chemical endpoints of nanomaterials, i.e. on surface area, particle size and size distribution, dissolution rate in biological media, surface chemistry, surface hydrophobicity, and dustiness. See the Work plan for Test Guidelines Programme, https://www.oecd.org/chemicalsafety/testing/Test_Guidelines_Workplan_2020.pdf

characterisation information for nanomaterials in their pristine form, in exposure media and in various biological or environmental compartments of their life cycle. Also, the form of the nanomaterial tested is often not representative of the form to which cells and organisms are exposed. As a consequence, risk assessors should ensure that the test material being examined: a) has been adequately characterised, b) represents a realistically conservative form of the material to which an individual or organism has been exposed; and c) is adequately representative of the test material used in effects testing. In addition, multiple dose groups and sufficient dose ranges are needed to adequately characterize the dose-response relationship.

35.   A number of steps have been taken to ensure that data is of high quality in risk assessment. For example, the German BMBF project DaNa2.0 (2013-2019) built a Literature Criteria Checklist[10] to serve as quality criteria for the toxicological publications used as a knowledge basis of the project. In addition, Card et al. (2010) built a two-step system based on the ToxRTool for assessing the quality of toxicity studies with nanomaterials, and Hartmann et al. (2017) built the NanoCRED web tool for the evaluation of ecotoxicity studies with nanomaterials for regulatory purposes on the basis of reliability and relevance criteria (http://www.scirap.org/). More recently, as part of the OECD WPMN project on 'Advancing Adverse Outcome Pathway (AOP) Development for Nanomaterial Risk Assessment and Categorisation', a systematic process for searching and mining the toxicity literature was established to identify key events (KEs) and adverse outcomes of relevance to nanomaterials. This project also established a database called NanoAOP, to enable gathering of biological plausibility or weight of evidence specifically for assessing tissue inflammation and tissue injury KEs induced by nanomaterials (OECD, 2020a; OECD, 2020b; Halappanavar et al., 2019; Halappanavar et al., 2021). These studies also demonstrated the challenges associated with using the existing toxicology data derived from *in vitro* and *in silico* methods as these methodologies are not formally validated for MNs.

2.2.2. *Analogues, Grouping and Read-across*

36.   When experimental data for the NM being assessed are not available or limited, existing data from one or more analogous nanomaterials may be considered (i.e. the "read-across" approach). Read-across from one MN to another (nano-to-nano) or from a bulk substance to a MN requires strong evidence and justification. In view of the diversity of MNs (or 'nanoforms') possible within one chemical composition or molecular identity, there is a 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). It is important to develop an understanding, and demonstrate the relationship between, biological effects and the physico-chemical properties (e.g. size, dissolution, surface property and functionalization, shape, aspect ratio), toxicokinetics, and toxicodynamics of the MN to build categories and enable QSAR approaches (OECD, 2016a; OECD, 2016b; Afantitis et al., 2018; Varsou et al., 2019; European Commission, 2014).

37.   The OECD published guidance in 2014 for grouping of chemicals with general principles that can be followed when assessing the validity of an analogue (OECD, 2014a). This includes a Section (6.9) specific to MNs titled 'initial considerations applicable to manufactured nanomaterials'. A workshop was held in Brussels in 2016 to discuss among experts in the field the specific aspects to be considered in a regulatory context when applying grouping and read-across to the hazard assessment of MNs (OECD, 2016b). The field of grouping for MNs has advanced significantly since 2014 and it is widely recognized by industry and regulators that grouping strategies for MN are urgently needed (European Commission, 2019).

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[10] https://nanopartikel.info/en/knowledge/literature-criteria-checklist/

38.   The EU REACH as amended in 2018 introduces the concepts of "nanoform" and "sets of nanoforms". It requires manufacturers and/or importers to submit the necessary information on certain intrinsic and extrinsic properties for each registered nanoform, whether on its own or for a set of similar nanoforms to which it belongs. It also expects that the information requirements under REACH are fulfilled for all nanoforms covered by the registration, with the relevance of the data provided explicitly established. A guidance document *Appendix R.6-1 for nanoforms applicable to the Guidance on QSARs and Grouping of Chemicals, Version 2.0* (ECHA, 2019a) was published in order to assist users in complying with their obligations under the amended REACH Regulation. The guidance addresses important considerations regarding grouping and read-across for NMs and aims to provide scientifically justified approaches and guidance for read-across between nanoforms under REACH. It describes a tiered strategy to justify read-across between different nanoforms of the same substance and outlines six steps: 1) identification of the nanoforms according to physico-chemical parameters; 2) initial grouping according to similarities in physico-chemical parameters, fundamental behaviour and reactivity; 3) identification of available data and data gaps; 4) identification of possible source of NMs to fill in data gaps; 5) definition of a testing strategy to validate the hypothesis; and 6) performance of additional experiments, where needed.

39.   The EFSA guidance on Technical Requirements (TR) for conventional materials containing a fraction of particles at the nanoscale is published in 2020. This guidance provides technical details for assessing under which conditions safety studies conducted with conventional materials can be extrapolated and used for assessing the risk of the fraction of nanoparticles.

40.   Information from microscale particulate materials can be used as a reference point in comparative potency assays with nanoscale materials. While use of microscale materials may be informative to the size-dependency of effects, they typically cannot be used in place of information specific to the MN. However, in circumstances where hazard data on the non-nanomaterial indicates a concern[11] it may be possible to use data for read-across to the nanomaterial. In cases of soluble materials, if it is established that the observed toxicity is due to the particle solubility, then information on related bulk material may be used in read-across. 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, volume, or number). For some subclasses of nanomaterials a relationship between the responses of nanoparticles 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, 2011; GRACIOUS[12]). Using the available data on such a relationship may assist in the risk assessment for those specific subclasses.[13]

41.   The state-of-the-art in grouping approaches for the hazard assessment of MN is included in a review by Lamon et al. (2019). The aim of this review was to classify the different existing approaches with respect to REACH that consider grouping for the purposes of read-across, and identification of relevant physico-chemical properties for the different approaches. Giusti et al., (2019) reviewed available approaches to grouping and read-across. Their recommendations include a focus on the importance of harmonized data storage systems, the application of harmonized scoring systems for comparing biological responses, and the use of high-throughput and other screening approaches.

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[11] An example of important properties are solubility and aspect ratio.

[12] See https://www.h2020gracious.eu

[13] A good example of this approach is the substance evaluation of the environmental effects of nano silver performed by the Netherlands.
See: https://echa.europa.eu/documents/10162/13628/SEV-231-131-3-2_conclusion_and_report_public_15577_en.pdf/

42.   In addition, a comprehensive review of EU legislation addressing the safety of chemicals, and possibilities within each piece of legislation for applying grouping and read-across approaches for the assessment of nanomaterials was also performed (Mech et al., 2019a). This review considers both the overarching regulation of chemical substances under REACH and CLP and the sector-specific pieces of legislation for cosmetic, plant protection and biocidal products, and legislation addressing foods, novel foods, and food contact materials.

43.   Tools or frameworks for grouping of NMs have also been developed:

- **DF4nanoGrouping** [14] : decision-making framework for the grouping and testing of nanomaterials, is a functionality-driven concept to group and test nanomaterials. Overall, the DF4nanoGrouping aims to group NMs by their specific mode-of-action that results in an apical toxic effect (Arts et al., 2015; Arts et al., 2016);

- **NANoREG II project**: ended in February 2019 and was funded under EU Horizon 2020 (H2020). The project has further developed grouping concepts and explored coupling of them with safe-by-design principles to the NM regulatory process (EU Commission, 2019);

- **GRACIOUS Framework**: GRACIOUS is a project funded under EU H2020 establishing governance frameworks to guide read-across and grouping of nanomaterials for the purposes of supporting risk assessments and to inform safe-by-design principles for industry and regulatory stakeholders. The GRACIOUS [15] project has developed the framework "*based on physico-chemical, release, exposure, environmental fate, toxicokinetic and toxicological information…[and] builds upon currently available approaches by collating, curating and assimilating existing and new knowledge on intrinsic and extrinsic physico-chemical properties in relation to their (environmental) health risk*" (European Commission, 2021). "*The initial collection of basic information allows selection of an appropriate pre-defined grouping hypothesis and a tailored Integrated Approach to Testing and Assessment (IATA), designed to generate new evidence to support acceptance or rejection of the hypothesis"* (Stone at el., 2020);

- **ECETOC NanoApp**: helps registrants follow ECHA's new registration requirements for nanomaterials under the EU's REACH legislation. It does this by creating and justifying 'sets of similar nanoforms' for a joint human health and environmental hazard, exposure and safety assessment (Janer et al., 2021).

2.2.3. Nanoinformatics and QSAR

44.   Quantitative structure activity relationships (QSARs) are mathematical relationships that link the structure of a chemical compound to an activity of interest (e.g. toxicity) in a quantitative manner. QSAR and computational informatics approaches are used to fill gaps in physico-chemical, environmental fate and biological effects data and advance our ability to categorize and group nanomaterials for decision making and predict toxicity. With respect to nanomaterials, attempts have been made to leverage the predictive capability of QSAR modelling for toxicity screening, prioritisation of nanomaterials for more advanced testing and for correlating the characteristics of nanomaterials to their biological responses (Chen, 2017; Oksel, 2017). The published nano-QSAR models to date are mostly relevant to metallic nanomaterials (i.e. metals and metal oxides), owing to the large amount of toxicity and characterization data that is available for modelling (Chen, 2017; Oksel, 2017). Cellular uptake and cell death are the two endpoints routinely used in QSAR modelling (Zhang et al., 2012; Singh and Gupta, 2014; Shin et

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[14] The DF4nanogrouping framework focuses on human inhalation toxicity only

[15] See www.h2020gracious.eu

al., 2017). While these studies demonstrated the possibility of QSARs to predict biological activity for nanomaterials, it is recognized that this field is still in its infancy.

45.   A recent study performed within the H2020 project ProSafe reviewed more than 20 QSAR models for their potential to be used in regulatory assessments of nanomaterials. The study concluded that QSAR techniques can be applied for read-across, analysing the outcome of functional assays, outlining AOPs and Modes of Action (MoA) and for supporting the design of safe nanomaterials during the Research and Development (R&D) phase. However, the study also concluded that the majority of models analysed do not fully comply with all five of the OECD validation principles and/or do not consider relevant endpoints. As such, the use of QSAR models for regulatory purposes is currently proposed only as part of a weight of evidence approach in conjunction with *in vitro* and *in vivo* information (Burello, 2017).

46.   Another study by Furxhi et al., 2020a compared the results of machine learning models with data from published empirical studies for nano (eco)-toxicological endpoints. It was concluded that these machine learning techniques were applied in the field of nano(eco)toxicology with very encouraging results. Machine learning was demonstrated to be effective in identifying the features of nanomaterials affecting toxicity and for predicting possible adverse effects (Furxhi et al., 2020b).

47.   Before QSAR models can be used in risk assessment processes, some challenges have to be overcome. Among these are the urgent needs for empirical physico-chemical, mammalian toxicity and ecotoxicity datasets for robust model development. The available datasets currently used for development of nanomaterial QSARs are limited and lack quality and reliability (Afantitis, 2020; Chen, 2017; Oksel, 2017) and so current QSAR models are not highly robust. There is a need to generate structure-activity data and organize them into databases to categorize and group materials for decision-making. These databases will facilitate the prediction of toxicity and support the weight-of-evidence to validate other empirical data being generated. Proof of concept models have been developed (e.g. Gernand and Casman, 2014; Gernand and Casman, 2016; Drew et al., 2017) for QSARs, but physico-chemical data are generally still too limited across a range of MN to predict hazard potency based on physico-chemical information alone. In addition, information on mode of action may facilitate development of category-based hazard and risk characterisation (OECD, 2014a).

48.   Another important consideration is available nano-QSAR tools under common analysis platforms (Afantitis et al., 2020). This will allow for benchmarking and validation of available models which is crucial for their adoption for risk assessment purposes.

49.   A major effort was made towards developing and implementing databases for NMs – both in terms of structuring the information and the data itself to support the application of *in silico* methods. For physico-chemical characterisation and toxicological studies, data and knowledge bases have been created and are currently in use (e.g. eNanoMapper[16] and NanoCommons[17]). Besides their role in long term storage of research data from EU-funded projects, they play an active role in the implementation of FAIR (Findable, Accessible, Interoperable and Reusable) data principles. FAIR principles relate to the reuse of data in nanoinformatics to maximize information generated as well as standardizing data collection and storage. The various nano governance projects play an important role in advancing the FAIR principles, tool development and promotion (e.g. AdvancedNano Implementation Network[18]).

50.   Progress has been made by different projects internationally to develop QSARs, modelling frameworks and IATAs for nanomaterials, such as by NanoSolveIT and NanoInformaTIX, and by the US National Toxicology Program's (NTP) Integrated Chemical Environment (ICE). Within H2020-

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[16] https://search.data.enanomapper.net/

[17] https://www.nanocommons.eu/

[18] https://www.go-fair.org/implementation-networks/overview/advancednano/

NanoSolveIT nanomaterial knowledge bases described above, which include data generated from OECD test guidelines and the scientific literature, were '*analysed for their suitability for integration with nanoinformatics approaches and for the development of NM-specific IATAs for human and environmental risk assessment*' (Afantitis et al., 2020). These data have been integrated with nanoinformatics methods to model the relationships between NM properties and their adverse effects and to predict the effects of other NMs for which less data is available (Afantitis et al., 2020). The first iteration of the NanoSolveIT cloud platform is expected to be released in 2023. In the NanoInformaTIX project, a modelling framework for exposure & toxicity of nanomaterials is expected. This framework will incorporate PBPK modelling, nano-QSARs, systems biology modelling, and *in vitro/in vivo* extrapolation to predict biological effects of nanomaterials at various stages in their life cycles.

51.   All of these initiatives, platforms and tools are supporting computational modelling, AOPs and IATAs development, grouping and read-across approaches and finally the risk assessment and decision-making processes.

2.2.4. Metrics

52.   Preparation of samples and dose administration are critical considerations for tests involving nanomaterials. The OECD has published 'Guidance on sample preparation and dosimetry for the safety testing of manufactured nanomaterials' (OECD, 2012b). Empirical test results for chemicals are primarily expressed in mass-based metrics (e.g. mg/L, mg/m^3, mg/kg body weight), which is also the conventional unit used to describe dose in particle toxicology and is currently used to set the occupational exposure limits for nanomaterials. As described by Donaldson and Poland (2013), mass explains the delivered dose, but not necessarily the biologically active dose. While increasing mass suggests an increasing delivered dose, only a fraction of it may be biologically effective in inducing adverse effects such as tissue inflammation and cell death. Although the necessary analytical methods for accurately measuring the biologically effective dose in experiments are still being developed, where possible, attempts must be made to differentiate between the delivered dose, the tissue retained dose and the biologically effective dose. To describe dose-response relationships across a range of particle sizes, the use of mass-based concentration alone may be insufficient if size-specific particle number or surface area metrics are more closely related to the biological effect (Aitken et al., 2011; Hankin et al., 2011; Huk et al., 2014; Oberdörster, 2018). The OECD recommends that dose is represented by mass, particle number and surface area (OECD, 2012b). While surface area and particle number per unit mass may be the more appropriate dose metrics for nanomaterials, uncertainties associated with their use should be considered in risk assessment.

53.   Dose and exposure estimates need to be expressed in the same metric to enable quantitative risk characterizations, so the choice of metrics for hazard require consistency with metrics used for the corresponding exposure and risk estimation (Simkó et al., 2014). As such, careful consideration must be given to the choice(s) of metric(s) for definition of the limit value for effects, exposure measurements or estimates, and to reliable methods for conversion of units/metrics if required (OECD, 2009a). For example, some studies show that the use of a particle number basis for expressing concentrations in air may be relevant for inhalation of nanofibres for systemic and local effects. In contrast, particle volume may be the most appropriate dose metric to describe effects of coated metal and metal oxide NPs in aquatic organisms and mammalian and piscine cell lines (Simko et al. 2014; Verschoor et al. 2019). NIOSH determined that the higher toxicity of nano titanium dioxide (TiO$_2$) particles compared to larger TiO$_2$ particles may be related to differences in surface area. NIOSH bases their recommended airborne exposure limits on mass (2.4 mg/m^3 for fine TiO$_2$ and 0.3 mg/m^3 for ultrafine TiO$_2$) with a lower permissible limit for the smaller particle (ultrafine size range) (NIOSH 2011). This provides an example of how particle surface area is a factor in toxicity and potency, supporting its use as a metric (e.g. cm^2/g) in risk assessment. In any given case, to support use of surface area as a dose metric, additional

considerations are necessary to determine its appropriateness as not all biological responses can be explained by particle surface area.

54.   Although dose units other than mass may better describe the dose-response relationships across a range of particle sizes, the airborne concentration (e.g. µg/m^3) remains the metric generally used in airborne particulate exposure monitoring and OELs. Knowledge about the mechanisms underlying the observed effect would assist in identifying the most scientifically appropriate dose metrics for MNs or groups of MNs. Implications of using nonstandard dose metrics should be also considered, including consequences for the international Mutual Acceptance of Data, classification and labelling of substances, and reporting in risk assessments. If empirical results are reported in terms of mass-based units, risk assessments may need to include a discussion of any limitations this metric may present.

2.3.   Issues Related to Nanomaterial Identification, Characterization and Physico-chemical properties

2.3.1. *Physico-chemical Properties and Characterization*

55.   Identification and characterization of NMs is required at the outset of a risk assessment to determine if it fits within the definition of a nanomaterial and to inform the scope of the assessment. Nanomaterials are described by their physical as well as their chemical properties, which are not the same as those relevant for conventional chemicals, and often have variable compositions with and between production batches in one or more of these properties. Properties of relevance for nanomaterials include size (and size distribution), shape, chemical composition, crystallinity, surface treatments and coatings, and morphology (ECHA, 2019b[19]; OECD, 2009b; Stefaniak et al., 2013).

56.   The OECD WPMN has published several reports arising from expert discussions and completed projects, including the results of the testing programme initiated in 2007 that involved testing of 11 representative nanomaterials. The OECD examined possible physico-chemical properties in published dossiers of the WPMN Testing and Assessment Programme (OECD, 2016c). Properties examined included: agglomeration/aggregation, chemical composition, water solubility/dispersibility[20], crystalline phase, dustiness, crystallite size, particle size distribution, specific surface area, zeta potential (surface charge), surface chemistry (where appropriate), photocatalytic activity, porosity, octanol-water partition coefficient (where relevant[21]), redox potential, radical formation potential, biodurability and shape (of individual and agglomerate particles). Several important conclusions were drawn from the Testing and Assessment Programme, which include: 1) more than one method may be required for characterizing different types of nanomaterials (e.g. carbon based materials such as graphene, metal oxides, etc.); 2) standardized and non-standardized methods are available for different endpoints, and 3) a detailed description of the methodology and sample preparation should be included when reporting physico-chemical endpoints.

57.   As an outcome of the WPMN Testing and Assessment Programme, detailed descriptions of the relevant physico-chemical endpoints for nanomaterials were published, including available OECD and non-OECD test methods to measure them (OECD 2016c; Rasmussen et al., 2018). The OECD has also

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[19] In the EU, REACH defines chemical composition, particle size number distribution, shape and other morphological characterisation, surface area, and surface chemistry as properties to be determined to be able to identify one nanoform and distinguish it from other nanoforms of the same substance.

[20] Water solubility has been proposed to be substituted with solubility in different media

[21] OECD WPMN has concluded that K_{OW} is not pertinent for MNs, but other parameters such as hydrophobicity may

published Guiding Principles and a corresponding Decision Framework providing details on the steps required to identify physico-chemical properties and material characteristics most needed for testing and interpretation of toxicity results and the most relevant test methods for measuring them for a given nanomaterial (OECD, 2019a; OECD, 2019b). The framework is intended to provide a process to enable decision-making on data needs in consideration of the purposes for which the data is generated, and to support fit-for-purpose risk assessments by reducing uncertainty in the applicability of test methods or strategies (OECD, 2019b). In addition, the EU project NanoDefine has developed a methods manual for size determination to assist in their identification and characterization (Mech et al., 2019b; Mech et al., 2020).

58.   Gao et al. (2018) comprehensively reviewed the progress towards standardisation and validation of methods used to characterise MNs for risk assessments, including limitations and accessibility for each method. The methods evaluated include OECD test guidelines, US EPA documents, and technical reports, SOPs or protocols developed within the EU projects NanoDefine, NanoValid, NanOximet or NANoREG, and protocols and methods included in peer-reviewed publications. In this paper the authors assessed the reliability of the available tools, methods, and protocols to characterize nanomaterials produced as powders, or when dispersed into aqueous and/or biological media for environmental fate and toxicity testing.

59.   Additional guidelines and platforms are available, supporting the generation of high-quality physico-chemical characterisation data (e.g. "Guideline for Method and Protocol Standardization (physico-chemical characterisation methods)" (Cornelis et al., 2018) and the toolbox developed within Horizon 2020 project ACEnano (https://www.acenano-project.eu/acenano-toolbox) that aims to support the work on nanomaterial analytics. This toolbox consists of a Decision Tool for the choice of optimal analytical techniques and a Knowledge Infrastructure for the storage and retrieval of SOPs, coupled with data from interlaboratory comparison studies, and in selected cases demonstrations of the use of techniques through video protocols.

60.   While the methods and tools for accurately characterizing NMs are now in place, a large number of published studies available in the public domain cannot be used for regulatory oversight. This is due to incomplete characterisation and inconsistency in how the results are recorded and reported. In addition to describing the properties of the primary pristine nanoparticles, interactions between nanoparticles and the matrix or media they are situated in for a given environment or formulation must also be considered. As such, nanomaterials should be characterized in their dry state, in suspension in relevant media, and in tissues and cells post-exposure. While dry particle characterisation reporting is improving, a lack of harmonization remains in sampling and preparation methods for characterisation of particles in media relevant to the exposure system (e.g. cell culture medium) and to the biological system studied (e.g. gut fluid, lung fluid, etc.). Moreover, the nanotoxicology field still has an incomplete understanding of which physico-chemical properties are relevant for specific adverse outcomes. Moving forward, a fit-for-purpose list of characteristics specific to the type of NM or to the adverse outcomes that they induce, should be developed.

61.   In the food and feed area, the EFSA (2018) guidance already provides details for performing the physico-chemical characterisation of nanomaterials and their residues in food. An update of the guidance has been published in 2021.

62.   During the life cycle of a nanomaterial, its properties may change depending on the conditions of the environmental and biological media or formulation they are situated in (Abbott et al. 2014). Such 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.). Physico-chemical characteristics subject to change may include agglomeration and aggregation, surface charge, surface modification (e.g. capping agents, coatings, functionalisations, bioconjugations, etc.), dissolution, degradation, adsorption of different species, among others. Therefore, methods used in hazard identification and assessment may

also need to be augmented to include the above considerations. 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 (Mitrano, 2015).

63.   For soluble particles, the determination of particle dissolution or solubility is key as it is integral to biopersistence or tissue retention and clearance of soluble nanomaterials. This information is critical for regulators as some nanomaterials that dissolve rapidly and completely may no longer fall under nano-specific regulation.

64.   Another life cycle aspect is nanomaterials being coated by proteins and other biomolecules in suspension forming what is referred to as a corona. The composition of the corona is complex and dynamic, meaning that the number, types and relative amounts of proteins or other biomolecules on the surface of the nanomaterial change across different environmental and biological media or compartments. The surface adsorption of biomolecules such as proteins alters the intrinsic properties of the NM and plays an important role in the interaction between NMs and biological structures, and their uptake and fate in cells and tissues. This adsorption may either increase their bioavailability and reactivity within biological systems or dampen it. While it has been postulated that knowledge of the corona can aid in identifying a Molecular Initiating Event (MIE), which can then be used to build predictive models of toxicity, it has been difficult to generate such data because of the complexity of these interactions.

2.3.2. *Material Heterogeneity, Batch-to-Batch Variation and Nanoform Variants*

65.   It may be identified during the nanomaterial characterization step that there is substantial variation in the properties of a given material from producer to producer and/or from batch to batch[22] (Izak Nau et al., 2015; Mülhopt et al., 2018). Such variations are important to understand for risk assessment, because of the influence of material properties on toxicity. For example, for multi-walled CNTs variation in length, metal content, aggregation and surface chemistry is known to influence toxicity (Johnston et al., 2010; Allegri et al., 2016) and for fullerenes, heterogeneity in the degree of surface modification and/or aggregation was reported to influence their toxicity (Chae, 2010). Variability in the composition or properties of the specific nanomaterial may occur between batches or formulations due to changes in manufacturing processes. Such variability may cause (quantitative) differences in toxicological effects and affect the outcome of hazard characterizations.

66.   The amended European REACH regulation (European Commission, 2006; European Commission, 2018), as discussed above (section 2.2.2), has introduced the concept of sets of similar nanoforms in which "almost identical" characterizers, as well as forms that differ beyond batch-to-batch variation, can be grouped together as a single entity given appropriate justification and clearly defined boundary criteria in which hazard, exposure and risk assessment can be performed jointly for all endpoints and exposure scenarios. In this context under REACH, a modification in process parameters (e.g. starting materials, solvents, temperature, order of manufacturing steps, purification steps, etc.) is within batch-to-batch variation to the extent that variability of these parameters is minimized. Any other modification in these or other parameters results in a different nanoform and would be considered to differ beyond batch-to-batch variation (ECHA, 2019b).

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[22] 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 section 2.3.1).

2.3.3. Adverse Abiotic Effects

67.   In the context of nanomaterial interaction with the abiotic environment (non-living components of the environment or the environment on which life depends), the release of a nanomaterial may affect the environment in a physico-chemical property dependent manner. Adverse abiotic effects can include altering the chemical make-up of natural waters, e.g. metal content, pH changes, or in soil, chemical-mediated compaction, etc. Considerations can include the potential reactions and interactions of the nanomaterial in the environment. These effects may adversely impact the ability of organisms to inhabit the environment.

2.4.   Issues Related to Exposure Assessment

2.4.1. Exposure Assessment

68.   Exposure assessment provides an evaluation of the extent to which humans and/or the environment are exposed to nanomaterials. Ideally, exposure assessments are quantitative, but in certain cases may be qualitative with descriptors (e.g. "no exposure", "negligible exposure", "minimal exposure" or "significant exposure"). Exposure assessments should consider all relevant sources of potential exposure, including indirect exposure (e.g. from exposure to materials released into the environment from manufacturing, disposal or use of consumer products containing nanomaterials) and direct exposure (e.g. exposure to workers in manufacturing facilities or to consumers using products). The general population may be continuously exposed to MNs over their lifetime. The increasing market for MNs adds to the potential for higher and more widespread exposures to occur, leading to the need for consideration of risks from cumulative and aggregate nanoparticle exposure.

69.   For many applications, the principal route of potential human exposure to nanomaterials is by inhalation. Inhalation is also generally regarded as the route with the highest potential for concern from nanomaterial exposure due to observed effects to the lung and pulmonary system from poorly soluble particulates (Oberdörster et al., 2005; ECHA, 2016). MNs are increasingly being used in consumer products, pharmaceutical preparations and food technology where dermal, gastrointestinal, and parenteral routes of exposure are becoming more significant. Studies examining migration/leaching/release of the MN from relevant matrices (e.g. in products or during disposal/incineration, etc.) improves the current level of knowledge on the relevance of exposure sources and routes, and biomonitoring studies inform our understanding on the levels of aggregate or cumulative exposure to nanomaterials (Kendall and Lynch, 2016).

70.   During exposure assessment, information on how a nanomaterial comes into contact with the body or is released into the environment is integrated with information on its fate in order to establish the degree of exposure that occurs, or may occur, for the receptor. As with chemical risk assessments, the main steps are:

- Considerations on exposure pathway, release and contact potential (Entry or release characterisation) – to understand if, how, and in what quantities a nanomaterial may come into contact with the human body or is released into the environment throughout its life cycle (from manufacture or importation through to disposal);
- Characterisation of fate and transport/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
- Quantification of exposure – to estimate exposure levels of human tissues/(sub)populations 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).

2.4.2. Entry or Release Characterisation

71.   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 characterization of the quantity, frequency, and duration of these releases. 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.

72.   Characterization of human exposure involves identification of the relevant route(s) and extent (e.g. quantity, frequency, duration) of exposure. A critical source of nanomaterial exposure to consumers is through the use of consumer products. In products materials may be embedded in solid matrices, or they may be dispersed in liquids (e.g. for spray application) or exist as powders. Nanomaterials may be released during product use/application, or from physical and chemical processes (e.g. due to mechanical abrasion, UV irradiation, rain or wash water leaching) during their life cycle.

73.   Koivisto et al. (2017) conducted a review of 96 peer-reviewed scientific publications reporting release of inorganic and carbon-based nanomaterials from products and articles to form the basis of a release library to support quantitative exposure assessments and modelling. The review includes studies examining the effects of artificial weathering (UV irradiation, rain leaching), mechanical treatment, spraying, washing and incineration on release of nanomaterials from textiles, thermosets, thermoplastics, coated surfaces, sprays/aerosols and other products (Koivisto et al., 2017). The studies showed releases of ions, free nanomaterial, matrix associated particles (e.g. matrix with protruding NM) and matrix particles with fully embedded NMs. The studies further showed that nanomaterials were *mainly* released embedded in or associated with the matrix or in ionic from; and to a lesser degree as free nanoparticles (Halappanavar et al., 2015; Koivisto et al. 2017 referencing Nowack et al., 2012; Nowack et al. 2013; Schlagenhauf et al., 2014; Shandilya et al., 2014; Kingston et al., 2014; Froggett et al., 2014; Duncan and Pillai, 2015; Duncan, 2015; Mackevica and Hansen, 2016). Quantitative release rates are reported in the Koivisto et al. (2017) review in a harmonized format with consistent units that serve as critical inputs to particle exposure models to support human exposure assessments from products and articles. The review included recommendations for information that should be reported and considered in release studies including the properties of the matrix and nanomaterial released, the technique for incorporation of the NM in the matrix, the concentration and level of dispersion of the NM in the matrix, the release scenario and process parameters and concentration gradients in the sampling volume.

74.   Kovochich et al. (2018) conducted another significant literature review and compiled published data on release of carbon nanotubes from nanocomposites to support quantitative exposure assessment and human risk assessment. The review included 21 published studies evaluating airborne release of CNTs subjected to mechanical (sanding, cutting, etc.) and physical (weathering, combustion, etc.) stressors. The authors found that methods used across the studies varied greatly, and factors such as composite type, CNT functionalization and energy input may affect release. Similar to the review by Koivisto et al. (2017), the Kovochich et al. (2018) review found that most studies reported release of the CNTs mainly associated with or fully embedded in the matrix, but that release of free fibres under reasonable conditions also occurred and is possible.

75.   Both reviews (Koivisto et al. 2017 and Kovochich et al. 2018) identified the need for better and more consistent experimental sampling and design, reporting and improved analytical techniques in

release studies to support nanomaterial exposure and risk assessment. Such studies should characterize and quantify the amount of free versus matrix-bound nanoparticles and provide sufficient detail on the experimental conditions leading to release as well as the characteristics of the base matrix.

76.   The ISO has developed a Technical Report (ISO/PFR TR 22293) under Technical Committee (TC) 229 Nanotechnologies, which reviews methods for assessing the release of nanomaterials from commercial nanomaterial-containing polymer composites. The work is in recognition of the importance of understanding release for safe development and use of products containing manufactured nanomaterials. The report provides guidance on the use of methods for the identification and evaluation of NM release from matrices as a framework to support decision making. It also identifies opportunities for further development of standards in this area.

2.4.3. Environmental Fate and Exposure

77.   Over the past decade, the understanding of sources, fate, and effects of NMs in the environment has made significant progress. The achievements in the field of nano-ecotoxicology in both aquatic and terrestrial systems refer to the pathways, measurements and predictions of NMs in natural ecosystems, as well as their environmental fate, transformation, stability, mechanisms of toxicity and their effects on individuals and populations (Bundschuh et al., 2018). A review by Lead et al. (2018) examines the progress gained since 2008 in the knowledge of nanomaterial environmental fate and exposure, particularly in aquatic and terrestrial systems, and discusses new questions arising from the research. The review covers wide-reaching developments in the research on nanomaterial fate and behaviour, metrology, transformation, bioavailability, mechanisms of toxicity and environmental impacts. A review by Baun et al. (2017) addresses the regulatory relevance and reliability of methods and data for determining the environmental fate of manufactured nanomaterials. A review by Quik et al. (2020) funded by EFSA evaluates the environmental risk assessment principles, focusing on feed additives and pesticides, highlighting the needs for updating the current guidance documents in order to cover the specific characteristics of nanomaterials. In addition, the H2020 NanoFASE project[23] includes a rich collection of publications that cover several aspects of NMs fate in the environment and GRACIOUS has developed IATAs for environmental compartments.

Transformation, Degradation and (Bio)durability

78.   As with chemicals, MN transformation can influence distribution within an organism or in the environment. Transformation, degradation and dissolution are determinants of a MN's (bio)durability, which is analogous to persistence, in biological and environmental media. Assessing transformations will need to consider the core material, as well as any influences functionalization or surface coatings may have on the MN's properties, and consequently its transformation or distribution pattern. Transformation includes the impact of aggregation/agglomeration on the biological and environmental fate of MNs and the degree to which dis-aggregation/dis-agglomeration is likely to occur in tissues or compartments. Transformation of MNs is also viewed in the context of corona formation within biological fluids because protein, lipid or other (bio)molecule coronas can influence their behaviour and toxicity (Canady, 2010). The potential for aggregation/agglomeration and corona formation should be considered in designing or evaluating experimental conditions in *in vitro* and *in vivo* tests. Transformation of MNs can also occur through dissolution (by release of ions and molecules) in biological and environmental media (OECD, 2018d). When generating degradation information on MNs, biotic degradation tests based on organic carbon should only be considered in instances where the MN can

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[23] https://cordis.europa.eu/project/id/646002/results

serve as an "organic" carbon source, and their application to inorganic materials should be approached with caution.

79.   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.

80.   Several cases on copper oxide (nano-CuO; included in pesticides formulations and relevant for agriculture applications) were developed within ERA-NET SIINN-NanoFARM that demonstrated the application of different techniques for determination of properties on dissolution rates (Vencalek et al., 2016), persistence and bioavailability (Gao et al., 2017) and ageing processes (Sekine et al., 2017) under environmentally relevant conditions.

Distribution and Compartmentalisation

81.   The distribution and compartmentalization of MNs in environmental and biological media can be predicted based on their physico-chemical properties and media-specific factors such as hardness, pH and presence of humic acids. However more work is needed to develop the knowledge and tools to facilitate this. Risk assessors are encouraged to use existing knowledge linking properties and conditions to environmental and biological fate as part of a weight-of-evidence. However, in the absence of such data, assessments should assume a reasonable worst-case behaviour; for example, that materials are agglomerated during transport, but dissociated or deagglomerated once present in a compartment or organism.

Bioaccumulation

82.   For neutral organic chemicals (that may partition to lipids) there is a direct relationship between its octanol water partition coefficient (K_{OW}) and bioaccumulation or bioconcentration factors (BAF/BCF). Evidence suggests that particles in dispersion do not exhibit this relationship (Isaacson et al., 2017: Utembe et al., 2018). K_{OW} is generally not applicable to MNs as non-soluble particles are not subject to equilibrium partitioning and cannot reach thermodynamic equilibrium when distributed between two phases (ECHA 2019c). Consequently, it is not recommended that risk assessors make attempts to predict MN bioaccumulation on the basis of existing chemical modelling programmes using K_{OW} as an input parameter. Empirical BAF/BCF tests may be conducted for MN, considering their potential for transformation (e.g. corona effects), however these tests are still considered to be flawed as they also are based on the assumption of equilibrium partitioning (Hou et al., 2013; van den Brink, 2019). Dynamically derived bioaccumulation factors, based on kinetic exposure experiments (e.g. physiologically based pharmacokinetic- PBPK or biodynamic models), may be more appropriate, because they are not based on equilibrium between the organism and exposure medium (Isaacson et al., 2017; Utembe et al., 2018; van den Brink, 2019). Empirical bioaccumulation studies should further consider the relevance of the MN's uptake and potential to cross cellular membranes, embed in tissues, release ions and be excreted. To this effect, Petersen et al. (2019) mentions that for many multicellular organisms, it is essential to differentiate between the MNs adsorbed to external surfaces or in the digestive tract and the amount absorbed across epithelial tissues.

83.   Considerable knowledge gaps remain with respect to the effects of agglomeration on bioavailability, uptake and intracellular compartmentalization (von Moos et al., 2014). There is also a critical need for further analytical method development to identify and quantify MNs in complex matrices for bioaccumulation studies (Petersen et al., 2019). Finally, Utembe et al. (2018) recommend taking biotic and abiotic factors into considerations when modelling bioaccumulation and interpreting bioaccumulation results. In the absence of some or all of this information, reasonable worst-case assumptions based on the size and chemistry might provide some insight into the potential for bioaccumulation.

84.   Traditionally bioaccumulation testing under OECD TG 305 is carried out using chronic exposure of fish over about a four-week period via the water or food where steady-state uptake and water and tissue concentrations are measured to calculate a BCF. However, K_{OW} is used to trigger or waive BCF tests and is not a relevant physico-chemical property for sparingly soluble and insoluble nanoparticles. Under the current OECD TG 305, if the K_{OW} test is not practical or feasible, the work proceeds directly to the *in vivo* fish test. Discussions are ongoing internationally within the EU-project NanoHarmony[24] and the OECD to explore modification of the bioaccumulation testing strategy, for example as proposed in Handy et al. (2018). The proposal and discussions include alternative triggers for TG 305 when log K_{OW} is not appropriate, the use of earthworm or other invertebrate bioaccumulation tests as alternative tests to *in vivo* fish tests and the possible use of *in vitro* alternative tests for screening. In addition alternative physico-chemical triggers, such as size, aggregation state and dissolution are being explored to replace K_{OW} for MN bioaccumulation testing.

2.4.4. Human Exposure

Occupational exposure

85.   A research need identified in 2010 was related to exposure of workers at different stages of the materials life cycle and progress has been made in this regard. Several systems for monitoring occupational exposure, data reporting and databases with information on occupational exposure to MN have been developed. For example the Nano Exposure & Contextual Information Database (NECID) was developed by the German IFA (Institut für Arbeitsschutz) in conjunction with the occupational health and safety institutions of the Partnership of European Research in Occupational Safety and Health (PEROSH) group to systematically capture, process and store scientific data on nanomaterial exposure.

86.   NanoMONITOR (LIFE project) "*developed an integrated approach to overcome current data gaps on the concentration of manufactured NMs in indoor workplaces and urban areas by combining a long series of robust data measures by a new wireless sensor network of monitoring stations and a tailor-designed data management application*" (NanoMonitor, 2018). The project includes:

- "An online software application to support data processing in real-time;
- A database containing information on the concentration of manufactured MNs, designed and structured according to the information requirements laid down in REACH and relevant monitoring programs:
- A proven low-cost monitoring station prototype; and
- A complete guidance on the use of environmental monitoring data under REACH, including detailed decision trees to support the use of monitoring data." (NanoMonitor, 2018).

87.   In 2015, the OECD 'Harmonized tiered approach to measure and assess the potential exposure to airborne emissions of MNs and their agglomerates and aggregates at workplaces' was published (OECD, 2015a). This three-tiered approach is based on a systematic evaluation of previously proposed and used strategies, which mainly aims to deal with the problem that many of the instruments used for nanoparticle measurement are non-specific (i.e. cannot distinguish engineered MNs from ambient nanoparticles). Other studies concluded that safe(r)-by-design (SbD) approaches are considered important tools for risk mitigation and prevention for workers, and, potentially, also for consumers exposed to manufactured NM (Bianchi et al. 2019).

88.   In the case of carbon nanotubes (CNT) exposure, work has begun to harmonize occupational exposure assessment and results reporting and there is a recognized need to identify companies for

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[24] https:// nanoharmony.eu

where exposure and health can be assessed and monitored in real-life settings. This will enable the risks from occupational exposure of MNs to be evaluated objectively and realistically, supporting appropriate risk management (Canu et al., 2020). See Annex for a case study on CNT that provides further context on occupational risk assessment.

89.   The Safe Nano Worker Exposure Scenario (SANOWORK) project which ended in February 2015 and was funded by the European FP7-NMP was designed to identify safe occupational exposure scenarios in real conditions and at all stages of the MN life cycle including product manufacture, use and disposal. The project identified factors contributing to MN release in workplaces and proposed remediation strategies.

90.   Dustiness is a key parameter in occupational exposure assessments, providing critical information about the potential for movement of powders to the air and inhalation as well as the identification of hazards such as dispersion, fire and explosion. Existing dustiness tests (e.g. EN 17199, EN15051) were not specifically designed for particles at the nanoscale. There are two ongoing projects funded by EU H2020 under NanoHarmony to develop the scientific basis for dustiness testing of highly reactive and high aspect ratio nanomaterials (HARN) and under Gov4Nano for non-HARN MN. It is expected that this work will result in two new guidance documents, one for use of dustiness data in exposure assessment modelling and one for its use in industrial risk analysis for explosive atmospheres. A new OECD TG is expected as well for generation of dustiness data and method-specific dustiness ranking schemes for regulatory risk management of powder. An official call for intra- and inter-laboratory comparison of dustiness test methods for HARN has been launched.

Consumer exposure

91.   A critical source of exposure of the general population to MNs is through the use of consumer products. The nanotechnology industry continues to see rapid growth with a total global market of $39.2 billion in 2016, 54 billion in 2020 and a projected 126.8 billion by 2027[25]. MNs are used in a wide range of applications and consumer products including medicinal products, textiles, paints, food packaging and personal care products (Mackevica and Hansen, 2016). Consumer exposure assessment requires clearly defined exposure scenarios including identification of relevant uses and types of exposure with reliable information on who is exposed, how often and via which routes. This information can be used to estimate exposure for different populations (Mackevica and Hansen, 2016).

92.   One of the important issues in consumer exposure assessment is identification and inventorying of consumer products that contain MNs, including the concentration of the MN in the product, the manner in which it is embedded or dispersed in the product matrix and how the product is used. Unless disclosed, this information is not available and high throughput techniques that allow screening of products for the presence of MNs can help fill gaps in this area. Standard methods for product monitoring/surveillance of MNs are lacking, however some studies have attempted to identify MN in subsets of products. For example, a recent study by Boyadzhiev et al. (2020) used enhanced darkfield hyperspectral imaging to detect MN in consumer personal care products available in the Canadian market.

93.   Inhalation is a particularly relevant route of exposure for MNs from the perspective of toxicity and exposure potential. Consumer exposure can happen through the inhalation of sprays, aerosols or powders that may occur in air as a result of direct product use or application (e.g. hair spray, dry shampoo or spray products intended for skin application) or as a result of release from solid coatings or articles later in the life cycle due to mechanical or physical process (e.g. sanding a cured paint containing MN, weathering, incineration of waste, etc.).

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[25]https://www.globenewswire.com/news-release/2020/07/16/2062964/0/en/Global-Nanotechnology-Industry.html

94.   Consumers are also exposed to NMs through the oral and dermal routes. Dermal exposure of consumers can occur through direct contact of the skin with products or articles containing the NM, through particle or aerosol deposition on the skin from the air or contact with residues after product use (Mackevica and Hansen, 2016). Consumers may also be exposed orally to MNs from their presence in household products, books, textiles, and in food and food packaging (Mackevica and Hansen, 2016; Bianchi et al., 2019). A number of studies have looked at release of MNs from food packaging (e.g. Su et al., 2017; Liu et al., 2016; Lin et al., 2014; Simon et al., 2008; Diaz et al., 2013; Doudrick et al., 2012; Xia et al., 2017 reviewed in Yan et al., 2019). Studies have found differing mechanisms of release from food packaging, either from the interior of the packaging or from the surface/interface. A better understanding is needed about the mechanisms of migration (Yan et al. 2019).

95.   Release measurement and estimation is a critical component for consumer exposure assessments of MNs, and over 100 peer-reviewed scientific studies have been published reporting release of MNs from products at different stages of their life cycle (Koivisto et al., 2017; Kovovich et al., 2018). As also discussed in Section 2.3.2. the evidence is showing that the majority of released MN are fully or partially embedded in matrix particles, but release of free MN is also possible and product release studies should take these differences into account and provide sufficient detail on the experimental conditions of the study and characteristics of the MN and matrix particles as exposed/released.

2.4.5. Models and Tools for Quantifying Exposure

96.   Traditionally for exposure assessments of conventional chemicals, models are frequently used to estimate exposures to the environment (e.g. EUSES), consumers (e.g. ConsExpo) and workers (e.g. ECETOC's Targeted Risk Assessment (TRA) tool[26]). Methods for quantifying exposure to MN follow the same general paradigm employed in chemical risk assessments, utilizing both monitoring (directly measured) and modelled data. For either type of data it is important to understand the form of the MN (e.g. free particles, aggregates or agglomerates, ions, etc.) and ensure that the units are in the same metrics as other parts of the assessment (i.e. for hazard/effects assessment). Recent activities within the OECD and other projects, such as the EU H2020 CaLIBRAte[27] are addressing the evaluation, validation and applicability of currently available tools and models used for occupational, general population/consumer and environmental exposure assessments of MN.

97.   The NANoREG Toolbox (Jantunen et al, 2017; Jantunen et al. 2018) provides an overview of available exposure tools and where to find them. The toolbox, developed with support from the EU FP7 project, lists tools that are primarily publicly available with links to webpages or other sources where the tool can be downloaded or otherwise accessed. These tools include those for characterizing environmental, occupational and consumer exposure to MNs, including available models, guidance, reports, experimental methods and data management and decision support tools. The tools in the toolbox are not to be considered validated by their presence there, rather it is a compilation of available tools. However, Jantunen et al. (2018) indicate that a fraction of tools have undergone validation.

98.   The OECD WPMN has been working on three complementary projects[28] under its Steering Group on Exposure Measurement and Exposure Mitigation, which are: i) Assessing the global readiness of regulatory and non-regulatory models for assessing occupational exposure to MNs (led by Denmark); ii) Compilation of Available Tools and Models Used for Assessing Consumer Exposure to MNs and Evaluation of their Applicability in Exposure Assessments (led by Canada); and iii) Compilation of

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[26] https://www.ecetoc.org/tools/targeted-risk-assessment-tra/

[27] http://www.nanocalibrate.eu/home

[28] The three projects address occupational, consumer and environmental exposure tools/ models, respectively, and were finalised in 2021

Available Tools and Models Used for Assessing Environmental Exposure to MNs and Evaluation of their Applicability in Exposure Assessments (led by Canada). These projects focused on existing tools and models with a view to evaluate their regulatory applicability for exposure assessments of MNs for three target population groups: workers, consumers and the environment. The National Research Centre for the Working Environment (NRCWE) in Denmark and Health Canada were collaborating on the occupational and consumer projects, because about one-third of the tools and models compiled in these respective projects were determined to be mutually applicable to both occupational and consumer exposure. In order to conduct the evaluations of the models, the WPMN collected/generated exposure data in a structured format. At the WPMN meeting held in February 2019, the group discussed ways to structure the exposure data so that it can be collected by existing OECD Harmonised Templates(OHTs), as well as potential areas of work to be considered in collaboration with the OECD Working Party on Exposure Assessment (WPEA). Results from the three projects were published in 2021 (OECD, 2021a, 2021b, 2021c, 2021d).

Environmental exposure models

99.　　Due to the difficulties in analysing MNs in complex environmental matrices, environmental fate assessment continues to be largely based on modelling. Many of the stages/pathways of fate (e.g. behaviour, persistence, transport, transformation pathways/products, bioaccumulation, and effects) for conventional chemicals are quite well predicted in existing models. However, most of these models were not designed for MNs and some of their assumptions, such as the amount of time a particle remains airborne or its solubility/insolubility in environmental media, may have to be adjusted for MNs[29].

100.　　To define conceptual environmental models for MNs it is necessary to understand their intrinsic physico-chemical properties and how these relate to their interaction with the surrounding environment. Properties such as chemical composition, particle size range, surface charge and others are fundamental starting points for understanding the exposure scenario for any given MN. MN-specific processes represented in existing aquatic fate models are mainly limited to aggregation and dissolution, and mostly ignore or exclude transformation processes, the role of manufactured coatings and particle dimensions (Williams et al., 2019). In addition, inputs for environmental exposure models for conventional chemicals are often based on Quantitative Structure Property Relationship (QSPR) calculations using physico-chemical properties of the substance, mainly K_{Ow} and K_p values, and these properties are not relevant for sparingly soluble and insoluble MN.

101.　　In addition to understanding the relationship between physico-chemical properties and MN fate, the following information is essential in adapting environmental exposure models:

- understanding of the life cycle of the specific MN, especially for the nanoform to which humans or the environment may be exposed (e.g. 'as manufactured', 'as used', 'pristine', 'transformed', 'in situ', etc.);
- the distribution of different nanoforms among different environmental compartments (i.e. partition coefficients for sediment/water, soil/water and air/water may have to be measured);
- fraction of particles absorbed.

102.　　The development and subsequent validation of conceptual models of MN exposure can contribute to reducing uncertainty in the assessment of exposure and risk. An example of a conceptual model, for a dynamic multimedia environmental fate and transport model ('nanoFate') for MN is illustrated in Figure 2.1 below (Garner et al., 2017). Different models may consider different processes. Some conceptual models 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. MN waste disposal;

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[29] 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

Köhler et al., 2008). Others can be used to develop emission scenarios and predict mass flows of MNs in the environment (Blaser et al., 2008). Such models should, however, be developed with the acknowledgement that MN behaviour in natural systems is complex and hypotheses should be established for empirical testing and research to support model validation.

Figure 2.1 Conceptual model with compartments, major transfers, and transformations used in 'nanoFate'. Reprinted (adapted) with permission from Garner et al. (2017). Copyright (2017) American Chemical Society.
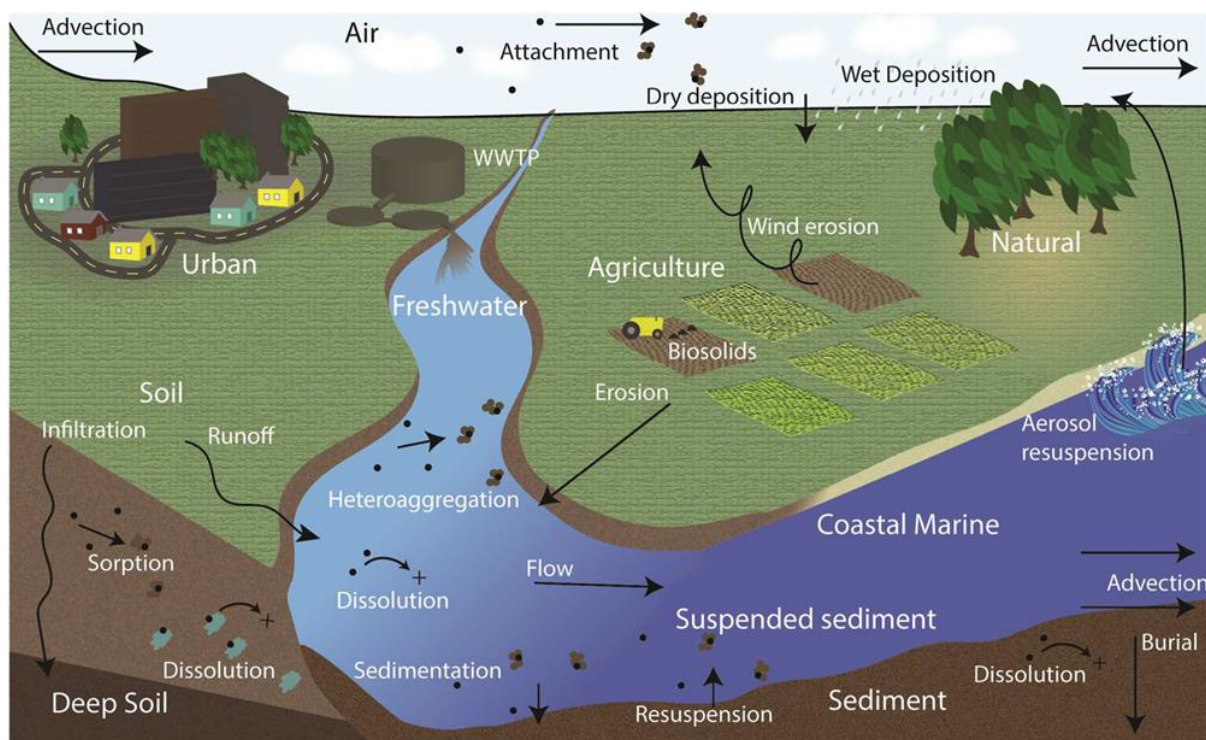
103. The OECD WPMN project to collect and evaluate available tools and models for MN environmental exposure assessment (described above) has undertaken a functional assessment of the compiled models involving an uncertainty analysis using Monte Carlo Simulations and a sensitivity analysis using a systematic one-at-a-time approach. The work of the project is presented in a report that discusses the results of these analyses and presents recommendations on the usage of the tools and models evaluated (OECD, 2021d).

104. SimpleBox4nano (SB4N; RIVM, 2019), one of the models evaluated in the WPMN environmental model project, is a modification of the multimedia mass balance model SimpleBox that includes nano-specific processes like aggregation. SB4N estimates long-term MN concentrations in air, surface waters, deep waters, sediments, soils, and other biological compartments. SB4N tracks three different species of MN: (i) freely dispersed, (ii) heteroaggregated with natural colloids (< 450 nm), and (iii) heteroaggregated with coarse natural particles (> 450 nm). The model's steady state functionality is easy-to-use in a well-designed spreadsheet that includes default parameters for a number of MNs. The model also offers dynamic functionality through R scripting (RIVM, 2019).

105. Van den Brink et al. (2019) have reviewed the available approaches for modelling the uptake of NMs by biota under environmentally relevant conditions. The authors evaluated current modelling approaches, including underlying assumptions and their applicability to MNs, for the uptake of solutes by organisms. Also, exemplification within case studies as well as guidance for the selection of modelling approaches is provided.

Occupational and consumer (human) exposure models

106.   The main objective of quantifying exposure is to determine the concentrations or amount of the nanomaterial that reaches the target organism, system, or (sub)population in a specific frequency for a defined duration. Methods for quantifying human exposure to MNs follow the same general paradigm employed in chemical risk assessments, utilizing both monitoring (directly measured) and modelled data. For either type of data it is important to understand the form of the MN (e.g. free particles, aggregates or agglomerates, ions, etc.) and ensure that the units are in the same metrics as other parts of the assessment (i.e. for hazard/effects assessment).

107.   Exposure models depend on, as key inputs, either predicted or measured release rates of the MN from its matrix under specified conditions (see Section 2.2.1 and 2.4.2). The ConsExpo Nano model estimates inhalation exposure to low or non-volatile substances released as an aerosol in consumer spray products. It is based on the normal ConsExpo spray model which is adapted to combine it with the International Commission on Radiological Protection (ICRP) deposition and clearance model. This enables it to estimate inhaled and deposited (alveolar load) doses which may be expressed using different metrics (RIVM, 2021). ConsExpo Nano is being evaluated under the WPMN project described above. It also has been recently evaluated against measured data by Delmaar and Meesters (2020) in a peer reviewed study where they found that the spray model in the regular ConsExpo and ConsExpo nano describe experimental (chamber) air concentrations reasonably well, especially for air space applications and less-so for surface applications where there were more uncertainties (Delmaar and Meesters, 2020). The authors identify uncertainties in the model and its assumptions and provide recommendations where improvements could be made.

108.   Inhalation exposure of consumers to manufactured MNs in consumer products often happens in indoor environments. Models for particle dynamics in outdoor and indoor air taking into account air flow and particle transport have been developed, both conceptually (e.g. Nazaroff et al., 1989; Nazaroff et al., 2004) and in the area of particulate matter/ultrafine particles and air quality (Jacobson, 1994; Rim et al. 2013). NIST in partnership with the US Consumer Product Safety Commission has developed two models that predict air concentrations for nanoparticles (expressed as mass and other metrics): a single size particle tool and a size-resolved tool (NIST, 2018). The single size tool is based on the CONTAM multizone modelling software[30], which has a history of use in whole-building indoor air quality analysis. The size-resolved tool accounts for changes in behaviour of particles in air from interacting with other particles of different number and size. For the latter, an important component is consideration of coagulation and its effect on removal rates associated with deposition and air change. Inputs to the models include parameters on the zone geometry (room dimensions), ventilation system (air flow, etc), particle properties, particle source, particle deposition and resuspension velocity. It calculates air concentrations, initial zone concentrations and surface loadings over a defined exposure time and 24-hour period when released as a burst or constant release (NIST 2018). The NIST model is an example of air exposure model for nanoparticles that can be used for a wider range of scenarios than releases from spray application, given that a meaningful release rate and particle characteristics can be defined as inputs.

109.   As described above, the OECD WPMN project to collect and evaluate available tools and models for MN occupational and consumer exposure assessment (described above) has been finalised in 2021 (OECD, 2021a, 2021b, 2021c).

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[30] https://www.nist.gov/services-resources/software/contam

2.5.   Issues Related to Hazard Assessment

110.   The overall objective of effects characterisation is to identify the type and severity of adverse effects to human health or the environment. Following exposure to a MN or its transformation product(s), effects can occur either directly or indirectly. SCENIHR (2006) considered the first priority in hazard assessment to be identification of the toxic principle of a given MN. The toxic principle describes the constituent, property or substructure of a given material that is responsible for the toxic effects of that material. Examples of toxic principles of MNs may include:

- the toxicological properties of the chemical(s) that comprise the core of the nanoparticle, or the influence of functionalization of the nanoparticle surface;
- the aspect ratio, surface charge, or surface area of a nanoform that may have greater reactivity potential than bulk substances;
- the potential for other chemicals of concern to be absorbed onto the nanoparticles due to the enhanced surface area and possible surface reactivity; and/or
- impurities and/or by-products related to nanoparticle production (e.g. metal catalysts).

111.   Four important issues identified for effects characterisation include:

- the appropriateness of test species
- the appropriateness of test methods
- where there is an adverse effect, the use of uncertainty factors (also called 'adjustment factors) or extrapolation factors to estimate a no-effect level associated with long-term exposure, and
- Accurate and comprehensive characterisation of the material tested.

112.   Additional considerations when characterizing MNs in toxicity studies are:

- 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
- context/media – how material properties change in different media.

113.   A generic overview of nanotoxicity following exposure to experimental animals that relate to human health focuses on inflammation, oxidative stress and cytotoxicity (see Figure 2.2). The interplay between these three effects is causal to injury at the tissue level and eventual tissue dysfunction. At the cellular level, acute interaction of MNs with cells (mechanical, physical, receptor-mediated, etc.) can lead to cellular injury, which activates host defence mechanisms involving immune and pro-inflammatory responses. The metabolic activity of pro-inflammatory cells and the action of some enzymes and cytokines secreted during this process leads to the synthesis of Reactive Oxygen Species (ROS), imbalances in anti-oxidant/oxidant levels and oxidative damage of biomolecules, resulting in propagation of cell injury and eventually to cytotoxicity. At the tissue level, persistent cell injury can result in tissue dysfunction. The surface reactivity of MNs can directly activate oxidative stress mechanisms. The entire process is driven by the concentration and duration of exposure, and specific physico-chemical properties of MNs, which affect the extent of response.

Figure 2.2. Schematic of the known key events and underlying mechanisms of MN-induced toxicity (modified from Halappanavar et al. 2021). The solid arrows indicate the main sequence of events leading to tissue injury. Dashed arrows represent parallel events and cyclic arrows represent feedback loops.
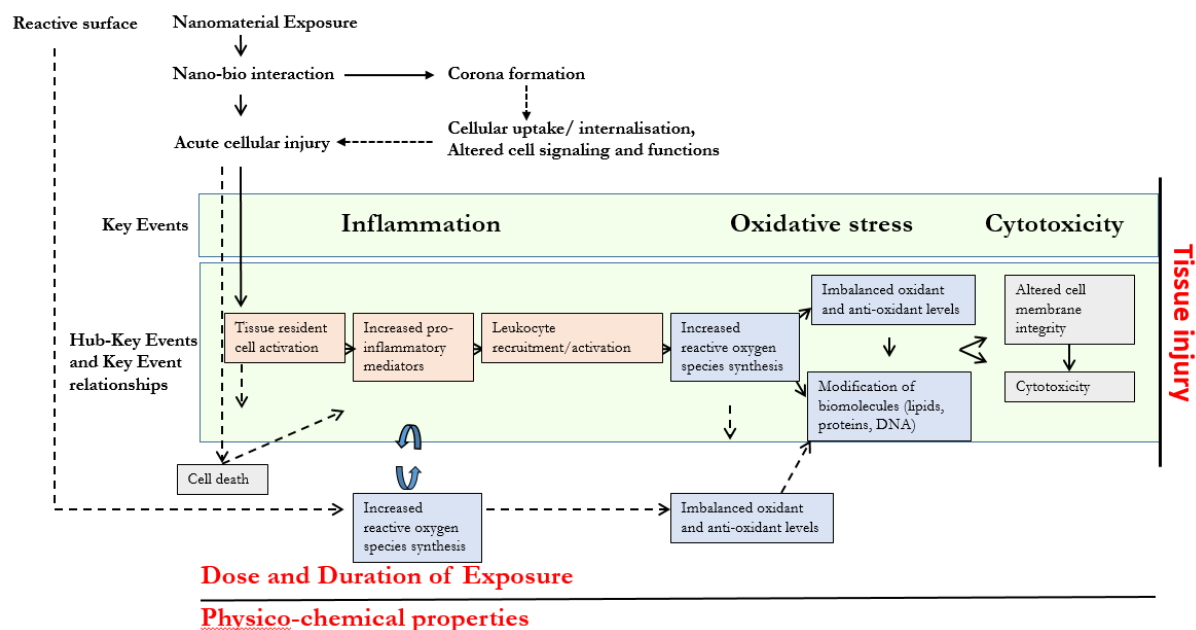
2.5.1. *Factors influencing toxicity*

114.   There is a significant body of evidence in the literature attributing higher toxicity to smaller nanoparticles (Fujirawa et al., 2008; Kim et al., 2010; Lipovsky et al., 2011; Park et al., 2011; Osborne et al., 2013; Lopes et al., 2014; Schultz et al., 2014; Bhuvaneshwari et al., 2015; Li et al., 2016; Kim et al., 2017). This holds true for both human health and ecological (environmental) receptors for various types of MNs, especially metals and metal oxides. Mechanistically, their smaller size enables easy cellular uptake, deeper tissue access and translocation to other organs. The large relative surface area enhances their reactivity and interaction at the molecular level with metabolites, proteins, and individual structures of cells (such as lipids and nucleic acids, in particular, DNA), by which they can potentially cause damage (Tomilina, 2011; Huang et al., 2017). An additional explanation may be that the increase in the rate of solubility with decreasing size might result in an increased concentration of ions, i.e. a bigger dose. If the ions are toxic the bigger dose will have a greater effect.

115.   In addition to size, other factors such as shape, aspect ratio, surface chemistry and surface charge also play a role in toxicity (McCracken et al., 2016; Sukhanova et al., 2018; Forest et al., 2019). 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 (Schinwald et al., 2012). This demonstrates the need to understand such complex interactions when predicting toxicity and pathogenicity for MNs. Without such knowledge, the descriptors chosen for MN identification may be unsuitable, leading to under- or overestimation of hazard and consequently, mislabelling of MNs for their potential to induce hazards.

116.   Abiotic factors may play critical roles in the bioavailability, distribution, bioaccumulation and, ultimately, toxicity of MNs when exposure occurs in natural settings, in particular. One factor in determining particle behaviour is how the particular natural environment or biological fluid influences

important physico-chemical characteristics such as surface charge or agglomeration and aggregation. In some cases, MNs stably adsorb specific environmental or biological components, such as small and large biopolymers to the particle surface (Handy, 2008). This phenomenon is referred to as the formation of a "protein corona" (Cedervall et al., 2007; Maiorano, 2010) or "lipid corona" (Raesch et al., 2015; Olenick et al., 2018). 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 MNs, but the specific factors of relevance and their variability and effect may be different from non-particulate chemicals.

2.5.2. Internal Exposure

117.   Internal exposure is dependent on many factors including the route of exposure, toxicokinetics, and toxicodynamics of a MN. For human health hazard assessments, the inhalation route of exposure is of primary concern because of potential airborne exposure at the production facilities during synthesis, packaging, etc. or later in the life cycle of the MN. The general pathways for the mechanical clearance of insoluble particles deposited in the pulmonary region typically involve either phagocytosis by alveolar macrophages and clearance via the mucociliary escalator into the gastrointestinal tract or alternatively, active or passive transport through the respiratory epithelium (Schlesinger, 1995; SCENIHR, 2006). This knowledge could be used for derivation of refined adjustment factors in risk assessment of particulates, as described by Pauluhn (2010a) and Pauluhn (2010b). For improved quantitative hazard assessment, methods and mathematical tools similar to PBPK/TK[31] models for "conventional" chemicals to describe pathway(s) as a whole have been proposed (Carlander et al., 2018). The OECD Inhalation Toxicity Test Guidelines 412 (Subacute Inhalation Toxicity) and 413 (Subchronic Inhalation Toxicity) were updated in 2018 to accommodate MNs. The main focus of the revisions was to include: i) Bronchoalveolar lavage (BAL) measurements; ii) Particle-size distribution for test atmospheres; iii) Post-exposure recovery period; and iv) Lung burden measurements (OECD, 2018a; OECD, 2018b).

118.   Presently, the understanding of the molecular and cellular barriers as well as the potential for translocation of MNs across such barriers is limited. It is acknowledged, however, that body fluids play a key role in modulating the behaviour and toxicity of MNs in the body, although the stability of MNs in various fluids *in situ* is still unclear. Evidence suggests that absorption of MNs by the oral routes is possible with potential disruption of the small intestine from their presence in these tissues. Dermal studies demonstrate a high potential for MNs to penetrate the skin, however their penetration is mostly limited to crossing the epidermis though follicles or damaged skin (ECHA, 2020).

119.   The rapidly increasing use of manufactured nanoparticles in e.g. 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 relevance of these pathways.

120.   Studies indicate that translocation of MNs from the site of exposure to secondary organs is possible; however, it is not very significant. For example, less than 1% of total applied inhalation dose of insoluble particles is expected to translocate to other organs. Translocation of particles deposited in lungs by other modes of deposition is high; ~ 10% of administered dose is suggested to translocate to other organs after intratracheal instillation and pharyngeal and intranasal aspiration. However, the results from studies using the latter techniques for depositing particles are not used to support risk assessment decisions. Translocation of MNs deposited in lung to the lung interstitium and other distal

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[31] Physiologically Based Pharmacokinetics / Toxicokinetic Pharmacokinetics / Toxicokinetic Modelling

organs (including the liver, spleen and possibly to the foetus in pregnant females, as well as to the brain), have been noted and the translocation processes described (MacNee et al, 2000; Oberdörster et al, 2000; Oberdörster et al, 2002; Semmler-Behnke et al., 2014). Following oral or parenteral exposure, MNs can be detected in blood, liver, spleen or kidneys (Chen, 2009; Wang, 2007). Nanoparticle translocation to the brain is suggested to occur via neuronal transport, a "novel" pathway relative to the processes known for translocation of larger particles (Oberdörster et al., 2004). There are limited qualitative and quantitative data available on these pathways. In biodistribution studies using isotope-tagged or fluorescence labelled particles, less than 1% of nano-gold (2–40 nm), nano-TiO$_2$ (22 nm), ultrafine iridium (15 and 80 nm), and carbon (25 nm) particles deposited in lungs were shown to translocate to systemic circulation and reach distal organs including heart and liver (Kreyling et al., 2002; Nemmar et al., 2002a; Nemmar et al., 2002b; Oberdorster et al., 2002; Geiser et al., 2005; Muhlfeld et al., 2007; Sadauskas et al., 2007; Sadauskas et al., 2009; Geiser & Kreyling, 2010). A study by Husain et al. (2015), showed traces of nano-TiO$_2$ in the blood and heart following intratracheal instillation in mice.

121.   Current OECD Test Guidelines in principle enable the assessment of different possible target organs affected by an exposure to a MN (OECD, 2009c). Toxicokinetic studies may provide useful information in this context, including for example on barrier penetration that can inform the degree of similarity between MNs or between MNs and bulk materials. More specifically, given that some nanoparticles have the potential to migrate from the respiratory tract to circulation and on to the brain (or translocate directly via the olfactory nerves), there is a need to develop quantitative assays to determine the presence of nanoparticles in different human and animal tissues. To date, toxicokinetic studies have usually relied on measurement of the primary matter, bound residues of metal catalysts or radiolabelling rather than the MN as such (e.g. Ti for nano-TiO$_2$ or cobalt for Baytube CNTs; Chen, 2009; Pauluhn, 2010). Taking into account the slow body clearance observed for some MNs (e.g. Chen, 2009; Pauluhn, 2010, Gustafson et al., 2015), local accumulation may play an important role. ISO provides a review of issues to consider for performing toxicokinetics studies with MNs (ISO/TR 22019, 2019).

2.5.3. Representative Test Species and Populations

122.   The selection of relevant test species for human health and environmental risk assessment requires consideration. For MNs, not many studies have investigated strain or species-specific effects. The strains and the species recommended in standard test guidelines may be a good place to start; however, the sensitivity of these to assessing nano-specific toxicological effects is questioned. For human health effects, most studies have used mice or rats. The rat is usually considered as the most sensitive species for inflammatory changes in the lung in repeated dose inhalation toxicity testing of MNs (Becker, 2011; Bevan et al., 2018). Other species may include guinea-pigs and hamsters. For the environmental effects, two possible avenues exist – either to use the most sensitive species or the species most frequently exposed to MNs (e.g. filter, suspension, or 'conveyor-belt' feeders including daphnids and earthworms) (Quik et al. 2020). The choice of species may also influence the number 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. How (qualitatively and quantitatively) respective findings from inhalation toxicity studies of MNs in animals should be extrapolated to humans, is a question that is actively debated in the field.

123.   Adverse environmental effects of a substance can occur at an individual, species, sub-population, community, or ecosystem level (e.g. reduced survival and reproductive impairments in ecological risk assessment). Clarification of the scope of risk to target species or populations may help in information gathering of the MNs life cycle and potential hazard. Information on persistence and bioaccumulation 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 of MNs between species. Empirical trophic transfer experiments may be necessary to measure food chain exposure to MNs. For uses with potential for residues in food (e.g. pesticides, feed additives, veterinary medicines) the identification of the nature and amount of residues should cover not only the chemical composition, but also determine if the residues are expected to be in ionic or molecular form or as particles. EFSA provides some guidance for facilitating this assessment (EFSA, 2021a).

124.   Generation of epidemiological data as a basis for hazard identification and assessment requires development and follow-up of MN worker cohorts and exposure registries. Practical experience suggests that case reports need timely validation to corroborate exposure conditions and material identity in order to be useful for risk assessment purposes.

2.5.4. Appropriateness of Test Methods

125.   For regulatory purposes, hazards are commonly identified based on standard acute and chronic toxicity tests. Such standard requirements may need adaptation for MNs, as in the revised annexes of the EU REACH Regulation[32], to ensure that relevant data pertinent to MNs enabling robust risk assessment is made available. Of importance, the annexes include the following provisions for nanoforms:

- registering the nanoforms characteristics (e.g. size, shape, surface chemistry);
- requirements and consideration for appropriate sample preparation, route of exposure and characterisation of test material, including the level of dispersion/agglomeration;
- consideration of appropriate metrics for reporting results and specific physico-chemical properties to support safety assessments (e.g. dissolution rate, dispersion stability of nanoforms)
- qualification of existing adaptation options for test methods and modification of information requirements where the test method is not applicable/informative for nanoforms (e.g. OECD TG 117 and TG 123 octanol-water partition coefficient tests (K_{OW}), OECD TG 471 Bacterial reverse mutation (Ames) test).

126.   In 2017, the "Malta Initiative"[33] (MI), led by Germany, triggered the development of a number of OECD TGs and Guidance Documents for a number of endpoints relevant to MNs, including physico-chemicals endpoints, as well as their environmental and human health toxicity. In some cases, OECD Test Guidelines have been modified to account for MNs and in others further modification is still needed (OECD, 2017, 2018a, 2018b; See Table 2.1). Specifically, the OECD has already updated test guidelines for subacute and subchronic inhalation toxicity testing (TG 412 and TG 413) to include considerations specific to MNs. In other cases, e.g. for genotoxicity assessment, the Ames test TG (OECD TG 471) was not deemed a good indicator of genotoxicity for MNs (OECD, 2014c). An overview of on-going work to adapt OECD test guidelines is given in Rasmussen et al. (2019). Using standard toxicity test methods has the additional benefit that it can allow for comparisons between chemical and MN hazard. Specific adaptations for conducting oral studies on MNs, including the integration of toxicokinetics in the 90 days repeated dose oral study are provided in the EFSA guidance (EFSA, 2021a). In addition, a new TG on toxicokinetics to accommodate testing of nano-particles is under development.

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[32] See https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment

[33] See https://www.nanosafetycluster.eu/international-cooperation/the-malta-initiative/ and https://www.nanosafetycluster.eu/international-cooperation/the-malta-initiative/

Table 2.1: OECD TGs published[34] and in preparation for nanomaterials

Number	TG Name (link)	Section of RA (media/details)	Publication Stage (estimate)
TG 318	Test Guideline 318: Dispersion Stability of Nanomaterials in Simulated Environmental Media	Fate (Environmental Media)	Published 2017
GD 318	GD for the Testing of Dissolution and Dispersion Stability of Nanomaterials and the Use of the Data for Further Environmental Testing and Assessment Strategies		Published 2020
TG 412	Test Guideline 412: 28 days (Subacute) Inhalation Toxicity Study	Hazard (Human Health)	Published 2018
TG 413	Test Guideline 413: 90 days (Subchronic) Inhalation Toxicity Study		Published 2018
Project 1.3	New TG on Determination of the (Volume) Specific Surface Area of Manufactured Nanomaterials	Physico-chemical Properties (NA)	Expected in 2022
Project 1.4	New Test Guideline on Particle Size and Size Distribution of Manufactured Nanomaterials		Expected in 2022
Project 1.5	GD on Determination of Solubility and Dissolution rate of NMs in Water and Relevant Synthetic Biological Media		Ongoing
Project 1.6	GD on Identification and Quantification of the Surface Chemistry and Coatings on Nano- and Microscale Materials		Ongoing – laboratory testing step
Project 1.7	New TG on Determination of Surface Hydrophobicity of Manufactured nanomaterials		Expected in 2023
Project 1.8	TG on Determination of the Dustiness of MNs		Ongoing
WPMN	Adaptation of OECD Test Guidelines 201, 202, and 203 for the Determination of Ecotoxicity of MNs	Hazard (Environmental, Effects on Biotic Systems)	Ongoing

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[34] Previously published GD can be found in the Series on the Safety of Manufactured Nanomaterials.

Number	TG Name (link)	Section of RA (media/details)	Publication Stage (estimate)
GD 317	GD on Aquatic and Sediment Toxicological Testing of Nanomaterials		Published 2020
Project 3.10	TG on Dissolution Rate of NMs in Aquatic Environment	Fate and Behaviour (Environmental)	Ongoing
	Study Report on a test for removal in wastewater treatment plants of gold manufactured nanomaterial		Published 2021
Project 3.12	GD on Assessing the Apparent Accumulation Potential for NMs		Ongoing
GD 342	GD to Support Implementation of TG 312 for NMs Safety Testing		Published 2021
Project 3.16	GD Environmental Abiotic Transformation of NMs		Ongoing – planning and determining methods step
Project 3.17	TG on *Hyalella azteca* Bioconcentration Test (HYBIT)		Ongoing – laboratory testing and analysis step
Project 3.18	Anaerobic Transformation of Chemicals in Liquid Manure		Ongoing – ad hoc expert group formation step
WPMN	Scoping Review for a Tiered Approach for Reliable Bioaccumulation Assessment of MNs in Environmental Organisms Minimising Use of Higher Tier Vertebrate Tests		Ongoing
Project 4.95	GD on the Adaptation of *In Vitro* Mammalian Cell Based Genotoxicity TGs for Testing of MNs	Hazard (Health Effects)	Ongoing
Project 4.133	Applicability of Key Event Based TG 442D for *In Vitro* Sensitisation Testing of NMs		Ongoing
Project 4.146	TG on Toxicokinetics to Accommodate Testing of nanoparticles		Ongoing

2.5.5. Dose Descriptor

127.   With sufficient dose-response data available, benchmark dose (BMD) estimation is a standard risk assessment method used to identify and more statistically rigorous point of departure compared to a no or lowest observed adverse effect level (NOAEL or LOAEL respectively; Crump, 1984; Crump, 1995; EPA, 2005). BMD methods have been used in cancer and noncancer risk assessments, including for pulmonary responses to inhaled nanoscale (ultrafine) particles (Kuempel et al., 2006; Dankovic et al., 2007; NIOSH, 2011; NIOSH, 2013; Weldon et al., 2016; NIOSH, 2018). A benchmark dose lower confidence limit (BMDLx) 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 BMDLx 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). BMDLx 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 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. For example, using this approach of estimating BMDs, in addition to NOAEL or LOAEL values as the critical effect level in animals, U.S. NIOSH has published a Reference Exposure Limit (REL) of 1 µg/m^3 for carbon nanotubes and nanofibers (NIOSH, 2013), and also proposed a draft REL for nano-silver of 0.9 µg/m^3 (NIOSH, 2018). Thus, standard methods for dose-response modelling that are used in general risk assessment methods are also applicable to the risk assessment of MNs.

2.5.6. In vitro/Alternative Approaches

128.   New approach methodologies (NAMs) are rapidly being developed as alternatives to animal testing. "Both the EU and other OECD member states have understood the increasing need for dedicated research focussed on assessing the potential environmental and health risks of commercial MNs. As a result, significant amounts of funding have been allocated in Europe by national governments as well as the Commission on an EU level, while the US have promoted EHS integration in its NNI programme since its inception." (Steinhäuser et al., 2018).

129.   In 2018, the United States (US) Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) published "A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States." It describes a framework for safety testing that will provide more human-relevant toxicology data while reducing the use of animals.

130.   In 2018, OECD (2018e) published a *Guidance Document on Good* In Vitro *Method Practices (GIVIMP)* No. 286. However, it is important to determine how valid these alternatives are for safety evaluation and for human health risk assessment.

131.   In order for their acceptance as animal replacements, the NAMs have to be able to provide an equivalent level of protection to that derived from animal testing and their performance in terms of sensitivity, specificity and predictive value, has to be evaluated, which is not currently met. Since *in vitro*

assays are not sophisticated enough to assess the complex adverse outcomes manifested at a tissue level, they are instead used to predict an adverse outcome by targeting the key biological events at lower levels of biological organisation (e.g. molecular, cellular) responsible for the initiation and manifestation of an adverse outcome.

132.   It is acknowledged that one single *in vitro* test or measurement may not be enough to predict a tissue or an organism level response and that multiple *in vitro* tests targeting multiple key events involved in a disease process may be needed to achieve the required predictive efficiency. For this, a thorough understanding of the *in vivo* biology perturbed and the underlying mechanisms leading to such perturbations is necessary.

133.   As stated above, a large number of *in vitro* endpoints are routinely assessed to understand the toxicity potential of a MN; however, their relevance to *in vivo* toxicity observed following exposure to the same material is seldom defined (Halappanavar et al., 2021). There is a clear disconnect between what is observed *in vivo* and what is assessed *in vitro*. At present, not all adverse outcomes induced by MNs are known. So, before an *in vitro* method is considered for toxicity testing, its relevance, purpose or usefulness to describing an *in vivo* toxicity endpoint has to be described. In other words, *in vitro* tests have to be anchored to an observed effect *in vivo* before using them as *in vivo* test replacements.

134.   The other issue with current *in vitro* test methods is that a single *in vitro* toxicity end point is assessed by several different assays and methods, which may be mainly based on the expertise of the specific laboratory and resources and the accessibility to the assay protocols. For example, *in vitro* cytotoxicity is assessed using a wide variety of cell types and assays targeting different mechanisms or stages of cytotoxicity. Some assays measure membrane permeability as indicative of loss of cell viability and others measured expression levels of enzymes such as caspases as markers of cell death. However, the relevance of the positive disruption of the cellular survival pathways resulting in cell death to what is observed *in vivo* for that material is not made clear in these studies. Also, the methodology used for the same assay in a given cell type differs widely. Assay heterogeneity is observed for almost all *in vitro* endpoints analysed, which can pose a serious impediment to establishing an *in vitro* method (Halappanavar et al., 2020).

135.   From the literature review, it is likely that a number of *in vitro* methods to assess specific adverse outcomes induced by MNs already exist; however, selection of the most appropriate and complete assays that exhibit the best predictive efficiency is a prerequisite for their further development and validation, which has been challenging.

136.   It is also important to define the MN domain of application, the MNs for which the *in vitro* assays are applicable. For example, more than one type of cytotoxicity assay may be needed to cover the different MN types; some MNs interfere with the components of certain colorimetric assays and thus, one specific assay may not be applicable to all.

137.   As part of the EU FP7 Nano-Valid project, in a pan-European inter-laboratory study, Piret et al. (2017) evaluated three different cell viability assays including MTT, ATP content and Caspase3/7 activity with absorbance, luminescence and fluorescence readouts, respectively, for their suitability and reliability for the assessment of nanosafety using three different cell types reflecting lung, liver and gut organs. The study investigated nanoparticles of silver and copper oxide. The authors concluded that MNs interfere with the assay readouts and therefore testing for MN interference should be routine for all methods. MTT and ATP content assays showed little variability. In contrast, Caspase 3/7 activity showed high inter-laboratory variability (Piret et al., 2017). Similarly, many other studies have investigated the suitability of *in vitro* assays for application in nanosafety assessment with different goals of establishing standard operating protocols, evaluation of suitability of cell types, variability in readouts, *in vivo – in vitro* response correlations, determination of *in vitro* dose metrics, *in vitro* exposure protocols and others.

138.   A recent EU Horizon2020 project PATROLS aimed to develop advanced *in vitro* models for predicting nanomaterial-induced adverse effects in lung, liver and the gut, and developed standard operating protocols for the selected methods. Thus, a large database of *in vitro* studies with details on specific *in vitro* assays already exists.

139.   The articles compiled in the NanoImpact special issue on Reliability of Methods and Data for Regulatory Assessment of Nanomaterial Risks[35] shows a set of available methods for endpoints. These methods are either validated to be used for regulatory decisions, or at least have some degree of demonstrated reliability and are therefore promising for near-term regulatory use (Steinhäuser et al., 2018), e.g.:

- Functional assays that are relevant for the characterisation of MNs (surface affinity, ROS generation, dissolution rate, etc.), but also to predict their fate and effects;
- Assessment methods for nanomaterials' ecotoxicity and toxicity, including the aquatic and sediment toxicity;
- Methods for mammalian hazard assessment by using *in vitro* assays (Drasler et al., 2017) or by applying *in silico* approaches.

140.   Progress has been made at the OECD where work is underway to investigate how certain TGs/GDs may not be appropriate to test NMs compared to bulk material. Work is being developed to see how alternate testing can be done so that whole organisms do not have to be used to test each individual NM, which is not cost effective and does not keep in line with the 3Rs for animal testing (Replacement, Reduction and Refinement).

141.   ISO/TR 21624:2020 provides information regarding the systems available for exposure and assessment of nano-objects and their aggregates and agglomerates (NOAA) for *in vitro* air exposure studies. It provides an overview of the various exposure systems and *in vitro* cell systems used to perform *in vitro* studies that simulate an inhalation toxicology study design.

142.   However, unlike for chemicals, a reliable database of *in vivo* studies is not available for MNs. The question then is how one should go about performing formal validation of an *in vitro* test method in the absence of reference gold standard animal toxicity data. While the nanotoxicology community is actively engaged in developing *in vitro* methods, it may be necessary for the community to come together to agree upon the validation process for *in vitro* test methods lacking *in vivo* data.

2.5.7. *Adverse Outcome Pathways*

143.   Adverse Outcome Pathways (AOPs) are disease or toxicity road maps (see Figure 2.3). They reveal the complex biology at play at different levels of biological organisation starting from molecular and cellular levels to organism and population levels, and they enable visualisation of how these various levels are interconnected. AOPs enable systematic and modular presentation of diverse historical and new data including data derived from novel assays involving key events (KEs). This organization of information helps identify knowledge gaps, data needs and guide future research priorities. AOPs provide a means of anchoring mechanistic information to human and environmental health effects.

144.   In the context of animal alternatives, AOPs help anchor *in vitro* tests and measurements to *in vivo* outcomes. By providing the biological and mechanistic context, they aid in the selection and development of the most relevant *in vitro* tests targeting KEs progressing to an adverse health or environmental outcome. In this way, AOPs support the strategic design and development of evidence-

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[35] https://www.sciencedirect.com/journal/nanoimpact/special-issue/10MHRPK1HMK

based testing tools and strategies, for the targeted generation of 'fit for purpose' data and their interpretation.

Figure 2.3. Example of Generic AOP Pathway and Networks and their Connections

This figure presents a generalised AOP (Panel A) and an example of an AOP network showing shared MIEs or KEs (Panel B), representing the complex mechanism and progression of toxicity processes. KEs are used to develop targeted bioassays and endpoints, potentially predictive of the eventual adverse outcome. Single or multiple assays can be developed for measuring individual KEs. As stated above, AOPs represent mechanistic biological processes leading to an adverse outcome and therefore, are not substance specific (Villeneuve et al., 2014). However, future development of AOPs that contain KEs relevant for MNs in support of MN risk assessment, are needed. This figure is adopted from Halappanavar et al., 2019.
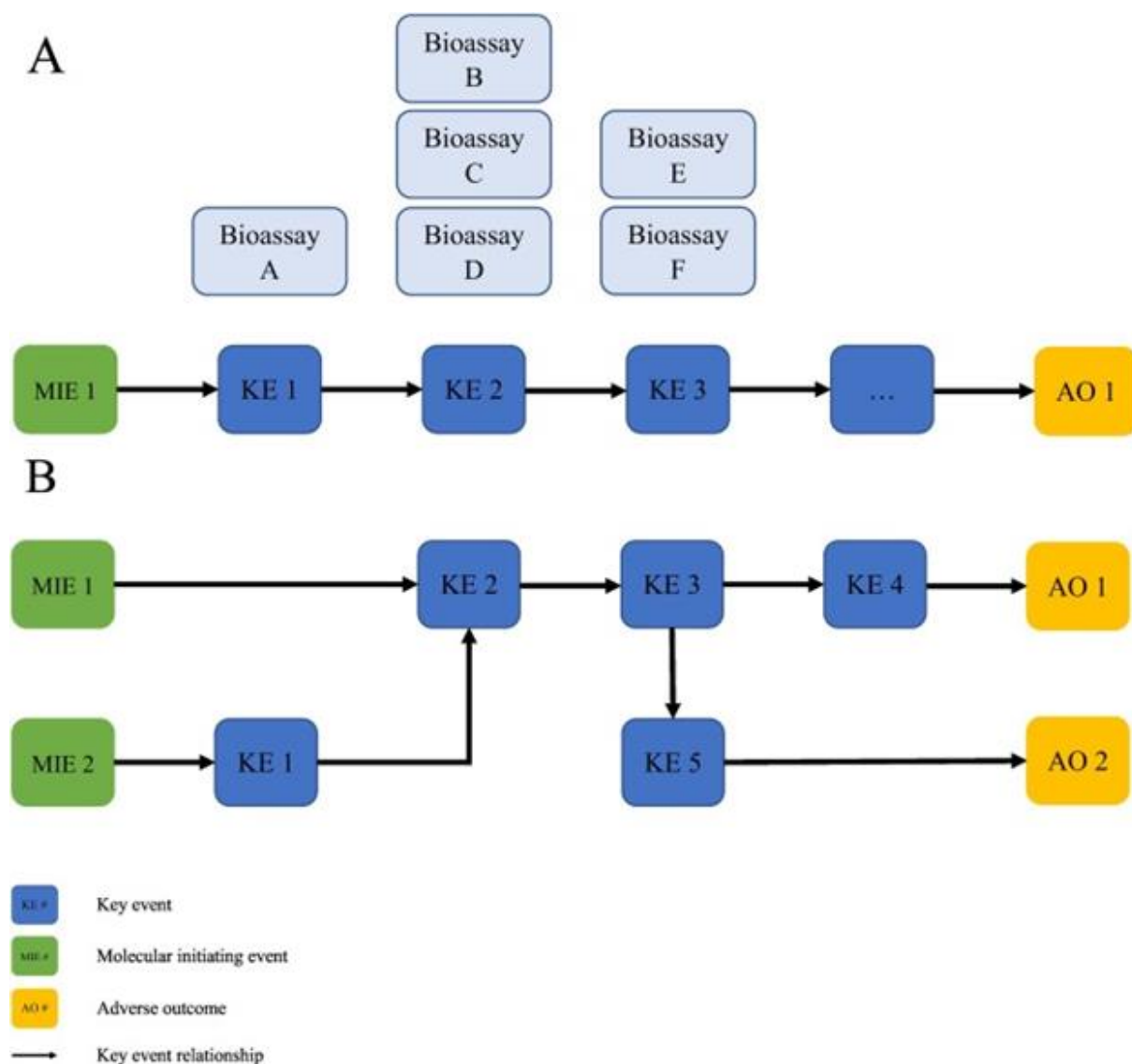
145.   By providing the mechanistic backbone, they support the novel approaches and strategies in risk assessment including Integrated Approaches to Testing and Assessment (IATAs) and Intelligent Testing Strategies (ITSs). Some principles of AOPs include:

- AOPs are chemical agnostic and multiple chemicals may initiate one AOP. They allow grouping of substances or MNs by the adverse outcomes they induce.

- AOPs are linear and depict one sequence of KEs. AOPs describe the relationship that an individual KE shares with another KE upstream or downstream to it, enabling the derivation of dose-response relationships and determinations of toxicity thresholds, which can then be used to build predictive models.

- Multiple linear AOPs can be connected in a network, revealing complexity of the toxicity process (Figure 2.3), which can help build quantitative and predictive models[36]. A list of AOPs that are endorsed by OECD or currently under development is available[37].

146.   In the context of nanotoxicology, the role of AOPs in furthering risk assessment activities is appreciated. The chemically agnostic paradigm of AOPs still needs to be confirmed for relevance to MNs. The physico-chemical properties of MNs may induce deviations in the known pathways, especially in creating new molecular initiating events (MIEs). A recent publication by Halappanavar et al. (2020) listed a number of linear AOPs describing the mechanisms of adverse outcomes relevant for inhalation toxicity of MNs. A network of linear AOPs highlighted shared MIEs and KEs across these AOPs. Furthermore, how multiple KEs from an AOP can be used to inform a tiered testing strategy in support of screening MNs for lung toxicity was shown. The role of AOPs in regulatory risk assessment continues to be developed, with challenges due to the lack of quality data or limited mechanistic knowledge of all adverse outcomes induced by MNs.

2.5.8. Historical Data

147.   Similar to existing chemicals, some MNs have been on the market for decades, including for example the use of nanosilver for their antibacterial properties and their toxicity has been previously tested, as reported in the scientific literature or provided to meet regulatory requirements (OECD, 2010). It is generally agreed that the accumulated historic safety information should be taken advantage of, but it remains to be determined how to integrate this data in current hazard assessments. As concluded at the Risk Assessment of Manufactured Nanomaterials in a Regulatory Context Workshop, 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 had different degrees of precision (OECD, 2010). Today's techniques and equipment for determining nanoparticle properties (e.g. BET surface[38], zeta potential, Scanning Mobility Particle Sizing (SMPS), etc.) and dosage (e.g. Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), particle counting) may not have been available or as sensitive at the time, which could hamper identification of dose equivalence and whether the historical and recent data were determined for the same material. Even if a certain historical MN is still on the market today and so can be analysed with today's techniques, one cannot be certain that the techniques to manufacture the nanomaterial are still the same. Approaches to provide adequate scientific proof for equivalence may be developed.

148.   A similar uncertainty can be expected with regard to the historic equivalence of 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 not specific to MN, but it should be taken into account, that measurement endpoints with particular relevance for MN toxicity (e.g. BAL parameters, parameters indicative for

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[36] https://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm

[37] https://aopwiki.org/

[38] 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).

immunotoxic effects, or measurements of lung burden providing clarity on the retained dose[39]) may have been included at a different schedule than other enhancements. For the applicability of OECD TGs reference is also made to the "Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials" (OECD, 2009c) as well as to Rasmussen et al. (2019).

2.5.9. *Extrapolation and Use of Uncertainty Factors*

149.   The use of chronic data is generally recommended, however, maintaining stable concentrations of MNs is a practical challenge leading to scarce chronic and multigenerational toxicity data (Quik et al., 2020). If these data are not available, uncertainty factors (also called adjustment factors, assessment factors etc.) can be used and reported in the risk assessment. Agency or jurisdiction-specific procedures and practices may also be applicable to risk assessments of MNs. Generally, uncertainty factors can be used to identify no effect levels by:

- extrapolating temporally (i.e. estimating chronic toxicity based on the results of acute toxicity testing, acute to chronic toxicity value ratios (ACRs));
- accounting for intra- and interspecies extrapolation (e.g. animal to human dose extrapolation); and
- accounting for laboratory to human or field conditions.

150.   ACRs are often used for extrapolation from acute (single, high dose) effects to chronic (repeated, lower dose) effects in environmental risk assessment. The same applies to subacute-to-subchronic and subchronic-to-chronic extrapolation in human health risk assessment. A lack of understanding of the acute and the chronic mechanism of action of specific MNs is a challenge for determining ACRs and can lead to testing on a case-by-case basis (Quik et al., 2020).

151.   Use of uncertainty factors is widely accepted when data are scarce or poor in quality and has been applied in scientific reviews of the health and environmental safety of different classes of nanomaterials (EPA, 1993; Chapman et al., 1998; WHO, 2005; Blaser, 2008; ECHA, 2019d; ENRHES, 2010; Dankovic et al., 2015;). 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 applying a higher overall assessment factor (on a case-by-case basis) depending on the information available (e.g. up to x1000 instead of x100).

152.   Empirical studies have yet to examine the scientific basis for the use of uncertainty factors in nanomaterial risk assessments. Considering the unique properties of certain classes of nanomaterials such as toxicokinetics (e.g. possible slow clearance), formation of protein coronas, etc., there is a need for additional research on the 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. In 2010, the OECD proposed that there is no current need for a separate additional nano-specific uncertainty factor, as uncertainty factors are best employed for specific area of uncertainty, rather than trying to compensate for a broad unknown (OECD, 2010; Canady, 2010). More recently, the Canadian Risk Assessment Framework on Manufactured Nanomaterials has suggested that there may be additional considerations when assessing NMs, such as uncertainties associated with read-across of NMs (within different nanoforms or bulk to nanoscale). Qualitative approaches could also be used until supporting empirical data becomes available for evidence-based adjustment factors for specific classes of nanomaterials.

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[39] Included in revised OECD TG 412 and 413: Guideline for the Testing of Chemicals: Sub acute/chronic Inhalation Toxicity: 28/90-Day Study, updated in 2018)

153.   Extrapolation can occur from one nanomaterial to another (nano-to-nano), from a bulk substance to a nanomaterial, inter- or intraspecies, or time related. Each extrapolation requires strong evidence and justification for nanomaterials and may be quantified by uncertainty factors. Between substances, reliable chemical-specific data, accompanied by a good understanding of the biological response to the material in the form of physico-chemical properties, toxicokinetics, and toxicodynamics, are needed for read-across and to predict hazard. An initiative to advance grouping approaches for nanomaterials is the H2020 EU funded project GRACIOUS[40].

154.   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 induced more inflammation than the same mass of larger particles of the same chemical composition (Bermudez et al., 2002; Bermudez et al., 2004; Elder et al., 2005). In these cases, hazard/risk grouping strategies may be considered for particles with the same mode of action. For example, approaches to determine Chemical-Specific Adjustment Factors have been suggested for CNTs (Nakanishi, 2009a; Pauluhn, 2010). Interspecies extrapolation (e.g. rodents to humans) factors are typically established based on in-depth knowledge of the mechanisms influencing dosing and toxicity of conventional chemicals. Further research into potential differences between species in deposition (pattern), clearance (including capacity), and sensitivity are needed for nanomaterials.

155.   Standard intraspecies extrapolation assessment factors between 3 and 10 have been established to account for inter-individual differences in workers and the general population (ECHA, 2012a; ECHA, 2012b; ECHA, 2019d; EPA, 1993). The Scientific Committee of the European Food Safety Authority (EFSA) concluded that the scientific literature current as of 2011 and 2018 does not indicate a need for different assessment/uncertainty factors for nanomaterials (EFSA, 2021a). A challenge identified regarding intraspecies extrapolation is that expensive and time-consuming vertebrate tests typically use inbred animals, for which non-animal alternatives are not identified.

156.   Large assessment factors have implications for risk management. The use of large assessment factors to derive limit values may prove to be over-precautionary, and such low PNECs or Maximum Residue 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 analytical challenges can pose significant feasibility concerns).

2.5.10. Considerations for Manufactured and Transformed Nanoforms

157.   The interactions between nanomaterials and exposure factors are complex. Recommended standard test protocols (e.g. a daphnid test with exposure to a single nanoform in standardised de-ionised water) may have only limited relevance when compared with the natural environment. Abiotic and biotic environmental factors can affect the structure, form, behaviour and fate of nanomaterials. Transformed nanomaterials can affect bioavailability and toxicity that may not be easily predictable from standard tests for bulk substances. The relevance of abiotic and biotic factors is likely to vary with both the method chosen (*in vitro / in vivo*) and exposure route (e.g. water, air). An example is the enhanced uptake and retention of nanoparticles by *Daphnia magna* in the presence of secreted corona (Briffa et al., 2018; Nasser and Lynch, 2016). Test conditions can be altered to provide a more realistic exposure scenario. For example, filter feeders may need to be fed during the exposure to displace previously ingested NMs providing a more realistic quantity of NMs retained.

158.   Poor or inadequate material physico-chemical characterisation has been a major barrier to interpreting and comparing studies addressing the human or ecological toxicity of MNs. In response to

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[40] See www.h2020gracious.eu

this, a number of international organisations have proposed indicative materials characterisation parameters, which should be determined when toxicity tests are undertaken (OECD, 2012a; OECD, 2019b; ISO/TR 13014:2012; Stefaniak et al., 2013). REACH was amended to take into account the characterisation of nanoforms for a number of physico-chemical parameters (European Commission, 2018).

2.6. Risk Assessment Strategies

2.6.1. Exposure Minimization

159.   Minimizing[41] 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. Examples of applicable circumstances may be where the material:

- is manufactured and processed at facilities which are designed to avoid 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 environmental monitoring is needed to confirm release measures are effective.
- 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).

160.   Minimizing or eliminating exposure may need to be assured using risk management measures. 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. As more data become available, the scope of the risk assessment could be extended to evaluate potential risk under other conditions of use.

2.6.2. Lack of Bioavailability or Toxicity

161.   Another strategy for addressing risk involves developing weight-of-evidence addressing bioavailability. For example, characterisation data may show substantive and unequivocal evidence that nanomaterials will rapidly and irreversibly form large aggregates that are not easily internalised by the cells. Then for aerosols, the size of the aggregates should be evaluated to determine if they are inhalable. In addition, stability of MN suspension must be verified. If a MN rapidly and completely dissolves, it may be investigated as a non-nano substance. This characterisation information in combination with biological effects data showing no evidence of toxicity for the aggregated MN, may

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[41] 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.

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.

162.   Alternatively, circumstances may exist where an exposure assessment based on comprehensively derived exposure scenarios 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 indicate that the material would not pose a risk under reasonably anticipated exposure scenarios, if those exposures are considerably lower than the exposure concentration 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 (oral repeat dose test), the limit test is described as follows:

163.   "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."

2.6.3. *Quantifying Risk*

164.   Some applications of MNs have an inherently dispersive use (e.g. paints, fertilizers, wastewater treatment) where release/exposure is difficult to be determined. Risk characterization might be qualitative and quantitative for various uses. Where such dispersive materials have a potential for biological effects, then quantification of risk would be appropriate.

165.   As discussed above, risk quantification may require the use of uncertainty factors. Currently there is policy support for use of existing default uncertainty factors for risk assessments of MNs (EFSA, 2021a), although, there is a lack of empirical evidence supporting the application of these standard uncertainty factors to MNs. Consequently, the use of 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.

2.6.4. *Iterative Risk Assessments*

166.   One approach addressing limited data is "adaptive management", based on a plan-do-check-act (PDCA) cycle (Nakanishi 2009b). 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. 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 could be taken when there is uncertainty in quantification of hazard or exposure (Schulte and Salamanca-Buentello, 2007).

3 Research and Risk Assessment

167.   Manufactured nanomaterials are becoming ubiquitous in consumer products and as a consequence, in the environment. There are different types of nanomaterials synthesised for different applications that vary in size and physico-chemical properties. At the start of the nanotechnology development, a single type of nanomaterial was incorporated in consumer products such as paints, textiles, food packaging, skin care products, etc., which are now referred to as first generation nanotechnology enabled products. In the last two decades more sophisticated applications have been found for nanomaterials, where more than one type of nanomaterials of different properties are combined to manufacture more complex nanomaterials and incorporated into nanostructures and nanosystems. The latter are sometimes categorised into second, third and fourth generation products. Some examples include, smart fertilisers, synthetic organs, self-assembling materials, gene therapy devices and others. However, human/environment population exposure and toxicity are not entirely characterised.

168.   Toxicologically, it is well acknowledged that their small size and larger surface area renders an advantage over bulk materials of similar chemical composition with respect to evading biological surveillance, deeper infiltration of tissues, translocation across barriers and reactivity with surrounding medium. Although new adverse outcomes unique to nanomaterials are not identified so far, they do induce effects in tissues and organisms that are not routinely captured under the current regulatory system. For example, tissue inflammation and tissue fibrosis are frequently reported after exposure of animals to different types of nanomaterials, which are not routinely targeted in regulatory decision-making. Moreover, apart from size, how unique physico-chemical properties of nanomaterials affect individual exposure outcomes in experimental models is not completely revealed. Even though significant progress has been made in the context of generating toxicological data for human and environmental health risk assessment, the 'gold standard' data used in risk assessments, including ADME and dose-response data are not available for nanomaterials. Because of their versatility, sheer number and the current toxicology testing system that is long and animal laborious, it may not even be possible to generate such data for all of the different types of nanomaterials that are presently in use. As a result, to date, effective risk assessment of nanomaterials has not been conducted.

169.   Development of risk assessment and risk management decisions in the absence of complete and comprehensive datasets or in-depth scientific understanding of underlying toxicity mechanisms is not a scenario unique to nanomaterials. However, the issue at hand requires switching from traditional animal-based bioassays to novel toxicology paradigms involving non-animal approaches for investigating exposure, hazard identification and risk assessment. Moving forward, effective cooperation between risk assessors and researchers is necessary to progress nanomaterials risk assessment. Chapter 3 addresses critical research needs that are required to improve risk assessment and to reduce the uncertainty for effective occupational, public health, and environmental risk management of nanomaterials. Specific research needs pertinent to physico-chemical properties characterisation and reporting, exposure assessment, dose metrics, predictive computational models; toxicokinetics, animal alternatives, novel approach methodologies, nanoinformatics needs, non-standard data, ecological effects and other topics are presented below. This Chapter is not intended to be exhaustive and does not cover all areas of research conducted in the field of nanotoxicology. This is a living Chapter and is expected to change as the identified needs are met and new ones arise.

3.1.   Physico-chemical properties characterisation and reporting (For details, refer to Sections 2.2.1, 2.2.2, 2.3, 2.5.4, and 2.5.10)

In the context of nanomaterials, their potential to induce harm is determined by their physico-chemical parameters such as, size, shape, chemical structure, surface properties including charge and their ability to interact with the surrounding milieu (biological or environmental). In addition, route, level and duration of exposure play an important role in determining the effects. Although research conducted so far has identified some characteristics of nanomaterials that can be used to group them based on their structural or chemical properties, a clear understanding of how any modification to the original material may induce changes in its biological or toxicological behaviour has not fully been considered in the interpretation of results. Such changes include changes in post-exposure fate and toxicokinetics. This is due to lack of detailed physico-chemical data on the nanomaterials tested and inconsistency in reporting standards. As a result, there is a critical need for research to identify the necessary measurands to assess in a context-specific manner and need for establishing harmonised reporting standards.

Research needs

- The development of a repository of reference nanomaterials reflective of real-life applications, for investigation in toxicological studies. Consideration of next generation nanomaterials or advanced materials for inclusion in this repository as their uses in products is steadily increasing. Current repositories of JRC and Fraunhofer consist of a wide variety of nanomaterials that are accessible by all researchers. However, these may not be reflective of nanomaterials in the environment.

- Identification of 'fit for purpose' physico-chemical properties, generation of thorough and complete data on physico-chemical properties of nanomaterials prior to exposure, during the exposure in the relevant medium of exposure and post-exposure in the biological or environmental matrices. For example, characterisation of aerosol, degree of agglomeration in the associated medium, etc. Where necessary, this should also include characterisation of temporal stability of materials through storage, handling, preparation, etc.

- Identification of the most relevant (specific to nanomaterials and/or exposure conditions, type of endpoints), and reliable techniques for characterisation. The choice of technique may depend on how widely a method has been used for characterising nanomaterials, for which a large literature base exists and/or analytical techniques that have well developed protocols, are validated in interlaboratory studies and applicable to a broad variety of nanomaterials classes.

- Development of standardised protocols for characterization techniques and harmonised standards for reporting physico-chemical data (e.g. templates for recording similar level of details and type of information). Where possible, inclusion of strategies to compare results from different techniques measuring the same measurand.

3.2.   Nanomaterial exposure assessment – workers, consumers, environment (For details, refer to Sections 2.4 and 2.6.1)

In general, exposure assessments in realistic conditions are lacking; exposure of workers at the

production facilities during different stages of the materials life cycle, population exposure via releases to the environment from industrial facilities, and data on nanomaterials present in nano-enabled products as well as on their potential exposure.

Owing to lack of labelling requirements, it has been challenging to identify products containing nanomaterials. High throughput techniques that allow screening of multitude of products for nanomaterials' presence are lacking as well. In addition, data on concentrations in and releases from consumer products into environment and transportation, translocation and persistence of nanomaterials in the environmental media, is lacking.

Therefore, understanding how nanomaterials' physico-chemical properties (e.g. morphology, heteroagglomeration, surface chemistry, size, etc.) impact their transformation, translocation and clearance during its journey through different environmental and biological media, and how this compares to non-nanomaterials, is an important area requiring immediate attention.

Moreover, the detection limit of currently available conventional methods to measure particles in the environment and workplace is limited. Thus, development of more sensitive and reliable methodologies to measure and characterize nanoparticles with lower detection limits is required. In addition, decision on logic models for exposure assessment based on particle morphology needs to be developed; and new or improvement of existing simulation approaches is also needed.

Research needs

- For humans, the occupational setting is the most likely situation in which (low-dose) chronic exposure to nanomaterials occurs, usually through inhalation. To date, no occupational nanomaterial-related diseases has been reported, however reliable exposure biomarkers still have to be identified, and also robust methodologies to characterise exposure have to be implemented.

- Development of tools and models to estimate, predict and quantify release or emission of nanoparticles to the environment and, human (workers and consumers) and environmental exposure during the normal use of products and across the life cycle of products, is an urgent need to comply with regulatory requirements. While generalisation in methodologies is preferred, for now, for nanomaterials a case-by-case approach is envisioned.

- Generation of data for model validation which will support environmental exposure assessments; development of trends in behaviour of nanomaterials including: i) comparing how specific properties of nanomaterials relate to biological effects; and ii) how different media affect these properties.

- Studies characterising and quantifying free versus matrix-bound nanomaterials are needed. These studies should detail the experimental conditions that lead to release of nanomaterials and the characteristics of the matrix.

  Example: better understanding of nanomaterials release from food packaging and understanding of mechanisms of migration to inform product and process design.

- Development or optimisation of techniques for rapid screening of products on the market that may contain nanomaterials is needed. An inventory of products that potentially contain nanomaterials will help guide the research priorities.

- It is assumed that substantive human exposures to nanomaterials is in its early stages. It is, therefore, important to identify the likelihood of population exposure to evaluate and validate initial risk estimates, e.g. confirm that estimated no-effect scenarios indeed do not lead to adverse impacts. This requires advancing epidemiological approaches and developing

biomonitoring techniques.

- Although not necessary for risk assessment, development of tools and techniques to characterise bio-corona and understanding the ways that secreted biomolecules or NOM may alter the stability, identity and toxicity of nanomaterials towards organisms. A question could be whether a bio- or eco-corona increases or decreases toxicity of nanomaterials towards organisms, e.g. by impacting passive uptake, active uptake by consumption, cell-mediated endocytosis, etc.

3.3. Toxicity assessment, Dose metrics and dosimetry (For details, refer to Section 2.2.4)

Mass is the universal metric used for describing chemical exposure. For nanomaterials, this can be tricky, and mass may not be sufficient to describe the dose for different nanomaterials of the same chemical composition. Other metrics have been suggested, which include particle number or surface area. However, sensitive analytical methods are not available to effectively measure the particle number, and surface area may not be applicable to all nanomaterials. Thus, as stated in the previous chapter, the metric used may be subjected to the type of experiment or nanomaterial used and, in some cases, a combination of different metrics may be necessary. For example, mass may be the correct unit of measurement for soluble metal oxides, while particle number may be relevant for high aspect ratio fibres. Moreover, understanding of delivered versus biologically active dose is also critical for risk assessments. Effective methods/techniques to measure these are not available at present.

Research needs

- Comparison of different dose metrics (e.g. mass, particle number, surface area). This is particularly important where exposure metrics differ from those used in hazard assessment.

- The required analytical methods for effective measurement of biologically active dose are still under development. Research in this area is needed as differentiating between the delivered, tissue retained and biologically active dose is important.

3.4. Toxicity assessment, Toxicokinetics – study design (For details, refer to Sections 2.5 and especially 2.5.1, 2.5.2, and also 2.6.2)

The preliminary results from the nanotoxicology research conducted so far suggest that ADME of nanomaterials differs from that of chemical substances. ADME of chemical substances is governed by diffusion, active transport, metabolism by enzymes and excretion. In contrast, the ADME of nanomaterials is dependent on their interaction with cells and internalisation, surface adsorption/opsonisation or binding to biomolecules and size-associated properties. An OECD workshop on toxicokinetics suggested that OECD TG 417 for chemicals may be applied to nanoparticles, but provides several specific recommendations for consideration. A new TG is under development to specifically address minimum requirements of the study design, which will be based on the expected presence or retention of nanomaterials in the different target tissues and the ability to detect the nanomaterials, or in case of labelled nanomaterials, detection of the radiolabel, fluorescent functional group or chemical components of the nanomaterials in tissues.

Many factors, such as dissolution kinetics of the particles and sensitivity of the techniques used for the detection, will influence the interpretation of results.

In a recent review, Oberdörster and Kuhlbusch further elaborate on specific biokinetics study design

requirements for nanomaterials that should be included in the specified new TG as well as in TGs 412 and 413. Key considerations for the study design will include dosing regimen, duration of the recovery period and critical samples for the analysis. In addition, the study design will include minimum material characterisation requirements, which may be tailored to different nanomaterials and their properties.

Research needs

- Understanding the properties of nanomaterials, including particle kinetics in biological systems (i.e. absorption, distribution, metabolism, and excretion – ADME), which influence the internal dose, biopersistence and bioaccumulation. This will assist risk assessors in interpreting results from toxicology studies and can be used to inform predictive toxicokinetic modelling.

- Quantitative analysis of nanomaterials post-exposure in different biological compartments including tissue, organs, and excretes to determine the distribution, fate and clearance of nanomaterials.

- Clarification on the dependence of persistency and tissue concentration in time on the dissolution rate in physiological media.

- Clarification of the impact of administration mode (inhalation vs. intratracheal, diet vs. gavage) on toxicokinetics.

- Clarification of the impact of dispersion and characteristics of MNs within the administration matrix on the toxicokinetics of MNs.

3.5. Ecological Effect Research Needs (For details, refer to Sections 2.5 and especially 2.5.3 and 2.5.9)

For environmental risk assessment lack of knowledge on the behaviour and fate processes (including transformations) of nanomaterials in the environment induce the main uncertainties. This lack of knowledge extends to all levels, including fate and behaviour towards and in the different environmental compartments, fate and behaviour in test systems, and in environmental organisms. For specific effect assessments this should be taken into account, including the choice of test species.

Research needs

- Identification of representative species for use in species sensitivity distributions for different compartments, 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 quantifying risk. In identifying the representative test species, exposure frequency (related to environmental compartment and organism's behaviour), and any differences in sensitivity in different life stages should be considered as well.

- Development of methods for predicting bioaccumulation and the potential for food chain transfer to occur. Identify mechanisms of bioaccumulation within a whole organism and working towards quantifying relevant ADME processes.

- Generation of data to support validation of extrapolations and uncertainty factors for regulatory decision-making, potentially including acute-to-chronic ecological toxicity or chronic toxicity data considering trophic levels and environmental habitat zones.

3.6.   Predictive computational models - Validated models for predicting properties of nanomaterials responsible for harm and for prediction of adverse effects (For details, refer to Sections 2.4.5, 2.5.6, and 2.5.7)

At present, generation of nanomaterial structure and activity models, development of databases to facilitate modelling, QSAR and computational approaches that enable categorization and grouping of materials for prioritisation for toxicity testing or for decision-making, is not fully achieved. This is mainly due to the lack of a minimum number of physico-chemical and toxicological datasets representing several nanomaterial variants. One example where progress is made, is the National Toxicology Program's Integrated Chemical Environment (ICE). These tools will facilitate the prediction of toxicity and provide weight-of-evidence to validate other empirical data being generated.

Research Needs

- Further standardisation of methodologies and protocols for toxicity testing, which will further improve generation of high-quality data.

- A large database with high-quality data for a wide variety of materials with diverse properties. Design of individual *in vivo* or *in vitro* studies involving a number of materials varying in one or two physico-chemical properties to understand how biological or toxicological behaviour of nanomaterial is influenced by their properties. Detailed measurement and consistent reporting of various physico-chemical properties is very critical for such studies, which is linked to research needs described above.

- Studies involving life cycle analysis of nanomaterials. Life cycle analysis in this context refers to the journey of nanomaterials from the stage of their preparation for exposure to the stage of excretion from the organisms in *in vivo* experiments or subcellular localisation in *in vitro* cellular models.

- One crucial knowledge gap for (Q)SARs is the lack of computational methodologies for the calculation of nano-specific descriptors. For example, due to the size and structural complexity of nanomaterials and the presence of heavy atoms, it is currently difficult to obtain a realistic description of a nanomaterial surface structure that is supposed to play a role in toxicity and fate mechanisms (Burello 2018). Given that determination of surface characterisation for umpteen number of nanomaterials is not feasible in the short term, prioritisation of nanomaterials for inclusion in such studies is needed. Prioritised nanomaterials could be those that pose the highest risk or have highest economic benefit.

- Collection and organisation of the available nano-QSAR tools under common analysis platforms (Afantitis 2020), which enables benchmarking and validation of available models. This is crucial for their adoption for risk assessment purposes.

- Development of strategies and models that allow inclusion of non-standard data (e.g. high-content omics data) and integration of heterogeneous types of data (combination of standard and non-standard data from different sources) in predictive modelling.

3.7.   Animal alternatives, novel approach methodologies (For details, refer to Sections 2.1, 2.5.6, and 2.5.7)

The investment and time required to test the number of nanomaterials that require assessment

necessitates consideration of non-testing or tiered testing approaches involving *in vitro*, *in silico* and *in chemico* approaches. Unlike for chemicals, a reliable database of *in vivo* studies is not available for nanomaterials. The question then is how to perform formal validation of a test method or an approach in the absence of gold standard animal toxicity data. Risk assessors are encouraged to stay informed of on-going developments in this field, in particular the increasing number of validated non-animal methods, e.g. the European Union Reference Laboratory for alternatives to animal testing (EURL-ECVAM), the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), or the latest OECD Test Guidelines for the latest updates.

Approaches such as Intelligent Testing Strategies (ITSs) are proposed and are expected to promote efficient assessment of nanomaterials without the need for testing each size or variant individually. A research need is to implement ITSs in case studies to a set of representative nanomaterials and demonstrate its effectiveness in decision making. Similarly, Integrated Approaches for Testing and Assessment (IATAs) form another approach that integrates and weights information from multiple and all available sources (properties, *in silico* models, *in vivo* and *in vitro* data, epidemiology data and other sources) to derive conclusions on the hazard of substances. Significant work is underway for use of IATAs in informing grouping of nanomaterials. However, more case studies are needed to evaluate IATAs in regulatory decision making for nanomaterials.

For these approaches to be successful, the foundation has to be mechanisms based. Integration of AOP thinking in research at the stage of problem formulation is critical as it will enable grounding of the scientific issue being investigated and provide an experimentally supported decision backbone. Construction of AOPs identifying the KEs at the molecular, cellular and tissue level organisation that are predictive of effects *in vivo* at the organism and population levels is important. These AOPs will help identify lower-tier *in vitro* tests that can be used to predict higher-tier *in vivo* results, eliminating the need to use organisms/animals during testing. Anchoring endpoints, assays or tests to KEs will allow evaluation of test relevance to assessing *in vivo* effects of nanomaterials.

Research needs

- Identifying nanomaterial-specific and nanomaterial relevant toxicological endpoints, or nano-specific considerations for the toxicological endpoints employed to assess chemicals. This line of research will ensure that risk assessors are identifying all appropriate biological responses that may lead to adverse outcomes (OECD, 2019a; OECD, 2019b). While there is consensus in the field that nanomaterial-specific adverse outcomes may not exist, the underlying mechanisms of toxicity induced by nanomaterials may be different from those known for chemicals. Moreover, as stated earlier, some toxicity endpoints are not routinely investigated for chemicals.

- Development and implementation of AOPs in nanomaterial safety screening

- A standardised template for recording and communicating data that uses consistent terminology should be developed. Data interpretation standards must be developed. Although *in vitro* assays have been routinely used for testing chemicals and nanomaterials alike, they were mainly employed for the purposes of identifying toxicity mechanisms. However, for the data to be relevant in regulatory decision making, the test and the results have to be anchored to a regulatory endpoint, which is traditionally a disease or an adverse outcome. Thus, all *in vitro* experimental designs must be prepared to answer questions such as,

  ▪ What do the selected endpoints assess? Are the *in vitro* endpoints anchored to a Key Event or multiple Key events in an AOP?

- What is the endpoint predicting in the context of a response at the organism level or toxicity of interest?

- What are the most appropriate assays available to measure the endpoint?

- How is the new assay comparable to an old assay?

- Are harmonised protocols and readily available? What are the limitations of the approach and methodology?

- How do exposure (duration and quantity), selection of test systems and models impact the mechanism depicted in an AOP?

- Does reporting of *in vitro* study results address questions such as, why is the *in vitro* study being conducted and what is the scientific problem being investigated?

- Who are end users? Who should be involved/ Are the right experts identified?

- The future research should focus on further evaluating the assays for their predictive performance (sensitivity, reproducibility and reliability) and establish data interpretation strategies for each of the assays/endpoints developed. A base dataset using representative nanomaterials must be generated for each of the methods to assess the reproducibility and reliability of each of the assays developed. The results can be verified against a benchmark *in vivo* dataset (where available), or by conducting limited *in vivo* experiments for a targeted set of nanomaterials that were positive hits in the *in vitro* testing.

- Strategies and guidance need to be developed for formal validation of a test method in the absence of gold standard animal toxicity data.

3.8.   Nanoinformatics (For details, please refer to Sections 2.2.3, and 2.5.8)

With the urge to reduce or replace animal tests with mechanisms-based lower-tier animal alternatives or computational methods, more and more studies are generating data that is non-standard such as high content (omics), high throughput, use of organoids, etc. However, interpretation of such data for regulatory uptake has been challenging. It is important to note that these types of data will become more and more common.

Research needs

- Standard operating protocols (SOPs), and data reporting and data analysis, quality control including suitable standards or benchmarks, and analysis algorithms have to be developed, established, standardised and/or harmonised. The regulatory acceptance criteria have to be developed and areas of regulatory applications have to be identified. Appropriate training courses to analyse these novel non-standard data in a consistent manner must be established.

- Appropriate data management strategies are a fundamental requirement for efficient nano-bioinformatics. For example, databases for storing omics data in standardised formats are available and provide access to nanomaterials associated omics data. However, metadata and associated toxicological and physico-chemical data requires nanomaterial specific databases capable of linking to the external omics databases. In addition, sustainable plans to store and share data widely for the common good (i.e. FAIR data), have to be established.

- Consistent ontology and reporting standards: Many different types of instruments, methods, and protocols are employed to generate data and inconsistent terminology is used to interpret and describe results. This poses challenges in integrating data from diverse sources and studies towards deriving a unified interpretation. This has also resulted in inconsistent ontology, which makes cross comparison of study results and methods challenging. There is a need for standardised data collection and reporting templates in hazard assessment.

Annexes

Annex 1. Case Studies on Risk Assessment of Manufactured Nanomaterials: Carbon Nanotubes

170.   This section presents risk assessment case studies on CNTs, including a review of available data, knowledge gaps, and risk assessment results.

171.   This section aims to provide further context on issues in hazard, exposure and risk assessments and monitoring in real-life settings by presenting risk assessment case studies. It currently presents risk assessment case studies on CNTs that were originally published as part of the first version of this document on "Important Issues on Risk Assessment of Manufactured Nanomaterials" [ENV/JM/MONO(2012)9]. In the future new risk assessment case studies may be added to this Annex.

Risk Assessment for Carbon Nanotubes.

172.   Carbon nanotubes (CNTs) are an example of manufactured nanomaterials that have been the subject of several recent risk assessments (e.g. Aschberger et al, 2010; Amenta & Aschberger, 2014, Fatkhutdinova et al, 2015). 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 OECD workshop on inhalation toxicity testing (OECD, 2012a) included presentations on acute and subchronic inhalation studies to form the basis for assessing risk. The issue of dose metric was raised at the workshop, where data were presented showing dose-response relationships with CNT particle mass or specific density (Pauluhn, 2010b). Other studies suggest that particle surface area or fibre number may be more relevant to the biological effect (OECD, 2010). 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.

Occupational Exposure Limit for specific CNTs

173.   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, 2010). 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 that formed the basis for interspecies extrapolation. When accounting for differences in alveolar deposition, ventilation parameters and particle clearance, the authors derived 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, for example to account for intraspecies variability, however, were not applied.

Estimation of a "no effect" concentration for a multi-walled CNT

174.   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 and Luizi, 2009).

Derivation of a Reference Exposure Limit for CNTs

175.   NIOSH in the U.S. published a Current Intelligence Bulletin (CIB) on Occupational Exposure to Carbon Nanotubes and Nanofibers which included a risk assessment and a Reference Exposure Limit (REL) of 1 µg/m^3 (8-hr time-weighted average (TWA) concentration) for CNTs and CNFs (NIOSH, 2013). 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.

176.   Risk estimates were derived by assuming either no clearance of the estimated deposited lung dose of CNT or normal clearance based on spherical particle overload 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 physico-chemical properties of the various CNT types and the lung responses. The NIOSH REL of 1 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) (NIOSH, 2013). The risk estimates indicate a greater than 10% excess risk of early-stage lung effects if exposed at the LOQ over a working lifetime.

177.   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.

IARC classification of MWCNT-7

178.   Since that time, the International Agency for Research on Cancer (IARC) published an evaluation of cancer hazard based on data published through October 2014. Based on that evidence, one type of MWCNT MWCNT-7) was classified as possibly carcinogenic to humans (IARC Group 2B). Most types of CNTs were considered to be not classifiable as to their carcinogenicity to humans (IARC Group 3) (IARC, 2017), i.e. there is insufficient evidence to permit a conclusion on carcinogenicity[42]. Later, a 2-year inhalation study was published that showed an increased incidence of lung cancer in rats following exposure to MWCNT-7 (Kasai et al., 2016).

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[42] It should be noted that 'not classifiable' is related to a lack of data and should not be interpreted as 'not carcinogenic'.

Annex 2. Conclusions from the WPMN Workshop on Risk Assessment of Manufactured Nanomaterials in a Regulatory Context

179.   This workshop was held in Washington DC in 2009. The following conclusions were produced (OECD, 2010):

a)   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;

b)   As with any risk assessment, extrapolation approaches for nanomaterials should be based on mechanistic data whenever 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;

c)   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;

d)   There does not appear to be a scientific rationale 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

e)   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.

180.   The conclusions from the workshop triggered a number of OECD projects, which allowed the development of tools supporting risk assessment of nanomaterials. For example: guidance on inhalation toxicity testing (OECD, 2018a, 2018b, 2018c), workplace exposure (OECD, 2015a), sample preparation and dosimetry (OECD, 2012b), methods for determining physico-chemical properties (OECD, 2014b; OECD, 2016c), genotoxicity (OECD, 2014c), toxicokinetics (OECD, 2016d), interspecies variability in life cycle assessment (OECD, 2015b), human health assessment (OECD, 2015c) and use of dissolution as a function of surface chemistry to evaluate environmental behaviour (OECD, 2015d).

181.   In addition, many international regulatory projects have advanced our readiness to assess risk of manufactured nanomaterials, including ProSafe [43], NANoREG [44], NanoReg2 [45], GRACIOUS [46], SmartNanoTox [47], NanoFASE [48], caLIBRAte [49], DF4nanoGrouping [50], nanoGRAVUR [51], NanoMILE, ACEnano.

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[43] Promoting the Implementation of Safe by Design (https://www.rivm.nl/en/international-projects/prosafe)

[44] A common European approach to the regulatory testing of Manufactured Nanomaterials (Promoting the Implementation of Safe by Design) (https://www.rivm.nl/en/international-projects/nanoreg)

[45] Development and implementation of Grouping and Safe-by-Design approaches within regulatory frameworks (http://www.nanoreg2.eu/)

[46] Grouping, Read-Across, CharacterIsation and classificatiOn framework for regUlatory risk assessment of manufactured nanomaterials and Safer design of nano-enabled products (https://www.h2020gracious.eu/)

[47] Smart Tools for Gauging Nano Hazards (http://www.smartnanotox.eu/)

[48] Nanomaterial FAte and Speciation in the Environment (http://nanofase.eu/)

[49] Performance testing, calibration and implementation of a next generation system-of-systems Risk Governance Framework for nanomaterials (http://www.nanocalibrate.eu/home)

[50] A Decision-making framework for grouping and testing of nanomaterials (ECETOC, European Centre for Ecotoxicology and Toxicology of Chemicals) (https://doi.org/10.1016/j.yrtph.2015.03.007)

[51] Nanostructured materials - Grouping for occupational health and consumer and environmental protection and risk mitigation. (http://nanofase.eu/)

Reference

Afantitis A, Melagraki G, Tsoumanis A, Valsami-Jones E, Lynch I (2018). A nanoinformatics decision support tool for the virtual screening of gold nanoparticle cellular association using protein corona fingerprints. Nanotoxicology 12(10): 1148-1165. https://doi.org/10.1080/17435390.2018.1504998.

Afantitis A, Melagraki G, Isigonis P, Tsoumanis A, Varsou DD, Valsami-Jone E, Papadiamantis A, Ellis L-JA, Sarimveis H, Doganis P, Karatzas P, Tsiros P, Liampa I, Lobaskin V, Greco D, Serra A, Kinaret PAS, Saarimäki LA, Grafström R, Kohonen P, Nymark P, Willighagen E, Puzyn T, Rybinska-Fryca A, Lyubartsev A, Alstrup Jensen K, Brandenburg JG, Lofts S, Svendsen C, Harrison S, Maier D, Tamm K, Jänes J, Sikk L, Dusinska M, Longhin E, Rundén-Pran E, Mariussen E, El Yamani N, Unger W, Radnik J, Tropsha A, Cohen Y, Leszczynsk J, Ogilvie Hendren C, Wiesner M, Winkler D, Suzuki N, Yoon TH, Choi J-S, Sanabria N, Gulumian M, Lynch I (2020). NanoSolveIT Project: Driving nanoinformatics research to develop innovative and integrated tools for *in silico* nanosafety assessment. Computational and Structural Biotechnology Journal: 18: 583-602. https://doi.org/10.1016/j.csbj.2020.02.023.

Aitken RA, Bassan A, Friedrichs S, Hankin SM, Hansen SF, Holmqvist J, Peters SAK, Poland CA, Tran CL (2011). Specific Advice on Exposure Assessment and Hazard/Risk Characterisation for Nanomaterials under REACH (RIP-oN 3) – Final Project Report, RNC/RIP-oN3/FPR/1/FINAL.

Allegri M, Perivoliotis DK, Bianchi MG, Chiu M, Pagliaro A, Koklioti MA, Trompeta A-FA, Bergamaschi E, Bussolati O, Charitidis CA (2016). Toxicity determinants of multi-walled carbon nanotubes: The relationship between functionalization and agglomeration. Toxicology Reports 3: 230-243. https://doi.org/10.1016/j.toxrep.2016.01.011.

Amenta V and Aschberger K (2014). Carbon nanotubes: Potential medical applications and safety concerns. Wiley Interdisciplinary Reviews Nanomedicine and Nanobiotechnology. 7(3). https://doi.org/10.1002/wnan.1317.

Arts JE. Hadi M, Irfan M-A, Keene AM, Kreiling R, Lyon D, Maier M, Michel K, Petry T, Sauer UG, Warheit D, Wiench K, Wohlleben W, Landsiedel R (2015). A decision-making framework for the grouping and testing of nanomaterials (DF4nanoGrouping). Regulatory Toxicology and Pharmacology 71(2): S1-S27. https://doi.org/10.1016/j.yrtph.2015.03.007.

Aschberger K, Johnston HJ, Stone V, Aitken RJ, Hankin SM, Peters SAK, Tran CL, Christensen FM (2010). Review of carbon nanotubes toxicity and exposure--appraisal of human health risk assessment based on open literature. Critical Reviews in Toxicology. 40(9): 759-790. https://doi.org/10.3109/10408444.2010.506638.

Arts JHE, Irfan M-A, Keene AM, Kreiling R, Lyon D, Maier M, Michel K, Neubauer N, Petry T, Sauer UG, Warheit D, Wiench K, Wohlleben W, Landsiedel R (2016). Case studies putting the decision-making framework for the grouping and testing of nanomaterials (DF4nanoGrouping) into practice. Regulatory Toxicology and Pharmacology 76: 234-261. https://doi.org/10.1016/j.yrtph.2015.11.020.

Bastian S, Busch W, Kühnel D, Springer A, Meissner T, Holke R, Scholz S, Iwe M, Pompe W, Gelinsky M, Potthoff A, Richter V, Ikonomidou C, Schirmer K (2009). Toxicity of tungsten carbide and cobalt-doped tungsten carbide nanoparticles in mammalian cells in vitro. Environ Health Perspect. 117(4): 530-536. https://doi.org/10.1289/ehp.0800121.

Baun A, Sayre P, Steinhäuser KG, Rose J (2017). Regulatory relevant and reliable methods and data for determining the environmental fate of manufactured nanomaterials. NanoImpact 8: 1-10. https://doi.org/10.1016/j.impact.2017.06.004.

Becker H, Herzberg F, Schulte A, Kolossa-Gehring M (2011). The carcinogenic potential of nanomaterials, their release from products and options for regulating them. Int J Hyg Environ Health 214(3): 231-238. https://doi.org/10.1016/j.ijheh.2010.11.004.

Bermudez E, Mangum JB, Asgharian B, Wong BA, Reverdy EE, Janszen DB, Hext PM, Warheit DB, Everitt JI (2002). Long-term pulmonary responses of three laboratory rodent species to subchronic inhalation of pigmentary titanium dioxide particles. Toxicol Sci 70(1): 86-97. https://doi.org/10.1093/toxsci/70.1.86.

Bermudez E, Mangum JB, Wong BA, Asgharian B, Hext PM, Warheit DB, Everitt JI (2004). Pulmonary responses of mice, rats, and hamsters to subchronic inhalation of ultrafine titanium dioxide particles. Toxicol Sci 77(2):347-357. https://doi.org/10.1093/toxsci/kfh019.

Bevan RJ, Kreiling R, Levy LS, Warheit DB (2018). Toxicity testing of poorly soluble particles, lung overload and lung cancer. Reg. Tox. Pharma. 100: 80-91. https://doi.org/10.1016/j.yrtph.2018.10.006.

Bhuvaneshwari M, Iswarya V, Archanaa S, Madhu GM, Suraish Kumar GK, Nagarajan R, Chandrasekaran N, Mukherjee A. (2015). Cytotoxicity of ZnO NPs towards fresh water algae Scenedesmus obliquus at low exposure concentrations in UV-C, visible and dark conditions. Aquat. Toxicol. 162: 29-38. https://doi.org/10.1016/j.aquatox.2015.03.004.

Bianchi MG, Bussolati O, Chiu M, Taurino G, Bergamaschi E (2019). Evaluation of potential engineered nanomaterials impacts on human health: from risk for workers to impact on consumers (Chapter 10) *IN* Exposure to Engineered Nanomaterials in the Environment. Elsevier, Amsterdam Netherlands.

Blaser SA, Scheringer M, MacLeod M, Hungerbühler K (2008). Estimation of cumulative aquatic exposure and risk due to silver: Contribution of nano-functionalized plastics and textiles. Sci. Total Environ. 390: 396-409. https://doi.org/10.1016/j.scitotenv.2007.10.010.

Boyadzhiev A, Trevithick-Sutton C, Wu D, Decan N, Bazin M, Shah GM, Halappanavar S (2020). Enhanced Dark-Field Hyperspectral Imaging and Spectral Angle Mapping for Nanomaterial Detection in Consumer Care Products and in Skin Following Dermal Exposure. Chem Res Toxicol. 33(5): 1266-1278. https://doi.org/10.1021/acs.chemrestox.0c00090.

Briffa SM, Nasser F, Valsami-Jones E, Lynch I (2018). Uptake and impacts of polyvinylpyrrolidone (PVP) capped metal oxide nanoparticles on Daphnia magna: role of core composition and acquired corona. Environ. Sci.: Nano 5(7): 1745-1756. https://doi.org/10.1039/C8EN00063H.

Bundschuh M, Filser J, Lüderwald S, McKee MS, Metreveli G, Schaumann GE, Schulz R, Wagner S (2018). Nanoparticles in the environment: where do we come from, where do we go to? Environmental Sciences Europe 30(1): art. no. 6. https://doi.org/10.1186/s12302-018-0132-6.

Burello E, Worth AP (2011). QSAR modelling of Nanomaterials. WIREs Nanomed Nanobiotechnol 3: 298–306. https://doi.org/10.1002/wnan.137.

Burello E (2017). Review of (Q)SAR models for regulatory assessment of nanomaterials risks. NanoImpact 8: 48-58. https://doi.org/10.1016/j.impact.2017.07.002.

Burin GJ, Saunders DR (1999). Addressing human variability in risk assessment--the robustness of the intraspecies uncertainty factor. Regul Toxicol Pharmacol. 30(3): 209-216. https://doi.org/10.1006/rtph.1999.1351.

Canady R (2010). The Uncertainty of Nanotoxicology: Report of a Society for Risk Analysis Workshop. Risk Analysis 30(11): 1663-1670. https://doi.org/10.1111/j.1539-6924.2010.01512.x.

Canu GI, Batsungnoen K, Maynard A, Hopf NB (2020). State of knowledge on the occupational exposure to carbon nanotubes. International Journal of Hygiene and Environmental Health 225: art. no. 113472. https://doi.org/10.1016/j.ijheh.2020.113472.

Card, J.W., Magnuson, B.A (2010). A method to assess the quality of studies that examine the toxicity of engineered nanomaterials. International Journal of Toxicology 29(4): 402-410. https://doi.org/10.1177/1091581810370720.

Carlander U, Moto TP, Desalegn AA, Yokel RA, Johanson G (2018). Physiologically based pharmacokinetic modelling of nanoceria systemic distribution in rats suggests dose- and route-dependent biokinetics. Int J Nanomedicine 13: 2631-2646. https://doi.org/10.2147/IJN.S157210.

Cedervall T, Lynch I, Lindman S, Berggard T, Thulin E, Nilsson H, Dawson KA, Linse S (2007). Understanding the nanoparticle-protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles. Proceedings of the National Academy of Sciences of the United States of America 104: 2050–2055. https://doi.org/10.1073/pnas.0608582104.

Chae SR, Badireddy AR, Farner Budarz J, Lin S, Xiao Y, Therezien M, Wiesner MR (2010). Heterogeneities in fullerene nanoparticle aggregates affecting reactivity, bioactivity, and transport. ACS Nano 4(9): 5011-5018. https://doi.org/10.1021/nn100620d.

Chapman PM, Fairbrother A and Brown D (1998). A Critical Evaluation of Safety (Uncertainty) Factors for ecological Risk Assessment. Env Sci & Tech 17(1): 99-108. https://doi.org/10.1002/etc.5620170112.

Chen J, Dong X, Zhao J, Tang G (2009). *In vivo* acute toxicity of titanium dioxide nanoparticles to mice after intraperitioneal injection. J Appl Toxicol. 29(4): 330-7. https://doi.org/10.1002/jat.1414.

Chen H, Zhao R, Wang B, Cai C, Zheng L, Wang H, Wang M, Ouyang H, Zhou X, Chai Z, Zhao Y, Feng W (2017). The effects of orally administered Ag, TiO2 and SiO2 nanoparticles on gut microbiota composition and colitis induction in mice. NanoImpact 8: 80-88. https://doi.org/10.1016/j.impact.2017.07.005.

Commission Regulation (EU) 2018/1881 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards Annexes I, III,VI, VII, VIII, IX, X, XI, and XII to address nanoforms of substances; OJ L 308 of 4.12.2018, p.1-20.

Cornelis G, Tuoriniemi J, Heinrich T, Johnston C (2018). Analytical and Characterisation Excellence in nanomaterial risk assessment: A tiered approach. https://cordis.europa.eu/project/id/720952.

Crump KS (1984). A new method for determining allowable daily intakes. Fund Appl Toxicol 4:854-871. https://doi.org/10.1016/0272-0590(84)90107-6.

Crump KS (1995). Calculation of benchmark doses from continuous data. Risk Analysis 15(1):79-89. https://doi.org/10.1111/j.1539-6924.1995.tb00095.x.

Dankovic D, Kuempel E, Wheeler M (2007). An approach to risk assessment for titanium dioxide. Inhal Toxicol 19(Suppl.1): 205-212. https://doi.org/10.1080/08958370701497754.

Dankovic DA, Naumann BD, Maier A, Dourson ML, Levy LS (2015). The scientific basis of uncertainty factors used in setting occupational exposure limits. J Occup Environ Hyg 12 (Suppl. 1): S55-S68. https://doi.org/10.1080/15459624.2015.1060325.

Davis JM (2007). How to assess the risks of nanotechnology: Learning from past experience. Journal of Nanoscience and Nanotechnology 7:402–409. https://doi.org/10.1166/jnn.2007.152.

Delmaar C, Meesters J (2020). Modeling consumer exposure to spray products: an evaluation of the ConsExpo Web and ConsExpo nano models with experimental data. Journal of Exposure Science and Environmental Epidemiology 30(5): 878-887. https://doi.org/10.1038/s41370-020-0239-x.

Diaz CA, Xia Y, Rubino M, Auras R, Jayaraman K, Hotchkiss J (2013). Fluorescent labeling and tracking of nanoclay. Nanoscale 5(1): 164–168. https://doi.org/10.1039/C2NR32978F.

Donaldson K, Poland CA (2013). Nanotoxicity: Challenging the myth of nano-specific toxicity. Current Opinion in Biotechnology 24(4): 724-734. https://doi.org/10.1016/j.copbio.2013.05.003.

Doudrick K, Herckes P, Westerhoff P (2012). Detection of carbon nanotubes in environmental matrices using programmed thermal analysis. Environmental Science & Technology 46(22): 12246–12253. https://doi.org/10.1021/es300804f.

Drasler B, Sayre P, Steinhäuser KG, Petri-Fink A, Rothen-Rutishauser B (2017). *In vitro* approaches to assess the hazard of nanomaterials. NanoImpact 8: 99-116. https://doi.org/10.1016/j.impact.2017.08.002.

Drew NM, Kuempel ED, Pei Y, Yang F (2017). A quantitative framework to group nanoscale and microscale particles by hazard potency to derive occupational exposure limits: proof of concept evaluation. Regul Toxicol Pharmacol 89:253–267. https://doi.org/10.1016/j.yrtph.2017.08.003.

Duncan TV (2015). Release of engineered nanomaterials from polymer nanocomposites: the effect of matrix degradation. ACS Appl. Mater. Interfaces 7: 20–39. https://doi.org/10.1021/am5062757.

Duncan TV, Pillai K (2015). Release of engineered nanomaterials from polymer nanocomposites: diffusion, dissolution, and desorption. ACS Appl. Mater. Interfaces 7: 2–19. https://doi.org/10.1021/am5062745.

Dunn KH, Eastlake AC, Story M, Kuempel ED (2018). Control Banding Tools for Engineered Nanoparticles: What the Practitioner Needs to Know. Annals of Work Exposures and Health 62(3): 362-388. https://doi.org/10.1093/annweh/wxy002.

Dusinska M, Tulinska J, El Yamani N, Kuricova M, Liskova A, Rollerova E, Rundén-Pran E, Smolkova B (2017). Immunotoxicity, genotoxicity and epigenetic toxicity of nanomaterials: New strategies for

toxicity testing?. Food and Chemical Toxicology 109: 797-811. https://doi.org/10.1016/j.fct.2017.08.030.

ECETOC (1993). European Centre for Ecotoxicology and Toxicology of Chemicals. Environmental hazard assessment of substances. Technical Report 51. Brussels, Belgium. https://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-051.pdf.

ECHA (2012a). Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health, version 2.1. European Chemicals Agency, Helsinki, November 2012. https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment.

ECHA (2012b). Appendix R8-15 Recommendations for nanomaterials applicable to Chapter R.8 Characterisation of dose [concentration] - response for human health, European Chemicals Agency, Helsinki, May 2012. https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment.

ECHA (2016). Guidance on Information Requirements and Chemical Safety Assessment, Part E: Risk Characterisation, version 3.0. European Chemicals Agency, Helsinki, May 2016. https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment.

ECHA (2019a) Guidance on information requirements and chemical safety assessment Appendix R.6-1 for nanomaterials applicable to the guidance on QSARs and grouping of chemicals Version 2.0. European Chemicals Agency, Helsinki, December 2019. https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment.

ECHA (2019b). Appendix for nanoforms applicable to the Guidance on Registration and Substance Identification Version 1.0. European Chemicals Agency, Helsinki, December 2019. https://echa.europa.eu/guidance-documents/guidance-on-reach.

ECHA (2019c). Guidance on information requirements and chemical safety assessment Appendix R7-1 for nanomaterials applicable to Chapter R7a Endpoint specific guidance Version 2.0. https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment.

ECHA (2019d). Appendix to Chapter R.8: Guidance for preparing a scientific report for health-based exposure limits at the workplace Version 1.0, European Chemicals Agency, Helsinki, August 2019. https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment.

ECHA (2020). A critical review of the factors determining dermal absorption of nanomaterials and available tools for the assessment of dermal absorption - Final report. https://op.europa.eu/en/publication-detail/-/publication/7043ca63-82e3-11eb-9ac9-01aa75ed71a1.

EFSA (2021a). EFSA Scientific Committee; Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: human and animal health. http://doi.org/10.2903/j.efsa.2021.6768.

EFSA (2021b). EFSA Scientific Committee; Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles. http://doi.org/10.2903/j.efsa.2021.6769.

Elder A, Gelein R, Finkelstein JN, Driscoll KE, Harkema J, Oberdörster G (2005). Effects of subchronically inhaled carbon black in three species. I. Retention kinetics, lung inflammation, and histopathology. Toxicol Sci 88(2): 614-629. https://doi.org/10.1093/toxsci/kfi327.

Elliott JT, Rösslein, SongN, Toman B, Kinsner-Ovaskainen A, Maniratanachote, Salit ML, Petersen J, Sequeira F, Romsos EL, Kim SJ, Lee J, Von Moos NR, Rossi F, Hirsch C, Krug HF, Suchaoin W, Wick P (2017). Toward achieving harmonization in a nanocytotoxicity assay measurement through an interlaboratory comparison study. Altex 34(2): 201-218. https://doi.org/10.14573/altex.1605021.

EN 15051-1:2013. Workplace exposure - Measurement of the dustiness of bulk materials - Part 1: Requirements and choice of test methods

EN 17199-1:2019. Workplace exposure - Measurement of dustiness of bulk materials that contain or release respirable NOAA and other respirable particles - Part 1: Requirements and choice of test methods

ENRHES (2010). Engineered Nanoparticles: Review of Health and Environmental Safety. Project Final Report. http://ihcp.jrc.ec.europa.eu/whats-new/enhres-final-report.

EPA (1993). Reference Dose (RfD): Description and Use in Health Risk Assessments, Background Document 1A. U.S. Environmental Protection Agency Washington, DC. https://www.epa.gov/iris/reference-dose-rfd-description-and-use-health-risk-assessments.

EPA (2005). Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum. U.S. Environmental Protection Agency Washington, DC. EPA/630/P-03/001F. https://www.epa.gov/sites/default/files/2013-09/documents/cancer_guidelines_final_3-25-05.pdf.

European Commission (2006). Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC; OJ L 396 of 30.12.2006.

European Commission (2008). Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

European Commission (2014). SCCS/1524/13: Memorandum on "Relevance, Adequacy and Quality of Data in Safety Dossiers on Nanomaterials". https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_142.pdf.

European Commission (2018). Regulation (EU) 2018/1881 of 3 December 2018 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards Annexes I, III,VI, VII, VIII, IX, X, XI, and XII to address nanoforms of substances. European Commission, EU.

European Commission (2019). CORDIS EU research results. Horizon2020 Development and implementation of Grouping and Safe-by-Design approaches within regulatory frameworks. https://cordis.europa.eu/project/id/646221.

European Commission (2019). Horizon2020 Development and implementation of Grouping and Safe-by-Design approaches within regulatory frameworks, https://cordis.europa.eu/project/id/646221.

European Commission (2021). GRACIOUS: Grouping, Read-Across, CharacterIsation and classificatiOn framework for regUlatory risk assessment of manufactured nanomaterials and Safer design of nano-enabled products, https://cordis.europa.eu/project/id/760840.

FAO WHO (2009). FAO/WHO Expert Meeting on the Application of Nanotechnologies in the Food and Agriculture Sectors: Potential Food Safety Implications. MEETING REPORT http://www.fao.org/ag/agn/agns/files/FAO_WHO_Nano_Expert_Meeting_Report_Final.pdf.

Fatkhutdinova LM, Khaliullin TO & Shvedova AA (2015). Carbon nanotubes exposure risk assessment: From toxicology to epidemiologic studies (Overview of the current problem). Nanotechnol Russia 10, 501–509. https://doi.org/10.1134/S1995078015030064.

Forest V, Pourchez J, GuibertC, Bitounis D, Leclerc L, Sarry G, Vergnon JM. (2019). Nano to micron-sized particle detection in patients' lungs and its pathological significance. Environ. Sci. Nano 6: 1343–1350. https://doi.org/10.1039/C8EN01301B.

Froggett SJ, Clancy SF, Boverhof DR, Canady RA (2014). A review and perspective of existing research on the release of nanomaterials from solid nanocomposites. Part. Fibre Toxicol. 11: 17. https://doi.org/10.1186/1743-8977-11-17.

Fujirawa K, Suematsu H, Kiyomiya E, Aoki M, Sato M, Moritoki N (2008). Size-dependent toxicity of silica nano-particles to Chlorella kessleri. Journal of Environmental Science and Health Part A 43: 1167–1173. https://doi.org/10.1080/10934520802171675.

Furxhi I, Murphy F, Mullins M, Arvanitis A, Poland CA (2020a). Nanotoxicology data for *in silico* tools: a literature review. Nanotoxicology 14(5): 612-637. https://doi.org/10.1080/17435390.2020.1729439.

Furxhi I, Murphy F, Mullins M, Arvanitis A, Poland CA (2020b). Practices and trends of machine learning application in nanotoxicology. Nanomaterials 101: art. no. 116. https://doi.org/10.3390/nano10010116.

Gao X, Lowry GV (2018). Progress towards standardized and validated characterizations for measuring physicochemical properties of manufactured nanomaterials relevant to nano health and safety risks. NanoImpact 9: 14-30. https://doi.org/10.1016/j.impact.2017.09.002.

Garner KL, Suh S, Keller AA (2017). Assessing the Risk of Engineered Nanomaterials in the Environment: Development and Application of the nanoFate Model. Environmental Science and Technology 51(10): 5541-5551. https://doi.org/10.1021/acs.est.6b05279.

Geiser M, Kreyling WG (2010). Deposition and biokinetics of inhaled nanoparticles. Part Fibre Toxicol 7:2. https://doi.org/10.1186/1743-8977-7-2.

Geiser M, Rothen-Rutishauser B, Kapp N, Schurch S, Kreyling W, Schulz H, Semmler, M., Im Hof V, Heyder J, Gehr P (2005). Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. Environ Health Perspect 13(11): 1555-1560. https://doi.org/10.1289/ehp.8006.

Gernand JM, Casman EA (2014). A meta-analysis of carbon nanotube pulmonary toxicity studiesdhow physical dimensions and impurities affect the toxicity of carbon nanotubes. Risk Anal 34(3):583-597. https://doi.org/10.1111/risa.12109.

Gernand JM, Casman EA (2016). Nanoparticle characteristic interaction effects on pulmonary toxicity: a random forest modeling framework to compare risks of nanomaterial variants. ASCE-ASME J Risk Uncertain Eng Syst Part B Mech Eng 2: 021002-1 to 021002-13. https://doi.org/10.1115/1.4031216.

Giusti A, Atluri R, Tsekovska R, Gajewicz A, Apostolova, Battistelli CL, Bleeker EAJ, Bossa C, Bouillard J, Dusinska M, Gómez-Fernández P, Grafström R, Gromelski M, Handzhiyski Y, Jacobsen NR, Jantunen P, Jensen KA, Mech A, Navas JM, Nymark P, Oomen AG, Puzyn T, Rasmussen K, Riebeling C, Rodriguez-Llopis I, Sabella S, Sintes JR, Suarez-Merino B, Tanasescu S, Wallin H, Haase A (2019). Nanomaterial grouping: Existing approaches and future recommendations. NanoImpact, 16: article 100182. https://doi.org/10.1016/j.impact.2019.100182.

Gordon SC, Butala JH, Carter JM, Elder A, Gordon T, Gray G, Sayre PG, Schulte PA, Tsai CS, West J (2014). Workshop report: strategies for setting occupational exposure limits for engineered nanomaterials. Regul. Toxicol. Pharmacol. 68(3): 305–311. https://doi.org/10.1016/j.yrtph.2014.01.005.

Gustafson HH, Holt-Casper D, Grainger DW, Ghandehari H (2015). Nanoparticle Uptake: The Phagocyte Problem. Nano Today 10(4): 487-510. https://doi.org/10.1016/j.nantod.2015.06.006.

Halappanavar S, Saber AT, Decan N, Jensen KA, Wu D, Jacobsen NR, Guo C, Rogowski J, Koponen IK, Levin M, Madsen AM, Atluri R, Snitka V, Birkedal RK, Rickerby D, Williams A, Wallin H, Yauk CL, Vogel U (2015). Transcriptional profiling identifies physicochemical properties of nanomaterials that are determinants of the *in vivo* pulmonary response. Environ Mol Mutagen. 56(2): 245-64. https://doi.org/10.1002/em.21936.

Halappanavar S, Ede JD, Shatkin JA, Krug HF (2019). A systematic process for identifying key events for advancing the development of nanomaterial relevant adverse outcome pathways. NanoImpact 15: Art. No. 100178. https://doi.org/10.1016/j.impact.2019.100178.

Halappanavar S, van den Brule S, Nymark P, Gaté L, Seidel C, Valentino S, Zhernovkov V, Høgh Danielsen P, De Vizcaya A, Wolff H, Stöger T, Boyadziev A, Poulsen SSø, Sørli JB, Vogel U (2020). Adverse outcome pathways as a tool for the design of testing strategies to support the safety assessment of emerging advanced materials at the nanoscale. Part Fibre Toxicol 17(1): Art. No. 16. https://doi.org/10.1186/s12989-020-00344-4.

Halappanavar S, Ede JD, Mahapatra I, Krug HF, Kuempel ED, Lynch I, Vandebriel RJ, Shatkin JA (2021). A methodology for developing key events to advance nanomaterial-relevant adverse outcome pathways to inform risk assessment. Nanotoxicology 15(3): 289-310. https://doi.org/10.1080/17435390.2020.1851419.

Handy RD, Owen R, Valsami-Jones E (2008). The ecotoxicology of nanoparticles and nanomaterials: Current status, knowledge gaps, challenges, and future needs. Ecotoxicology 17: 315-325. https://doi.org/10.1007/s10646-008-0206-0.

Hankin SM, Peters SAK, Poland CA, Foss Hansen S, Holmqvist J, Ross BL, Varet J, Aitken RJ (2011). Specific Advice on Fulfilling Information Requirements for Nanomaterials under REACH (RIP-oN 2) – Final Project Report, RNC/RIP-oN2/FPR/1/FINAL. https://ec.europa.eu/environment/chemicals/nanotech/pdf/report_ripon2.pdf.

Hartmann NB, Ågerstrand M, Lützhøft H-CH, Baun A (2017). NanoCRED transparent framework to assess' the regulatory adequacy of ecotoxicity data for nanomaterials – Relevance and reliability revisited. NanoImpact 6: 81-89. https://doi.org/10.1016/j.impact.2017.03.004.

Hou WC, Westerhoff P, Posner JD (2013). Biological accumulation of engineered nanomaterials: a review of current knowledge. Environ. Sci.: Processes Impacts 15: 103–122. https://doi.org/10.1039/C2EM30686G.

Huang YW, Cambre M, Lee HJ (2017). The Toxicity of Nanoparticles Depends on Multiple Molecular and Physicochemical Mechanisms. Int. J. Mol. Sci. 18: 2702. https://doi.org/10.3390/ijms18122702.

Huk A, Izak-Nau E, Reidy B, Boyles M, Duschl A, Lynch I, Dušinska M (2014). Is the toxic potential of nanosilver dependent on its size? Part Fibre Toxicol. 11: 65. https://doi.org/10.1186/s12989-014-0065-1.

Husain M, Wu D, Saber AT, Decan N, Jacobsen NR, Williams A, Yauk CL, Wallin H, Vogel U, Halappanavar S (2015). Intratracheally instilled titanium dioxide nanoparticles translocate to heart and liver and activate complement cascade in the heart of C57BL/6 mice. Nanotoxicology 9(8): 1013-22. https://doi.org/10.3109/17435390.2014.996192.

IARC (2017). Some Nanomaterials and Some Fibres. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 111 International Agency for Research on Cancer, Lyon, France. https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identification-Of-Carcinogenic-Hazards-To-Humans/Some-Nanomaterials-And-Some-Fibres-2017.

Isaacson CW, Sigg L, Ammann AA, Stadbicka-Michalak J, Schimer K (2017). Interactions of TiO_2 nanoparticles and the freshwater nematode Plectus aquatilis: particle properties, kinetic parameters and bioconcentration factors. Environ. Sci.: Nano 4: 712-719. https://doi.org/10.1039/C6EN00495D.

ISO/PFR TR 22293 (2021). Evaluation of methods for assessing the release of nanomaterials from commercial, nanomaterial-containing polymer composites.

ISO/TR 13014 (2012). Nanotechnologies - Guidance on physico-chemical characterisation of engineered nanoscale materials for toxicologic assessment.

ISO/TR 18637 (2016). Nanotechnologies — Overview of Available Frameworks for the Development of Occupational Exposure Limits and Bands for Nano-objects and Their Aggregates and Agglomerates (NOAAs). International Organization for Standardization (ISO) Technical Report. ISO, Geneva, Switzerland ISO/TR 18637, Nov. 21.

ISO/TR 22019 (2019). Nanotechnologies -- Considerations for performing toxicokinetic studies with nanomaterials.

ISO/TR 21624 (2020). Nanotechnologies — Considerations for in vitro studies of airborne nano‐objects and their aggregates and agglomerates (NOAA)

ISO/TS 12901-2: (2014). Nanotechnologies – occupational risk management applied to engineered nanomaterials – Part 2: Use of the control banding approach.

Izak-Nau E, Huk A, Reidy B, Uggerud H, Vadset M, Eiden S, Voetz M, Himly M, Duschl A, Dusinska M, Lynch I (2015). Impact of storage conditions and storage time on silver nanoparticles' physicochemical properties and implications for their biological effects. RSC Advances 5: 84172-84185. https://doi.org/10.1039/C5RA10187E.

IMPORTANT ISSUES ON RISK ASSESSMENT OF MANUFACTURED NANOMATERIALS

Jacobson MZ (1994). Developing, Coupling, and Applying a Gas, Aerosol, Transport, and Radiation Model to Study Urban and Regional Air Pollution. Thesis, University of California at Los Angeles. https://dl.acm.org/doi/book/10.5555/221819.

Janer G, Landsiedel R, Wohlleben W (2021). Rationale and decision rules behind the ECETOC NanoApp to support registration of sets of similar nanoforms within REACH. Nanotoxicology 15(2): 145-166. https://doi.org/10.1080/17435390.2020.1842933.

Jantunen P, Gottardo S, Crutzen, H (2017). NANoREG Toolbox for the Safety Assessment of Nanomaterials. European Commission, Joint Research Centre (JRC) [Dataset] PID: http://data.europa.eu/89h/jrc-nano-ehs-ring-nanoreg-tb.

Jantunen P, Gottardo S, Rasmussen K, Crutzen HP (2018). An inventory of ready-to-use and publicly available tools for the safety assessment of nanomaterials. NanoImpact. 2018. https://doi.org/10.1016/j.impact.2018.08.007.

Johnston HJ, Hutchison GR, Christensen FM, Peters S, Hankin S, Aschberger K, Stone V (2010). A critical review of the biological mechanisms underlying the *in vivo* and *in vitro* toxicity of carbon nanotubes: The contribution of physico-chemical characteristics. Nanotoxicology 4:207-246. https://doi.org/10.3109/17435390903569639.

Kasai T, Umeda Y, Ohnishi M, Mine, T, Kondo H, Takeuchi T, Matsumoto M, Fukushima S (2016). Lung carcinogenicity of inhaled multi-walled carbon nanotube in rats. Part. Fibre Toxicol. 13(1): 53. https://doi.org/10.1186/s12989-016-0164-2.

Kendall M, Lynch I (2016). Long-term monitoring for nanomedicine implants and drugs. Nature Nanotechnology 11: 206-210. https://doi.org/10.1038/nnano.2015.341.

Kim KT, Kaline SJ, Cho J, Kim SH, Kim SD (2010). Oxidative stress responses of Daphnia magna exposed to TiO_2 nanoparticles according to size fraction. Sci. Tot. Env. 408: 2268-2272. https://doi.org/10.1016/j.scitotenv.2010.01.041.

Kim DH, Park JC, Jeon GE, Kim CS, Seo JH (2017). Effect of the Size and Shape of Silver Nanoparticles on Bacterial Growth and Metabolism by Monitoring Optical Density and Fluorescence Intensity. Biotechnology and Bioprocess Engineering 22: 210-217. https://doi.org/10.1007/s12257-016-0641-3.

Kingston C, Zepp R, Andrady A, Boverhof D, Fehir R, Hawkins D, Roberts J, Sayre P, Shelton B, Sultani Y, Vejinsj V, Wohlleben W (2014). Release characteristics of selected carbon nanotube polymer composites. Carbon 68: 33–57. https://doi.org/10.1016/j.carbon.2013.11.042.

Köhler AR, Som C, Helland A, Gottschalk F (2008). Studying the potential release of carbon nanotubes throughout the application life cycle. Journal of Cleaner Production 16: 927-937. https://doi.org/10.1016/j.jclepro.2007.04.007.

Koivisto AJ, Jensen ACØ, Kling KI, Nørgaard A, Brinch A, Christensen F, Jensen KA (2017). Quantitative material releases from products and articles containing manufactured nanomaterials: Towards a release library. NanoImpact 5: 119-132. https://doi.org/10.1016/j.impact.2017.02.001.

Kovochich M, Fung CCD, Avanasi R, Madl AK (2018). Review of techniques and studies characterizing the release of carbon nanotubes from nanocomposites: Implications for exposure and human health

risk assessment. Journal of Exposure Science and Environmental Epidemiology 28(3): 203-215. https://doi.org/10.1038/jes.2017.6.

Kreyling WG, Semmler M, Erbe F, Mayer P, Takenaka S, Schulz H, Oberdörster G, Ziesenis A (2002). Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. J Toxicol Environ Health A 65: 1513–30. https://doi.org/10.1080/00984100290071649.

Kuempel ED, Tran CL, Castranova V, Bailer AJ (2006). Lung dosimetry and risk assessment of nanoparticles: evaluating and extending current models in rats and humans. Inhal Toxicol 18(10):717-724. https://doi.org/10.1080/08958370600747887.

Kuempel ED, Castranova V, Geraci CL, Schulte PA (2012). Development of risk-based nanomaterial groups for occupational exposure control. J. Nanoparticle Res. 14(9): 1029. https://doi.org/10.1007/s11051-012-1029-8.

Larsen PB, Christophersen DV, Andersen DN (2019). The applicability of the GHS classification criteria to nanomaterials. Copenhagen: Nordisk Ministerråd, p. 114. https://doi.org/10.6027/NA2019-907.

Lamon L, Aschberger K, Asturiol D, Richarz A, Worth A (2019). Grouping of nanomaterials to read-across hazard endpoints: a review. Nanotoxicology 13(1): 100-118. https://doi.org/10.1080/17435390.2018.1506060.

Lead JR, Batley GE, Alvarez PJJ, Croteau M-N, Handy RD, McLaughlin MJ, Judy JD, Schirmer K (2018). Nanomaterials in the environment: Behavior, fate, bioavailability, and effects—An updated review. Environmental Toxicology and Chemistry 37(8): 2029-2063. https://doi.org/10.1002/etc.4147.

Lecloux AJ, Luizi F (2009). The safe use of carbon nanotubes in coating applications. 3rd Annual Nanotechnology Safety for Success Dialogue, Brussels, 3-4 November 2009.

Li Q, Hu H, Jiang L, Zou Y, Duan J, Sun Z (2016). Cytotoxicity and autophagy dysfunction induced by different sizes of silica particles in human bronchial epithelial BEAS-2B cells. Toxicol. Res. 5: 1216-1228. https://doi.org/10.1039/c6tx00100a.

Lin Q, Li H, Zhong H, Zhao Q, Xiao D, Wang Z (2014). Migration of Ti from nano-TiO_2-polyethylene composite packaging into food simulants. Food Additives & Contaminants Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment: 31(7): 1284–1287. https://doi.org/10.1080/19440049.2014.907505.

Lipovsky A, Nitzan Y, Gedanken A, Lubart R. (2011). Antifungal activity of ZnO nanoparticles- the role of ROS mediated cell injury. Nanotechnology 22: 105101 (5pp). https://doi.org/10.1088/0957-4484/22/10/105101.

Liu F, Hu C, Zhao Q, Shi Y, Zhong H (2016). Migration of copper from nanocopper/LDPE composite films. Food Additives & Contaminants: Part A 33(11): 1741–1749. https://doi.org/10.1080/19440049.2016.1237779.

Lopes S, Ribeiro F, Wojnarowicz J, Lojkowski W, Jurkschat K, Crossley A, Soares AMVM, Loureiro S (2014). Zinc oxide nanoparticles toxicity to Daphnia magna: Size-dependent effects and dissolution. Environmental Toxicology and Chemistry 33(1): 190-198. https://doi.org/10.1002/etc.2413.

Mackevica A, Hansen S (2016). Release of nanomaterials from solid nanocomposites and consumer exposure assessment – a forward-looking review. Nanotoxicology 10: 641–653. https://doi.org/10.3109/17435390.2015.1132346.

MacNee W, Li XY, Gilmour P, Donaldson K (2000). Systemic effect of particulate air pollution. Inhal. Toxicol. 12: 233-244. https://doi.org/10.1080/08958378.2000.11463218.

Ma-Hock L, Treumann S, Strauss V, Brill S, Luizi F, Mertler M, Wiench K, Gamer AO, van Ravenzwaay B, Landsiedel R (2009). Inhalation toxicity of multiwall carbon nanotubes in rats exposed for 3 months. Toxicol Sci. 112(2): 468-81. https://doi.org/10.1093/toxsci/kfp146.

Maiorano G, Sabella S, Sorce B, Brunetti V, Malvindi MA, Cingolani R, Pompa PP (2010). Effects of cell culture media on the dynamic formation of protein-nanoparticle complexes and influence on the cellular response. ACS Nano 4(12): 7481-91. https://doi.org/10.1021/nn101557e.

McCracken C, Dutta PK, Waldmann WJ. (2016). Critical assessment of toxicological effects of ingested nanoparticles. Environ. Sci.: Nano 3:256-282. https://doi.org/10.1039/C5EN00242G.

Mech A, Rasmussen K, Jantunen P, Aicher L, Alessandrelli M, Bernauer U, Bleeker EAJ, Bouillard J, Di Prospero Fanghella P, Draisci R, Dusinska M, Encheva G, Flament G, Haase A, Handzhiyski Y, Herzberg F, Huwyler J, Jacobsen NR, Jeliazkov V, Jeliazkova N, Nymark P, Grafström R, Oomen AG, Polci M L, Riebeling C, Sandström J, Shivachev B, Stateva S, Tanasescu S, Tsekovska R, Wallin H, Wilks MF, Zellmer S, Apostolova MD. (2019a). Insights into possibilities for grouping and read-across for nanomaterials in EU chemicals legislation. Nanotoxicology 1(2): 119-141. https://doi.org/10.1080/17435390.2018.1513092.

Mech A, Rauscher H, Babick F, Hodoroaba V, Ghanem A, Wohlleben W, Marvin H, Weigel S, Brüngel R, Friedrich C, Rasmussen K, Loeschner K, Gilliland D (2019b). The NanoDefine Methods Manual, EUR 29876 EN, Publications Office of the European Union, Luxembourg, 2020, ISBN 978-92-76-12336-1, doi:10.2760/58586, JRC117501.

Mech A, Wohlleben W, Ghanem A, Hodoroaba V-D, Weigel S, Babick F, Brüngel R, Friedrich C M, Rasmussen K, Rauscher H (2020). Nano or Not Nano? A Structured Approach for Identifying Nanomaterials According to the European Commission's Definition. Nano Micro Small 16: 2002228. https://doi.org/10.1002/smll.202002228.

Meesters JAJ, Peijnenburg WJGM, Hendriks AJ, Van De Meent D, Quik JTK (2019). A model sensitivity analysis to determine the most important physicochemical properties driving environmental fate and exposure of engineered nanoparticles. Environmental Science: Nano 6(7): 2049-2060. https://doi.org/10.1039/C9EN00117D.

MINChar Initiative (2008). Recommended Minimum Physical and Chemical Parameters for Characterizing Nanomaterials on Toxicology Studies available at: http://characterizationmatters.org/parameters / Accessed September 25, 2010.

Mitrano DM, Motellier S, Clavaguera S, Nowack B. (2015). Review of nanomaterial aging and transformations through the life cycle of nano-enhanced products. Environ Int. 77: 132-47. https://doi.org/10.1016/j.envint.2015.01.013.

Muhlfeld C, Geiser M, Kapp N, Gehr P, Rothen-Rutishauser B (2007). Re-evaluation of pulmonary titanium dioxide nanoparticle distribution using the "relative deposition index": evidence for clearance through microvasculature. Part Fibre Toxicol 4: 7. https://doi.org/10.1186/1743-8977-4-7.

Mülhopt S, Diabaté S, Dilger M, Adelhelm C, Anderlohr C, Bergfeldt T, Adelhelm C, Anderlohr C, Bergfeldt T, de la Torre JG, Jiang Y, Valsami-Jones E, Langevin D, Lynch I, Mahon E, Nelissen I, Piella J, Puntes V, Ray S, Schneider R, Wilkins T, Weiss C, Paur H-R (2018). Characterization of Nanoparticle Batch-To-Batch Variability. Nanomaterials 8(5): E311. https://doi.org/10.3390/nano8050311.

Nakanishi J (2009a). Risk Assessment of Manufactured Nanomaterials: Carbon Nanotubes (CNTs). NEDO project "Research and Development of Nanoparticle Characterisation Methods" (P06041). Interim report issued on October 16, 2009. http://www.aist-riss.jp/main/modules/product/nano_rad.html.

Nakanishi J (2009b). The principles and basic approaches to risk assessment of manufactured nanomaterials. NEDO project "Research and Development of Nanoparticle Characterisation Methods" (P06041). Interim report issued on October 16, 2009.

Nanocyl (2009). Responsible Care and Nanomaterials Case Study Nanocyl. Presentation at European Responsible Care Conference, Prague, 21-23rd October, 2009. http://www.cefic.be/Files/Downloads/04_Nanocyl.pdf.

NanoMONITOR Consortium (2018). Guidance on the sampling methods and analytical techniques for the measurement and monitoring of engineered nanomaterials in the environment. ISBN: 978-92-9495-699-6. http://www.lifenanomonitor.eu/wp-content/uploads/2018/07/NM-guidance-final-for-web.pdf.

Nasser F, Lynch I (2016). Secreted protein eco-corona mediates uptake and impacts of polystyrene nanoparticles on Daphnia magna. J Proteomics 137: 45-51. https://doi.org/10.1016/j.jprot.2015.09.005.

Nazaroff WW, Cass GR (1989). Mathematical Modeling of Indoor Aerosol Dynamics. Environmental Science and Technology 23(2): 157-166. https://doi.org/10.1021/es00179a003.

Nazaroff WW (2004). Indoor particle dynamics. Indoor Air 14: 175-183. https://doi.org/10.1111/j.1600-0668.2004.00286.x.

Nemmar A, Hoet PH, Vanquickenborne B, Dinsdale D, Thomeer M, Hoylaerts MF, Vanbilloen H, Mortelmans L, Nemery B (2002a). Passage of inhaled particles into the blood circulation in humans. Circulation 105: 411–14. https://doi.org/10.1161/hc0402.104118.

Nemmar A, Hoylaerts MF, Hoet PH, Dinsdale D, Smith T, Xu H, Vermylen J, Nemery B (2002b). Ultrafine particles affect experimental thrombosis in an *in vivo* hamster model. Am J Respir Crit Care Med 166: 998–1004. https://doi.org/10.1164/rccm.200110-026OC.

NIOSH (2011). NIOSH Current Intelligence Bulletin 63: Occupational Exposure to Titanium Dioxide. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Cincinnati, OH, 140 pp. DHHS (NIOSH) Publication No. 2011–160. https://www.cdc.gov/niosh/docs/2011-160/.

NIOSH (2013). NIOSH Current Intelligence Bulletin 65: Occupational Exposure to Carbon Nanotubes and Nanofibers, U.S. Department of Health and Human Services, Public Health Service Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Cincinnati, OH, 184 pp. DHHS (NIOSH) Publication No. 2013–145. https://www.cdc.gov/niosh/docs/2013-145/.

NIOSH (2017). Draft Current Intelligence Bulletin: the Occupational Exposure Banding Process: Guidance for the Evaluation of Chemical Hazards. Department of Health and Human Services (HHS), Centers for Disease Control and Prevention (CDC), National Institute for Occupational Safety and Health (NIOSH), Cincinnati, OH, 141 pp. https://www.cdc.gov/niosh/docket/archive/docket290.html.

NIOSH (2018). Revised External Review Draft Current Intelligence Bulletin: Health Effect of Occupational Exposure to Silver Nanoparticles. Department of Health and Human Services (HHS), Centers for Disease Control and Prevention (CDC), National Institute for Occupational Safety and Health, Cincinnati, OH, 503 pp. https://www.cdc.gov/niosh/docket/archive/docket260a.html.

NIST (2018). Development of Airborne Nanoparticle Exposure Modeling Tools, Technical Note (NIST TN), National Institute of Standards and Technology, Gaithersburg, MD. https://www.nist.gov/publications/development-airborne-nanoparticle-exposure-modeling-tools.

NNI (2011). Draft National Nanotechnology Initiative 2011 Environmental, Health, and Safety Research Strategy: http://strategy.nano.gov/blog/generic/page/draft-nni-ehs-strategy.

Nowack B, Ranville JF, Diamond S, Gallego-Urrea JA, Metcalfe C, Rose J, Horne N, Koelmans AA, Klaine SJ (2012). Potential scenarios for nanomaterial release and subsequent alteration in the environment. Environ. Toxicol. Chem. 31: 50–59. https://doi.org/10.1002/etc.726.

Nowack B, David RM, Fissan H, Morris H, Shatki JA, Stintz M, Zepp R, Brouwer D (2013). Potential release scenarios for carbon nanotubes used in composites. Environ. Int. 59: 1–11. https://doi.org/10.1016/j.envint.2013.04.003.

NRC (1983). Risk Assessment in the Federal Government: Managing the Process. Committee on the Institutional Means for Assessment of Risks to Public Health, Commission on Life Sciences, National Research Council. Washington, D.C.: National Academy Press, pp. 191.

NRC (2009). Science and Decisions: Advancing Risk Assessment. Committee on Improving Risk Analysis Approaches Used by the U.S. EPA, Board on Environmental Studies and Toxicology, Division on Earth and Life Studies, National Research Council of the National Academies. Washington, D.C.: The National Academies Press, 424 pages. ISBN-10: 0-309-12046-2.

Oberdörster G, Finkelstein JN, Johnston C, Gelein R, Cox C, Baggs R, Elder AC (2000). Acute pulmonary effects of ultrafine particles in rats and mice. Res Rep Health Eff Inst. 96: 5-86.

Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Lunts A, Kreyling W, Cox C (2002). Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. Journal of Toxicology and Environmental Health - Part A 65: 1531-1543. https://doi.org/10.1080/00984100290071658.

Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, Cox C (2004). Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol 16(6-7): 437-445. https://doi.org/10.1080/08958370490439597.

Oberdörster G, Oberdörster E, Oberdörster J (2005). Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect. 113: 823–839. https://doi.org/10.1289/ehp.7339.

Oberdörster G (2018). *In vivo* effects: Methodologies and biokinetics of inhaled nanomaterials. NanoImpact 10: 38-60. https://doi.org/10.1016/j.impact.2017.10.007.

OECD (2003). OECD Series on Testing and Assessment No. 44: Descriptions of Selected Key Generic Terms Used in Chemical Hazard/Risk Assessment, ENV/JM/MONO(2003)15.

OECD (2008). Working Party on Manufactured Nanomaterials: List of Manufactured Nanomaterials and List of Endpoints for Phase One of the OECD Testing Programme, ENV/JM/MONO(2008)13/REV.

OECD (2009a). Report of an OECD Workshop on Exposure Assessment and Exposure Mitigation: Manufactured Nanomaterials, ENV/JM/MONO(2009)18.

OECD (2009b). Guidance Manual for the Testing of Manufactured Nanomaterials: OECD Sponsorship Programme: First Revision, ENV/JM/MONO(2009)20/REV.

OECD (2009c). Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials, ENV/JM/MONO(2009)21.

OECD (2010). Report of the Workshop on Risk Assessment of Manufactured Nanomaterials in a Regulatory Context, ENV/JM/MONO(2010)10.

OECD (2012a). Inhalation Toxicity Testing: Expert Meeting on Potential Revisions to OED Test Guidelines and Guidance Document, ENV/JM/MONO(2012)14.

OECD (2012b). Guidance of Sample Preparation and Dosimetry for The Safety Testing of Manufactured Nanomaterials, ENV/JM/MONO(2012)40.

OECD (2013a). Co-operation on Risk Assessment: Prioritisation of Important Issues on Risk Assessment of Manufactured Nanomaterials – Final Report, ENV/JM/MONO(2013)18.

OECD (2013b). Draft Recommendation of the Council on the Safety Testing and Assessment of Manufactured Nanomaterials, C(2013)107.

OECD (2014a). Guidance on Grouping of Chemicals, Second Edition, ENV/JM/MONO(2014)4.

OECD (2014b). Report of The OECD Expert Meeting on The Physical Chemical Properties of Manufactured Nanomaterials and Test Guidelines, ENV/JM/MONO(2014)15.

OECD (2014c). Genotoxicity of Manufactured Nanomaterials: Report of The OECD Expert Meeting, ENV/JM/MONO(2014)34.

OECD (2015a). Harmonized Tiered Approach to Measure and Assess The Potential Exposure to Air borne Emissions of Engineered Nano-Objects and Their Agglomerates and Aggregates at Workplaces, ENV/JM/MONO(2015)19.

OECD (2015b). Guidance Manual Towards The Integration of Risk Assessment into Life Cycle Assessment of Nano-Enables Applications, ENV/JM/MONO(2015)30.

OECD (2015c). Preliminary Guidance Notes on Nanomaterials: Interspecies Variability Factors in Human Health Risk Assessment, ENV/JM/MONO(2015)31.

OECD (2015d). Considerations for Using Dissolution as A Function of Surface Chemistry to Evaluate Environmental Behaviour of Nanomaterials in Risk Assessments, ENV/JM/MONO(2015)44.

OECD (2016a). Categorisation of Manufactured Nanomaterials: Workshop Report Series on the Safety of Manufactured Nanomaterials No. 66, ENV/JM/MONO(2016)9.

OECD (2016b). Grouping and Read Across for the Hazard Assessment of Manufactured Nanomaterials Report from the Expert Meeting, ENV/JM/MONO(2016)59.

OECD (2016c). Physical-Chemical Properties of Nanomaterials: Evaluation of Methods Applied in The OECD-WPMN Testing Programe, ENV/JM/MONO(2016)7.

OECD (2016d). Toxicokinetics of Manufactured Nanomaterials: Report from The OECD Expert Meeting, ENV/JM/MONO(2016)24.

OECD (2017). Test No. 318: Dispersion Stability of Nanomaterials in Simulated Environmental Media, OECD Guidelines for the Testing of Chemicals, Section 3, https://doi.org/10.1787/9789264284142-en.

OECD (2018a). Test No. 412: Subacute Inhalation Toxicity: 28-Day Study, OECD Guidelines for the Testing of Chemicals, Section 4, https://doi.org/10.1787/9789264070783-en.

OECD (2018b). Test No. 413: Subchronic Inhalation Toxicity: 90-day Study, OECD Guidelines for the Testing of Chemicals, Section 4, https://doi.org/10.1787/9789264070806-en.

OECD (2018c). Guidance Document on Inhalation Toxicity Studies, Series on Testing and Assessment No. 39 (Second Edition), ENV/JM/MONO(2009)28/REV1.

OECD (2018d). Assessment of Biodurability of Nanomaterials and their Surface ligands, Series on the Safety of Manufactured Nanomaterials No. 86, ENV/JM/MONO(2018)11.

OECD (2018e). Guidance Document on Good In Vitro Method Practices (GIVIMP), Series on Testing and Assessment, No. 286, https://doi.org/10.1787/9789264304796-en.

OECD (2019a). Guiding Principles for Measurements and Reporting for Nanomaterials: Physical Chemical Parameters, Series on the Safety of Manufactured Nanomaterials No. 91, ENV/JM/MONO(2019)13.

OECD (2019b). Physical-Chemical Decision Framework to Inform Decisions for Risk Assessment of Manufactured Nanomaterials, Series on the Safety of Manufactured Nanomaterials No. 90, ENV/JM/MONO(2019)12.

OECD (2020a). Advancing Adverse Outcome Pathway (AOP) Development for Nanomaterial Risk Assessment and Categorisation/ PART1: Final Project Report and Recommendations with Methodology to Prioritise Key Events (KEs) Relevant for Manufactured Nanomaterials, Series on the Safety of Manufactured Nanomaterials, No. 93, ENV/JM/MONO(2020)33.

OECD (2020b). Advancing Adverse Outcome Pathway (AOP) Development for Nanomaterial Risk Assessment and Categorisation/ PART2: Case Study on Tissue Injury, Series on the Safety of Manufactured Nanomaterials, No. 94, ENV/JM/MONO(2020)34.

OECD (2020c). Guidance Document on Aquatic and Sediment Toxicological Testing of Nanomaterials, Series on Testing and Assessment, No. 317, ENV/JM/MONO(2020)8.

OECD (2020d). Guidance Document for the Testing of Dissolution and Dispersion Stability of Nanomaterials and the Use of the Data for Further Environmental Testing and Assessment Strategies, Series on Testing and Assessment, No. 318, ENV/JM/MONO(2020)9.

OECD (2021a). Evaluation of Tools and Models for Assessing Occupational and Consumer Exposure to Manufactured Nanomaterials – Part I: Compilation of tools/models and analysis for further evaluation, Project 1: Assessing the Global Readiness of Regulatory and Non-regulatory Models for Assessing Occupational Exposure to Manufactured Nanomaterials, Project 2: Compilation of Available Tools and Models Used for Assessing Consumer Exposure to Manufactured Nanomaterials, ENV/CBC/MONO(2021)27.

OECD (2021b). Evaluation of Tools and Models for Assessing Occupational and Consumer Exposure to Manufactured Nanomaterials – Part II: Performance testing results of tools/models for occupational exposure, Project: Assessing the Global Readiness of Regulatory and Non-regulatory Models for Assessing Occupational Exposure to Manufactured Nanomaterials, Series on Testing and Assessment, No. 347, ENV/CBC/MONO(2021)28.

OECD (2021c). Evaluation of Tools and Models for Assessing Occupational and Consumer Exposure to Manufactured Nanomaterials – Part III: Performance testing results of tools/models for consumer exposure, Project: Compilation of Available Tools and Models Used for Assessing Consumer Exposure to Manufactured Nanomaterials and Evaluation of their Applicability in Exposure Assessments, Series on Testing and Assessment, No. 348, ENV/CBC/MONO(2021)29.

OECD (2021d). Evaluation of Tools and Models Used for Assessing Environmental Exposure to Manufactured Nanomaterials, Functional Assessment and Statistical Analysis of Nano-Specific Environmental Exposure Tools and Models, Series on Testing and Assessment, No. 345, ENV/CBC/MONO(2021)23.

OECD (2021e). Study Report on a test for removal in wastewater treatment plants of gold manufactured nanomaterial (MN): Activated Sludge Sorption Isotherm, Series on Testing and Assessment, No. 340, ENV/CBC/MONO(2021)15.

OECD (2021f). Guidance Document on testing Nanomaterials using OECD TG No. 312 "Leaching in soil columns", Series on Testing and Assessment, No. 342, ENV/CBC/MONO(2021)17.

Oksel C, Ma CY, Liu JJ, Wilkins T, Wang XZ (2017). Literature Review of (Q)SAR Modelling of Nanomaterial Toxicity. Chapter (pp 103-142) in Modelling the Toxicity of Nanoparticles. Volume 947 of the Series Advances in Experimental Medicine and Biology. Springer Nature. https://doi.org/10.1007/978-3-319-47754-1_5.

Olenick LL, Troiano JM, Vartanian A, Melby ES, Mensch AC, Zhang L, Hong J, Mesele O, Qiu T, Bozich J, Lohse S, Zhang X, Kuech TR, Millevolte A, Gunsolus I, McGeachy AC, Doğangün M, Li T, Hu D, Walter SR, Mohaimani A, Schmoldt A, Torelli MD, Hurley KR, Dalluge J, Chong G, Feng ZV, Haynes CL, Hamers RJ, Pedersen JA, Cui Q, Hernandez R, Klaper R, Orr G, Murphy CJ, Geiger FM (2018). Lipid Corona Formation from Nanoparticle Interactions with Bilayers. Chem. 4(11): 2709-2723. https://doi.org/10.1016/j.chempr.2018.09.018.

Osborne OJ, Johnston BD, Moger J, Balousha M, Lead JR, Kudoh T, Tyler CR (2013). Effects of particle size and coating on nanoscale Ag and TiO_2 exposure in zebrafish (Danio rerio) embryos. Nanotoxicology 7(8): 1315-1324. https://doi.org/10.3109/17435390.2012.737484.

Park MVDZ, Neight AM, Vermeulen JP, de la Fonteyne LJJ, Verharen HW, Breidé JJ, Loveren H, de Jong WH (2011). The effect of particle size on the cytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles. Biomaterials 32(36): 9810-9817. https://doi.org/10.1016/j.biomaterials.2011.08.085.

Pauluhn J (2010a). Multi-walled carbon nanotubes (Baytubes): Approach for derivation of occupational exposure limit. Regul Toxicol Pharmacol. 57(1): 78-89. https://doi.org/10.1016/j.yrtph.2009.12.012.

Pauluhn J (2010b). Subchronic 13-week inhalation exposure of rats to multiwalled carbon nanotubes: toxic effects are determined by density of agglomerate structures, not fibrillar structures. Toxicol Sci. 113(1):226-242. https://doi.org/10.1093/toxsci/kfp247.

Persoone G and Janssen CR (1994). Field validation of predictions based on laboratory toxicity tests. In Hill IR, Heimbach F. Leeuwangh P, Matthiesen P, eds, Freshwater Field Tests for Hazard Assessment of Chemcials. Lewis, Boca Raton, FL, USA, pp 379-397.

Petersen EJ, Mortimer M, Burgess RM, Handy R, Hanna S, Ho KT, Jonhson M, Loureiro S, Selck H, Scott-Fordsmand JJ, Spurgeon D, Unrine J, van den Brink NW, Wang Y, White J, Holden P (2019). Strategies for robust and accurate experimental approaches to quantify nanomaterial bioaccumulation across a broad range of organisms. Environ. Sci.: Nano 6, 1619-1656. https://doi.org/10.1039/C8EN01378K.

Piret J-P, Bondarenko OM, Boyles MSP, Himly M, Ribeiro AR, Benetti F, Smal C, Lima B, Potthoff A, Simion M, Dumortier E, Leite PEC, Balottin LB, Granjeiro JM, Ivask A, Kahru A, Radauer-Preiml I, Tischler U, Duschl A, Saout C, Anguissola S, Haase A, Jacobs A, Nelissen I, Misra SK, Toussaint O (2017). Pan-European inter-laboratory studies on a panel of *in vitro* cytotoxicity and pro-inflammation assays for nanoparticles. Archives of Toxicology 91(6): 2315-2330. https://doi.org/10.1007/s00204-016-1897-2.

Quik JTK, Meesters JAJ, Peijnenburg WJGM, Brand W, Bleeker EAJ (2020). Environmental Risk Assessment (ERA) of the application of nanoscience and nanotechnology in the food and feed chain. European Food Safety Authority (EFSA). https://efsa.onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2020.EN-1948.

Raesch SS, Tenzer S, Storck W, Rurainski A, Selzer D, Ruge CA, Perez-Gil J, Schaefr UF, Lehr CM (2015). Proteomic and Lipidomic Analysis of Nanoparticle Corona upon Contact with Lung Surfactant Reveals Differences in Protein, but Not Lipid Composition. ACS Nano 9: 11872-85. https://doi.org/10.1021/acsnano.5b04215.

Rasmussen K, González M, Kearns P, Riego Sintes J, Rossi F, and Sayre P (2016) Review of Achievements of the OECD Working Party on Manufactured Nanomaterials' Testing and Assessment Programme. From Exploratory Testing to Test Guidelines. Regulatory Toxicology and Pharmacology 74: 147–160. https://doi.org/10.1016/j.yrtph.2015.11.004.

Rasmussen K, Rauscher H, Mech A, Sintes JR, Gilliland D, González M, Kearns , Moss , Visser M, Groenewold M, and Bleeker EAJ (2018). Physico-chemical properties of manufactured nanomaterials

- Characterisation and relevant methods. An outlook based on the OECD Testing Programme. Regulatory Toxicology and Pharmacology 92: 8-28. https://doi.org/10.1016/j.yrtph.2017.10.019.

Rasmussen K, Rauscher H, Kearns P, González M, Riego Sintes J (2019). Developing OECD Test Guidelines for Regulatory Testing of Nanomaterials to ensure mutual acceptance of test data. Regulatory Toxicology and Pharmacology 104:74-83. https://doi.org/10.1016/j.yrtph.2019.02.008.

Rim D, Persily A, Emmerich S, Dols WS, Wallace L (2013). Multi-zone modeling of size-resolved outdoor ultrafine particle entry into a test house. Atmospheric Environment 69: 219-230. https://doi.org/10.1016/j.atmosenv.2012.12.008.

RIVM (2019). SimpleBox4nano model, v20190830. http://www.rivm.nl/en/soil-and-water/simplebox4nano. Accessed 20 May 2021.

RIVM (2021). ConsExpo Nano model, Version 2.0. https://www.consexponano.nl. Accessed 20 May 2021.

Roco, M.C. (2011). The long view of nanotechnology development: the National Nanotechnology Initiative at 10 years. J Nanopart Res 13, 427–445. https://doi.org/10.1007/s11051-010-0192-z.

Roco, M.C. (2017). Overview. In Nanotechnology Commercialization (eds T.O. Mensah, B. Wang, G. Bothun, J. Winter and V. Davis). https://doi.org/10.1002/9781119371762.ch1.

Royal Society (2004). Nanoscience and nanotechnologies: opportunities and uncertainties. The Royal Society & The Royal Academy of Engineering, London, UK. https://royalsociety.org/~/media/royal_society_content/policy/publications/2004/9693.pdf.

Sadauskas E, Wallin H, Stoltenberg M, Vogel U, Doering P, Larsen A, Danscher G (2007). Kupffr cells are central in the removal of nanoparticles from the organism. Part Fibre Toxicol 4: 10. https://doi.org/10.1186/1743-8977-4-10.

Sadauskas E, Danscher G, Stoltenberg M, Vogel U, Larsen A, Wallin H (2009a). Protracted elimination of gold nanoparticles from mouse liver. Nanomedicine 5: 162–9. https://doi.org/10.1016/j.nano.2008.11.002.

Sadauskas E, Jacobsen NR, Danscher G, Stoltenberg M, Vogel U, Larsen A, Kreyling W, Wallin H (2009b). Biodistribution of gold nanoparticles in mouse lung following intratracheal instillation. Chem Central J 3: 16. https://doi.org/10.1186/1752-153X-3-16.

SCENIHR (2006). Modified Opinion (after public consultation) on: The appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies. Scientific Committee on Emerging and Newly Identified Health Risks. SCENIHR/002/05. 10 March 2006.

Schinwald A, Murphy FA, Prina-Mello A, Poland CA, Byrne F, Movia D, Glass JR, Dickerson JC, Schultz DA, Jeffree CE, Macnee W, Donaldson, K (2012). The threshold length for fiber-induced acute pleural inflammation: shedding light on the early events in asbestos-induced mesothelioma. Toxicol Sci 128: 461-70. https://doi.org/10.1093/toxsci/kfs171.

Schlagenhauf L, Nüesch F, Wang J (2014). Release of carbon nanotubes from polymer nanocomposites. Fibers 2: 108–127. https://doi.org/10.3390/fib2020108.

Schlesinger RB (1995). Deposition and clearance of inhaled particles (pp. 191-224) *IN* Concepts in Inhalation Toxicology, McClellan RO, Henderson RF (Editors). Taylor & Francis, Washington DC, USA.

Schulte PA, Salamanca-Buentello F (2007). Ethical and scientific issues of nanotechnology in the workplace. Cien Saude Colet. 12(5): 1319-1332. https://doi.org/10.1590/S1413-81232007000500030.

Schultz AG, Boyle D, Chamot D, Ong KJ, Wilkinson KJ, McGeer JC, Sunahara G, Goss GG. (2014). Aquatic toxicity of manufactured nanomaterials: challenges and recommendations for future toxicity testing. Environ. Chem. 11: 207-226. https://doi.org/10.1071/EN13221.

Sekine R, Marzouk ER, Khaksar M, Scheckel KG, Stegemeier JP, Lowry GV, Donner E, Lombi E (2017). Aging of dissolved copper and copper-based nanoparticles in five different soils: Short-term kinetics vs. long-term fate. Journal of Environmental Quality 46(6): 1198-1205. https://doi.org/10.2134/jeq2016.12.0485.

Semmler-Behnke M, Lipka J, Wenk A, Hirn S, Schaffler M, Tian F, Schmid G, Oberdorster G, Kreyling W G (2014). Size dependent translocation and fetal accumulation of gold nanoparticles from maternal blood in the rat. Part Fibre Toxicol. 11: 33. https://doi.org/10.1186/s12989-014-0033-9.

Shandilya N, Bihan OL, Bressot C, Morgeneyer M (2014). Evaluation of the particle aerosolization from n-TiO$_2$ photocatalytic nanocoatings under abrasion. J. Nanomater. 2014: 1–11. https://doi.org/10.1155/2014/185080.

Shatkin J (2008). Nanotechnology Health and Environmental Risks. CRC Press, Boca Raton FL.

Shin HK, Kim KY, Park JW, No KT (2017). Use of metal/metal oxide spherical cluster and hydroxyl metal coordination complex for descriptor calculation in development of nanoparticle cytotoxicity classification model, SAR and QSAR in Environmental Research 28(11): 875-888. https://doi.org/10.1080/1062936X.2017.1400998.

Shipley HJ, Yean S, Kan AT, Tomson MB (2009). Adsorption of arsenic to magnetite nanoparticles: effect of particle concentration, pH, ionic strength, and temperature. Environ Toxicol Chem. 28(3): 509-15. https://doi.org/10.1897/08-155.1.

Simkó M, Nosske D, Kreyling WG (2014). Metrics, Dose, and Dose Concept: The Need for a Proper Dose Concept in the Risk Assessment of Nanoparticles. Int. J. Environ. Res. Public Health 11: 4026-4048. https://doi.org/10.3390/ijerph110404026.

Simon P, Chaudhry Q, Bakos D (2008). Migration of engineered nanoparticles from polymer packaging to food—A physicochemical view. Journal of Food and Nutrition Research 47(3): 105–113.

Singh KP, Gupta S (2014). Nano-QSAR modeling for predicting biological activity of diverse nanomaterials. RSC Adv. 4(26): 13215-13230. https://doi.org/10.1039/C4RA01274G.

Stefaniak AB, Hackley VA, Roebben G, Ehara K, Hankin S, Postek MT, Lynch I, Fu WE, Linsinger TP, Thunemann AF (2013). Nanoscale reference materials for environmental, health and safety measurements: needs, gaps and opportunities. Nanotoxicology 7: 1325–1337. https://doi.org/10.3109/17435390.2012.739664.

Steinhäuser KG, Sayre PG, Nowack B (2018). Reliability of methods and data for regulatory assessment of nanomaterial risks. NanoImpact 10: 68-69. https://doi.org/10.1016/j.impact.2017.11.005.

Stone V, Gottardo S, Bleeker EAJ, Braakhuis H, Dekkers S, Fernandes T, Haase A, Hunt N, Hristozov D, Jantunen P, Jeliazkova N, Johnston H, Lamon L, Murphy F, Rasmussen K, Rauscher H, Jiménez AS, Svendsen C, Spurgeon D, Vázquez-Campos S, Wohlleben W, Oomen AG (2020). A framework for grouping and read-across of nanomaterials- supporting innovation and risk assessment. Nano Today 35: art. no. 100941. https://doi.org/10.1016/j.nantod.2020.100941.

Su Q, Lin Q, Chen C, Wu L, Wang Z (2017). Effect of organic additives on silver release from nanosilver–polyethylene composite films to acidic food simulant. Food Chemistry 228: 560–566. https://doi.org/10.1016/j.foodchem.2017.01.148.

Sukhanova A, Bozvrova S, Sokolov P, Berestovoy M, Karaulov A, Nabiev I (2018). Dependence of Nanoparticle Toxicity on Their Physical and Chemical Properties. Nanoscale Research Letters 13: 44. https://doi.org/10.1186/s11671-018-2457-x.

The Toxic Substances Control Act (15 U.S.C. 2601–2697) consists of Public Law 94–469 (Oct. 11, 1976; 90 Stat. 2003) and the amendments made by subsequent enactments.

Tomilina II, Gremyachikh VA, Myl'nikov AP, Komov VT (2011). Changes in Biological Characteristics of Freshwater Heterotrophic Flagellates and Cladocerans under the Effect of Metal Oxide Nan- and Microparticles. Aquat. Tox. 4(4): 475-483. https://doi.org/10.1134/S1995082911040201.

UN (2019). Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Seventh revised edition. United Nations, New York and Geneva. https://unece.org/ghs-rev7-2017.

Utembe W, Wepener V, Yu IJ, Gulumian M (2018). An assessment of applicability of existing approaches to predicting the bioaccumulation of conventional substances in nanomaterials. Environ Toxicol Chem 37: 2972-2988. https://doi.org/10.1002/etc.4253.

Van den Brink NW, Kokalj AJ, Silva PV, Lahive E, Norrfors K, Baccaro M, Khodaparast Z, Loureiro S, Drobne D, Cornelis G, Lofts S, Handy RD, Svendsen C. Spurgeon D, van Gestel CAM (2019). Tools and rules for modelling uptake and bioaccumulation of nanomaterials in invertebrate organisms. Environ. Sci.: Nano 6: 1985-2001. https://doi.org/10.1039/C8EN01122B.

Varsou DD, Afantitis A, Tsoumanis A, Melagraki G, Sarimveis H, Valsami-Jones E, Lynch I (2019). A safe-by-design tool for functionalised nanomaterials through the Enalos Nanoinformatics Cloud platform. Nanoscale Advances 1(2): 706-718. https://doi.org/10.1039/C8NA00142A.

Vencalek BE, Laughton SN, Spielman-Sun E, Rodrigues SM, Unrine JM, Lowry GV, Gregory KB (2016). In Situ Measurement of CuO and Cu(OH)2 Nanoparticle Dissolution Rates in Quiescent Freshwater Mesocosms. Environmental Science and Technology Letters 3(10): 375-380. https://doi.org/10.1021/acs.estlett.6b00252.

Verschoor AJ, Harper S, Delmaar CJE, Park MVDZ, Sips AJAM, Vijver MG, Peijnenburg WJGM (2019). Systematic selection of a dose metric for metal-based nanoparticles. NanoImpact 13:70-75. https://doi.org/10.1016/j.impact.2019.01.002.

Villeneuve DL, Crump D, Garcia-Reyero N, Hecker M, Hutchinson TH, LaLone CA, Whelan M (2014). Adverse outcome pathway (AOP) development I: strategies and principles. Toxicol. Sci. 142(2): 312-320. https://doi.org/10.1093/toxsci/kfu199.

Von Moos N, Bowen P, Slaveykova VI (2014). Bioavailability of inorganic nanoparticles to planktonic bacteria and aquatic microalgae in freshwater. Environ. Sci.: Nano 1: 214–232. https://doi.org/10.1039/C3EN00054K.

Wang J, Zhou G, Chen C, Yu H, Wang T, Ma Y, Jia G, Gao Y, Li B, Sun J, Li Y, Jiao F, Zhao Y, Chai Z (2007). Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration. Toxicol Lett. 168(2): 176-85. https://doi.org/10.1016/j.toxlet.2006.12.001.

Weldon, BA, Faustman EM, Oberdorster G, Workman T, Griffith WC, Kneuer C, Yu IJ (2016). Occupational exposure limit for silver nanoparticles: considerations on the derivation of a general health-based value. Nanotoxicology 10(7): 945–956. https://doi.org/10.3109/17435390.2016.1148793.

WHO (2005). Chemical-specific adjustment factors for interspecies differences and human variability: guidance document for use of data in dose/concentration-response assessment. IPCS harmonisation project document no. 2, World Health Organization, Geneva. https://apps.who.int/iris/handle/10665/43294.

Williams RJ, Harrison S, Keller V, Kuenen J, Lofts S, Praetorius A, Svendsen C, Vermeulen LC, van Wijnen J (2019). Models for assessing engineered nanomaterial fate and behaviour in the aquatic environment. Current Opinion in Environmental Sustainability 36: 105-115. https://doi.org/10.1016/j.cosust.2018.11.002.

Xia Y, Uysal Unalan I, Rubino M, Auras R (2017). Carbon nanotube release from polymers into a food simulant. Environmental Pollution, 229 : 818–826. https://doi.org/10.1016/j.envpol.2017.06.067.

Yan J-W, Hu C, Chen K, Lin QB (2019). Release of graphene from graphene-polyethylene composite films into food simulants. Food Packaging and Shelf Life 20: art. no. 100310. https://doi.org/10.1016/j.fpsl.2019.100310.

Zhang H, Ji Z, Xia T, Meng H, Low-Kam C, Liu R, Pokhrel S, Lin S, Wang X, Liao Y-P, Wang M, Li L, Rallo R, Damoiseaux R, Telesca D, Mädler L, Cohen Y, Zink JI, Nel AE (2012). Use of metal oxide nanoparticle band gap to develop a predictive paradigm for oxidative stress and acute pulmonary inflammation. ACS Nano 6(5): 4349-4368. https://doi.org/10.1021/nn3010087.