A common European approach to the regulatory testing of nanomaterials

NANoREG

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D1.11 in the NANoREG context

0.1. Description of task 1.4

(According to the DoW)

Task 1.4: Framework development

The task will coordinate the establishment of an overall framework applicable for all/most types of legislations on how to address safety of nanomaterials, including, as appropriate, legislation/sector specific issues (although the primary focus will be on REACH, other regulations covering Cosmetics, Biocidal Products, Food and Feed, as well as Food and Feed Contact Materials, etc. may be considered). The framework development will be based on the results collected from the other WPs and the input from the regulators and stakeholders (via 7.2 and dedicated workshops in WP7). It will be iteratively developed by the WP1 working group. Specific answers / tools resulting from Task 1.3 (based on WPs 2-6) will be mapped against this framework. While JRC, TCD and TUKES will exploit their direct knowledge of regulations and their participation in regulatory and multi-stakeholder as well as their previous experience in developing, implementing and evaluating regulation and guidance, VN-Ecamricert and ENEA will contribute mainly their expertise in directly interacting with the industrial sectors.

(Lead JRC, contributors: TCD, TUKES, VN-Ecamricert, ENEA, NILU, ISS)

0.2. Description of the work done in T1.4 and main achievements

Summary

This deliverable is NANoREG's definitive framework for the safety assessment of nanomaterials (hereinafter NMs). This framework is the result of collaborative work among the many partners of this large-scale FP7 project. The overall goal of NANoREG is to support regulatory authorities, and also industry, in dealing with environmental, health and safety (EHS) issues of manufactured nanomaterials.

The proposed framework provides at the same time a detailed overview of how the safety of nanomaterials is being addressed / assessed in the context of the European REACH Regulation (Part I of the document), and forward-looking strategies aiming at making that safety assessment practical, economically efficient (Part II).

The framework analyses the applicability of the current EU regulatory framework to NMs and aims at giving concrete, practical guidance to industry and regulatory authorities on how to address NMs in a legislative context, with focus on REACH. Part I of the FW illustrates step-by-step how REACH

applies to NMs, highlights the differences between NMs and conventional chemicals and the hurdles in the safety assessment of nanomaterials under REACH.

The FW recognises that a change of paradigm in the safety assessment of NMs is advocated by several stakeholders to address adequately the hurdles identified in Part I. Hence, Part II examines forward-looking strategies proposed by those actors (e.g. integration of Safe-by-Design concepts) and discusses their benefits and potential limitations. Those approaches are not yet accepted by the relevant authorities. However, the FW emphasises the need to use the flexibility built into REACH, such as options for adaptation of standard information requirements in Annex XI, and discusses possible ways to (partially) implement those forward-looking strategies under existing conditions. Take-home messages and final considerations have been condensed in chapter 7.

Being a collaborative effort between scientists, regulators and industrial partners, this document is expected to be useful for all stakeholders in the nanoEHS arena, in Europe and beyond.

0.3. Background of task 1.4

The overall goal of NANoREG is to support regulatory authorities, and also industry, in dealing with environmental health and safety (EHS) issues of manufactured nanomaterials.
Based on questions and requirements of regulatory relevance identified by the NANoREG partners with those stakeholders (T1.1 / D1.1), the project shall, among others, i) propose options for solutions to EHS issues of NMs based on existing data and information (collected and generated by NANoREG in the 'scientific' WPs 2-6 and in T1.3 and used in developing this FW), complemented with new knowledge; ii) for the short to medium term, provide a set of fully developed tools applicable for NMs covering all steps of the risk assessment process including physicochemical characterisation, (eco)toxicity testing, and exposure monitoring and control (Part I of this FW and Toolbox in T1.7); iii) for the long-term, develop new testing strategies for NMs adapted to innovation requirements (Part II); and iv) establish a close collaboration among authorities, industry and science to create the basis for common approaches, mutually acceptable datasets and risk management practices (indeed: the collaborative in this task 1.4).
All data, information and tools addressing EHS aspects of the selected NANoREG NMs, which are generated and/or evaluated during the project, form the knowledge base for developing the NANoREG Framework (task 1.4) and related NANoREG Toolbox (task 1.7).

0.4. Description of the work carried out

This document is the result of extensive collaboration between NANoREG partners, not only those committed to deliver under WP1 as per DoW, but also many others from the other project WPs volunteering to contribute and report information generated or collected in those other compartments of this large FP7 initiative.

Under the leadership of the task leader, the work has started in May 2014 and ended in early November 2016 with the release of the final framework (D1.11). Partners from Industry, expert scientists and representatives of regulatory authorities have worked together. 19 teleconference calls, 2 dedicated workshops, 4 Consortium-wide meeting sessions and other WP1 sessions were held on this subject, involving not only the core WP1 partners, but all interested NANoREG participants. Hence, the views and proposals in this document rely on a wide consensus in the large NANoREG community.

All partners involved in the FW drafting process have been assigned to and have accepted one or more sections to be developed in small dedicated teams, based on their expertise. Those NANoREG partners are: AIT, BfR, CEA, CEREGE, ECAMRICERT, ECHA, ENEA, GAIKER, IOM, ISS, ITENE, JRC, LEITAT, NIA, NILU, RIVM, SOLVAY, TCD, TEMAS and TUKES.

Other NANoREG partners that have only edited / reviewed (parts of) the text: ISQ, FOPH and KI.

0.5. Results

See the framework document in the following chapters. The summary of the results is in chapter 7 on take-home messages.

0.6. Evaluation and conclusions.

Concluding remarks are in chapter 7.

0.7. Deviations from the work plan

The development of this definitive framework required more time and effort than foreseen at the start of the project. This certainly has to do with the breadth of subjects and communities covered by this ambitious deliverable: from physicochemical characterisation, to *in vivo* and *in vitro* testing and looking forward with new assessment strategies like decision trees, safe-by-design and life cycle assessment. The task leader had to devote a higher than expected amount of effort just to get the work up and running during the first 15 months of the roughly 24 months of activity.

Eventually the delay has been compensated by intense work during the last 6 months and the extension of the project was welcome. The deliverable has been submitted much ahead of the updated delivery deadline (09/11/16 compared to 28/02/17).

0.8. Performance of the partners

All core partners of T1.4 performed adequately.

NANoREG FRAMEWORK FOR THE SAFETY ASSESSMENT OF NANOMATERIALS

NOVEMBER 2016

Executive summary

The overall goal of NANoREG is to support regulatory authorities, and also industry, in dealing with environmental health and safety (EHS) issues of manufactured nanomaterials (NMs). Data, information and tools generated and/or evaluated in the project, as well as relevant publications from scientific literature form the knowledge base of the NANoREG Framework (FW) (task 1.4, Deliverable D1.11) and related NANoREG Toolbox (TB) (task 1.7, Deliverable D1.12). Both instruments (FW and TB) have been developed via a collective effort of project partners under JRC's leadership and are supported by a NANoREG-wide consensus.

The scope of the NANoREG FW is two-fold. First, the FW analyses the applicability of the current European regulatory framework to NMs, with focus on REACH (the ongoing review of REACH Annexes was not taken into account while drafting the deliverable; such a process is not concluded yet (in February 2017)). It aims at giving concrete, practical guidance to industry and regulatory authorities on how to address NMs in that legislative context.
Part I of the FW illustrates step-by-step how REACH applies to NMs. It highlights the differences between NMs and conventional or bulk substances, and discusses both applicability and regulatory acceptance of approaches that have been developed so far to address nanospecific issues (section 3). The findings are then linked with relevant sections in the second part of the FW.
Part II describes, from a scientist's point of view, three forward-looking strategies seeking to facilitate/accelerate the implementation of REACH for NMs, while discussing the strategies' benefits and potential limitations. Those three schemes include: i) the use of a nanospecific approach for prioritisation and risk assessment of NMs (NanoRA) (section 4); ii) the development and implementation of the NANoREG Safe-by-Design (SbD) concept (section 5); and iii) the integration of Life Cycle Assessment (LCA) and risk assessment in the case of NMs (section 6). Annexes II-V provide useful complementary information to several sections of this document. The FW conveys a list of take-home messages for both parts (section 7).

The European Commission, the European Chemicals Agency (ECHA), the Organisation for Economic Co-operation and Development (OECD) and the scientific community have worked closely together in recent years to improve the knowledge on EHS of NMs, remove hurdles and concretely help stakeholders in addressing regulatory requirements for NMs. In spite of those efforts, several nanospecific issues are still difficult to address efficiently and may hamper the NM safety assessment. NANoREG analysed means of overcoming these obstacles and partners consider NanoRA, SbD and LCA as valuable paths for exploration by scientists, industry and regulators to achieve a more efficient implementation of REACH principles for NMs in the near

future. Clearly, the three forward-looking strategies need to be further developed, tested and debated before a decision can be made on how far they are actually relevant for assessment methodology under REACH and, if so, how they can be properly implemented at both industrial and regulatory level.

The NANoREG FW serves as an overarching structure that indicates where and how to apply the tools collected in the NANoREG Toolbox (Deliverable D1.12). The TB is organised in worksheets that mirror the FW document structure. The TB contains a collection of tools – from NANoREG and several other initiatives – that may be used to deal with nanospecific aspects at different steps of the safety assessment process under REACH. Those available tools also address the need for tools under the forward-looking strategies described in Part II. The TB focuses on 'working tools', i.e. tools that are ready-to-use by industry and authorities (e.g. public guidance documents, fully developed models that are downloadable from the Internet). The TB also differentiates between tools that are already accepted at regulatory level, such as internationally accepted guidelines and standards, and tools that are products of research initiatives, which may have only limited use for industry and authorities.

Finally, a coordinated effort has been made to harmonise the use of specific wording within NANoREG. The NANoREG Harmonised Terminology for safety assessment of NMs (Annex I) is a NANoREG output expected to support regulatory-oriented discussions in various fora, beyond the remit of this project.

Abbreviations

AAN..... average agglomeration number
ADP..... average particle diameter
AE ..... assessment entity (reporting tool)
AF ..... assessment factor
ALI..... air-liquid interface
BAL ..... bronchoalveolar lavage
BALF ..... bronchoalveolar lavage fluid
BCF..... bioconcentration factor
BMF ..... biomagnification factor
BCOP..... bovine cornea opacity and permeability
CBMN ..... cytokinesis-blocked micronucleus (test)
CES..... contributing exposure scenario
CF ..... characterisation factor
CHO ..... Chinese hamster ovary (cells)
CMR..... carcinogenic, mutagenic or reprotoxic
CPC ..... condensation particle counter
CSA..... chemical safety assessment
CSR ..... chemical safety report
CTA..... cell transformation assay
D ..... diameter
DCFDA..... dichlorofluorescin diacetate
DLS ..... dynamic light scattering
DMEL..... derived minimal effect level
DNEL ..... derived no effect level
E..... effect
EC ..... engineering control
EDX..... energy-dispersive X-ray spectroscopy
EEPS ..... engine exhaust particle sizer
EHS..... environmental health and safety
EINECS..... European inventory of existing commercial chemical substances
ELPI ..... electrical low pressure impactor
ENM ..... engineered nanomaterial
ES ..... exposure scenario
FFF-ICPMS... field-flow fractionation coupled to inductively coupled plasma mass spectrometry
FMPS ..... fast mobility particle sizer
FRAS ..... ferric reducing ability of serum
FU ..... functional unit

GES ..... generic exposure scenario
GHS ..... globally harmonized system (of classification and labelling of chemicals)
GLP ..... good laboratory practice
GT ..... gastrointestinal tract
HARN ..... high aspect ratio nanoparticles (/nanomaterials)
HEPA ..... high efficiency particulate air (filter)
HPRT ..... hypoxanthine guanine phosphoribosyltransferase
ICE ..... isolated chicken eye
ICP-MS ..... inductively coupled plasma mass spectrometry
INEC ..... indicative no effect concentration
IU ..... identified use
IUCLID ..... international uniform chemical information database
K_d ..... distribution coefficient
K_{oc} ..... organic carbon-water partitioning coefficient
K_{ow} ..... octanol-water partitioning coefficient
L ..... length
LAS ..... laser aerosol spectrometer
LCA ..... life cycle assessment
LCI ..... life cycle inventory
LCIA ..... life cycle impact assessment
L/D ..... length/diameter (ratio)
LD50 ..... lethal dose, 50 %
LDH ..... lactate dehydrogenase
LEV ..... local exhaust ventilation
LIBS ..... light-induced breakdown spectroscopy
LOAEC ..... lowest observed adverse effect concentration
LOAEL ..... lowest observed adverse effect level
MLA ..... mouse lymphoma (thymidine kinase (TK) gene mutation) assay
MNO ..... manufactured nano object
MoA ..... mode of action
MPS ..... Mini Particle Sampler
MTS ..... 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
MWCNT ..... multi-wall(ed) carbon nanotube
NM ..... nanomaterial
NO ..... nitrogen oxide
NOAA ..... nano objects, aggregates and agglomerates
NOAEC ..... no observed adverse effect concentration
NOAEL ..... no observed adverse effect level
NOM ..... natural organic material
NSAM ..... nanoparticle surface area monitor

OC..... operational condition
OEL..... occupational exposure limit
OPS ..... optical particle sizer
OSOR ..... one substance, one registration (principle)
P..... probability
PBPK ..... physiologically based pharmacokinetic (model)
PBT ..... persistent, bioaccumulative and toxic
PC ..... particle concentration/counter
PE ..... polyethylene
PEC..... predicted environmental concentration
PIR ..... post implementation review
PNC ..... particle number concentration
PNEC ..... predicted no effect concentration
PPE..... personal protective equipment
PPORD ..... product and process orientated research and development
PROC..... process category
PSLT ..... poorly soluble low-toxicity
PSSD ..... probabilistic species sensitivity distribution
PVC..... polyvinyl chloride
(Q)SAR ..... (quantitative) structure-activity relationship
(Q)NAR ..... (quantitative) nanostructure-activity relationship
R ..... risk
R&D ..... research and development
RC..... risk characterization
RCR ..... risk characterization ratio
RHE ..... reconstructed human epidermis
RMM ..... risk management measure
ROS ..... reactive oxygen species
SAXS ..... small-angle x-ray scattering
SbD ..... safe-by-design
SEM ..... scanning electron microscopy
SMEs ..... small and medium-sized enterprises
SMPS..... scanning mobility particle sizer
SNAP ..... strategic nanotechnology action plan
SOP ..... standard operating procedure
SpERC..... specific environmental release category
SRD ..... scientific research and development
SSD..... species sensitivity distribution
STIS ..... short-term inhalation study
STP ..... sewage treatment plant

SWCNT..... single-wall carbon nanotube
TEM ..... transmission electron microscopy
TER..... transcutaneous electrical resistance
TG ..... test guideline
Tk ..... thymidine kinase
TNF-alpha ..... tumour necrosis factor alpha
TRA..... targeted risk assessment
U ..... uncertainty
UA ..... uncertainty analysis
UVCB ..... (substance of) unknown or variable composition, complex reaction products or biological materials
vPvB..... very persistent and very bioaccumulative
VSSA ..... volume specific surface area
WoE ..... weight of evidence
XRD ..... X-ray diffraction

European Union legislation

BPR..... Biocidal Products Regulation (EU) 528/2012 concerning the making available on the market and use of biocidal products
CLP ..... Regulation (EC) 1272/2008 on the classification, labelling and packaging of substances and mixtures
FIC ..... Regulation (EU) 1169/2011 on provision of food information to consumers
REACH ..... Regulation (EC) 1907/2006 concerning the registration, evaluation, authorisation and restriction of chemicals

Organisations, committees, and groups

CEN ..... European Committee for Standardization
DTI ..... Danish Technological Institute
EC ..... European Commission
ECETOC ..... European Centre for Ecotoxicology and Toxicology of Chemicals
ECHA ..... European Chemicals Agency
ECVAM ..... European Centre for the Validation of Alternative Methods
EEA..... European Environment Agency
GAARN ..... Group Assessing Already Registered Nanomaterials
ICG..... Informal Correspondence Group
ISO..... International Standardisation Organisation
JRC ..... Joint Research Centre
NMWG ..... Nanomaterial Working Group
OECD..... Organisation for Economic Co-operation and Development
SCENIHR..... Scientific Committee on Emerging and Newly Identified Health Risks

UNSCEGHS . United Nations Economic and Social Council's Sub-Committee on Experts on Globally Harmonized System of Classification and Labelling of Chemicals

WPMN..... Working Party of Manufactured Nanomaterials

1. INTRODUCTION

1.1. Scope

The overall goal of NANoREG is to support regulatory authorities, and also industry, in dealing with environmental health and safety (EHS) issues of manufactured nanomaterials (NMs[1]).

Based on questions and requirements of regulatory relevance identified by the NANoREG partners in co-operation with those stakeholders (NANoREG Deliverable D1.1), the project shall: i) scientifically evaluate data and test methods that already exist or are becoming available and for which the regulatory relevance is still unclear or unproven; ii) propose options for solutions to EHS issues of NMs based on existing data and information, complemented with new knowledge; iii) for the short to medium term, provide a set of fully developed tools applicable to NMs covering all steps of the risk assessment process including physicochemical characterisation, (eco)toxicity testing, and exposure monitoring and control; iv) for the long-term, develop new testing strategies for NMs adapted to innovation requirements; and v) establish a close collaboration among authorities, industry and science to create the basis for common approaches, regulatory acceptable datasets and risk management practices.

Data, information and tools addressing EHS aspects of NMs which have been generated and/or evaluated during the project, as well as relevant publications from scientific literature, form the knowledge base for developing the NANoREG framework (task 1.4, Deliverable D1.11) and related NANoREG toolbox (task 1.7, Deliverable D1.12 2016). Both instruments (the framework and the toolbox) have been developed via a collective effort of project partners under JRC's leadership and are supported by a NANoREG-wide consensus.

The scope of the NANoREG framework is two-fold. First, the framework analyses the applicability of the current European regulatory framework to NMs and aims at giving concrete, practical guidance to industry and regulatory authorities, such as European agencies, scientific committees and national competent authorities, on how to address NMs in that legislative context, with focus on REACH[2] (European Council and Parliament 2006). To this end, Part I of the framework

[1] Both acronyms NMs and MNMs refer to manufactured nanomaterials and are interchangeable within NANoREG.

[2] The ongoing review of REACH Annexes was not taken into account while drafting this deliverable. Such a process is not concluded yet. Moreover, at the time this deliverable was written, the Appendixes to the ECHA

(sections 2 and 3) illustrates step-by-step how REACH applies to NMs, highlights the differences between NMs and bulk substances, and discusses both applicability and regulatory acceptance of approaches that have been developed so far to address nanospecific issues. Part I of the framework thus serves as a frame of reference for the risk and safety assessment of NMs from a regulatory point of view in the REACH context. At the same time, Part I automatically highlights critical issues in the current safety assessment of NMs, such as physicochemical characterisation, applicability of (eco)toxicological tests, grouping and read-across between NMs and/or between NMs and correspondent bulk form(s).

These findings are then linked with relevant sections in Part II of the framework (sections 4, 5 and 6). The framework indeed recognises that a change of paradigm in the safety assessment of NMs is advocated by several stakeholders[3] to address adequately the critical issues identified in Part I. Part II of the framework therefore examines, from a scientist's point of view, three forward-looking strategies seeking to facilitate/accelerate the implementation of REACH objectives for NMs, while discussing the strategies' benefits and potential limitations. As depicted in figure 1.1, the three strategies include: 1) the use of a nanospecific approach for prioritisation and risk assessment of NMs (NanoRA); 2) the development and implementation of the NANoREG Safe-by-Design (SbD) concept; and 3) the integration of Life Cycle Assessment (LCA) and risk assessment in the case of NMs. NanoRA is described as a new strategy for prioritisation and risk assessment of NMs, where approaches for (Quantitative) Structure Activity Relationships ((Q)SARs), grouping and read-across are integrated and expanded, pointing to where and how a more efficient risk assessment of NMs can be performed and what type of information could be used to scientifically justify it in a REACH context. The NANoREG SbD concept is built upon the basic idea that safety should be considered and incorporated early on into the design and development of NMs. The integration of outcomes from LCA and risk assessment are considered as necessary for a more comprehensive evaluation of EHS aspects of NMs. These three approaches as well as their possible use within the REACH implementation process are currently debated in the scientific arena, and are not yet fully recognised or accepted by the relevant authorities. The relationship of each approach with REACH indeed still needs to be clearly defined. This is illustrated in figure 1.1 through four triangles that do

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guidance on implementation of REACH providing recommendations for nanomaterials were under consultation (https://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach).

[3] Industry, some national authorities, members of European scientific committees and part of the scientific community.

not touch or interlock yet, as their level and way of interaction with each other is not yet (fully) established.

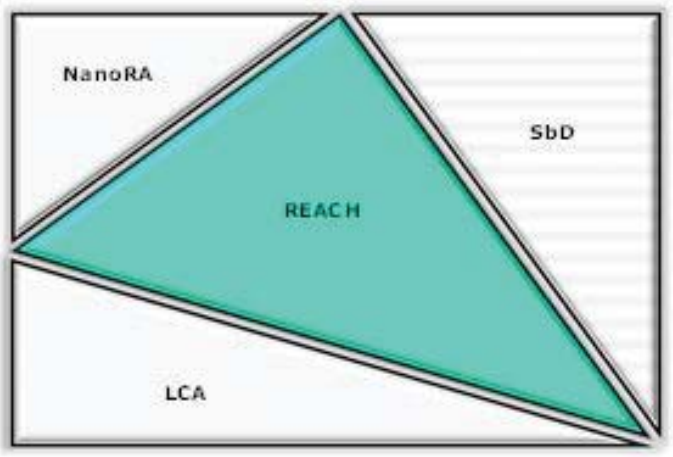

Figure 1.1: The four components of the NANoREG framework. The triangles do not touch or interlock with each other, since each relationship between a forward-looking strategy (grey triangles) and REACH (green triangle) is still to be defined. LCA = Life Cycle Assessment; NanoRA = Nanospecific Risk Assessment; SbD = Safe-by-Design.

In this context, the framework emphasises the need to use the flexibility built into REACH, such as options for adaptation of standard information requirements in Annex XI, and discusses possible ways to (partially) implement those forward-looking strategies under existing conditions.

The framework addresses the needs of both regulatory authorities and industry as it helps the regulatory authorities to formulate those legislative questions that need to be tackled by industry and themsolves for the practical implementation of the REACH requirements and guidance for NMs. Additionally, the framework helps industry to identify what information is currently required and what may be required in the near future by regulatory authorities for the NMs safety assessment.

The NANoREG toolbox (Deliverable D1.12) contains a collection of tools that may be used to address nanospecific aspects at different steps of the safety assessment process under REACH or other legislative frameworks. Those available tools also address the need of tools under the forward-looking strategies discussed in Part II of the framework. The toolbox focuses on 'working tools', i.e. tools that are ready-to-use by industry and authorities, such as public guidance documents, e.g. published by ECHA, or fully developed models that are downloadable from the Internet. The toolbox also differentiates between tools that are already accepted at regulatory level, such as internationally accepted guidelines and standards, and tools that are products of research initiatives, which may have only limited use for industry and authorities.

The framework hence serves as an overarching structure that indicates where and how to apply the assembled tools. The toolbox is organised in worksheets that mirror the structure of the framework document.

A very important achievement was made by reaching a consensus within the NANoREG scientific community on the scope and contents of both the framework and toolbox. ECHA has been involved from the initial stages of development, thus ensuring a strong link with current REACH requirements and possibilities to fulfil those.

1.2. Terminology

Consistent use of terminology is important in any field to ensure common understanding of concepts and tools among experts and stakeholders, such as regulatory authorities, industry, and consumers.

The definition of the terms 'nanotechnology' and 'nanomaterial' has been the subject of many discussions in the last 10 years, resulting in the publication of peer reviewed papers, reports, legislative initiatives, and international standards. For instance, ISO/TC 229 (Nanotechnologies) has published a number of Technical Specifications, i.e. the ISO/TS 80004 series[4], which define an ISO vocabulary in relation to nanotechnology and its applications. A recommended, though not legally binding, definition of a 'nanomaterial' has also been provided by the European Commission (2011). Several other terms in the field of nanomaterials safety have been defined or used by various organisations such as OECD, ECHA and the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), as well as in the scientific community.

Terminology hence plays an important role in the NANoREG internal process of developing both the framework and the toolbox in a collaborative effort across all relevant NANoREG tasks. Moreover, the framework and toolbox are addressed to a large audience of scientists, industry and regulatory bodies, which may extend beyond Europe. A coordinated effort has been made to harmonise the use of specific wording within NANoREG and this is reflected in the terms list of annex I. Definitions have been developed after reaching a consensus among the project partners involved in the drafting of the present document. The sources of information with which these definitions are aligned are primarily European legislation (e.g. REACH, Regulation (EC) N°

[4] http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=68058

1272/2008 on classification, labelling and packaging of substances and mixtures (European Parliament and Council 2008)), documents from international organisations like OECD, ECHA and ECETOC, publicly available documents dealing with terminology produced by other EU projects or shared with NANoREG by individual project partners, and peer-review publications. The terms defined in annex I represent the NANoREG harmonised terminology for safety assessment of NMs, which has been published by JRC as a NANoREG output (Gottardo et al. 2016)[5].

Finally, terminology has to be streamlined and agreed upon also for experimental work. Often, scientists from different fields, and sometimes even from the same field, but from different laboratories, use the same term with a different understanding, or use different terms for the same concept or item. This specific issue has been addressed in the scientific community by creating ontologies. 'Ontology' is a formal and explicit representation of knowledge belonging to a subject area (Thomas et al. 2011). Accordingly, ontology descriptors have been developed within NANoREG in close collaboration with eNanoMapper project (task 1.5) to support the generation of an experimental database where project partners can log their experimental results and thus share data and information in a coherent way. However, this work is beyond the scope of the present document and ontology descriptors are not reported in annex I.

1.3. Sources of information

The framework builds upon the knowledge generated by project partners. In addition, input from: i) other European projects, such as ITS-NANO, MARINA, SUN, GUIDEnano, NanoDefine, NanoValid, ProSafe and NanoReg2, ii) European institutions (e.g. ECHA), iii) international organisations (e.g. OECD), iv) industry-led organisations (e.g. ECETOC) and v) peer-reviewed scientific literature, has been considered to ensure consistency at European level and alignment to the state-of-the-art.

More specifically, input to the analysis of the NMs case under REACH has mainly come from: i) ECHA guidance documents and related appendices containing recommendations for NMs[6], which implement the results of the RIPoN projects[7], and ii) JRC NANO-SUPPORT reports (Task I and

[5] http://publications.jrc.ec.europa.eu/repository/bitstream/JRC100906/jrc%20technical%20report-nanoreg%20terminology%20ehs%20assessement%20nms.pdf

[6] http://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment

[7] http://ec.europa.eu/environment/chemicals/nanotech/reach-clp/ripon_en.htm

II)[8], including proposals of amendment of the REACH Annexes for NMs (still under discussion at EU level[9] at the time the present NANoREG Framework was issued). Input to the discussion on the European Commission's Recommendation on the definition of 'nanomaterial' has mainly come from publicly available JRC reports concerning implementation issues and on-going review process (Linsinger et al. 2012; Rauscher et al. 2014; Rauscher et al. 2015; Roebben et al. 2015). The work on development, adaptation, harmonisation and standardisation of physicochemical and (eco)toxicity testing protocols for NMs, which has been performed by the OECD Working Party on Manufactured Nanomaterials (WPMN)[10], ISO/TC 229[11] and CEN/TC 352[12], has also been considered in the development of the framework.

1.4. Structure of this document

The present document is structured into two parts. Part I focuses on the current regulatory framework and provides guidance on how to best implement the European Commission's Recommendation on the definition of 'nanomaterial' (section 2) and the REACH chemical safety assessment paradigm to NMs (section 3). Part II discusses the forward-looking strategies and the pros and cons of a potential integration into the REACH safety assessment paradigm of approaches that are considered more efficient for NMs by the scientific community. The three forward-looking strategies include: i) use of a nanospecific approach for prioritisation and risk assessment (section 4); ii) development and implementation of the NANoREG Safe-by-Design (SbD) concept (section 5); and iii) integration of Life Cycle Assessment (LCA) and risk assessment in the case of NMs (section 6). Annexes II-V provide useful complementary information to several sections of this document. The framework also conveys a list of take-home messages and final considerations for both Part I and II (section 7).

Each section of the present document is linked to the corresponding worksheet of the toolbox (NANoREG Deliverable D1.12 2016), where all publicly available 'working tools' for that specific step/component of the NMs safety assessment process are listed and briefly described.

[8] http://ec.europa.eu/environment/chemicals/nanotech/reach-clp/nano-support_en.htm
[9] https://ec.europa.eu/growth/sectors/chemicals/reach/nanomaterials_en
[10] http://www.oecd.org/science/nanosafety/
[11] http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_tc_browse.htm?commid=381983
[12] https://www.cen.eu/work/areas/Nanotech/Pages/default.aspx

1.5. References

European Commission 2011. Commission Recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU). Official Journal of the European Union, L 275, pp. 38-40

European Parliament and Council 2006. Regulation (EC) N° 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) N° 793/93 and Commission Regulation (EC) N° 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. 30.12.2006. Official Journal of the European Union, L 396/1

European Parliament and Council 2008. Regulation (EC) N° 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) N° 1907/2006. Official Journal of the European Union, L 353. 31.12.2008

Gottardo S, Quiros Pesudo L, Totaro S, Riego Sintes J, Crutzen H. 2016. NANoREG harmonised terminology for environmental health and safety assessment of nanomaterials. JRC Technical Reports, EUR 27808 EN, European Commission's Joint Research Centre, European Union

Linsinger T, Roebben G, Gilliland D, Calzolai C, Rossi F, Gibson N, Klein C. 2012. Requirements on measurements for the implementation of the European Commission definition of the term "nanomaterial". JRC Reference Report. Publications Office of the European Union. EUR 25404

NANoREG Deliverable D1.1. 2014. Deliverable report for NANoREG. Grant Agreement Number 310584. Report on a Virtual Workshop to identify, formulate and prioritize issues/questions. http://www.nanoreg.eu/media-and-downloads/deliverable-reports/247-deliverable-1-1-regulatory-questions-issues

NANoREG Deliverable D1.12. 2016. Deliverable report for NANoREG. Grant Agreement Number 310584. Toolbox

Rauscher H, Roebben G, Amenta V, Boix Sanfeliu A, Calzolai L, Emons H, Gaillard C, Gibson P, Linsinger T, Mech A, Quiros Pesudo L, Rasmussen K, Riego Sintes J, Sokull-Klüttgen B, Stamm H. 2014. Towards a review of the EC Recommendation for a definition of the term "nanomaterial"; Part 1: Compilation of information concerning the experience with the definition. JRC Scientific and Policy Report. Publications Office of the European Union. EUR 26567

Rauscher H, Roebben G, Boix Sanfeliu A, Emons H, Gibson P, Koeber R, Linsinger T, Rasmussen K, Riego Sintes J, Sokull-Klüttgen B, Stamm H. 2015. Towards a review of the EC Recommendation for a definition of the term "nanomaterial"; Part 3: Scientific-technical evaluation of options to clarify the definition and to facilitate its implementation. JRC Science for Policy Report. Publications Office of the European Union. EUR 27240

Roebben G, Rauscher H, Amenta V, Aschberger K, Boix Sanfeliu A, Calzolai L, Emons H, Gaillard C, Gibson P, Holzwarth U, Koeber R, Linsinger T, Rasmussen K, Sokull-Kluettgen B, Stamm H. 2014. Towards a review of the EC Recommendation for a definition of the term "nanomaterial"; Part 2: Assessment of collected information concerning the experience with the definition. JRC Science for Policy Report. Publications Office of the European Union. EUR 26744

Thomas DG, Pappu RV, Baker NA. 2011. NanoParticle Ontology for Cancer Nanotechnology Research. J Biomed Inform

PART I – CURRENT REGULATORY CONTEXT FOR NANOMATERIALS

Part I of the NANoREG FW illustrates in a stepwise manner how REACH applies to NMs. It highlights the differences between nanomaterials (NMs) and conventional or bulk substances and discusses both applicability and regulatory acceptance of approaches that have been developed so far to address nanospecific issues.

2. DEFINITION OF A NANOMATERIAL IN A REGULATORY CONTEXT

In the European Union, the term 'nanomaterial' has been defined in different documents with regulatory relevance, namely in an overarching non-binding Recommendation published by the European Commission in 2011 (section 2.1) as well as in several sector-specific pieces of legislation (section 2.2). The latter either has implemented the overarching definition provided by the European Commission or uses a dedicated and to some extent different definition of the term.
Outside the European Union, a regulatory definition of the term 'nanomaterial' does not exist yet. In most of the cases, working definitions or descriptions are used and public authorities provide industry with guidance on how to address nanomaterials (NMs) in a regulatory context (Amenta et al. 2015).

The currently available tools (e.g. guidance documents, models, protocols, decision trees), including those developed under NANoREG, for implementing the European Commission's Recommendation on the term 'nanomaterial' are listed in the NANoREG Toolbox (Deliverable D1.12, see worksheet "2 EC Nano Definition").

2.1 European Commission's Recommendation on the definition of 'nanomaterial'

In October 2011 the European Commission published a Recommendation on the definition of the term 'nanomaterial' (2011/696/EU), here subsequently referred to as the EC Definition (European Commission 2011). The purpose of this definition is to clarify when a material should be considered as a NM for regulatory purposes in the European Union. The definition covers natural, incidental and manufactured materials and is based solely on the size of the constituent particles of a material, without regard to specific functional or hazard properties or risks.

The European Commission (2011) recommends the following definition of the term 'nanomaterial':

"'Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm.
In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50% may be replaced by a threshold between 1 and 50%."

The Recommendation further specifies:

"By derogation […], fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.
[…] 'particle', 'agglomerate' and 'aggregate' are defined as follows:
(a) 'particle' means a minute piece of matter with defined physical boundaries;
(b) '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;
(c) 'aggregate' means a particle comprising of strongly bound or fused particles.
Where technically feasible and requested in specific legislation, compliance with the definition […] may be determined on the basis of the specific surface area by volume. A material should be considered as falling under the definition […] where the specific surface area by volume of the material is greater than 60 m^2/cm^3. However, a material which, based on its number size distribution, is a nanomaterial should be considered as complying with the definition […] even if the material has a specific surface area lower than 60 m^2/cm^3."

The salient points of this definition are:

- Legal status: Recommendation (legally non-binding);
- Scope: broad, generic, not limited to certain compositions or application fields;
- Origin of materials: all kinds of origin, i.e. natural, incidental, manufactured;
- Particulate *vs.* non-particulate materials: limited to particulate materials, nanostructured materials generally are not covered (but see the point below referring to constituent particles in agglomerates and aggregates);
- The definition is based on the size (external dimensions) of the particles as the only criterion;
- Size range: one or more external dimensions in the range 1–100 nm, lower limit to exclude large atoms and molecules;
- Threshold: if at least 50% of the particles in a material have one or more external dimensions in the range 1-100 nm, the material is a NM;
- The definition refers to the number fraction of particles in a material;
- Constituent particles are counted, either unbound or in agglomerates or aggregates
- The Volume Specific Surface Area (VSSA) criterion may be used if requested in specific legislation.

Practical guidance needs to be developed for implementing the definition in a regulatory context, as pointed out in several reports by the Joint Research Centre (JRC) of the European Commission

(Linsinger et al. 2012, Roebben et al. 2014). Furthermore, measurement methods must be available for manufacturers, to provide accurate information, and for authorities, to verify the accuracy of the information they receive. These measurements have to meet certain requirements in order to determine whether a material is a NM. Currently, these requirements cannot all be met by a single technique (Linsinger et al. 2012) and therefore a range of measurement methods is needed to test whether a material meets the EC Definition.

In 2013, the JRC started to develop a series of three scientific-technical reports with a common header: "Towards a review of the EC Recommendation for a definition of the term nanomaterial". The reports are based on a list of tasks addressing specific points of the EC Definition, which were agreed initially between the European Commission's policy services and the JRC. The first report collects information on scientific-technical issues that should be considered when reviewing the current EC Definition (Rauscher et al. 2014). In the second report, JRC assesses the information collected between August 2013 and April 2014 from scientists, research institutes, regulatory bodies, non-governmental organisations, and industry regarding implementation of the EC Definition (Roebben et al. 2014). In the third report of the series, JRC describes science and technology based options to clarify the wording and facilitate the implementation of the EC Definition (Rauscher et al. 2015). The options presented in the report are provided to the European Commission's policy services, which are assessing in 2017 whether and how the definition should be revised or supported with additional guidance.

The reports support that the scope of the EC Definition regarding the origin of NMs should remain unchanged, addressing natural, incidental as well as manufactured NMs. Moreover, there is little evidence to support deviating from size as the sole defining property of a nanoparticle or from the range of 1 to 100 nm as definition of the nanoscale.

Certain scientific-technical issues seem to deserve particular attention in terms of clarification of the definition and/or provision of additional implementation guidance:

- The term 'particle': this term should be defined more rigorously for the purposes of the EC Definition to leave less room for interpretation, or detailed guidance for the interpretation of the term should be provided.
- The terms 'particle size' and 'external dimension': 'particle size' and 'external dimension' or more precisely 'minimum external dimension' should be better defined, or more precise guidance on what is considered as (minimum) external dimension should be provided.
- The term 'constituent particle': this term is important for the understanding of the definition but does not appear in the definition itself; the term could be explicitly included in the definition and/or guidance could be issued on the meaning of the term.

- There is a conceptual difference between a threshold for the definition of a NM (number fraction of particles with external dimensions between 1 nm and 100 nm in a material, currently 50%) and the content threshold for such materials in a product; using the phrase *"mainly consisting of particles"* in the definition (rather than the currently used *"containing particles"*) could prevent the misunderstanding that products containing nanoparticles become NMs themselves.
- Consequences of the possibility of varying thresholds for the particle number fraction in the definition: variable thresholds may allow regulators to address specific concerns in certain application areas but may also confuse customers and lead to an inconsistent classification (as NM or not) of the same material based on the field of application.
- Ambiguity on the role of VSSA: the potential use of VSSA should be clarified and ambiguities arising from the current wording should be eliminated; VSSA could either be retained as a proxy or additional criterion but with clearer wording about its use in specific cases, or it could be moved from the text of the EC Definition into guidance as one screening method (among several) for practical implementation of the definition.
- The means and methods to prove that a material is not a NM: the definition makes it very difficult to prove that a material is not a NM; this implementation issue should be resolved, for example, by adding an additional criterion, which might be based on mass, VSSA, or additional size-based parameters.
- The alignment of the EC Definition with other international terminology, if relevant.
- The status of nanostructured materials.

According to the JRC reports, many of the above listed issues could in principle be clarified by developing new or improved guidance. Also the need for specific guidance beyond clarification of the definition itself is identified. JRC provides a number of suggestions on scientific-technical guidance documents that could help in facilitating the practical implementation of the definition.

The text of the EC Definition is currently included as such in the Biocidal Products Regulation (BPR) (European Parliament and Council 2012), in the European Commission's proposal for a Regulation on medical devices (COM(2012) 542 final) (European Commission 2012) and referred to in the Appendixes to the ECHA guidance for implementation of REACH containing recommendations for NMs (e.g. ECHA 2012).

2.2 Other regulatory definitions in the European Union

Specific attention should be given to the harmonisation of the EC Definition and other NM definitions included in European legislation, e.g. in the Regulation N° 1223/2009 on cosmetics products, Article 2 (1) (k), in which 'nanomaterial' is described as *"an insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm"* (European Parliament and Council 2009). Differently to the EC Definition, no specific guidance is provided so far for number size distribution thresholds and potential exceptions therefrom. Other legislation of relevance includes the new Novel Food Regulation 2015/2283 (European Parliament and Council 2015), which refers to the NM definition reported in Regulation 1169/2011 on provision of Food Information to Consumers (FIC Regulation) (European Parliament and Council 2011).

2.3 References

Amenta V, Aschberger K, Arena M, Bouwmeester H, Botelho Moniz F, Brandhoff P, Gottardo S, Marvin HJP, Mech A, Quiros Pesudo L, Rauscher H, Schoonjans R, Vettori MV, Weigel S, Peters RJ. 2015. Regulatory aspects of nanotechnology in the agri/feed/food sector in EU and non-EU countries. Regulatory Toxicology and Pharmacology, 73:463-476

ECHA 2012. Guidance on information requirements and chemical safety assessment. Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7a Endpoint specific guidance. European Chemicals Agency, Helsinki (Finland)

European Commission 2011. Commission Recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU). Official Journal of the European Union, L 275, pp. 38-40

European Commission 2012. Regulation of the European Parliament and of the Council on medical devices, and amending Directive 2001/83/EC, Regulation (EC) N° 178/2002 and Regulation (EC) N° 1223/2009. COM(2012) 542 final

European Parliament and Council 2009. Regulation (EC) N° 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. Official Journal of the European Commission, L 342, p. 59. 22.12.2009

European Parliament and Council 2011. Regulation (EU) N° 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. Official Journal of the European Commission, L 304/18. 22.11.2011.

European Parliament and Council 2012. Regulation (EU) N° 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. Official Journal of the European Union, L 167/1. 27.6.2012

European Parliament and Council 2015. Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. Official Journal of the European Union, L 327/1. 11.12.2015

Linsinger T, Roebben G, Gilliland D, Calzolai C, Rossi F, Gibson N, Klein C. 2012. Requirements on measurements for the implementation of the European Commission definition of the term "nanomaterial". JRC Reference Report. Publications Office of the European Union. EUR 25404

Rauscher H, Roebben G, Amenta V, Boix Sanfeliu A, Calzolai L, Emons H, Gaillard C, Gibson P, Linsinger T, Mech A, Quiros Pesudo L, Rasmussen K, Riego Sintes J, Sokull-Klüttgen B, Stamm H. 2014. Towards a review of the EC Recommendation for a definition of the term "nanomaterial"; Part 1: Compilation of information concerning the experience with the definition. JRC Scientific and Policy Report. Publications Office of the European Union. EUR 26567

Rauscher H, Roebben G, Boix Sanfeliu A, Emons H, Gibson P, Koeber R, Linsinger T, Rasmussen K, Riego Sintes J, Sokull-Klüttgen B, Stamm H. 2015. Towards a review of the EC Recommendation for a definition of the term "nanomaterial"; Part 3: Scientific-technical evaluation of options to clarify the definition and to facilitate its implementation. JRC Science for Policy Report. Publications Office of the European Union. EUR 27240

Roebben G, Rauscher H, Amenta V, Aschberger K, Boix Sanfeliu A, Calzolai L, Emons H, Gaillard C, Gibson P, Holzwarth U, Koeber R, Linsinger T, Rasmussen K, Sokull-Kluettgen B, Stamm H. 2014. Towards a review of the EC Recommendation for a definition of the term "nanomaterial"; Part 2: Assessment of collected information concerning the experience with the definition. JRC Science for Policy Report. Publications Office of the European Union. EUR 26744

3. SAFETY ASSESSMENT OF NANOMATERIALS UNDER REACH

In the European Union, manufacturing and importing of industrial substances is ruled by REACH, i.e. the Registration, Evaluation, Authorisation and Restriction of Chemicals Regulation (European Parliament and Council 2006).

REACH requires that a substance is registered before being placed into the market (section 3.1). The registration dossier must include, as a minimum, data covering the Standard Information Requirements specified in Annexes VII-X of REACH, which vary according to the volume of production (tonnage level) of the substance (section 3.2). The Standard Information Requirements can also be met via submission of data generated through alternative methods to animal testing, including grouping and read-across, *in vitro* methods, weight of evidence (section 3.3). If the substance exceeds 10 tons/year or is classified as having hazardous properties (e.g. carcinogenicity, acute toxicity in aquatic species), a Chemical Safety Assessment (CSA) needs to be performed. The CSA consists of three main steps: th hazard assessment (section 3.4), exposure assessment (section 3.5), and risk characterisation (section 3.6).

Each of the subsequent sub-sections illustrates: how REACH works for substances; how its provisions apply to nanomaterials (NMs)[13]; what the nanospecific issues are; and how these issues can be addressed based on the state-of-the-art on NMs[14].

The currently available tools (e.g. guidance documents, models, protocols, decision trees), including those developed under NANoREG[15], for addressing the nanospecific considerations discussed in the next sub-sections are listed in the NANoREG toolbox (Deliverable D1.12, see worksheets: "3.1 REACH Substance ID", "3.2 REACH Info Requirements", "3.3 REACH Adaptation rules", "3.4 REACH Hazard assessment", "3.5 REACH Exposure assessment", and "3.6 REACH Risk characterisation" – For convenience, the numbering of the worksheets mirrors the numbering of the next sub-sections).

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[13] The ongoing review of REACH Annexes was not taken into account while drafting this deliverable. Such a process is not concluded yet.

[14] At the time this deliverable is written, the Appendixes to ECHA guidance on implementation of REACH providing recommendations for nanomaterials are under consultation.

[15] For example: NANoREG deliverable D2.5 developed a revised substance identification scheme for nanomaterials; NANoREG deliverable D2.4 developed a procedure for identification and quantification of nanomaterial surface treatments; NANoREG deliverable D2.9 proposed nanospecific revisions to existing OECD Test Guidelines; NANoREG deliverable D2.12 further analysed the ongoing revision of ECHA guidance on substance identification and the physicochemical methods that can be used to support the revised procedure for nanomaterials.

3.1. Substance identification

A 'substance' is defined under REACH as *"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"* (European Parliament and Council 2006). The concept of substance therefore goes beyond a pure chemical compound defined by a single molecular structure and includes different constituents such as impurities and additives. Each manufacturer or importer of substances is required under REACH to include in his technical dossier sufficient information in line with REACH Article 10 "Information to be submitted for general registration purposes", which enables the correct and unambiguous identification of the composition(s) of the substance that he intends to register. Annex VI Section 2 of REACH lists the set of information that shall be used to identify a substance (Table 3.1).

Annex VI Section 2 explicitly states that the information provided for each substance shall be sufficient to enable the identification of each substance. Consequently, sufficient information relating to the identity of the substance, its composition(s) and the corresponding analytical data that enable the identity and composition verification needs to be included. Where additional identifiers/characterizers are needed to identify the substance, the information included according to this section needs to address them.

REACH does not define the rules for identifying and naming substances; however, the "Guidance for identification and naming of substances under REACH and CLP" (ECHA 2016a), hereinafter referred to as the Guidance on Substance Identification (Figure 3.1), outlines the principles of substance identification under REACH and provides the elements that can be considered relevant for substance identity. Thus, it is fundamental for proper implementation REACH on aspects related to substance identification. The methodology to be used for identifying and naming a substance must be carefully selected depending on the substance type. Substances can be divided into three main groups: well-defined substances, well-defined substances that require additional identifiers, and substances that qualify as UVCB (Unknown or Variable composition, Complex reaction products or Biological materials) substances.

Table 3.1: Standard information requirements concerning 'substance identification' under REACH Annex VI Section 2. CAS = Chemical Abstracts Service; EINECS = European INventory of Existing Commercial chemical Substances; ELINCS = European LIst of Notified Chemical Substances; IUPAC = International Union of Pure and Applied Chemistry; SMILES = Simplified Molecular-Input Line-Entry system.

INFORMATION REQUIREMENTS IN REACH ANNEX VI SECTION 2 FOR EACH SUBSTANCE	
2	**IDENTIFICATION OF THE SUBSTANCE**
	For each substance the information given shall be sufficient to enable each substance to be identified. If it is not technically possible or if it does not appear scientifically necessary to give information on one or more items below, the reason shall be clearly stated
2.1	**NAME OR OTHER IDENTIFIER OF EACH SUBSTANCE**
2.1.1	Name(s) in the IUPAC nomenclature or other international chemical name(s)
2.1.2	Other names (usual name, trade name, abbreviation)
2.1.3	EINECS or ELINCS number (if available and appropriate)
2.1.4	CAS name and CAS number (if available)
2.1.5	Other identity code (if available)
2.2	**INFORMATION RELATED TO MOLECULAR AND STRUCTURAL FORMULA OF EACH SUBSTANCE**
2.2.1	Molecular and structural formula (including SMILES notation, if available)
2.2.2	Information on optical activity and typical ratio of (stereo) isomer (if applicable and appropriate)
2.2.3	Molecular weight or molecular weight range
2.3	**COMPOSITION OF EACH SUBSTANCE**
2.3.1	Degree of purity (%)
2.3.2	Nature of impurities, including isomers and by-products
2.3.3	Percentage of (significant) main impurities
2.3.4	Nature and order of magnitude (... ppm, ... %) of any additives (e.g. stabilising agents or inhibitors)
2.3.5	Spectral data (ultra-violet, infra-red, nuclear magnetic resonance or mass spectrum)
2.3.6	High-performance liquid chromatogram, gas chromatogram
2.3.7	Description of the analytical methods or the appropriate bibliographical references for the identification of the substance and, where appropriate, for the identification of impurities and additives. This information shall be sufficient to allow the methods to be reproduced

Well-defined substances are substances with a defined qualitative and quantitative chemical composition that can be satisfactorily identified based on the identification parameters of REACH Annex VI Section 2. Rules for identification and naming of well-defined substances differ according to whether there is one main constituent present at concentration greater than 80% (mono-constituent substance) or the main constituent is present at concentration ≥ 10% (w/w) and < 80% (w/w) (multi-constituent substance). In addition to the substance identification parameters as described in Table 3.1, for some well-defined substances (either mono-constituent substances or multi-constituent substances) other information may need to be considered at the substance identity level to get their own, unequivocal substance identification. This could be the case for inorganic minerals, where additional information on crystal phase, size, shape, etc. may be

required. The additional identification parameters are to be chosen on a case-by-case basis depending on the substance type.

On the other hand, UVCB substances cannot be sufficiently identified based on the composition, as they have a large number of constituents and/or the composition is to a significant part unknown and/or the variability of the composition is relatively large. Advice on how to identify and name specific types of UVCB substances (in Figure 3.1: "Substances with variation in the carbon chain length", and "Substances obtained from oil or oil-like sources and enzymes") is provided in the Guidance on Substance Identification. While for simple substances the identification can therefore be straight-forward, for more complex substances identification needs to take factors such as variability in composition, unknown constituents or/and other parameters that are relevant for identification into account.

As an example, in the identification of inorganic substances the crystalline phase is a factor that needs to be taken into account and X-ray diffraction (XRD) is the typical method that is used to verify the crystal phase. Such observations are clearly made in the Guidance on Substance Identification (p. 25): *"For minerals, it is important to combine the results of the elemental composition with the spectral data to identify the mineralogical composition and crystalline structure. This is then confirmed by characteristic physicochemical properties like crystalline structure (as revealed by X-ray diffraction), shape, hardness, swelling capacity, density and/or surface area"* (ECHA 2016a). This is also in line with the general principle of Annex VI Section 2 that the information provided is required to be sufficient to enable the identity of the substance to be verified. Diamond and graphite represent a typical example, also mentioned in the Guidance on Substance Identification, of two substances with the same chemical composition (carbon) but with different crystalline structure. Information on crystalline structure is in this case essential for their appropriate identification and characterization.

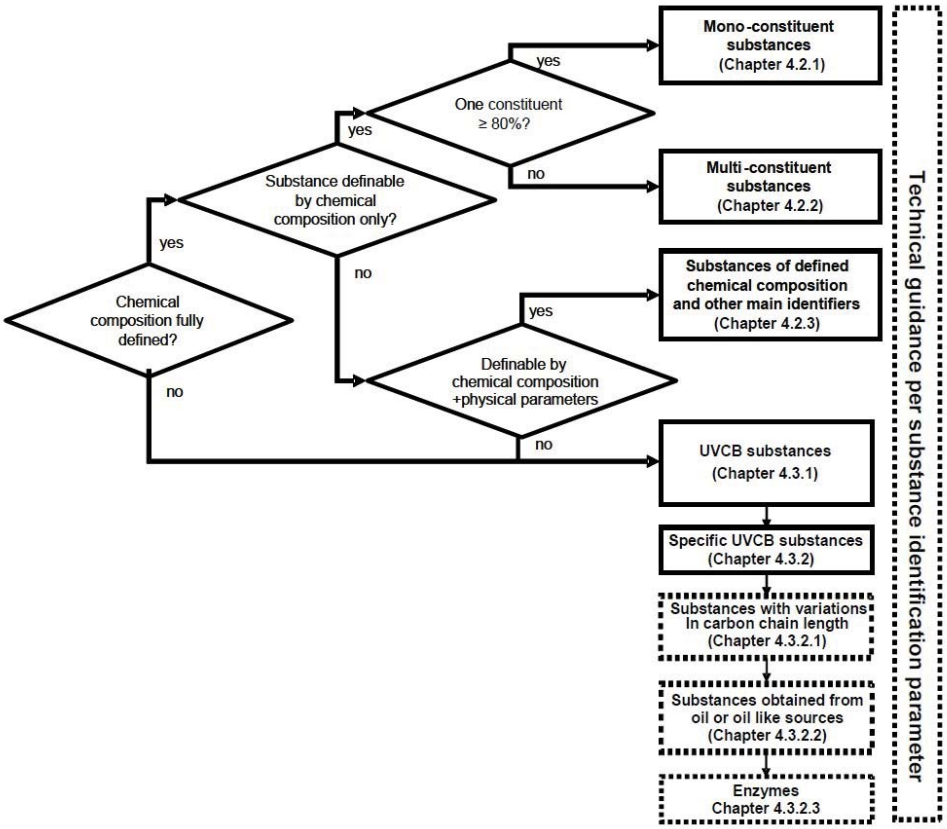

Figure 3.1: Key to relevant chapters of the Guidance on Substance Identification (ECHA 2016a) for various types of substances

Accurate identification of a substance underpins all REACH processes and allows the sharing of information among registrants, which prevents unnecessary animal testing and costs. REACH foresees that substances are registered jointly by parties that manufacture/import the same substance to ensure that costs are kept to a minimum and that animal testing is not duplicated. This is the “One Substance-One Registration” (OSOR) principle where all manufacturers/importers submit a joint registration for the same substance (ECHA 2012). Establishing substance sameness is the responsibility of these parties, and the name and other identifiers chosen by them collectively determine the scope of the registered substance. The criteria to be followed for checking whether or not substances from different manufacturer/importers can be regarded as the same are described in the Guidance for Substance Identification.

In terms of technical reporting in IUCLID dossiers, each registrant is required to include the substance identity information specific for its substance in his own dossier. The name and other identifiers refer to the registered substance and is the same for all registrants of that substance while the composition in a specific dossier refers to a composition of this substance as manufactured/imported by that specific legal entity.

3.1.1. Nanospecific considerations

The identification and naming of a substance under REACH may present challenges when it comes to nanomaterials (NMs). Such nanospecific considerations are illustrated in this sub-section. The currently available tools (e.g. guidance documents, models, protocols, decision trees), including those developed under NANoREG, for addressing the nanospecific considerations discussed in this sub-section are listed in the NANoREG Toolbox (Deliverable D1.12, see worksheet "3.1 REACH Substance ID").

REACH deals with substances in whatever size, shape or physical state they come. Substances at the nanoscale, i.e. NMs, are therefore covered by the definition of 'substance' under REACH and are subject to the same obligations as any substance, which means that sufficient information is required to be included in the dossier to enable safe use of the substance. REACH currently does not explicitly address NMs in the legal text (European Commission 2013), just like it does not explicitly refer to fibres, petroleum substances, enzymes, etc. In the second regulatory review on NMs, the European Commission concluded that REACH offers the best possible framework for the risk management of NMs, but also that within this framework, more specific requirements for NMs have proven necessary (European Commission 2012). As a consequence, a process of revision of the REACH Annexes is currently ongoing and explicit obligations both in the reporting and in the information requirements for NMs are foreseen in the near future (mid 2017). According to ECHA, the term 'nanoform' refers to a particular form of a substance that meets the criteria of the European Commission's Recommendation on the definition of 'nanomaterial' (2011/696/EU) (European Commission 2011), here subsequently referred to as the EC Definition (see section 2 for more information), as opposed to the 'bulk form(s)' of the same substance, i.e. (the) form(s) of the substance not meeting the criteria of the EC Definition. ECHA is preparing an appendix on recommendations for NMs applicable to the Guidance on Registration under REACH[16]. The aim is to define the term nanoform, the minimum criteria for distinguishing between different nanoforms, and the minimum set of parameters which must be reported to characterize a reported nanoform.

The Guidance on Substance Identification (ECHA 2016a) does not include any specific advice for the identification and naming of NMs. However, nanotechnology is mentioned in the chapter concerning "Substances of defined chemical composition and other main identifiers" (Section 4.2.3

[16] https://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach

of the Guidance on Substance Identification, p. 24), where it is stated that the current developments in nanotechnology may cause the need for additional information on size of substances in the future.

The EC Definition comprises the statement: *"Member States, the Union agencies and economic operators are invited to use the following definition of the term 'nanomaterial' in the adoption and implementation of legislation and policy and research programmes concerning products of nanotechnologies"* (European Commission 2011). ECHA applies the EC Definition when implementing REACH and registrants are advised to assess whether their substance or form of a substance meets the criteria outlined in the EC Definition. The EC Definition is at the moment under review (see section 2 for more information); if modified, ECHA guidance may need to be adjusted in the near future.

A registered substance may have compositions that have multiple shapes and sizes (including nanoforms). Some registered substances refer solely to one specific morphology (e.g. nanotubes) while others cover multiple shapes and sizes (e.g. bulk silver, powder silver, and nano silver; bulk copper, granulated copper, flake copper, powder copper, and nanocopper). The question whether substances in nanoform should be regarded as new or existing substances was answered in 2008 during the 6th Meeting of Competent Authorities for REACH and CLP, where it was agreed that *"the decisive criterion whether a nanomaterial is a new or existing substances is the same as for other substances, i.e. whether or not the substance is on EINECS. Thus, substances in nanoform which are in EINECS (e.g. titanium dioxide) shall be regarded as existing substances. Substances in nanoform which are not in EINECS (e.g. carbon allotropes other than those listed in EINECS) shall be regarded as new substances"* (European Commission 2008). Under REACH substances at the nanoscale listed on EINECS are considered as 'phase-in' substances and can benefit from the extended registration deadlines, while substance at the nanoscale which are not listed in EINECS are considered as 'non-phase-in' substances and need to be registered before manufacturing or importing (European Commission 2008). When an existing chemical substance, already placed on the market and registered under REACH as bulk substance, is introduced in the market in a NM form, the registration dossier has to be updated to include specific properties of the nanoform of the substance (Bleeker et al. 2013). Registrants can therefore register all the nanoforms of a substance under a same registration and together with the corresponding non-nanoform. This approach is well in line with the OSOR concept. A registration may cover compositions of a substance having different hazards profiles: UVCB substances may have more than one reported composition that is relevant for hazard assessment; the same applies to mono-constituent substances with different impurities triggering different classification. The situation of multiple nanoforms covered by one registration is analogous to the above mentioned situations, in the

sense that different nanoforms that may also trigger different hazard can be reported under the same registration. However, what is crucial under REACH is that the different compositions and/or the different nanoforms must be covered by the hazard information submitted to demonstrate the safe use of the registered substance.

One aspect that should be taken into particular consideration for nanoforms is the presence of surface treatment, i.e. the modification of the surface chemistry of the particle. The interaction of a particle with its environment is in fact strongly driven by its surface chemistry, and the effect becomes more prominent as the size of particles decreases and the ratio of the specific surface area to the mass increases. Thus, the modification of the surface chemistry of nano-sized particles can have a significant effect on their interaction with the environment and living organisms. Without thorough knowledge of the particle surface chemistry and all deliberate modifications, it is not possible to determine whether the interaction of NMs with their environment is underestimated or not and, consequently, if the hazard information provided for the different forms is applicable also to the modified counterparts.

The particular impact that surface treatment may have on the properties of compositions that fulfil the EC Definition is also explicitly reflected in the recent "Guidance on sample preparation and dosimetry for the safety testing of manufactured nanomaterials" published by the OECD (2012) where the relevance of surface treatment for hazard assessment of NMs is addressed: *"such modifications have been shown to significantly affect the chemical reactivity of a nanomaterial and thereby its potential effects on (or interactions with) living organisms and the environment […] therefore the surface functionality of a nanomaterial is likely to have a strong impact on its (eco)toxicological behaviour"*. In addition, the OECD Working Party on Manufactured Nanomaterials (WPMN) listed surface treatment as an endpoint for phase 1 testing of NMs at the level of "nanomaterial information/identification" in its sponsorship program for testing a number of representative manufactured NMs (OECD 2010).

In light of these considerations, the information included in registration dossiers needs to contain sufficient characterization of surface treated NMs and potential difference in hazard between surface treated and non-surface treated nanoforms should not be underestimated.

While no designated location for reporting surface treatment was available in IUCLID 5, IUCLID 6, which was released in April 2016, includes new "conditionally active" fields to describe composition-related information on NMs (particle number size distribution, shape and aspect ratio, specific surface area and surface treatment), therefore providing the opportunity for registrants to improve clarity when presenting information on nanoforms within their registration dossiers.

ECHA has developed requirements and new specifications for IUCLID 6 on the basis of test cases proposed by industry. A new reporting tool has been developed in this context, the Assessment Entity (AE) reporting tool. The AE has been defined as a wrapper for a set of substance property data (across endpoints) used for assessment purpose. When different compositions with different hazard potential (e.g. bulk *vs* nano) are covered by the same registration, the AE is meant to enable logical grouping of data to facilitate IT processing and a transparent documentation of the safety assessment in IUCLID and the Chemical Safety Report (CSR) (for complex assessment cases). These new different functionalities that have been included in IUCLID 6 therefore represents an opportunity for the assessor to present information on the substance that he intends to register in a transparent manner and to make disseminated information more understandable.
Detailed information on how to use the AE tool in IUCLID 6 and, more in general on all new IUCLID 6 features is available in the ECHA manuals on "Functionalities of IUCLID 6" (ECHA 2016b) and on "How to prepare registration and PPORD dossiers" (ECHA 2016c).

Moreover, a new appendix to the Guidance on Registration under REACH including recommendations for NMs has been prepared by ECHA in order to provide advice to registrants preparing their registration dossiers for NMs. The aim of this document is to provide the registrant with a definition of the term nanoform, the minimum criteria for distinguishing between different nanoforms within a registration dossier, and the minimum set of parameters which must be reported to characterize a nanoform. Such an appendix is currently under consultation. The draft (public) version is downloadable from ECHA website.[17]

3.1.2. References

Bleeker EAJ, Theodori D, Wijnhoven SWP. 2013. Exploring Building Blocks for Amending EU Regulation of Nanomaterials. RIVM report 601353003/2013. National Institute for Public Health and the Environment, The Netherlands

ECHA 2012. Guidance on data sharing. April 2012, v2.0. European Chemicals Agency, Helsinki (Finland)

ECHA 2016a. Guidance for identification and naming of substances under REACH and CLP. June 2016, v1.4. European Chemicals Agency, Helsinki (Finland)

ECHA 2016b. Functionalities of IUCLID 6. European Chemicals Agency, Helsinki (Finland).

ECHA 2016c. Manual. How to prepare registration and PPORD dossiers. April 2016. European Chemicals Agency, Helsinki (Finland)

[17] http://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach

European Commission 2008. Follow-up to the 6th Meeting of the REACH Competent Authorities for the implementation of Regulation (EC)1907/2006 (REACH). 15-16 December 2008. Centre A. Borschette, Rue Froissart 36, 1040 Brussels, Belgium. Nanomaterials in REACH. CA/59/2008 rev.1

European Commission 2011. Commission Recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU). L 275/38. 20.10.11

European Commission 2012. Communication from the Commission to the European and Parliament, the Council and the European Economic and Social Committee: Second Regulatory Review on Nanomaterials. COM(2012) 572 final. Brussels, 3.10.2012

European Commission 2013. Commission Staff Working Document. General Report on REACH. COM(2013) 49 final. Brussels, 5.2.2013

European Parliament and Council 2006. Regulation (EC) No 1907/2006 of the 957 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. Official Journal of the European Union, L396/1, pp. 1-849

OECD 2010. List of manufactured nanomaterials and list of endpoints for phase one of the sponsorship programme for the testing of manufactured nanomaterials: revision. Series on the Safety of Manufactured Nanomaterials N°. 27. ENV/JM/MONO(2010)46. Organisation for Economic Co-operation and Development, Paris (France)

OECD 2012. Guidance on sample preparation and dosimetry for the safety testing of manufactured nanomaterials. Series on the Safety of Manufactured Nanomaterials N°. 36. ENV/JM/MONO(2012)40. Organisation for Economic Co-operation and Development, Paris

3.2. Information requirements

Under REACH, all relevant and available information on the intrinsic properties of a substance must be collected. The type and minimum quantity of information on the intrinsic properties of a given substance that is required depends on the amount of the substance (tonnage level) that is manufactured, imported, or used in the EU. This minimum set of "Standard Information Requirements" is specified in Annexes VI-X of REACH. In this sub-section, a summary is presented for physicochemical properties (Table 3.2), toxicological properties (Table 3.3) (updated according to: European Commission 2016), and ecotoxicological properties (Table 3.4). It is, however, important to recognize that the registrant is required to collect all information that is relevant and available regardless of whether information on a given endpoint is required or not at the specific tonnage level (REACH Annex VII).

For each information requirement, specific rules are reported in Annexes VI-X allowing the registrant to omit, replace, or adapt the required information under particular circumstances (see sub-section 3.3). These rules refer to cases when the study does not need to be conducted, e.g. if the substance is highly insoluble in water or the substance is inorganic.

Table 3.2: Standard information requirements on physicochemical properties of substances to be provided in the REACH registration dossier according to the manufacture/imported substance tonnage level

REACH Standard Information Requirements Physicochemical properties	Annual tonnage level manufactured or imported (t/y)			
	≥1	≥10	≥100	≥1000
7.1. State of the substance at 20 °C and 101.3 kPa	+	+	+	+
7.2. Melting / freezing point	+	+	+	+
7.3. Boiling point	+	+	+	+
7.4. Relative density	+	+	+	+
7.5. Vapour pressure	+	+	+	+
7.6. Surface tension	+	+	+	+
7.7. Water solubility	+	+	+	+
7.8. Partition coefficient n-octanol/water	+	+	+	+
7.9. Flash-point	+	+	+	+
7.10. Flammability	+	+	+	+
7.11. Explosive properties	+	+	+	+
7.12. Self-ignition temperature	+	+	+	+
7.13. Oxidising properties	+	+	+	+
7.14. Granulometry	+	+	+	+
7.15. Stability in organic solvents and identity of relevant degradation products			+	+
7.16. Dissociation constant			+	+
7.17. Viscosity			+	+

Table 3.3: Standard information requirements on toxicological properties of substances to be provided in the REACH registration dossier according to the manufacture/imported substance tonnage level (Updated according to: European Commission 2016)

REACH Standard Information Requirements Toxicological properties	Annual tonnage level manufactured or imported (t/y)			
	≥1	≥10	≥100	≥1000
8.1. Skin corrosion/irritation				
8.1.1 *In vitro* skin corrosion	+	+	+	+
In vivo skin corrosion test to be performed if *in vitro* test not applicable or results not adequate for classification and risk assessment		+	+	+
8.1.2. *In vitro* skin irritation	+	+	+	+
In vivo skin irritation test to be performed if *in vitro* test not applicable or results not adequate for classification and risk assessment		+	+	+
8.2. Serious eye damage/eye irritation				
8.2.1. Serious eye damage/eye irritation, *in vitro*	+	+	+	+
In vivo eye corrosion/eye irritation test to be performed if *in vitro* test not applicable or results not adequate for classification and risk assessment		+	+	+
8.3. Skin sensitization	+	+	+	+
8.4. Mutagenicity				
8.4.1. *In vitro* gene mutation study in bacteria	+	+	+	+
8.4.2. *In vitro* cytogenicity study in mammalian cells or micronucleus study		+	+	+
8.4.3. *In vitro* gene mutation study in mammalian cells		+	+	+
8.5. Acute toxicity				
8.5.1. By oral route	+	+	+	+
8.5.2. By inhalation		+	+	+
8.5.3. By dermal route		+	+	+
8.6. Repeated dose toxicity				
8.6.1. Short-term repeated dose toxicity study (28 days)		+	+	+
8.6.2. Sub-chronic toxicity study (90-day)			+	+
8.6.3. Long-term repeated toxicity study (≥ 12 months)				+
8.6.4. Further repeated dose toxicity studies				+
8.7. Reproductive toxicity				
8.7.1. Screening for reproductive/developmental toxicity		+	+	+
8.7.2. Pre-natal developmental toxicity study			+	+
8.7.3. Extended One-Generation Reproductive Toxicity Study			+	+
8.8. Toxicokinetics				
8.8.1. Assessment of the toxicokinetic behaviour		+	+	+
8.9. Carcinogenicity				
8.9.1. Carcinogenicity study				+

Table 3.4: Standard information requirements on ecotoxicological properties of substances to be provided in the REACH registration dossier according to the manufacture/imported substance tonnage level

REACH Standard Information Requirements Ecotoxicological properties	Annual tonnage level manufactured or imported (t/y)			
	≥1	≥10	≥100	≥1000
9.1. Aquatic toxicity				
9.1.1. Short-term toxicity testing on invertebrates	+	+	+	+
9.1.2. Growth inhibition study aquatic plants	+	+	+	+
9.1.3. Short-term toxicity testing on fish		+	+	+
9.1.4. Activated sludge respiration inhibition testing		+	+	+
9.1.5. Long-term toxicity testing on invertebrates			+	+
9.1.6. Long-term toxicity testing on fish			+	+
9.1.6.1. Fish early-life stage (FELS) toxicity test			+	+
9.1.6.2. Fish short-term toxicity test on embryo and sac-fry stages			+	+
9.1.6.3. Fish, juvenile growth test			+	+
9.2. Degradation				
9.2.1. Biotic				
9.2.1.1. Ready biodegradability	+	+	+	+
9.2.1.2. Simulation testing on ultimate degradation in surface water			+	+
9.2.1.3. Soil simulation testing			+	+
9.2.1.4. Sediment simulation testing			+	+
9.2.2. Abiotic		+	+	+
9.2.2.1. Hydrolysis as a function of pH		+	+	+
9.2.3. Identification of degradation products			+	+
9.3. Fate and behaviour in the environment				
9.3.1. Adsorption/desorption screening		+	+	+
9.3.2. Bioaccumulation in aquatic species, preferably fish			+	+
9.3.3. Further information on adsorption/desorption			+	+
9.3.4. Further information on the environmental fate and behaviour				+
9.4. Effects on terrestrial organisms				
9.4.1. Short-term toxicity to invertebrates			+	+
9.4.2. Effects on soil micro-organisms			+	+
9.4.3. Short-term toxicity to plants			+	+
9.4.4. Long-term toxicity testing on invertebrates				+
9.4.5. Long-term toxicity testing on plants				+
9.5 effects on sediment organisms				
9.5.1. Long-term toxicity to sediment organisms				+
9.6 Toxicity to birds				
9.6.1. Long-term or reproductive toxicity to birds				+

ECHA has developed detailed guidance on REACH information requirements and endpoint-specific guidance (ECHA 2011, 2014, 2015, 2016)[18]. In the guidance, ECHA has specified what parameters need to be reported in the registration dossier for each property listed in Tables 3.2, 3.3 and 3.4 of this document (e.g. when providing information on water solubility (property required in Annex VII and listed in Table 3.2) the registrant is asked to report the value of the saturation

[18] Please note that at the time this document is drafted ECHA guidance is being updated. More information at: https://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach

mass concentration of the substance in water at a given temperature (parameter describing that property), specified in units of mass per volume of solution (kg/m^3)).

3.2.1. Nanospecific considerations

Fulfilling the Standard Information Requirements under REACH may present challenges when it comes to NMs. Such nanospecific considerations are illustrated in this sub-section. The currently available tools (e.g. guidance documents, models, protocols, decision trees), including those developed under NANoREG, for addressing the nanospecific considerations discussed in this sub-section are listed in the NANoREG Toolbox (Deliverable D1.12, see worksheet "3.2 REACH Info Requirements").

Standard Information Requirements (Annexes VII-X of REACH) in principle apply equally to bulk forms (i.e. non-nanoforms) and nanoform(s) of a substance (see sub-section 3.1 for a definition of these terms). While preparing a registration dossier, the registrant has to make sure that the data provided are representative for all the specified form(s) of that substance (sub-section 3.1).

The technical adequacy of the ECHA guidance for implementation of REACH for application to NMs was initially reviewed in the European "REACH Implementation Projects on Nanomaterials" (RIP-oNs) launched by the European Commission in 2009[19]. It provided specific advice on the key aspects of implementation of REACH with regard to NMs concerning Standard Information Requirements and Chemical Safety Assessment (CSA) (JRC 2011, Hankin et al. 2011, Aitken et al. 2011). Based on the outcomes of the RIP-oNs, in 2012 ECHA published a series of appendices to the guidance for implementation of REACH containing recommendations for NMs in relation to the Standard Information Requirements (ECHA 2012a, 2012b, 2012c)[20]. The main points addressed in those appendices are summarized in Tables 3.5, 3.6 and 3.7 of this document. The recommendations published by ECHA partly implement the advice generated by the RIP-oNs (Hankin et al. 2011). Specifically, the appendices implemented those points that were unanimously agreed and recommended to be changed in the review of the RIP-oNs. The appendices are currently under revision. ECHA has recently proposed updates that are under evaluation by the established expert groups[21].

[19] http://ec.europa.eu/environment/chemicals/nanotech/reach-clp/ripon_en.htm
[20] Please not that at the time this document is drafted ECHA guidance is being updated. More information at: https://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach
[21] http://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach

3.2.1.1. Sample preparation

ECHA guidance specifically addresses sample preparation and dosimetry when dealing with NMs (ECHA 2012a, 2012b). Various parameters related to sample preparation have been recognized as highly important for obtaining reliable and repeatable results. Issues that have been raised include, for example, methods to achieve a representative test aliquot from the particulate material, the degree of agglomeration, the difference between dispersed and dissolved particles, and the influence of contaminants and impurities on (eco)toxicological test results. Sample preparation is inherently linked to dosimetry, which together with the biokinetics of nanoparticles, determines the internal dose. Guidance on sample preparation and dosimetry has also been published by OECD (2012a).

3.2.1.2. Physicochemical properties

Table 3.5 illustrates the nanospecific considerations regarding the REACH Standard information Requirements for physicochemical properties.

Table 3.5: Nanospecific considerations regarding the Standard Information Requirements on physicochemical properties in the REACH registration dossier

NANOSPECIFIC CONSIDERATIONS ON REACH STANDARD INFORMATION REQUIREMENTS Physicochemical properties
7.1. State of the substance at 20 °C and 101.3 kPa No nanospecific recommendations provided in ECHA guidance. As no differences are detected between bulk forms and nanoforms, in most of the materials this endpoint is not considered as nanospecific. Nonetheless, in some cases e.g. colour may differ from bulk to nanosize (e.g. depending on the shape and size, gold nanocrystals have different colours ranging from blue-purple to red) (Daniel and Astruk 2004) and this information should be reflected in the dossier.
7.2. Melting / freezing point No nanospecific recommendations provided in ECHA guidance. Nanoparticles exhibit lower melting point temperatures as compared to their bulk counterpart (temperature depression phenomena) because of the large fraction of (more reactive) surface atoms. The melting temperature in nanoparticles is inversely proportional to the radius of the nanoparticles (Goldstein et al. 1992, Burda et al. 2005). For example, the melting point of bulk silver is 962 °C but for a 2 nm diameter silver nanocrystal the melting point drops about 800 degrees below that of the bulk form, i.e. to 127 °C (experimentally) (Little et al. 2012). This information should be properly addressed when characterising the substance in the nanoform and when applying endpoint specific rules. OECD has concluded that the Test Guideline relevant to characterising melting point/melting range (i.e. OECD TG 102) is considered to be applicable to NMs (OECD 2009).
7.3. Boiling point No nanospecific recommendations provided in ECHA guidance. ECHA Guidance (ECHA 2015) advices to use OECD TG 103 for testing boiling point of a substance. OECD (2009) concluded that TG 103, though applicable for determining the boiling point of manufactured NMs, is probably not relevant to existing solid NMs. It should be noted that REACH does not require the determination of boiling point for solids which either melt above 300 °C or decompose before boiling. This in practice means that determination of boiling point may not be required for certain NMs. Yet, the dependency of melting temperature from the radius of a nanoparticle should be properly addressed when characterising the substance in the nanoform and when applying endpoint specific rules. Although the boiling temperature (like the melting temperature) of NMs is expected to decrease when the particle is below a critical size, liquid nanoparticles (or, more accurately, nanodrops formed in the melting process) are expected to coalesce very rapidly to produce a single melt, thus destroying the structure of the NM, which is not expected to be re-established during the cooling process. Consequently, the boiling point determination is extremely unlikely to be a characteristic of the manufactured NM, per se, but of the generic material composition (OECD 2009). In the case of liquid manufactured NMs (nanoemulsions), the act of heating to the boiling point may again destroy the structure of the NM, which is also unlikely to re-establish on condensation; hence, the boiling point determination is for a material in a different form. Furthermore, the multiphase nature of a nanoemulsion means that it is unlikely to have a characteristic boiling point but rather a boiling range (OECD 2009).
7.4. Relative density No nanospecific recommendations provided in ECHA guidance. OECD has concluded that the Test Guideline relevant to characterising relative density (i.e. OECD TG 109) might be applicable under some circumstances or to some classes of manufactured NMs, although further work is required to determine this and adjust the Test Guideline, if necessary (OECD 2009).

7.5. Vapour pressure

No nanospecific recommendations provided in ECHA guidance.

It should be noted that determination of vapour pressure is not required under REACH for substances that have a melting point above 300 °C. This in practice means that determination of vapour pressure may not be required for certain NMs, regardless of any nanospecific changes in the vapour pressure compared to the bulk material.

ECHA Guidance (ECHA 2015) advices to use OECD TG 104 for testing vapour pressure of a substance. The OECD has concluded that TG 104 might be applicable under some circumstances or to some classes of manufactured NMs, hence no further work was planned on adjusting this Test Guideline (OECD 2009).

Although vapour pressure is not considered to be a nanospecific property, nanoparticles can have a significantly higher vapour pressure than that of its bulk counterparts (Cao and Wang 2004) and this information should be addressed when registering a nanoform of a substance.

7.6. Surface tension

No nanospecific recommendations provided in ECHA guidance.

It should be noted that REACH requires information on surface tension only if the substance's structure indicates that surface activity is expected, or if surface activity is a desired property of the material. This in practice means that information on surface treatment may be needed for those nanoforms that meet the aforementioned criteria.

Generally speaking, surface tension is not relevant for NMs, except for the special sub-classes of Janus particles which may exhibit domains of different hydrophilicity (Granick et al. 2009).

In its preliminary review of OECD Test Guidelines and their applicability to NMs, OECD concluded that the Test Guideline relevant to characterising surface tension (i.e. OECD TG 115) might be applicable under some circumstance or to some classes of manufactured NMs. It was stated that this TG is applicable to solutions, but it is not known how the results might be impacted by the presence of a colloidal suspension, which might be present if the sample of manufactured NM does not completely dissolve. Hence, further work is required to determine this and to adjust the TG, if necessary (OECD 2009).

7.7. Water solubility

Nanosized materials may be more soluble than the same substance in bulk form. ECHA guidance (ECHA 2012a) further defines 'solubility' as*: "the degree to which a material (the solute) can be dissolved in another material (the solvent) such that a single, homogeneous, temporally stable phase (a suspension down to the molecular level) results, and is relevant to solids, liquids and gases".* ECHA further specifies that "*the three properties, solubility, hydrolytic stability and acid dissociation constant are inter-related. It is not possible to measure any of these without some knowledge of the other two*", and that, in the case of NMs, the preliminary test assessing solubility might need to be performed by instrumental means rather than visual.

Current methods for solubility assessment of a bulk material could in principle be used for NMs; specific nano-tailored protocols and guidelines are under development (Hartmann et al. 2015, Tantra 2016).

A review of OECD TG 105 (water solubility), with respect to NM testing, is ongoing (OECD 2016a). OECD (2014a) previously concluded that TG 105 is not appropriate for NMs and a new TG should be created to address the dissolution behavior of NMs. OECD suggested that the measurand of interest (beginning with a pre-determined unit of particles in a standardised solution and temperature) is the mass proportion of NMs held in solution. OECD also advised that, whether this mass diminishes after a set period of time or not, the amount of time required for mass to diminish by X % needs to be determined (OECD 2009).

It is important to distinguish between water solubility, as defined by ECHA, and other parameters (e.g. dispersibility, dissolution rate, aggregation, etc.). While such parameters may be of importance to NMs, they may not fall under the definition of water solubility in the ECHA guidance. Both ECHA (2012a) and OECD (OECD 2012a) highlight that it is important not to confuse solubility, which occurs at molecular level, with dispersibility, which occurs at particle level. The distinction between the two can be difficult in case of a colloidal suspensions of NMs. ECHA (based on OECD 2012a) defines dispersibility as the degree to which a particulate material can be uniformly distributed in another material (the dispersing medium or continuous phase).

The state of dispersion is typically assessed using comparative particle size measurements (ECHA 2012a), which requires a reliable method of measuring the baseline particle size distribution of the material. By comparing changes in particle size distribution (including agglomeration/aggregation state), a qualitative assessment or proxy measure of the state of dispersion can be made. Zeta potential measurement, combined with Dynamic Light Scattering (DLS) also enables the stability of nanoparticles dispersions to be monitored and a qualitative understanding of the agglomeration process (ECHA 2012a).

7.8 Partition coefficient n-octanol/water

The current Test Guidelines for n-octanol/water partition coefficient (OECD TG 107, 117, 123) might be applicable under some circumstances or to some classes of NMs, although further work is required to determine this and modify the TGs, if necessary (ECHA 2012a, OECD 2009). Results might be impacted by the formation of a colloidal suspension if manufactured NMs do not dissolve completely (OECD 2009). In case of NMs, it can be difficult to distinguish if a sample is dissolved or dispersed due to the small particle size.

Measurement of the water-octanol partition coefficient for NMs turned out not to be meaningful, as the coefficient relates to distribution of dissolved material between the two phases. NMs, however, are not dissolved but dispersed as particles (or if dissolved, they become bulk forms without the need to apply nanospecific considerations) (OECD 2014a).

7.9. Flash-point

No nanospecific recommendations provided in ECHA guidance.

It should be noted that measurement of the flash-point is not required for inorganic substances, which may exclude a large number of NMs. Furthermore, as the flash-point is a property of liquids, the property may not be relevant for many NMs.

OECD concluded that the Test Guideline relevant to characterising the flashpoint (i.e. OECD TG 113) is considered applicable to NMs (OECD 2009).

7.10. Flammability

No nanospecific recommendations provided in ECHA guidance.

Flammability and explosive properties may differ between nano and bulk form of a same substance (Bouillard 2008). According to Bouillard (2008), most nanopowders display high reactivity characteristics that can lead to fire or explosion accidents, providing support to the suggestion that the REACH information requirements on explosive properties are as relevant for NMs as for bulk materials. The following properties have been defined as important for estimating the explosive risk of NMs: i) particle size, size distribution and shape; ii) surface area and surface charge; and iii) particle and surface composition. The author further reports that several commonly applied methods for explosivity studies are unsuitable for nanopowders, namely: i) current modified, open-ended Hartmann tubes (used to visualise ignition of powders and measure the minimal ignition energy), due to the potential release of nanoparticles during the experiment; ii) current falling hammer equipment used to measure mechanical stability with regards to shock/impact. Both methods were adapted for NMs by the NANOSAFE2 project.

Bouillard (2008) highlights that there is not enough supporting evidence available in the literature to judge whether there may be the potential for read-across of explosivity data from bulk equivalents to NMs. In addition, it is suggested by others that read-across of explosivity data from bulk materials to NMs is not possible, since NMs may have explosive properties which are solely due to the small particle size (RIVM 2009).

7.11. Explosive properties

No nanospecific recommendations provided in ECHA guidance.

See 7.10. Flammability.

7.12. Self-ignition temperature

No nanospecific recommendations provided in ECHA guidance.

7.13. Oxidising properties

No nanospecific recommendations provided in ECHA guidance.

7.14 Granulometry

In ECHA guidance (ECHA 2012a), granulometry is defined as the determination of particle size distribution. When a group of particles are of differing sizes, they may be described by a particle size distribution. The guidance further specifies that in the case of NMs, shape and specific surface area are inseparable parts of granulometry. Thus, additional information on these two properties should also be provided. Available techniques specific for the determination of particle size distribution of NMs are summarized in ECHA guidance (ECHA 2012a). The OECD Test Guideline 110, Method B (electron microscopy), is considered applicable to NMs, but not Method A (sedimentation of centrifugation) (ECHA 2012a). Additional information on sizing techniques has been published by OECD (2014a, 2016a, 2016b) and CEN/TC 352[22].

The ECHA guidance (ECHA 2012a) further specifies that the data on particle size distribution should contain information on: suspending medium, concentration (relevant to particles or fibres), representative image(s) from microscopy, particle size distribution histogram from the applied measurement technique, average particle size(s) for resolvable peaks in the distribution, as mass number and surface area per unit volume as appropriate, among others. It is also assumed that particle size distribution is available as a histogram.

Particle size distribution should not only be measured for the material under investigation but also for the airborne dust (dustiness), where appropriate, as it may influence the decision regarding which route of administration is most appropriate for the acute toxicity and repeat dose toxicity animal studies. A number of methods are provided for determining the particle size fractions, which are then used to assess the possible health effects resulting from inhalation of airborne particles in the workplace. Generally, dustiness, which is the propensity of a material to become suspended in air, is of interest when NMs are manufactured or handled. As highlighted by the OECD, *"the methods that are readily available were generally developed with an aim to assess the likelihood of workplace exposures to powders and were not designed with nanomaterials in mind. An additional challenge is that many methods require a large mass of material which is often not available for nanomaterials"* (OECD 2012a). As identified in the RIP-oN 2 report, some methods alter the pristine NMs and can fracture aggregated/agglomerated NMs into smaller entities (NanoCare 2009). The methods chosen needs to take these factors into account. The Vortex Shaker and the Rotating drum method have been specifically developed for NMs (Nanogenotox 2012, Rasmussen et al. 2013). Both methods are currently under standardisation at CEN level.

7.15. Stability in organic solvents and identity of relevant degradation products

No nanospecific recommendations provided in ECHA guidance.

7.16. Dissociation constant

No nanospecific recommendations provided in ECHA guidance.

This endpoint should be taken into consideration especially when dealing with surface treated nanoparticles. OECD (OECD 2012a) highlighted that surface acidity (related to dissociation constants of surface ionisable sites) is an aspect of surface chemistry that may be particularly relevant, noting that: ionisable sites may influence the surface charge, which has been considered significant in toxicological studies; and surface ionisation may also play a major role in colloidal particle stability and even inhibit migration into hydrophobic phases (e.g. octanol/water partition coefficients).

OECD concluded that the Test Guideline relevant for dissociation constant characterization (i.e. OECD TG 112) might be applicable under some circumstances or to some classes of manufactured NMs. It stated that this TG is applicable to solutions but it is not known how the results might be impacted by the presence of a colloidal suspension, which might be present if the sample manufactured NM does not completely dissolve. Hence, further work is required to determine this and to modify the TG, if necessary (OECD 2009). This TG is currently referenced in the ECHA guidance (ECHA 2015), and it is the only one suggested for testing.

7.17. Viscosity

No nanospecific recommendations provided in ECHA guidance.

OECD concluded that the Test Guideline on relevant viscosity characterization (i.e. OECD TG 114) is not applicable to manufactured NM or, if applicable, provides no useful information. TG 114 is only applicable to liquids and does not refer to solutions, suspensions or emulsions (OECD 2009). Although the viscosity of a solution can be measured, standardized preparation procedures are needed to be included but are not given in TG 114. Additionally, it is unknown what impact a colloidal suspension may have on the results. It is not clear yet what the importance of this property might be for the behaviour of NMs, both in the environment and in living organisms. At the same time, there is the need to define the medium or media in which such suspensions should be assessed.

[22] https://standards.cen.eu/dyn/www/f?p=204:32:0::::FSP_ORG_ID,FSP_LANG_ID:508478,25&cs=18E152154F73BA190A16C4D279047F5FD

Nanospecific considerations on other physicochemical properties and endpoints (e.g. shape, surface area, agglomeration/aggregation, adsorption/desorption), which are not explicitly mentioned in REACH Annexes VII-X but are considered relevant when fulfilling the Standard Information Requirements, can be found in annex II of this document.

3.2.1.3. Toxicological properties

Table 3.6 illustrates the nanospecific considerations regarding the REACH Standard Information Requirements for toxicological properties.

Nanospecific considerations on other toxicological properties and endpoints (e.g. respiratory tract corrosion and irritation, oxidative stress, short-term inhalation studies), which are not explicitly mentioned in REACH Annexes VII-X but are considered relevant when fulfilling the Standard Information Requirements, can be found in annex II of this document.

Table 3.6: Nanospecific considerations regarding the Standard Information Requirements on toxicological properties in the REACH registration dossier

NANOSPECIFIC CONSIDERATIONS ON REACH STANDARD INFORMATION REQUIREMENTS Toxicological properties
8.1 Skin corrosion/irritation The standard test methods are applicable also for testing the effects of NMs. However, non-testing approaches (e.g. (Q)SAR, grouping and read-across) can be applied on a case-by-case basis only, and require detailed scientific justification (ECHA 2012a).
8.2 Serious eye damage/eye irritation The standard test methods are applicable also for testing the effects of NMs. However, non-testing approaches (e.g. (Q)SAR, grouping and read-across) can be applied on a case-by-case basis only, and require detailed scientific justification (ECHA 2012a).
8.3 Skin sensitization The standard test methods are applicable also for testing the effects of NMs. However, non-testing approaches (e.g. (Q)SAR, grouping and read-across) can be applied on a case-by-case basis only, and require detailed scientific justification (ECHA 2012a).
8.4. Mutagenicity The majority of the test methods are applicable also for testing the effects of NMs. However, the bacterial reverse mutation test (Ames test) is not considered reliable for the assessment of NMs and should not be used for mutagenicity testing on NMs (ECHA 2012a, 2013a, OECD 2014b). Non-testing approaches (e.g. (Q)SAR, grouping and read-across) can be applied on a case-by-case basis only and require detailed scientific justification (ECHA 2012a).
8.5. Acute toxicity When selecting the exposure route, it is important to remember that the route of exposure should reflect the most likely route of human exposure. For NMs, inhalation may be the most likely route of exposure. ECHA may require testing by inhalation when the substance is a solid with inhalable particle size (ECHA 2015). When performing acute inhalation toxicity studies with NMs, it is important to include aspects on lung overload in the interpretation of the study results (ECHA 2012a). Non-testing approaches (e.g. (Q)SAR, grouping and read-across) can be applied on a case-by-case basis only and require detailed scientific justification (ECHA 2012a).
8.6. Repeated dose toxicity Inhalation may be the most likely route of exposure for NMs. When performing repeated dose inhalation toxicity studies with NMs, it is important to include aspects on lung overload in the interpretation of the study results. For details, see ECHA (2012a). Non-testing approaches (e.g. (Q)SAR, grouping and read-across) can be applied on a case-by-case basis and this requires scientific justification (ECHA 2012a). The RIPoN-2 report recommended the analysis of the bronchoalveolar lavage fluid (BALF) (cell count and total protein) as an additional endpoint or measurand (Hankin et al. 2011). The addition of this endpoint and lung burden measurements were also discussed at the OECD inhalation toxicity testing expert meeting (OECD 2012b). Currently, OECD TG 412 and 413 are under review to address NMs by including evaluation of bronchoalveolar lavage (BAL) when testing gases, vapours, and aerosols, and lung burden measurements.
8.7. Reproductive toxicity ECHA guidance does not provide nanospecific recommendations on testing reproductive toxicity for NMs. The available standard test methods can be considered as applicable to NMs. Non-testing approaches (e.g. (Q)SAR, grouping and read-across) can be applied on a case-by-case basis and this requires scientific justification (ECHA 2012a).

8.8. Toxicokinetics Physicochemical characteristics of a substance may be modified in the test systems because of metabolism transformations or other physicochemical changes. These potential modifications may change the toxicokinetics behaviour of the substance compared to what is expected from the parent substance, before being tested. In the case of NMs, ECHA guidance recommends paying special attention to these potential modifications during the toxicokinetics evaluation. ECHA guidance also underlines the consideration of translocation of nanoparticles across the gastrointestinal wall in the models to predict absorption and bioavailability. Nanoparticles' translocation may also occur for other uptake routes (ECHA 2012a, 2012c). In a discussion on best practices for hazard assessment of NMs, the ECHA Group Assessing Already Registered Nanomaterials (GAARN) encouraged evaluating toxicokinetic data for grouping and read-across as well as for extrapolation of information from in vitro to in vivo. Such data should also be considered when defining the testing strategy for ecotoxicological endpoints (ECHA 2013a). In the working document by ECHA/RIVM/JRC (2016), information on toxicokinetic behaviour is also considered important in the substantiation of read-across between nanoforms.

3.2.1.4. Ecotoxicological properties

Table 3.7 illustrates the nanospecific considerations regarding the REACH Standard Information Requirements for ecotoxicological properties.

Table 3.7: Nanospecific considerations regarding the Standard Information Requirements on ecotoxicological properties in the registration dossier

NANOSPECIFIC CONSIDERATIONS ON REACH STANDARD INFORMATION REQUIREMENTS Ecotoxicological properties
9.1. Aquatic toxicity OECD (2014c) suggested three tiers of decision trees for establishing: firstly, how the stock/stem solution for a NM should be prepared; secondly, how the exposure solution should be prepared; and thirdly, how the actual aquatic toxicity test should be conducted. These tiers involve various pilot tests, aiming at stability and realism of the testing conditions. It was also suggested that grouping on basis of material properties and characteristics, mode of action etc. is used for identifying NMs for which the same testing protocol can be used. When performing toxicity testing on fish with NMs, it is recommended to collect data on the following parameters as supportive evidence: fish ventilation rate, gill pathologies, mucus secretion, brain pathology, animal behaviour and activity levels of enzymes (catalase, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase) (ECHA 2012b). Regarding algal tests, OECD (2014c) recommends that the assay to be used is tested in advance for lack of interference due to particle presence, which has been reported to confound the measurement of algal cell counts. NM photoreactivity and effect on the availability on solved nutrients should also be considered in algal tests.
9.2. Degradation OECD (2014c) identified degradation (abiotic and biotic) of NMs among the important pieces of information to be known before further tests in water compartments are conducted, and included it in a planned decision tree, stating that TGs need to be developed for appropriate degradation tests. ECHA (2012b) clarified that a majority of OECD TGs on biodegradability are applicable for those NMs that are of organic nature. Moreover, despite the fact that many NMs are inorganic and even carbon-based NMs tend to be of inorganic nature, surface coating and functionalization might be organic and consist of biodegradable materials. If several conclusive aerobic tests indicate very low or negligible degradation, it may be concluded that the substance is not biodegradable, without performing further tests (ECHA 2012b).

9.3.1. Adsorption/desorption screening

With regard to NMs, the distribution coefficient K_d has to be based on actual testing using one of the methods for the measurement of adsorption, since estimations of K_d derived from the organic carbon-water partition coefficient (K_{OC}) and the octanol-water partition coefficient (K_{ow}) have no or questionable merit when it comes to NMs (ECHA 2012a).

OECD (2014c) concluded that TG 106 (Adsorption -- Desorption Using a Batch Equilibrium Method) is not appropriate for testing the adsorption/desorption of NMs, and that a new adsorption test should be developed, also as a pre-test for TG 312 (Leaching in soil columns).

9.3.2. Bioaccumulation in aquatic species, preferably fish

It is not possible to estimate BCF values from log K_{ow} for those NMs that are dispersed as particles and not in solution (ECHA 2012c) (see also property 7.8 in Table 3.5). For the same reason, OECD concluded that BCF (TG 305) is an inappropriate endpoint for NMs that do not dissolve; however, further research is needed to determine which alternative endpoint (including internalisation rate, attachment efficiency, bioavailable fraction) may be appropriate for those NMs. Dietary exposure route should therefore be used for testing NMs (as a worst-case situation), and the test procedure used should be described in detail. OECD guidance on assessing the apparent accumulation potential for NMs (which provides information on how to test NMs via dietary exposure and how to quantify the accumulation potential in fish) is under development. ECHA guidance (2012c) is also being updated to reflect this.

For BCF measurements of dissolving NMs, information on the form of the substance present in the animal tissue is important (ECHA 2012c).

Non-testing approaches (e.g. (Q)SAR, read-across) can be applied on a case-by-case basis and require scientific justification (ECHA 2012c).

According to OECD (2014c), nanospecific guidance should also be developed for TG 315 (Bioaccumulation in sediment-dwelling benthic oligochaetes) and TG 317 (Bioaccumulation in terrestrial oligochaetes).

9.4. Effects on terrestrial organisms

OECD (2014c) recommended continuing with both wet and dry spiking of soils in order to identify which procedure is the most suitable for testing. It was also recommended to use the same stock solution as in aquatic toxicity tests. The amount of NM accumulating in organisms is seen as likely the key for regulatory policies. It was stated that guidance on detection techniques for NMs in soil is needed, and understanding the state of the NM in soils was considered critical for interpreting results.
Estimates based on partitioning may not be relevant, as substances may be distributed in the environment as particles (ECHA 2012c). Non-testing approaches (e.g. (Q)SAR, grouping and read-across) can be applied on a case-by-case basis only, and require detailed scientific justification (ECHA 2012c).

9.5. Effects on sediment organisms

OECD (2014c) recommended continuing with both wet and dry spiking of sediments in order to identify which procedure is the most suitable for testing. It was also recommended to use the same stock solution as in aquatic toxicity tests. The amount of NM accumulating in organisms is seen as likely the key for regulatory policies. It was stated that guidance on detection techniques for NMs in sediment is needed, and understanding the state of the NM in sediments was considered critical for interpreting results. Estimates based on equilibrium partitioning methods may not be relevant, as substances may be distributed in the environment as particles (ECHA 2012b).

9.6. Effects on birds

No nanospecific recommendations provided in ECHA guidance.

Nanospecific considerations on ecotoxicological properties and endpoints (e.g. NM aging, transformation, detection), which are not explicitly mentioned in REACH Annexes VII-X but are considered relevant when fulfilling the Standard Information Requirements, can be found in annex II of this document.

3.2.2. References

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

Bouillard J. 2008. How to estimate nanoaerosol explosion risk? Dissemination report. October 2008. NANOSAFE DR-152/423-200810-4

Burda C, Chen X, Narayanan R, El-Sayed MA. 2005. Chemistry and Properties of Nanocrystals of Different Shapes. Chem. Rev., 105: 1025-1102

Cao G, Wang Y. 2004. Nanostructures and Nanomaterials Synthesis, Properties and Applications. World Scientific. ISBN: 978-1-86094-415-4

Daniel MC, Astruc D. 2004. Gold nanoparticles: Assembly, supramolecular chemistry, quantum – size-related properties and application toward biology, catalysis and nanotechnology. Chem. Rev., 104: 293-346

ECHA 2011. Guidance on information requirements and chemical safety assessment. Chapter R.2: Framework for generation of information on intrinsic properties. Version 2.1, December 2011. European Chemicals Agency, Helsinki

ECHA 2012a. Guidance on information requirements and chemical safety assessment. Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7a Endpoint specific guidance. April 2012. European Chemicals Agency, Helsinki

ECHA 2012b. Guidance on information requirements and chemical safety assessment. Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7b Endpoint specific guidance. April 2012. European Chemicals Agency, Helsinki

ECHA 2012c. Guidance on information requirements and chemical safety assessment. Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7c Endpoint specific guidance. April 2012. European Chemicals Agency, Helsinki

ECHA 2013a. Assessing human health and environmental hazards of nanomaterials - Best practice for REACH Registrants. Second GAARN meeting Helsinki, 21-22 January 2013. European Chemicals Agency, Helsinki

ECHA 2014. Guidance on information requirements and chemical safety assessment. R7c Endpoint specific guidance. Version 2.0, November 2014. European Chemicals Agency, Helsinki

ECHA 2015. Guidance on information requirements and chemical safety assessment. R7a Endpoint specific guidance. Version 4.1, October 2015. European Chemicals Agency, Helsinki

ECHA 2016. Guidance on information requirements and chemical safety assessment. R7b Endpoint specific guidance. Version 3.0, February 2016. European Chemicals Agency, Helsinki

ECHA/RIVM/JRC 2016. Usage of (eco)toxicological data for bridging data gaps between and grouping of nanoforms of the same substance – Elements to consider. Technical and Scientific Report ED-02-16-228-EN-N. European Chemicals Agency (ECHA), Joint Research Centre (JRC), Dutch National Institute for Public Health and the Environment (RIVM), Helsinki, Finland.

European Commission 2016. Commission Regulation (EU) 2016/863 of 31 May 2016 amending Annexes VII and VIII to Regulation (EC) N° 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards skin corrosion/irritation, serious eye damage/eye irritation and acute toxicity (Text with EEA relevance). Official Journal of the European Union, 1.6.2016, L 144/27

Goldstein AN, Echer CM, Alivisatos AP. 1992. Melting in semiconductors nanocrystals. Science, 256: 1425-1427

Granick S, Shan J, Qian C. 2009. Janus particles. Physics Today, 62 (7): 68

Guadagnini R, Halamoda Kenzaoui B, Walker L, Pojana G, Magdolenova Z, Bilanicova D, Saunders M, Juillerat-Jeanneret L, Marcomini A, Huk A, Dusinska M, Fjellsbø LM, Marano F, Boland S. 2015. Toxicity screenings of nanomaterials: challenges due to interference with assay processes and components of classic in vitro tests. Nanotoxicology, 9 sup1: 13-24

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

Hartmann NB, Jensen KA, Baun A, Rasmussen K, Rauscher H, Tantra R, Cupi D, Gilliland D, Pianella F, Riego Sintes JM. 2015. Techniques and Protocols for Dispersing Nanoparticle Powders in Aqueous Media – is there a Rationale for Harmonization? J Tox Environ Health, Part B, 00: 1-28

JRC 2011. REACH Implementation Project Substance Identification of Nanomaterials (RIP-oN 1). AA N°070307/2009/D1/534733 between DG ENV and JRC. Advisory Report. March 2011. Joint Research Centre, Ispra (Italy)

Little SA, Begou T, Collins RW, Marsillac S. 2012. Optical detection of melting point depression for silver nanoparticles via in situ real time spectroscopic ellipsometry. Applied Physics Letters, 100(051107): 1-3. doi: 10.1063/1.3681367

NanoCare 2009. Health-related aspects of synthetic nanomaterials – Final Scientific Report. DECHEMA e.V., Germany

Nanogenotox 2012. Deliverable 4.6: Dustiness of NANOGENOTOX nanomaterials using the NRCWE small rotating drum and the INRS Vortex shaker

OECD 2009. Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials. ENV/JM/MONO(2009)21. Organisation for Economic Co-operation and Development, Paris

OECD 2012a. Guidance on sample preparation and dosimetry for the safety testing of manufactured nanomaterials. OECD Environment, Health and Safety Publications. Series on the Safety of Manufactured Nanomaterials N° 36. ENV/JM/MONO(2012)40. Organisation for Economic Co-operation and Development, Paris

OECD 2012b. Inhalation toxicity testing: expert meeting on potential revisions to OECD test guidelines and guidance document. OECD Environment, Health and Safety Publications. Series on the Safety of Manufactured Nanomaterials N° 35. ENV/JM/MONO(2012)14. Organisation for Economic Co-operation and Development, Paris

OECD 2014a. Report of the OECD expert meeting on the physical chemical properties of manufactured nanomaterials and test guidelines. OECD Environment, Health and Safety Publications. Series on the Safety of Manufactured Nanomaterials N° 41. ENV/JM/MONO(2014)15. Organisation for Economic Co-operation and Development, Paris

OECD 2014b. Genotoxicity of Manufactured Nanomaterials: Report of the OECD expert meeting. OECD Environment, Health and Safety Publications. Series on the Safety of Manufactured Nanomaterials N° 43. ENV/JM/MONO(2014)34. Organisation for Economic Co-operation and Development, Paris

OECD 2014c. Ecotoxicology and Environmental Fate of Manufactured Nanomaterials: Test Guidelines. Expert Meeting Report. OECD Environment, Health and Safety Publications. Series on the Safety of Manufactured Nanomaterials N° 40. ENV/JM/MONO(2014)1. Organization for Economic Co-operation and Development, Paris

OECD 2016a. Physical-chemical parameters: Measurements and methods relevant for the regulation of nanomaterials. OECD Environment, Health and Safety Publications. Series on the Safety of Manufactured Nanomaterials N° 63. ENV/JM/MONO(2016)2. Organisation for Economic Co-operation and Development, Paris

OECD 2016b. Physical-chemical properties of nanomaterials: Evaluation of methods applied in the OECD-WPMN testing programme. OECD Environment, Health and Safety Publications. Series on the Safety of Manufactured Nanomaterials N° 65. ENV/JM/MONO(2016)7. Organisation for Economic Co-operation and Development, Paris

Rasmussen K et al. 2013. Synthetic Amorphous Silicon Dioxide (NM-200, NM-201, NM-202, NM-203, NM-204): Characterisation and Physico-Chemical Properties. JRC Repository: NM-series of Representative Manufactured Nanomaterials. EUR report 26046

RIVM 2009. Nanomaterials under REACH: Nanosilver as a case study. RIVM Report

Tantra R, Sikora A, Hartmann N, Riego Sintes J. Comparison of the effects of different protocols on the particle size distribution of TiO2 dispersions. Particuology 19, 35–44. 2014

3.3. Rules for adaptation of the standard testing regime

Annex XI of REACH sets out the "General rules for adaptation of the standard testing regime set out in Annexes VII to X". The registrant may adapt the standard testing regime under REACH according to three general rules.

The first rule concerns the cases when (animal) testing does not appear to be scientifically necessary and data may be obtained through other approaches. The adaptations include:

- Use of existing data (from experiments not performed according to GLP or performed according to test methods not recognised by the European Commission or ECHA and from historic human data)
- Weight of Evidence (WoE) (when there is evidence from several independent sources leading to a certain conclusion on a property)
- Qualitative or Quantitative Structure-Activity Relationship ((Q)SAR) (when the substance falls within the applicability domain of the model and the obtained results are scientifically valid, adequate for the purpose of use, and adequately and reliably documented)
- *In vitro* methods (when the model is considered as 'suitable' i.e. well-developed according to internationally agreed ECVAM criteria)
- Grouping of substances and read-across (when substances have structural similarities and results are adequate for the purpose of use, have adequate and reliable coverage of the key parameters addressed in the corresponding test method, cover an exposure duration comparable to or longer than the corresponding test method, and are adequately and reliably documented)

The second rule applies when (animal) testing to fulfil a specific information requirement may be omitted/waived without the need of providing data from other approaches, if it is technically not possible to conduct the study because of the properties of the substance.

The third rule concerns the possibility of omitting/waiving (animal) testing based on the exposure scenarios (i.e. substance-tailored exposure-driven testing), for example when absence of or no significant exposure is demonstrated for all scenarios or when the substance is incorporated in an article and is demonstrated that no release is expected during its life cycle.

3.3.1. Nanospecific considerations

Using the rules for adaptations of Standard Information Requirements under REACH may present challenges when it comes to NMs. Such nanospecific considerations are illustrated in this sub-section. The currently available tools (e.g. guidance documents, models, protocols, decision trees), including those developed under NANoREG, for addressing the nanospecific considerations discussed in this sub-section are listed in the NANoREG Toolbox (Deliverable D1.12, see worksheet "3.3 REACH Adaptation rules").

3.3.1.1. Use of existing data

In many cases, existing data may not be available for the nanoform(s) but for the non-nanoform(s) of the substance (e.g. bulk form(s)) (see sub-section 3.1 for a definition of these terms).

As for any substance, data on a certain information requirement for a certain NM can be available from studies not performed according to GLP or mutually accepted guidelines. The use of such data must be carefully considered and relevant conditions must be met (e.g. that the method is adequate for the purpose, reliable, of sufficient duration, and well-documented).

The existing data should be considered and used as appropriate to develop a suitable testing strategy for NMs.

3.3.1.2. Weight of evidence

Where the Weight of Evidence (WoE) approach is particularly important for NMs is for the use of information from newly developed test methods that may not yet be fully validated. However, the experience on the use of WoE for NMs is limited and based on expert judgment.

Some scientists, e.g. Hristozov et al. (2014) and Cuddy et al. (2016), attempted to apply WoE principles to NMs for hazard and exposure screening.

3.3.1.3. Qualitative or Quantitative structure-activity relationship ((Q)SAR)

When applying (Q)SAR methods to NMs (sometimes referred to as Qualitative or Quantitative Nanostructure-Activity Relationship, (Q)NAR) there are in general no nanospecific adaptations as the general requirements are considered applicable to NMs (Tantra et al. 2015). Several (Q)SAR/(Q)NAR approaches are under development but their use as alternative methods in a regulatory context still needs to be accepted. Most of them are based on very small datasets and this limits their applicability.

3.3.1.4. In vitro methods

When performing risk assessment mostly based on *in vitro* test results, *in vitro* to *in vivo* extrapolation (IVIVE) shall be performed. One of the essential aspects of IVIVE is kinetic information. *In vitro* tests generally do not consider the kinetics of a body as animal tests do: the absorption in the gut, for example, is not considered in an *in vitro* test with liver cells. Thus, *in vitro* test results must be supplemented with kinetic data using kinetic models to enable IVIVE. This approach is valid for non-nano (molecular) substances and there is no reason why this general approach should not be valid for NMs as well.

Some NMs can dissolve into the molecular or ionic form. As a result, such NMs essentially lose their nanoparticle properties and can be dealt with using the same approach applied to the non-nano (molecular) substances. For the NMs that do not dissolve (and are thus durable), there is a high potential for accumulation as no other elimination pathways are currently known besides dissolution. In case of accumulation of molecular substances and NMs, the accurate determination of the kinetics becomes of greater importance for the correct estimation of human health risk as an extrapolation over time needs to be made.

Even though kinetic information in general is just as important for molecular substances as for NMs, the type of kinetic information that is necessary differs (Table 3.8). Available kinetic studies generally demonstrate a distribution pattern for nanoparticles that differs from molecular substances. Particles tend to disappear from the blood very rapidly and distribute to liver, spleen, and to a lesser extent to lung and testis (e.g. Geraets et al. 2014, van Kesteren et al. 2014). It is remarked that comparisons between molecular substances and nanoforms are not always possible as some of the most widely used nanoparticles are not available in molecular form. A few PBPK-

models[23] for nanoparticles have been published based on the paradigm that the distribution is not a diffusion-driven process (as for most organic molecular substances) but a process governed by the active uptake of the nanoparticles by macrophages (e.g. Bachler et al. 2015, Carlander et al. 2016, Lin et al. 2016). This implies that sampling plasma is not suitable to monitor nanoparticle exposure and kinetics.

Kinetic parameters important for IVIVE with NMs are:

- Dissolution rate in the various surroundings (including in macrophages);
- Protein corona composition and size in the various surroundings;
- Absorption (i.e. translocation over the barriers encountered, dependent on the exposure route);
- Some form of uptake rate by macrophages or by monocytes in tissues, which is a very new parameter and thus has a very high uncertainty associated with its determination.

Together with physiological information on macrophage content of tissues, knowledge on uptake rate by macrophages/monocytes should help determine the uptake rate into tissues.

Table 3.8 Comparison of kinetic aspects that distinguish nanoparticles from conventional (molecular) substances. PBPK = Physiologically Based Pharmaco-Kinetic models.

Kinetic aspect	Molecular substances	Nanoparticles
Type of kinetics	Dissolved substance kinetics	Particle kinetics
Substance form	Uniform	Pluriform, also during internal exposure
Linearity	Less/more than or equal to dose-proportional	Less than dose-proportional is observed at higher doses due to agglomeration
Barrier transport	Gradient driven	Against gradient is observed
	0-100%	Mainly low (<10%)
Proteins	Protein binding decrease free fraction, free fraction determines activity	Protein corona formation (may) affect kinetics
Metabolism (enzymatic degradation)	0-100%	Not relevant for metals; maybe for organic-metal combinations
Conjugation	Aids excretion	Probably not relevant
Distribution	Flow and extraction ratio dependent	Uptake by macrophages, thus distribution mainly to tissues with phagocytic capacity
Uptake into tissue	Diffusion driven, carrier mediated	In principle driven by active processes, but passive processes cannot be excluded
Excretion	Renal, hepatic, etc.	Clearance from tissues in general very low

[23] Physiologically Based Pharmaco-Kinetic models (PBPK)

	Renal, hepatic transporters	Mechanism of clearance not fully understood
Accumulation	Possible, both in plasma and tissues	Possible, merely in tissues, hardly in plasma
Mechanism of accumulation	Hydrophobic or bound to cellular structures or proteins	In vesicles
Interactions	Mechanisms known	Unknown
Route-to-route extrapolation	Basic understanding	Unknown, but route-dependent kinetics seem plausible related to changes in physicochemical properties or protein corona
Interspecies differences	Basic understanding	Not clear, some indications
PBPK models	Physiological parameterization is understood	Physiological parameterization is under development

According to Landsiedel et al. (2012) the distinct factors strongly influencing the specific kinetics of NMs (apart from those that also influence the kinetics of molecular substances) are:

- Protein binding to NMs;
- The size (primary particle and agglomeration) of NMs;
- The surface charge of NMs.

In vitro methods are prone to nanospecific issues. The exposure conditions in *in vitro* methods are susceptible to aggregation/agglomeration and subsequent sedimentation, flotation, and protein corona formation, which affect the fate of the NM. Also the read-out of *in vitro* methods can be influenced by NMs, e.g. for light scattering. As a consequence, the outcome of an *in vitro* method is often difficult to interpret for NMs. Efforts are ongoing to develop suitable dispersion protocols, analysis of cellular dose, and quality criteria.

3.3.1.5. Grouping of substances and read-across

The general approach for grouping and read-across (e.g. OECD 2014, ECHA 2015) are in principle applicable to NMs but several additional aspects shall be considered when relevant.

It is recognised that, when it comes to NMs, similarity cannot be based on structural or chemical composition only. The ECHA Group Assessing Already Registered Nanomaterials (GAARN) clarified that while read-across commonly involves substances of different chemical composition but structural similarity, read-across for NMs largely involves different nanoscale materials of the same chemical composition, i.e. different nanoforms of a certain substance addressed in the same REACH registration dossier (ECHA 2013). In addition to the Standard Information Requirements for a substance under REACH (Annexes VII-X, see sub-section 3.2), a full physicochemical characterisation of the NM is recommended, including other properties e.g.: solubility, (rate of) dissolution, specific surface area, particle size and particle size distribution, surface characteristics

(including surface chemistry coating, functionalization, surface charge), hydrophobicity, agglomeration and aggregation, crystalline phase, shape/morphology, rigidity, aspect ratio, photocatalytic properties, porosity and pour density, dustiness, dispersibility, zeta potential, flammability, explosivity, and reactivity (redox potential, radical formation) as well as cellular effects, kinetics, and fate parameters like biopersistence, biodegradation (of coating), biodurability, and (toxic) ion release.

Several recent scientific publications can provide additional details on how these different parameters can be used for grouping and read-across of NMs (e.g. Oomen et al. 2015).

Specific recommendations for NMs are provided by ECHA/RIVM/JRC (2016). This document intends to help the user to design a testing strategy that fulfils the REACH information requirements for the substance and is schematically presented as a stepwise approach. The read-across hypothesis can be substantiated by a toxicokinetic argument related to parameters under "where they go" and a hazard argument related to parameters under "what they do" (ECHA/RIVM/JRC 2016). ECHA is currently updating its guidance on (Q)SAR and grouping of chemicals with recommendations for NMs based on, among the others, the content of this document[24].

3.3.1.6. Adaptations when testing is technically not possible

There could be instances where the technical development is not sufficiently advanced to allow appropriate measurements to be performed (e.g. instrumentation or detection of organic NMs in certain organic matrices can pose such difficulties). Consequently, the testing of NMs should be adapted on a case-by-case basis.

3.3.1.7. Substance-tailored exposure-driven testing

REACH Annex XI describes the specific conditions in which exposure scenarios developed in the Chemical Safety Assessment (CSA) may be used as grounds to omit the testing required in Annexes VII-X. All the described conditions are relevant for NMs. For instance, absence of exposure or no significant exposure to the NM may be demonstrated due to the permanent embedding of the NM in a matrix, resulting in no release in the course of its life cycle.

[24] https://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach

3.3.2. References

Bachler G, von Goetz N, Hungerbuhler K. 2015. Using physiologically based pharmacokinetic (PBPK) modeling for dietary risk assessment of titanium dioxide (TiO_2) nanoparticles. Nanotoxicology, 9: 373-80

Carlander U, Li D, Jolliet O, Emond C, Johanson G. 2016. Toward a general physiologically-based pharmacokinetic model for intravenously injected nanoparticles. Int J Nanomedicine, 11: 625-40

Cuddy MF, Poda AR, Moser RD, Weiss CA, Cairns C, Steevens JA. 2016. A weight-of-evidence approach to identify nanomaterials in consumer products: a case study of nanoparticles in commercial sunscreens. J Expo Sci Environ Epidemiol, 26(1): 26-34. doi: 10.1038/jes.2015.51

ECHA 2013. Assessing human health and environmental hazards of nanomaterials - Best practice for REACH Registrants. Second GAARN meeting. Helsinki, 21-22 January 2013. European Chemicals Agency, Helsinki

ECHA 2015. Read-Across Assessment Framework (RAAF). European Chemicals Agency, Helsinki

ECHA/RIVM/JRC 2016. Usage of (eco)toxicological data for bridging data gaps between and grouping of nanoforms of the same substance – Elements to consider. Technical and Scientific Report ED-02-16-228-EN-N. European Chemicals Agency (ECHA), Joint Research Centre (JRC), Dutch National Institute for Public Health and the Environment (RIVM), Helsinki, Finland

Geraets L, Oomen AG, Krystek P, Jacobsen NR, Wallin H, Laurentie M, Verharen HW, Brandon EFA, de Jong WH. 2014. Tissue distribution and elimination after oral and intravenous administration of different titanium dioxide nanoparticles in rats. Part Fibre Toxicol, 11: 30

Hristozov DR, Zabeo A, Foran C, Isigonis P, Critto A, Marcomini A, Linkov I. 2014. A weight of evidence approach for hazard screening of engineered nanomaterials. Nanotoxicology, 8(1): 72-87. doi: 10.3109/17435390.2012.750695

Landsiedel R, Fabian E, Ma-Hock L, van Ravenzwaay B, Wohlleben W, Wiench K, Oesch F. 2012. Toxico-/biokinetics of nanomaterials. Archives of Toxicology, 86: 1021-1060

Lin Z, Monteiro-Riviere NA, Riviere JE. 2016. A physiologically based pharmacokinetic model for polyethylene glycol-coated gold nanoparticles of different sizes in adult mice. Nanotoxicology, 10: 162-172

OECD 2014. Guidance on grouping of chemicals. Second edition. Series on Testing and Assessment N° 194. Organisation for Economic Co-operation and Development, Environment Directorate, Joint Meeting of the Chemical Committee and the Working Party on Chemicals, Pesticides and Biotechnology, Paris. ENV/JM/MONO(2014)4. Unclassified. 14-April-2014

Oomen AG, Bleeker EAJ, Bos PMJ, van Broekhuizen F, Gottardo S, Groenewold M, Hristozov D, Hund-Rinke K, Irfan M-A, Marcomini A, Peijnenburg WJGM, Rasmussen K, Sánchez Jiménez A, Scott-Fordsmand JJ, van Tongeren M, Wiench K, Wohlleben W, Landsiedel R. 2015. Grouping and Read-Across Approaches for Risk Assessment of Nanomaterials. Int. J. Environ. Res. Public Health, 12: 13415-13434. doi:10.3390/ijerph121013415

van Kesteren PC, Cubadda F, Bouwmeester H, van Eijkeren JC, Dekkers S, de Jong WH, Oomen AG. 2015. Novel insights into the risk assessment of the nanomaterial synthetic amorphous silica, additive E551, in food. Nanotoxicology, 9: 442-52

Tantra R, Oksel C, Puzyn T, Wang J, Robinson KN, Wang XZ, Ma CY, Wilkins T. 2015. Nano(Q)SAR: Challenges, pitfalls and perspectives, Nanotoxicology, 9(5)

3.4. Hazard assessment

The objective of the hazard assessment under REACH is to identify the hazards of the substance with respect to human health and the environment. It encompasses: the collection of all relevant and available information on the intrinsic properties of the substance; the identification of critical effects; the classification and labelling of the substance based on Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures (European Parliament and Council 2008); the calculation of Derived No-Effect Levels (DNEL) for human health and Predicted No-Effect Concentrations (PNEC) for the environment from available testing results and other appropriate information on various endpoints; and the determination of whether the substance should be regarded as a persistent, bioaccumulative, and toxic (PBT) substance or as a (very) persistent, (very) bioaccumulative (vPvB) substance (ECHA 2014).

The hazard assessment includes the following 4 steps.

Step1. Hazard identification

For the identification of the hazards of a substance, all the relevant available data for each information requirement (see sub-section 3.2) must be assessed and integrated in order to determine whether the substance may have adverse effects on human health and/or the environment. Regarding the identification of physical hazards, information on at least flammable, explosive and oxidising properties are necessary. The major part of the hazard identification involves evaluating all existing toxicological and ecotoxicological data to identify the critical effects and estimate the dose descriptors (the relationship between a specific effect of a substance and the dose at which it takes place) for each critical adverse health or environmental effect. The outcome includes e.g. the derivation of No Observed Effect Level/Concentration (NOAEL/NOAEC), Lowest Observed Effect Level/Concentration (LOAEL/LOAEC), or Lethal Dose 50% (LD50) values. When a quantitative dose-response relationship cannot be defined, a semi-quantitative or qualitative analysis is performed (ECHA 2009).

Step 2. Classification and labelling

When the nature and severity of an identified hazard meets the classification criteria, hazard classification is the assignment of a standardised description of this hazard to a substance or a mixture causing harm to human health or the environment. The determination of the appropriate classification and labelling of a substance on its own, in a mixture or in an article is a requirement under REACH and has to be documented both in the registration technical dossier and in the Chemical Safety Report (CSR). Harmonised criteria for classification and labelling have been developed within the United Nations (UN) structure and are compiled in the "Globally Harmonised

System of Classification and Labelling of Chemicals" (GHS), which was adopted in 2002[25]. In the EU, the classification and labelling criteria for substances and mixtures, based on GHS, are provided in Annex I of the CLP Regulation (European Parliament and Council 2008). Guidance on the application of the CLP criteria has been provided by ECHA (2015a, 2015b).

For physical hazards, the hazard classes according to CLP Regulation include: Explosives, Flammable gases, Aerosols, Oxidising gases, Gases under pressure, Flammable liquids and solids, Self-reactive substances and mixtures, Self-heating substances and mixtures, Self-reactive substances and mixtures, Pyrophoric liquids and solids, Substances and mixtures which in contact with water emit flammable gases, Oxidising liquids and solids, Organic peroxides, and Corrosive to metals.

For health hazards, the hazard classes are: Acute toxicity, Skin corrosion/irritation, Serious eye damage/eye irritation, Respiratory or skin sensitisation, Germ cell mutagenicity, Carcinogenicity, Reproductive toxicity, Specific target organ toxicity, and Aspiration hazard.

For environmental hazards, the hazard class is Hazardous to the aquatic environment. One additional hazard class to be considered is Hazardous to the ozone layer.

The corresponding labelling is also stated in the CLP Regulation and guidance is provided by ECHA (2015a, 2015b).

Step 3. Derivation of the hazard threshold levels for human health and the environment

Based on the hazard identification (step 1), the threshold levels for exposure below which risks for human health and for the environment are considered to be controlled have to be derived.

Derived No-Effect Level (DNEL)

The DNEL is the level of exposure to a substance above which humans should not be exposed. For each health effect and each relevant exposure pattern, a DNEL needs to be established.

The DNELs are calculated by dividing the value of the health effect dose descriptor (see Step 1) by an assessment factor (AF). Dose descriptors identified in the hazard assessment are expressed as NOAEL/NOAEC, LD50, etc. Default AFs have been proposed by ECHA (ECHA 2012a) but it may also be appropriate to use other factors as long as justification is provided. Since dose descriptors are often obtained from experimental data, an AF is required to allow for extrapolation between test animals and humans. Furthermore, the AFs are addressing intraspecies differences among

[25] http://www.unece.org/trans/danger/publi/ghs/ghs_welcome_e.html

individuals, differences in exposure duration between experimental setting and real scenarios, issues related to dose-response and the quality of the whole database.

DNELs need to be derived for the different populations likely to be exposed to the substance, i.e. workers, consumers or humans exposed through the environment. In some cases, specific vulnerable subpopulations can be considered such as pregnant women or children. In addition, DNELs can be set for different durations of exposure, normally meaning single/short-term exposure and repeated/long-term exposure (e.g. calculated as worker exposure for 8 h/day). Furthermore, DNELs need to be derived for the relevant routes of exposure: oral, inhalation and/or dermal. After the values have been derived, the lowest DNEL for each exposure pattern is identified and used for risk characterisation (see sub-section 3.6).

In situations, where no safe threshold level can be obtained, it is not possible to derive a DNEL. This is the case for example, for non-threshold carcinogens. In these cases, a semi-quantitative value, known as the Derived Minimal Effect Level (DMEL), may be developed. The DMEL values represent exposure levels where the likelihood that the identified adverse effect occurs in a population is sufficiently low to be of no concern. DMELs can be used later on in the risk characterisation process in the same way as DNELs.

Guidance on the derivation of DNELs and DMELs has been published by ECHA (2012a).

Predicted No-Effect Concentration (PNEC)

The PNEC quantitatively assesses the effects of a substance in the environment by determining the concentration of the substance below which adverse effects in the environmental compartment of concern are not expected to occur. Three main environmental compartments are considered: aquatic (both freshwaters and marine waters), soil and air. In addition, adverse effects need to be assessed for predators exposed via the food chain and microorganisms in wastewater treatment plants. In aquatic environments, the main compartments are the water column and the sediment. In terrestrial ecosystems, the environment is divided into the soil and the 'above soil' compartments covering both e.g. earthworms living in soil and terrestrial organisms. Inland waters that are generally protected against wind (e.g. ponds) may develop a surface layer on top of the water column. This layer forms a special habitat with a special exposure pattern to chemicals, i.e. exposure is mainly via atmospheric deposition and not via the water column.

Because the conditions in laboratory tests differ from natural conditions, it is considered likely that ecosystems are more sensitive to chemicals than are individual organisms in the laboratory. Therefore, test results are not used directly for the assessment but as a basis for extrapolating the PNEC. Two different types of extrapolation methods exist: Species Sensitivity Distribution (SSD) and AF method. In the AF method the result from a laboratory test is divided by an appropriate AF. The sparser the available data, the higher is the AF applied. PNECs are estimated by dividing the

environmental effect dose descriptor with the lowest value by the relevant AF. Long-term tests are preferred over short-term tests, as long-term results give a more realistic picture of effects on the organisms in the course of their entire life cycle. When establishing the size of the AFs, a number of uncertainties have to be addressed to extrapolate from single-species laboratory data to a multi-species ecosystem. These are: intra- and inter-laboratory variation of toxicity data, intra- and inter-species variations (biological variance), short-term to long-term toxicity extrapolation, and laboratory data to field impact extrapolation. The SSD is a statistical distribution and usually requires experimentally determined NOEC values for a number of species from different taxonomic groups. These method aims at calculating a concentration, which is assumed to protect a certain percentage (e.g. 95%) of the species of the ecosystem against toxic effects. The method assumes that the species-specific NOEC values follow a certain distribution function and that this can be applied for other taxonomic groups of species in the environment. When the available data do not fulfil these requirements (which is most often the case), the AF method is used.
Guidance on the derivation of PNECs has been published by ECHA (2008).

Step 4. PBT and vPvB assessment

Substances that persist for long periods of time in the environment and have a high potential to accumulate in biota are of specific concern since their long-term effects are rarely predictable. Having once entered the environment, exposure to these substances is very difficult to reverse by the cessation of emissions. Protection of pristine remote areas from PBT/vPvB substances is particularly difficult, as these substances do not degrade close to their emission sources but may be gradually transported to remote areas.
Environmental persistence is expressed as degradation half-life, i.e. the time required for a 50% reduction of the initial concentration by degradation. Substances that are persistent in the environment, lipophilic, and slowly eliminated by organisms have an elevated tendency to bioaccumulate. Bioaccumulation is the process through which there is an increase of concentration of a substance in an organism compared to the concentration in the surrounding environment. The extent of bioaccumulation is quantitatively expressed by the Bioconcentration Factor (BCF), which is the ratio of the concentration of a contaminant in the organism and its average concentration in water. The transfer process of the contaminant through the food webs is called biomagnification and is measured by the Biomagnification Factor (BMF).
For PBT/vPvB substances, a 'safe' concentration in the environment cannot be established by using the currently available procedures.

The objective of the PBT/vPvB assessment of a substance is to determine if it fulfils the numerical criteria set up under REACH Annex XIII (European Parliament and Council 2011) for persistence, bioaccumulation and toxicity, which are:

Persistence (P)

- half-life in sea water > 60 days, or
- half-life in freshwater > 40 days, or
- half-life in marine sediments > 180 days, or
- half-life in freshwater sediments > 120 days, or
- half-life in soil > 120 days.

Bioaccumulation (B)

- BCF > 2000 L/Kg (aquatic species).

Toxicity (T)

- NOEC < 0.01 mg/l, aquatic species or
- Carc. (cat. 1 or 2), mut. (cat. 1 or 2), or reprotoxic (cat. 1, 2, or 3)

Very persistent (vP)

- half-life in water > 60 days, or
- half-life in sediments > 180 days, or
- half-life in soil > 180 days.

Very bioaccumulative (vB)

- BCF > 5000 l/Kg

The criteria for PBT/vPvB assessment apply to all organic substances, including organo-metals, and generally to any substance containing an organic moiety but are not applicable to inorganic substances (ECHA 2014).

PBT or vPvB substances give rise to 'very high concern' and can be proposed for inclusion in REACH Annex XV ("List of substances subject to authorisation"). The authorisation for a specific use of the substance can be granted only if risks resulting from the use are adequately controlled or if no economically and technically feasible alternative exists.

3.4.1. Nanospecific considerations

The hazard assessment under REACH may present challenges when it comes to NMs. Such nanospecific considerations are illustrated in this sub-section. The currently available tools (e.g. guidance documents, models, protocols, decision trees), including those developed under NANoREG, for addressing the nanospecific considerations discussed in this sub-section are listed in the NANoREG Toolbox (Deliverable D1.12, see worksheet "3.4 REACH Hazard assessment").

Under REACH, NMs are registered in the same dossier as the corresponding bulk substance. Only in cases where there are solid scientific grounds for considering the NM as a distinct substance, the NM can be registered in a separated dossier (see sub-section 3.1.1). For each form of a substance, safe use should be ensured. Additional data, potentially derived from NM specific testing, may thus be necessary to demonstrate the safety of NMs. The provisions that apply to the registration of NMs under REACH are the same that must to be fulfilled for any other chemical substance. However, in line with scientific developments, there are specific considerations that the registrant should report for specific endpoints to facilitate the evaluation of whether the tests performed and the data obtained are adequate for the safety assessment of NMs (e.g. sample preparation, solubility/dispersion, use of stabilisers) (ECHA 2013a). Nanospecific considerations regarding the different steps of the hazard assessment (as previously described) are addressed in the following paragraphs. Other nanospecific considerations (i.e. nanospecific intracellular pathways and effects, nanospecific protocols, and NM carcinogenicity *in vitro*) are reported in annex III of this document.

Step1. Hazard identification

Nanospecific considerations for hazard identification regarding human health and the environment have been analysed in detail as a part of the REACH consultation process under "Specific Advice on Fulfilling Information Requirements for Nanomaterials under REACH Implementation Project on Nanomaterials 2 (RIP-oN2)" (Hankin et al. 2011); nanospecific recommendations formulated during the 2nd GAARN meeting have been published by ECHA (2013a); and the appendixes to ECHA guidance for implementation of REACH containing recommendations for NMs are under consultation at the time the present document is drafted[26].

[26] https://echa.europa.eu/support/guidance/consultation-procedure/ongoing-reach

Nanospecific issues concerning information requirements and collection of data are discussed in sub-section 3.2. Among those, hazard-related nanospecific issues (ECHA 2012b, 2012c, 2013a) include:

- Lung overload

  Issues related to particle overload in rat inhalation studies performed with poorly soluble low-toxicity (PSLT) particles have been identified. It has been argued that observed effects may be a reflection of the experimental conditions and not of the intrinsic potential of the NM to cause, for instance, inflammation or fibrosis. When evaluating and interpreting inhalation studies with NMs, attention should be paid to the doses and any data indicating lung overload.
- Interference with assays

  It is important to note that studies have indicated that NMs may cause inhibition or enhancement in assays related to cytotoxicity. Examples of interference may include direct effects on absorbance or fluorescence or binding to assay components due to the large surface area of the particles. Such effects need to be considered when studies with NMs are evaluated in the hazard identification.
- Mutagenicity / Bacterial mutation assays

  Bacterial mutation assays are not recommended for studying the mutagenicity of NMs as several studies have indicated that NMs are not always capable of penetrating the cell wall of bacteria. The identification of the potential mutagenicity of a NM should therefore be based on data from other types of studies than the Ames test.
- Non testing data

  Currently there is a lack of comprehensive data that could be used as basis for approaches including grouping, read-across or (Q)SAR in the case of NMs. If such approaches are used, the hazard identification step should include, for each hazard endpoint, a critical evaluation of the scientific justification for using those approaches (see sub-section 3.3 for more information).

Step 2. Classification and labelling

The CLP Regulation (European Parliament and Council 2008) explicitly states that it applies to substances and mixtures in all physical states or forms (Art. 9(5)). In ECHA guidance (2015b), it is specified that: *"Putative forms comprise properties such as crystal structure, particle size, homogeneity (e.g. emulsions) and texture (e.g. viscosity or tablet form). Examples of physical state factors are: surface treatment (e.g. coating), state of aggregation, moisture content, residual solvent, activation or stabilisation"*. Accordingly, if the physical state or form of a substance is

changed it has to be evaluated whether this might affect the classification and whether re-testing is necessary. This means the nanoform of a substance can have a different classification compared to the correspondent bulk form if the available relevant information indicates a variation in the hazard properties (European Commission 2009).

The UN GHS Sub-Committee, in its 24th session (UNSCEGHS 2013), agreed to review the applicability of the GHS to manufactured NMs. In this framework, the Informal Correspondence Group (ICG) on NMs was established to make clear if nanoforms of a substance are within the scope of the GHS and to review whether the classification and labelling criteria of GHS are appropriate for nanoforms as well as bulk forms of a substance. In its 28th session (UNSCEGHS 2014) the UN GHS Sub-Committee agreed that the ICG should focus its work on a classification exercise for some selected NMs. This exercise includes review on physical, health and environmental hazard classes. The work is still ongoing[27] and no definite conclusions have yet been drawn. The progress of the work can be followed from the working and informal documents of the ECOSOC Sub-Committee of Experts on the GHS website[28].

One highly important issue in this context is the NM characterization and identification. It is very likely that in some cases there may be a need to classify a certain NM in a different way than the substance in a bulk form or in a slightly different nanoform. To be able to make conclusions on classification, the reports on studies on hazardous properties must contain detailed characterisation data on the material tested. In IUCLID 6 there is already a possibility to choose "nanoform/nanomaterial" from a pick list under the section "Classification and labelling".

Step 3. Derivation of the hazard threshold levels for human health and the environment

Derived No-Effect Level (DNEL)

ECHA published guidance addressing issues to be considered when deriving DNELs for NMs (ECHA 2012d). The guidance focuses on the following issues:

- Metrics

  The choice of metrics, or parameters, is of critical importance since it is not possible to establish a single metric that is applicable to all NM cases. There are many metrics, all of which include mass or number, which are currently used in the risk assessment of NMs (both regulatory and otherwise) across the three elements of exposure, toxicology,

[27] At the time this deliverable is written
[28] http://www.unece.org/trans/main/dgdb/dgsubc4/c4age.html

and risk. The most commonly used metrics have been identified and discussed by Hankin et al. (2011) and ECHA (2013b).

- Mode of action

  For the decision on whether identified hazards are based on threshold or non-threshold mechanisms, it is important to notice that carcinogenic/mutagenic effects may occur also via mechanisms secondary to a threshold effect. In the case of NMs, such situations could for example occur if exposure to poorly soluble nanoparticles results in particle overload and inflammation, triggering oxidative stress and as a final outcome tumour formation. In such cases it may be correct to derive a DNEL and not a DMEL.

- Route to route extrapolation

  If data originating from studies performed using the relevant route of exposure is lacking, REACH allows route-to-route extrapolation from studies using another exposure route. In the case of NMs, there is, however, not much experience from such extrapolations and therefore it is not advised to extrapolate from other exposure routes.

- AFs for interspecies differences

  If the default AFs are not used it is relevant to consider differences in ventilation rates, deposition and clearance between humans and experimental animals when deciding on specific factors to be derived.

- Differences in the duration of exposure

  It should be noticed that in the case of exposure to poorly soluble low toxicity particles by inhalation, exposure at high concentrations may result in local accumulation, further increasing the toxicity following long-term exposure.

- DNEL derivation when an occupational exposure limit value is available

  In some situations it may be justified to use an occupational exposure limit value (e.g. Indicative Occupational Exposure Limit and Binding Exposure Limit established at European level or a national occupational exposure limit) instead of deriving a DNEL. In the case of NMs, it is highly important to consider whether the route and duration of exposure as well as the physicochemical attributes (including size, shape, crystallinity and surface characteristics), which may affect the toxicity, are the same as for the substance for which the occupational exposure limit has been set. If not, the limit value cannot be used in place of a DNEL and a specific DNEL should be derived.

Predicted No Effect Concentration (PNEC)

The outputs concerning PNEC derivation for NMs agreed by Aitken et al. (2011) are implemented in ECHA guidance for implementation of REACH. The current version of the guidance (ECHA 2012e) addresses the following issues:

- Extrapolation methods

  The default AFs can sometimes be changed if properly justified. One of the plausible justifications is when evidence established by read-across from closely related substances can demonstrate the use of a higher or lower AF. In relation to NMs, where there is uncertainty due to the absence of available data, the use of read-across from available data on bulk or other forms of the material to the NM being assessed must be scientifically justified and may be associated with additional uncertainty.

- Equilibrium partitioning methods

  Estimates based on results from equilibrium partitioning methods are limited to the distribution of a substance in molecular form. As NMs may also be distributed in the environment as particles, extrapolation based on partitioning may not be relevant. In such a case, the equilibrium partitioning method may underestimate exposure of soil and sediment environments and overestimate the exposure in water. If the particle size is small, air distribution may also occur (ECHA 2012f).

  The equilibrium partitioning method uses the PNEC for the water compartment and the partitioning coefficient between soil or suspended matter and water as inputs to estimate the PNEC for freshwater sediment, marine sediment and soil. Several factors have to be taken into account when using this method for NMs, including the fact that the method considers only exposure via water phase and not, for example, ingestion of soil or sediment particles to which NMs have been adsorbed (ECHA 2008). To increase the reliability of PNEC for sediment or PNEC for soil estimates derived by using the equilibrium partitioning method, it is important to choose a realistic partitioning coefficient (K_d, K_{ow}, or K_{oc}). Normally, equilibrium partitioning can mainly be applied to neutral organic chemicals; as the method is based on a thermodynamic equilibrium of the concentrations of the substance in the solid and the aqueous phase and in the organism, and such a thermodynamic equilibrium generally does not apply to NMs in the environment, care should be taken when applying the method to NMs and interpreting the results (Praetorius et al. 2014).

  When deriving PNEC values for NMs, it is important to consider the relevance of potential indirect effects that may contribute to the adverse effects observed at environmentally-relevant concentrations or at concentrations that are considered to be safe for the environment (ECHA 2013b).

- Danish study (Lützhøft et al. 2015) on PNEC estimation of engineered NMs

  The key findings include the following:

  - Investigations have shown that currently accepted PNEC estimation approaches within the present European legislation (e.g. REACH) in principle can be used for

NMs as well. This concerns the AF and SSD methods. These methods do, however, not take nanospecific processes (such as aggregation) during the testing of NMs into account and the tests may therefore not always be representative of natural conditions. Based on a literature review performed by Lützhøft et al. (2015), three other methods were suggested: the Probabilistic SSD (PSSD), the dissolved metal ion, and the Indicative No Effect Concentration (INEC).

- The current approach to select data for PNEC estimation favors effect studies conducted according to Good Laboratory Practice (GLP) and accepted guidelines. A consequence is that effect studies conducted according to guidelines for soluble chemicals may be unreliable as they do not take into account the specific nature of engineered NMs.

Step 4. PBT and vPvB assessment

REACH and the associated guidance do not specifically address the PBT or vPvB assessment of NMs. However, considering the scope of the PBT and vPvB assessment, it is expected to be relevant for NMs that have an entirely or predominantly organic chemical nature as well as for NMs that contain (an) organic moiety/ies even if the main chemical structure of the NM is not organic.

The PBT/vPvB criteria are based on persistence, bioaccumulation and toxicity of chemicals, which are strictly related to their behaviour. The behaviour of NMs in the environment is related both to their physicochemical properties and to the environmental compartments where they are released. For this reason the applicability of existing environmental exposure or distribution models is limited. High surface area to volume ratio results in highly reactive and physicochemically dynamic materials in the environmental media. Mobility, stability and transformation are important aspects of NM behaviour in the environment.

NMs dispersed in water behave according to the mechanisms of colloid science. NMs may undergo a number of processes in water, including partitioning to sediment and suspended particulate matter and transformation through abiotic and/or biological degradation. The possibility of bioaccumulation depends on stability/reactivity, elimination and degradation rates of NMs and their degradation/transformation products. The stability of NMs in the aquatic environment depends on their chemical structure, other particle properties (e.g. size and surface coating), as well as environmental conditions. Surface properties of NMs, including hydrophobicity, are identified as critical in determining their transformation and aggregation behaviour, and thus for their mobility in aquatic environment and their ultimate interaction with and general bioavailability to organisms. Surface modifications, both intentional functionalization and modifications due to natural

processes, complicate interactions and ultimate fate and behaviour. In particular, polymeric surface coatings are identified as stabilizers reducing autoaggregation.

While evidence suggests that NMs released into the environment most likely end up in association with sediments and soils, very little is known about how NMs behave in these compartments. Environmental factors such as pH and ionic strength, together with the NM physicochemical properties and interactions with particles, determine whether they are bound within or transported out of soils and sediments. The lack of data is so pronounced, that no general conclusions can be drawn at the moment.

Preliminary information regarding the fate and behaviour of NMs in air can be provided by aerosol science. However, some major issues still require validation, including the effect of differing particle morphologies. There is a need for systematic studies on different types of airborne NMs using a range of physicochemical parameters to generate data and support the development of reliable models.

In conclusion, not much can be said about long-term forecasts related to persistent and bioaccumulative properties of NMs due to the lack of data. Anyway, it can be argued that a PBT substance in bulk form has to be considered as PBT also when it is in nanoform.

3.4.2. References

Aitken RA, Bassan A, Friedrichs S, Hankin SM, Hansen SF, Holmqvist J, Peters SAK, Poland CA, Tran CL. 2011. RNC/RIP-oN3/FPR/1/FINAL 07 July 2011. Specific Advice on Exposure Assessment and Hazard/Risk Characterisation for Nanomaterials under REACH (RIP-oN 3). Final Project Report

ECHA 2008. Guidance on information requirements and chemical safety assessment. Chapter R.10: Characterisation of dose [concentration]-response for environment. May 2008. European Chemicals Agency, Helsinki

ECHA 2009. Guidance in a Nutshell. Chemical Safety Assessment. 30/09/2009. European Chemicals Agency, Helsinki

ECHA 2012a. Guidance on information requirements and chemical safety assessment. Chapter R.8: Characterisation of dose[concentration]-response for human health. November 2015,v2.1. European Chemicals Agency, Helsinki

ECHA 2012b. Guidance on information requirements and chemical safety assessment. Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7a Endpoint specific guidance. April 2012. European Chemicals Agency, Helsinki

ECHA 2012c. Guidance on information requirements and chemical safety assessment. Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7c Endpoint specific guidance. April 2012. European Chemicals Agency, Helsinki

ECHA 2012d. Guidance on information requirements and chemical safety assessment. Appendix R8-15: Recommendations for nanomaterials applicable to Chapter R.8 Characterisation of dose[concentration]-response for human health. May 2012. European Chemicals Agency, Helsinki

ECHA 2012e. Guidance on information requirements and chemical safety assessment. Appendix R10-2: Recommendations for nanomaterials applicable to Chapter R.10 Characterisation of dose [concentration]-response for environment. May 2012. European Chemicals Agency, Helsinki

ECHA 2012f. Guidance on information requirements and chemical safety assessment. Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7b Endpoint specific guidance. April 2012. European Chemicals Agency, Helsinki

ECHA 2013a. Assessing human health and environmental hazards of nanomaterials - Best practice for REACH registrants. Second GAARN meeting Helsinki, 21-22 January 2013. European Chemicals Agency, Helsinki

ECHA 2013b. Human health and environmental exposure assessment and risk characterisation of nanomaterials - Best practice for REACH registrants. Third GAARN meeting Helsinki, 30 September 2013. European Chemicals Agency, Helsinki

ECHA 2014. Guidance on Information Requirements and Chemical Safety Assessment. Part C: PBT/vPvB assessment. November 2014, v2. European Chemicals Agency, Helsinki

ECHA 2015a. Introductory guidance to CLP. November 2015, v2.1. European Chemicals Agency, Helsinki

ECHA 2015b. Guidance on the application of the CLP criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. June 2015, v4.1. European Chemicals Agency, Helsinki

European Commission 2009. Classification, labelling and packaging of nanomaterials in REACH and CLP. Brussels, 3 December 2009. Doc.CA/90/2009 Rev2

European Commission 2011. Commission Regulation (EU) No 253/2011 of 15 March 2011 amending Regulation (EC) No 1907/2006 of the European Parliament and of the European Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards Annex XIII. Official Journal of the European Union. 16.3.2011

European Parliament and Council 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. Official Journal of the European Union, L 353. 31.12.2008

Hankin SM, Peters SAK, Poland CA, Foss Hansen S, Holmqvist J, Ross BL, Varet J, Aitken RJ. 2011. RNC/RIP-oN2/FPR/1/FINAL 01 July 2011. Specific Advice on Fulfilling Information Requirements for Nanomaterials under REACH (RIP-oN 2). Final Project Report

Lützhøft H-CM, Hartmann NB, Brinch A, Kjølholt J, Baun A. 2015. Environmental effects of engineered nanomaterials. Estimations of Predicted No-Effect Concentrations (PNECs). The Danish Environmental Protection Agency. Environmental project No. 1787

Oberdörster G, Oberdörster E, Oberdörster J. 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect, 113(7): 823-839

OECD 2015a. Analysis of the Survey on Available Methods and Models for Assessing Exposure to Manufactured Nanomaterials. Series on the Safety of Manufactured Nanomaterials No. 56. ENV/JM/MONO(2015)20. 03-Jul-2015. Organisation for Economic Co-operation and Development, Paris

OECD 2015b. Guidance document on the in vitro Syrian Hamster Embryo (SHE) cell transformation assay. Series on Testing & Assessment No. 214. ENV/JM/MONO(2015)18. 22-May-2105. Organisation for Economic Co-operation and Development, Paris

Praetorius A, Tufenkji N, Goss K-U, Scheringer M, von der Kammer F, Elimeleche M. 2014. The road to nowhere: equilibrium partition coefficients for nanoparticles. Environmental Science: Nano, 1(4): 317-323

UNSCEGHS 2013. Report of the Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals on its twenty-forth session held in Geneva from 12 to 14 December 2012. United Nations Secretariat, 3 January 2013

UNSCEGHS 2014. Report of the Sub-Committee of Experts on the Globally Harmonized System of Classification and Labelling of Chemicals on its twenty-eighth session held in Geneva from 10 to 12 December 2014. United Nations Secretariat, 23 December 2014

3.5. Exposure assessment

The text below illustrates how an exposure scenario is defined (sub-section 3.5.1) and exposure is estimated (sub-section 3.5.2) in the Chemical Safety Assessment (CSA) process under REACH. Nanospecific considerations are reported in sub-section 3.5.3.

3.5.1. REACH exposure scenarios

An Exposure Scenario (ES) is defined under REACH as a set of information describing the conditions under which the risks associated with the identified use(s) of a substance can be controlled. An ES includes Operational Conditions (OCs) and, if needed, Risk Management Measures (RMMs) (ECHA 2012):

- OCs include any action, use of tool or parameter state that prevails during the manufacture or use of a substance that may have an impact on exposure of humans and/or the environment (e.g. the duration and frequency of use, the amount used, the process temperature or pH).
- RMMs include any action, use of tool or technique, or change of parameter state that is introduced during the manufacture or use of a substance in order to prevent, control, or reduce exposure of humans and/or the environment (e.g. local exhaust ventilation or a certain type of glove, wastewater and gas treatment).

An ES is the cornerstone of the CSA and the related communication in the supply chains under REACH. As illustrated in Figure 3.2, ESs must be identified along the entire life cycle of the substance. Firstly, the different stages of the life cycle are defined considering the use of the substance on its own, in mixtures or in articles (i.e. manufacturing, functionalization, manufacturing of intermediates, manufacturing of end-products, use, and end-of-life). Secondly, a series of Identified Uses (IUs) is associated to each life cycle stage. Thirdly, Use Mapping is applied to each IU. The aim of the Use Mapping is the identification of those activities and processes (i.e. OCs)

that could pose a risk for the upstream or downstream users of the substance or for the environment. Fourthly, appropriate RMMs are assigned to each IU to ensure that any risk due to exposure is sufficiently controlled.

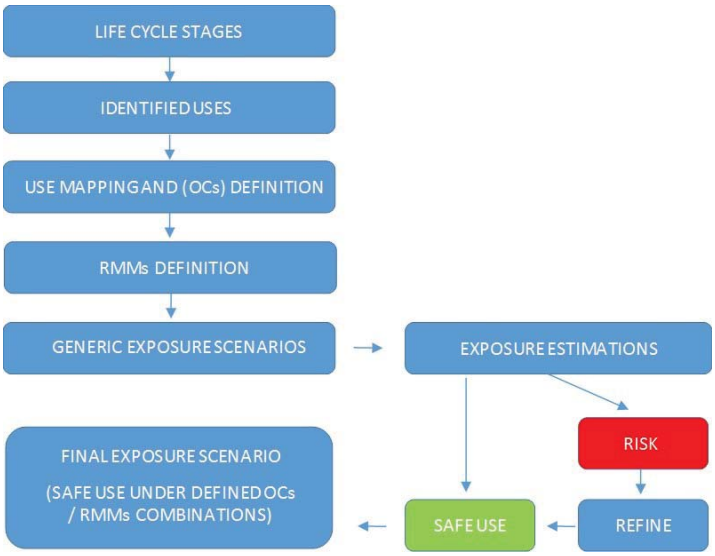

Figure 3.2: Step by step approach for the development of Exposure Scenarios under REACH. OCs = Operational Conditions. RMMs = Risk Management Measures.

In occupational ESs, OCs and RMMs for workers are described for each handling activity. ESs for consumers should include information on the population exposed (e.g. children, adults), particular conditions of use (e.g. in spray, in cream), body parts exposed, and any behavioural advice to reduce exposure. For environmental ESs, OCs (e.g. river flow rate, STP size, and annual number of working days) and RMMs (e.g. oil skimmer, carbon filter) are described as part of "Specific Environmental Release Categories" (spERCs) (ECHA 2015a).

Finally, Exposure is estimated for each Identified Use. The Exposure Estimation (described in detail in sub-section 3.5.2) is the input to the Risk Characterisation (see section 3.6).

3.5.2. REACH exposure estimation

Conceptually, exposure assessment is the process of measuring or estimating the dose or concentration of the substance to which the human population or the environment is or may be exposed, depending on the uses and consequent releases of the substance.

Figure 3.3 depicts the exposure assessment approach as required under REACH, including the scope of the exposure assessment in terms of human and environmental exposure. A comprehensive assessment of the potential exposure to chemicals should include all life cycle stages and take into account all exposure routes for human exposure and all environmental

compartments for environmental exposure. Human exposure include occupational exposure, which occurs at workplaces during the performance of the job duties, and consumer exposure, which refers to exposure of the general public to products that can be purchased in retail outlets. Consumer exposure includes exposure from the direct use of the product or as a bystander, due to being in the vicinity of the product being used indoors or in public areas (e.g. air fresheners). However, it does not include indirect exposure via the environment, i.e. through contaminated air, water, food or soil (ECHA 2016a).

The exposure assessment needs to be performed for each ES. The exposure assessment should preferably be based on quantitative measurements for each relevant target exposure route or environmental compartment.

The availability of reliable exposure data is generally very limited and mostly focused on the workplace. This dearth of data implies that in the vast majority of cases, exposure levels must be estimated by making use of exposure estimation models. There is a wide range of exposure estimation models that can be used under REACH to obtain an initial estimation of exposure based on conservative or worst-case exposure conditions. This estimation is usually defined as the Tier 1 estimation. A higher Tier estimation can be made using more sophisticated and detailed models or by carrying out exposure measurements or experiments.

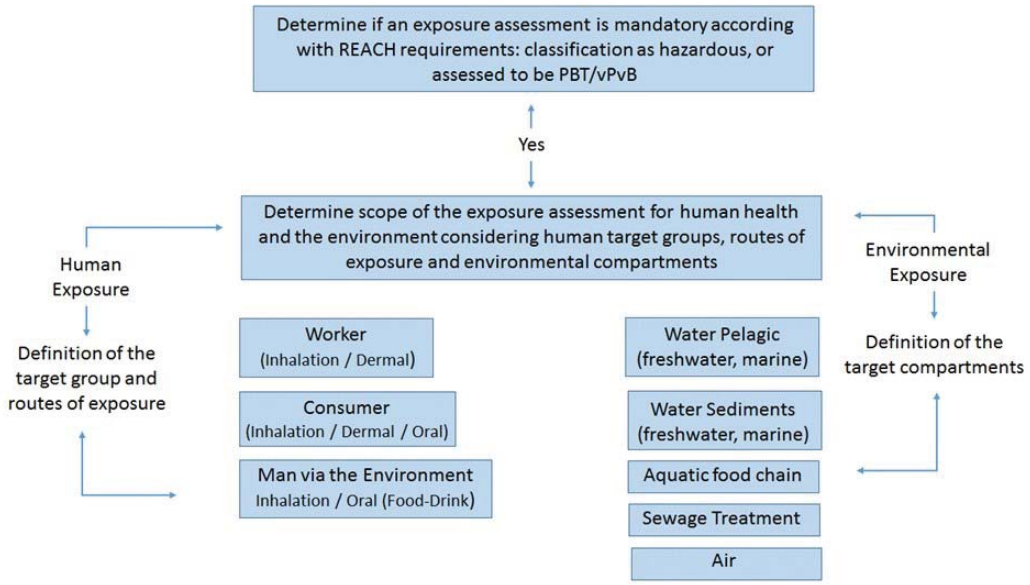

Figure 3.3 Exposure assessment approach as required under REACH. PBT/vPvB = Persistent, Bioaccumulative, Toxic/very Persistent, very Bioaccumulative.

ECHA guidance is available for occupational (ECHA 2015b), consumer (ECHA 2016a) and environmental (ECHA 2016b) exposure assessment, providing further details on the methods and models available to carry out the exposure assessments. It may be necessary to address

aggregated exposure, either across different routes of exposure (e.g. inhalation, dermal and ingestion) or across different ESs. The ECHA guidance specifies that for systemic health effects the Risk Characterisation Ratios (RCRs) for different routes of exposure needs to be summed up to obtain a total systemic RCR (ECHA 2016c) (see sub-section 3.6 for more details on RCRs). Assessment of risks due to combined exposure from different sources usually requires experimental data sets or more sophisticated (probabilistic) modelling approaches, e.g. using detailed information on distribution of use patterns of different products containing the same chemical agent.

3.5.3. Nanospecific considerations

The life cycles of many NMs are determined by their application within products. While the manufacturing stage of the life cycles are likely be in a controlled industrial setting, the use of nano-enabled products by consumers are decidedly less predictable and involve more variables. In particular, it is clear that the released fraction of NMs from nano-enabled products no longer represents the primary particles initially dispersed in the matrix, but rather, a variety of different fragments, agglomerates, and transformed products that may have significantly different physical and chemical properties than the original, as manufactured NMs (Mitrano et al. 2015). NM aging and transformation processes therefore need to be accounted for.

In the course of NANoREG, a selection was made of available Standard Operating Procedures (SOPs) that could be used to simulate, in controlled conditions, the release of nanoparticles from products and their subsequent transformation in the main compartments where exposure is likely to occur (i.e. indoor air, outdoor air, and water) (NANoREG Deliverable D3.3):

- Nanoparticle release from textiles to the water compartment during washing cycles;
- Nanoparticle release from polymers to the water compartment during accelerated aging;
- Nanoparticle release to the indoor air and/or outdoor air during sanding processes;
- Nanoparticle release to the indoor air and/or outdoor air as well as to the water compartment during environmental weathering/aging.

One of the most important added values of the SOPs described within NANoREG Deliverable D3.3 was to implement previously developed normalized ISO tests. It means that the described tests were accepted as tests that reproduce at best the aging or weathering of materials.

Qualitative and quantitative estimation of NM exposure is very complex, as these materials have very low mass, can be highly dynamic in terms of particle aggregation/agglomeration or reactivity and co-exist with ambient particles of the same size range. There are currently no agreed, standardized and validated methods for measuring personal exposure (i.e. measurements in the

breathing zone) to NMs. Furthermore, there are currently no validated models providing quantitative estimates of human (worker or consumer) or environmental exposure. The existing Tier 1 and higher Tier exposure models described in ECHA guidance are designed and evaluated for use with chemicals and should not be applied to obtain quantitative estimates of exposure to NMs, unless there is evidence that the models perform appropriately (i.e. that the NMs agglomerate into larger stable micron-sized particles).

Occupational Exposure

A number of control banding tools and semi-quantitative exposure assessment tools have been developed that can be used to determine if exposure needs to be controlled. For occupational exposure, the following tools are currently available:

- The Swiss Precautionary Matrix (Höck et al. 2013)
- The CB NanoTool[29] (Paik et al. 2008, Zalk et al. 2009)
- Stoffenmanager-Nano
- NanoSafer[30]

While these tools are useful for screening purposes, there is still a lack of information on the validity of the exposure models the tools use.

Exposure to airborne particles is generally assessed by measuring the individual exposure in the personal breathing zone, defined as a 30 cm hemisphere around mouth and nose. Measurements in the personal breathing zone require instruments that are small and lightweight. In recent years, novel samplers and monitors have been introduced that allow for an assessment of the more nanospecific personal exposure to airborne NMs. In particular, projects such as nanoIndEx[31] evaluated the performance of personal devices in laboratories but also in real case studies (NM production pilot lines and SMEs).

In 2015, the OECD published a "Harmonized tiered approach to measure and assess the potential exposure to airborne emissions of engineered nano-objects and their agglomerates and aggregates at workplaces" (OECD 2015). This three-tiered approach is based on a systematic evaluation of previously proposed and used strategies, which mainly address the fact that many of the instruments used for nanoparticle measurements are non-specific, i.e. they cannot distinguish

[29] http://www.controlbanding.net/Home.html
[30] http://NanoSafer.i-bar.dk/
[31] http://www.nanoindex.eu/

the engineered nanoparticles from ambient nano-sized particles. In Tier 1, information is gathered from the workplace, while in Tier 2 some basic measurements are carried out to determine the potential for nanoparticle release in the workplace. Tier 3 consists of a detailed and comprehensive survey to determine:

i) Whether or not exposure to nano-objects has the potential to occur;
ii) The level of exposure; and
iii) The need for additional risk management steps.

Figure 3.4 illustrates the OECD approach. It should be noted, however, that this approach was developed with the intention to be used as part of a risk management/mitigation rather than a risk assessment approach. CEN is also developing a standard named "Assessment of Inhalation Exposure to Nano Objects and their Agglomerates and Aggregates (NOAAs)" (CEN TR 137).[32]

There is a wide range of measurement and sampling devices for airborne NMs that have been used for measuring airborne concentrations. Table 3.9 summarizes the direct reading instruments most frequently cited in the literature for detecting nano-sized airborne particles. These direct reading instruments are able to generate real-time measurement data on particle size, number and/or surface area; however, as mentioned earlier, these instruments generally lack specificity, i.e. they do not distinguish between engineered nanoparticles and any background/ambient nano-sized particles. Therefore, the choice of instruments is affected by the measurement strategy. If, for example, task-based exposure with short-lived spikes in the concentrations is to be assessed, the use of personal monitors with high time resolution is inevitable. To the contrary, for the determination of shift-based averages samplers may also be used. If personal exposure to a certain chemical species need to be assessed, then with the currently available technology this can only be achieved by particle sampling and subsequent offline chemical analysis. Placement of the instruments for monitoring of the background or far-field concentrations is also an important component of the measurement strategy.

[32] http://www.cencenelec.eu/research/tools/Horizon2020/IndustrialLeadership/nanotech/Pages/default.aspx

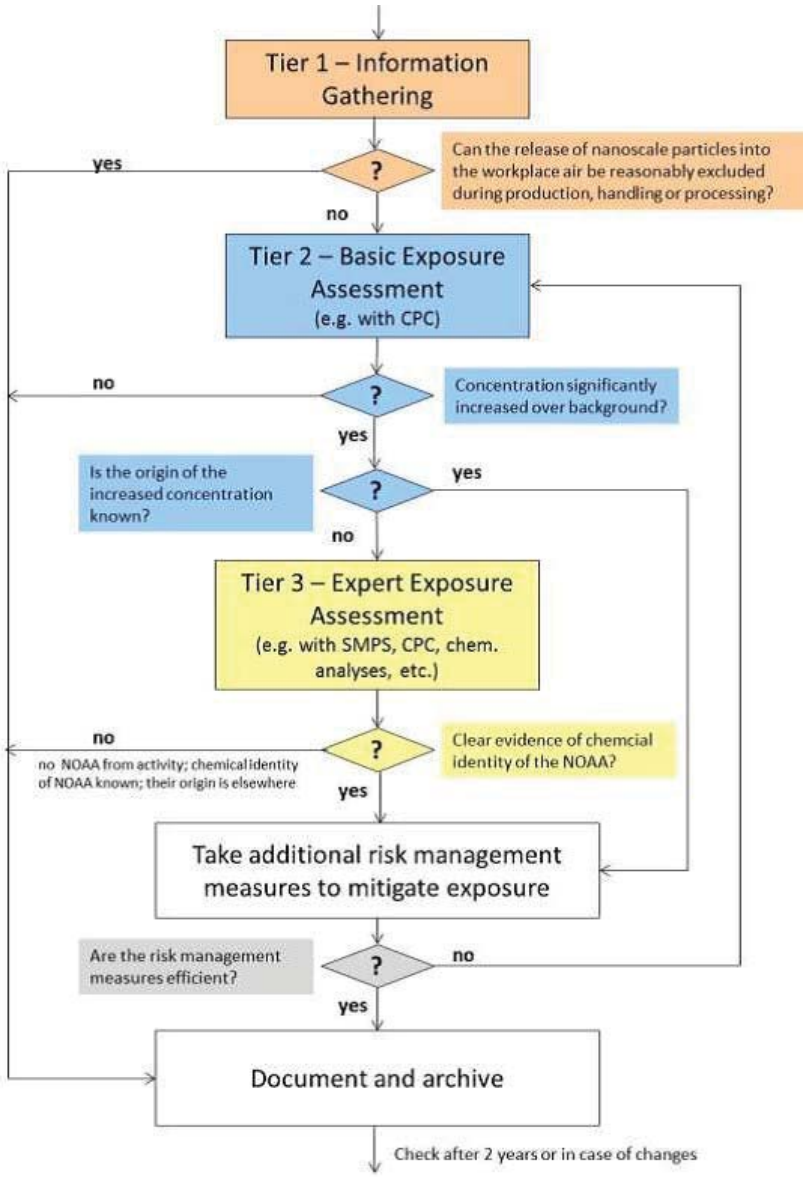

Figure 3.4: OECD tiered approach for exposure assessment (source: OECD 2015). CPC = Condensation Particle Counter; NOAA = Nano Objects and their Agglomerates and Aggregates; SMPS = Scanning Mobility Particle Sizer.

Table 3.9: Overview of direct reading instruments used for monitoring nanoparticles. CPC = Condensation Particle Counter; EEPS = Engine exhaust particle sizer; ELPI = Electrical Low Pressure Impactor; FMPS = Fast mobility particle sizer; LAS = Laser aerosol spectrometer; NSAM = Nanoparticle Surface Area Monitor; OPS = Optical Particle Sizer; SMPS = Scanning mobility particle sizer.

Instrument	Method	Type	Remarks
Particle Counters (PC)	Heated saturator	Size integrated CPC	Particle counter intended for measuring ultrafine particles (10 - 100 nm) Concentration range of 0 to 100,000 particles/cm^3 Metrics: particles number concentration (PNC)
	Diffusion charging	Size resolved Nanotracer, DiSCmini	Detects ultra-fine airborne particles (10 to 300 nm) Concentration range of 0 to 1.10^6 particles/cm^3 Measures both particle concentration (PC) and average particle diameter (APD)
Optical Particle Sizer/Laser aerosol spectrometer	Laser light scattering	Size resolved OPS LAS	Provides fast and accurate measurement of particle concentration and particle size distribution (300 nm - 10 µm) Metrics: number size distribution
Surface Area monitor	Diffusion charging	Size integrated NSAM, Partector	Provides fast and accurate measurement of active particle surface area / Size range: 10 nm - 1 µm) Concentration range: 0 to 10,000 $µm^2/cm^3$ Metrics: surface are reported as $µm^2/cm^3$
Scanning mobility particle sizer	Electrical mobility diameter	Size resolved SMPS	Provides fast and accurate measurement of particle concentration and particle size distribution 2.5 nm - 1000 nm Concentration range from 1 to 10^7 particles/cm3 Metrics: number size distribution
Fast mobility particle sizer/Engine exhaust particle sizer	Electrical mobility diameter / Unipolar diffusion charger	Size resolved FMPS, EEPS	Provides fast and accurate measurement of particle concentration and particle size distribution: 5.6 nm - 560 nm Metrics: number size distribution
Electrical low pressure impactor	Unipolar diffusion charger	Size resolved ELPI	Real-time particle size distribution and concentration in the size range of 6nm - 10µm Metrics: size distribution
Inertial spectrometer	Aerosol Time-of-Flight Mass Spectrometry	Size resolved	Provides accurate measurement of particle size distribution and chemical composition of individual particles Metrics: particle size distribution
Sampler	Filter sampling	Personal sampler NanoBadge	Light-weight, battery-operated and portable device, which can collect airborne particles directly in the breathing zone of a worker. The sampler is connected to a cassette, the filter of which is analysed offline by X-ray fluorescence spectroscopy providing a cumulative mass-based quantification of the chemical elements present on the filters.

In many cases, however, the study may be focused on workplace exposure to specific NMs and in those cases, the omnipresent, non-workplace related background of ultrafine particles must be properly addressed. How well this is done largely determines the quality of the whole study.

The following possibilities exist:

- Specific measurement of only the NM in question with direct discrimination of the background (e.g. chemical or morphological speciation)

- Spatial compensation of the background by measurements close to and during the relevant activity (near field) as well as at some distance from the activity (far field)
- Temporal compensation by measuring with and without the specific activities of the workplace
- A combination of the latter two

In addition, special consideration should be given to the 'outdoor' (i.e. outside the respective building) background, which may mostly be influenced by combustion engine exhaust.

In addition to direct reading instruments, it is also possible to collect air samples in adequate filter media for off-line chemical and microscopic analyses (e.g. using SEM or TEM with Energy Dispersive X-ray Spectroscopy).

In the course of NANoREG, a selection has been made of available instruments, tools and methods that can be used to assess occupational, consumer or environmental exposure (NANoREG Deliverable D3.6). A total of 14 different instruments, tools and methods were selected, and the corresponding SOPs were prepared in order to cover the three main compartments (soil, air, and water) and two exposure routes (inhalation and dermal exposure). The instruments tested apply different principles and aim at providing portable monitoring solutions and/or techniques for specific cases. Moreover, state-of-the-art direct reading instruments for occupational exposure assessment were directly used without further development in field measurements. The corresponding SOPs, when available, were collated from other projects (e.g. NanoGEM, nanoIndEx). In addition to air measurements for aerosol exposure assessment, biomonitoring tools were selected since they are essential for determining whether there is real individual exposure. The following instruments, tools and methods were covered:

- MiniParticleSampler (MPS)
- Light Induced Breakdown Spectroscopy (LIBS)
- NanoBadge Sampler
- Electrical Low Pressure Impactor (ELPI)
- Nasal paper flag
- Exhaled breath condensate
- Field-Flow Fractionation coupled to Inductively Coupled Plasma Mass Spectrometry (FFF-ICPMS)
- Transmission Electron Microscopy (TEM), Cryogenic mode
- X-Ray Tomography
- Small-angle X-ray scattering (SAXS)

- Surface swab and Tape stripping techniques

Consumer exposure

Modelling of consumer exposure to NMs is less advanced than that of worker exposure. A modification of the ConsExpo tool is available for estimating exposure to NMs from applying spray products[33]. There is also a control banding tool, NanoRiskcat (Hansen et al. 2014), which provides a ranking of the exposure risk for consumers and professional users (i.e. none, possible, expected, unknown). The assessment is based on the location of the NM in the product (e.g. embedded in a matrix, on the surface) and the description of the activity but not on the amount of the NM used in the product.

Environmental exposure

For environmental exposure, terrestrial and aquatic mesocosms are currently being developed (Auffan et al. 2014, Tella et al. 2014). Mesocosms are one of the rare biophysicochemical exposure characterization tools but their application extends to ecotoxicity testing in a realistic setting. Depending on the NM and the contamination scenarios, the mesocosms can operate with different physical and physicochemical features (e.g. soil properties, water quality and depth, sediment mineralogy and depth, current velocity, tidal reservoirs) and biota thanks to their high flexibility. The currently available data from mesocosms testing demonstrate the capability of this exposure testing strategy to characterize and distinguish acute and chronic exposure and account for varying surface chemistries (coated vs. uncoated) by quantifying the distribution of NMs and their alteration residues within the different compartments of an ecosystem.

The main obstacles to the characterization and quantification of NMs in the different environmental compartments are the very low environmental concentrations of NMs and the similar chemical composition of NMs in respect to other matter and particles commonly present in the environment. Moreover, the isolation of NMs from their environmental matrices by filtration, extraction and separation processes may alter their physicochemical properties compared to their original state in the system.

Another challenge is to understand if a NM, present at a certain time and in a certain space in the environment, originates directly from a point source (a production process or a NM-embedded product) or was transported there and underwent transformation in the environment. In fact, the

[33] http://www.rivm.nl/en/Topics/C/ConsExpo/Nano_tool

environment is a dynamic system in which NMs, as any chemical, can migrate from one environmental compartment to another and react with other entities present in the system with the possibility of disappearing from one compartment due to aggregation, sedimentation or dissolution phenomena or changing their identity due to chemical reactions, thus producing different NMs.

As the detection, characterization and quantification of NMs in the environment is usually challenging and often not feasible, modelling approaches have been developed for estimating the occurrence and concentration of NMs in the environment. One way to obtain estimates of existing environmental levels of NMs is to employ refined and updated models to predict their concentrations in the environment (Sun et al. 2016).

The first modelling attempts were proposed in 2008 employing NM flow analysis instead of hypothetical calculation (Mueller and Nowack 2008). These studies were based on deterministic models in which the NM flow was calculated considering as input the NM production quantity and as output the NM release rate. Certain natural and technical compartments were selected mainly for the availability of measurement data to be used in the validation of the predicted environmental concentrations (PECs). Most of these studies took into account only one application of the NM, were restricted to a single NM production event, and considered only a few NM transfers from one compartment to another (Blaser et al. 2008). Later on, the fate and behaviour of NMs in environmental compartments were introduced in the mass balance of the models and dynamic processes such as aggregation, sedimentation, and degradation were taken into account. A further improvement was achieved when probabilistic models were implemented to describe each NM transfer event from one compartment to another (Gottschalk et al. 2010). Further refinement was obtained by employing updated NM production values and correlating them with the NM release in the course of all the NM life stages from production, use (e.g. ageing, abrasion, washing) and end-of-life (e.g. waste water treatment, incineration) (Mueller and Nowack 2008). With the growing production of NMs and nano-enabled products more data became available and different ESs were investigated within the models. This allowed for the introduction of a further level of complexity that was represented by the temporal and spatial resolution of the NM releases (Ort et al. 2009), which also made it possible to consider multiple NM sources. More recent models include the *per capita* consumption of nanoproducts and the product lifetime (Sun et al. 2016) and implement physical theory in order to model process NM heterocoagulation with natural particles and reactions of NMs with organic matter (Arvidsson et al. 2012).

Even if current modelling approaches are able to predict environmental NM concentrations, more sophisticated environmental fate models, including mechanistic descriptions of fate processes and considering chemical reactions and physical changes of NMs (i.e. NM particle size), are needed in order to refine these models. On the other hand, more measured environmental exposure data are

required to assess, validate and improve the accuracy of the models and to further hone and improve them in order to obtain tools useful for NM environmental risk assessment.

Risk Management Measures

Risks should be reduced to the lowest reasonably practicable level by taking preventative measures in the order of priority. Therefore, wherever reasonably practicable, exposure to hazardous particles and liquids, including NMs, by all routes (inhalation, dermal and ingestion) should be eliminated or controlled to the lowest reasonably practicable level, following the principles of the hierarchy of controls. The hierarchy of controls involves the following steps:

- Elimination
- Substitution
- Technical measures - Engineering controls
- Organizational measures (use of administrative controls)
- Personal Protective Equipment (PPE)

Often a combination of RMMs is used to obtain the required level of protection. Although the hierarch of control dictates that elimination and substitution of hazardous materials should be considered first in controlling exposure, in practice occupational exposures are generally controlled with ventilation systems and PPE.

The data published so far and evaluated within NANoREG suggest a good level of performance for respiratory protective equipment. Most of the data retrieved from peer-reviewed publications showed efficiencies above the threshold levels defined in reference harmonized standards. In the case of protective clothing and chemical protective gloves, there are still a lot of unknowns as to whether or not traditional protective measures provide a proper level of protection against NM exposure. The information retrieved from the literature highlight two main challenges linked with protective suits and gloves. The first is to understand the external parameters that can influence the penetration of nanoparticles through commonly issued fabrics. The second is to consider the variations of the surface properties of the materials used in protective gloves and clothing, which results in a high variation of performance results.

All guidelines specific to NMs emphasize the need for technical exposure mitigation including physical and technical solutions in the work process in order to isolate, encapsulate and shield the process as well as using mechanical ventilation and filters (locally and/or centrally). Technical measures are likely the most effective and applicable control strategy for most processes involving NMs. Ventilation is the most common technical measure used for controlling occupational exposures to air contaminants including NMs. The use of general ventilation is limited to low

toxicity sources in circumstances where the sources are usually diffused throughout the workplace and the workers are at a sufficient distance from them. The use of Local Exhaust Ventilation (LEV) systems is preferred to general ventilation and should be considered when working with NMs[34]. Table 3.10 summarizes the degree of recommendation of different technical measures when dealing with NMs in the workplace according to NANoREG Deliverable D3.9.

Table 3.10: Recommended technical measures when working with nanomaterials (content from NANoREG Deliverable D3.9). HEPA = High-efficiency particulate arrestance; LEV = Local Exhaust Ventilation.

Protection level	*Technical measures*
Highly recommended (High protection)	Local exhaust enclosure (Glove Box) HEPA filtered down flow booth Custom-fabricated enclosures HEPA filtered down flow room Ventilated Laboratory Hood + built-in water wash down systems (sprays) Negative pressure rooms
Acceptable level of protection (non-hazardous nanomaterials)	Ventilated Laboratory Hood (partial enclosure) Biological safety cabinet (small amounts of nanomaterials) Walk-in hoods Ventilated collar-type exhaust hoods Movable LEV systems (extendable arms) Receiving hood (hot process) Work processes in furnaces (High cost)
Not recommended	Biological safety cabinet (amounts above 100 g) Ventilation by dilution

According to the hierarchy of controls, the use of PPE is the least desired option for controlling worker exposure, to be used when engineering and administrative controls are not feasible or effective in reducing exposures to acceptable levels. The respiratory protective equipment, chemical protective gloves and protective clothing used must offer a good barrier against hazardous particles in the nanometer scale (i.e. airborne nanoparticles), liquid splashes, nanoaerosols and liquids (i.e. jets). Table 3.11 summarizes the degree of recommendation of different PPE when dealing with NMs in the workplace according to the outcomes of NANoREG Deliverable D3.9.

[34] http://www.lifenanorisk.eu/index.php/interactive

Table 3.11: Recommended Personal Protective Equipment (PPE) when working with nanomaterials (content from NANoREG Deliverable D3.9). PVC = Polyvinyl chloride.

Protection level	*Personal Protective Equipment*
Highly recommended (High protection)	Full Face particulate respirators (P3) Half Face particulate respirators (P3) Nitrile gloves – Double glove for large exposure periods Full body protective coverall (EN type 4-6) made of PE laminated with built-in hood Tight-fitting dustproof (i.e. non-vented) safety goggles
Acceptable level of protection (non-hazardous NMs)	Half-Face particulate respirators (P2) Neoprene gloves/Butyl gloves Full body protective coverall (EN type 4-6) made of polypropylene with or without built-in hood Laboratory coats (Non-woven) Dustproof safety goggles
Not recommended	Filtering Facepiece (FFP3) Latex/Cotton/PVC gloves Laboratory coats (cotton/spunbonded polypropylene) Safety glasses

Finally, regarding emission control technologies, several of them intended to capture and remove NMs from air and water streams generated in occupational settings are starting to appear. These technologies are key to controlling unintended releases of NMs into the environment, especially the freshwater, soil and air compartments.

Current studies suggest that the use of current adsorption and filtration technologies can be effective in removing from wastewater a wide range of NMs, which may have different properties such as different zeta potentials, different surface charge and unpredictable behaviour under the operating conditions of the wastewater treatment system. Ultrafiltration, nanofiltration and reverse osmosis can be used to remove NMs from wastewater considering the specific properties of the NMs release, including particle size distribution, speciation, and surface chemistry (Yang and Tsai 2006, Lingxiangyu et al. 2013, Park et al. 2013, Tzu-Ming et al. 2014).

Concerning unintended emissions of NMs into air, common technologies aimed to collect and remove particulate matter are being re-designed. The electrostatic precipitator is used for removing particles and it has been satisfactorily used to trap and remove dust particles from the exhaust gas stream of industrial processes. However, conventional electrostatic precipitators cannot remove submicron particles, and the collection efficiency drops to less than 40% when the particle size is less than 1 μm. Scrubbers can also be used to remove some particulates and/or gases from industrial exhaust streams.

Finally, removal of NMs from soils is still a challenge. Several techniques are applied, including common techniques such as landfilling. Promising efficiencies of novel techniques, such as phytoremediation and fast crystal growth, have been retrieved from peer-reviewed publications (Mahmood et al. 2012, Jacob et al. 2013).

3.5.4. References

Arvidsson R, Molander S, Sandén BA. 2012. Particle Flow Analysis: Exploring Potential Use Phase Emissions of Titanium Dioxide Nanoparticles from Sunscreen, Paint, and Cement. Journal of Industrial Ecology, 16: 343-351

Auffan M, Tella M, Santaella C, Brousset L, Pailles C, Barakat M, Espinasse B, Artells E, Issartel J, Masion A, Rose J, Wiesner MR, Achouak W, Thiery A, Bottero J-Y. 2014. An adaptable mesocosm platform for performing integrated assessments of nanomaterial risk in complex environmental systems. Scientific Reports, 4: 5608. doi: 10.1038/srep05608

Blaser SA, Scheringer M, MacLeod M, Hungerbühler K. 2008. Estimation of cumulative aquatic exposure and risk due to silver: contribution of nanofunctionalized plastics and textiles. Science of the Total Environment, 390: 396-409

ECHA 2012. Guidance on information requirements and chemical safety assessment. Chapter R.13: Risk management measures and operational conditions. October 2012. European Chemicals Agency, Helsinki

ECHA 2015a. Guidance on information requirements and chemical safety assessment. Chapter R.12: Use description. Version 3.0, December 2015. European Chemicals Agency, Helsinki

ECHA 2016a. Guidance on information requirements and chemical safety assessment. Chapter R.15: Consumer exposure assessment. Version 3.0, July 2016. European Chemicals Agency, Helsinki

ECHA 2015b. Guidance on information requirements and chemical safety assessment. Chapter R.14: Occupational exposure assessment. Draft (Public) Version 3.0, November 2015. European Chemicals Agency, Helsinki

ECHA 2016b. Guidance on information requirements and chemical safety assessment. Chapter R.16: Environmental exposure assessment. Version 3.0, February 2016. European Chemicals Agency, Helsinki

ECHA 2016c. Guidance on information requirements and chemical safety assessment. Part D: Framework for exposure assessment. Version 2.0, August 2016. European Chemicals Agency, Helsinki

Gao P, Jaques PA, Hsiao TC, Shepherd A, Eimer BC, Yang M, Miller A, Gupta B, Shaffer R. 2011. Evaluation of nano- and submicron particle penetration through ten nonwoven fabrics using a wind-driven approach. J Occup Env Hyg, 8: 13-22. doi:10.1080/15459624.2010.515554

Gottschalk F, Sonderer T, Scholz RW, Nowack B. 2010. Possibilities and limitations of modelling environmental exposure to engineered nanomaterials by probabilistic material flow analysis. Environmental Toxicology and Chemistry, 29: 1036-1048

Hansen SF, Jensen KA, Baun A. 2014. NanoRiskCat: A conceptual tool for categorization and communication of exposure potentials and hazards of nanomaterials in consumer products. Journal of Nanoparticle Research, 16(1): 2195. doi: 10.1007/s11051-013-2195-z

Höck J, Epprecht T, Furrer E, Gautschi M, Hofmann H, Höhener K, Knauer K, Krug H, Limbach L, Gehr P, Nowack B, Riediker M, Schirmer K, Schmid K, Som C, Stark W, Studer C, Ulrich A, von Götz N, Weber A, Wengert S, Wick P. 2013. Guidelines on the Precautionary Matrix for Synthetic Nanomaterials. Federal Office of Public Health and Federal Office for the Environment, Berne. Version 3.0

Jacob DL, Borchardt JD, Navaratnam L, Otte ML, Bezbaruah AN. 2013. Uptake and translocation of Ti from nanoparticles in crops and wetland plants. Int J Phytoremediation, 15(2): 142-53

Lee SA, Hwang DC, Li HY, Tsai CF, Chen CW, Chen JK. 2016. Particle Size-Selective Assessment of Protection of European Standard FFP Respirators and Surgical Masks against Particles-Tested with Human Subjects. Journal of Healthcare Engineering, Vol. 2016, 12 pages

Lingxiangyu L, Hartmann G, Döblinger M, Schuster M. 2013. Quantification of Nanoscale Silver Particles Removal and Release from Municipal Wastewater Treatment Plants in Germany. Environ. Sci. Technol, 47(13): 7317-7323

Mahmood T, Malik SA, Hussain ST, Aamir S. 2012. Metallic phytoremediation and extraction of nanoparticles. International Journal of Physical Sciences, 7(46): 6105-6116

Mitrano DM, Motellier S, Clavaguera S, Nowack B. 2015. Review of nanomaterial aging and transformations through the life cycle of nano-enhanced products. Environmental International, 77: 132-147

Mueller NC, Nowack B. 2008. Exposure modelling of engineered nanoparticles in the environment. Environ. Sci. Technol., 42: 4447-4453

NANoREG Deliverable 3.3. Deliverable report for NANoREG. Grant Agreement Number 310584. Methods for the use of simulation approaches

NANoREG Deliverable 3.6. Deliverable report for NANoREG. Grant Agreement Number 310584. Improved measurement instruments, tools and methods (towards multi-metric, discriminating, etc.)

NANoREG Deliverable 3.9. Deliverable report for NANoREG. Grant Agreement Number 310584. Data on the effectiveness of Risk Management Measures

OECD 2015. Harmonized tiered approach to measure and assess the potential exposure to airborne emissions of engineered nano-objects and their agglomerates and aggregates at workplaces. Series on the Safety of Manufactured Nanomaterials N° 55. 17-Jun-2015. ENV/JM/MONO(2015)19. Organisation for Economic Development and Co-operation, Paris.

Ort C, Hollender J, Schaerer M, Siegrist H. 2009. Model-based evaluation of reduction strategies for micropollutants from wastewater treatment plants in complex river networks. Environmental Science & Technology, 43: 3214-3220

Paik SY, Zalk DM, Swuste P. 2008. Application of a Pilot Control Banding Tool for Risk Level Assessment and Control of Nanoparticle Exposures. Ann Occup Hyg, 52(6): 419-428

Park HJ, Kim HY, Cha S, Ahn CH, Roh J, Park S, Kim S, Choi K, Yi J, Kim Y, Yoon J. 2013. Removal characteristics of engineered nanoparticles by activated sludge. Chemosphere, 92(5): 524-8

Sun TY, Bornhöft NA, Hungerbühler K, Nowack B. 2016. Dynamic Probabilistic Modeling of Environmental Emissions of Engineered Nanomaterials. Environ. Sci. Technol., 50: 4701-4711

Tella M, Auffan M, Brousset L, Issartel J, Kieffer I, Pailles C, Morel E, Santaella C, Angeletti B, Artells E, Rose J, Thiery A, Bottero JY. 2014. Transfer, Transformation, and Impacts of Ceria Nanomaterials in Aquatic Mesocosms Simulating a Pond Ecosystem. Environ. Sci. Technol., 48: 9004–9013

Tzu-Ming C, Chuen-Jinn T, Shaw-Yi Y, Shou-Nan L. 2014. An efficient wet electrostatic precipitator for removing nanoparticles, submicron and micron-sized particles. Separation and Purification Technology, 136: 27-35

Yang GCC, Tsai C-M. 2006. Performance evaluation of a simultaneous electrocoagulation and electrofiltration module for the treatment of Cu-CMP and oxide-CMP wastewaters. Journal of Membrane Science, 286(1–2): 36-44

Zalk DM, Paik SY, Swuste P. 2009. Evaluating the Control Banding Nanotool: a qualitative risk assessment method for controlling nanoparticle exposures. Journal of Nanoparticle Research, 11: 1685

3.6. Risk characterization

The text below illustrates how risk is estimated (sub-section 3.6.1) and uncertainty is analysed (sub-section 3.6.2) in the last step of the Chemical Safety Assessment (CSA) under REACH. Nanospecific considerations are reported in sub-section 3.6.3.

3.6.1. Risk estimation

Under REACH, risk characterization is defined as the comparison of exposure levels of a certain substance and quantitative or qualitative hazard information to evaluate if risks are adequately controlled in each identified Exposure Scenario (ES) for different human populations and environmental compartments (see sub-sections 3.4 on hazard assessment and 3.5 on exposure assessment). ECHA published guidance on how to perform risk characterisation under REACH (ECHA 2016a). The reader is referred to that document for more detail.

The comparison of quantitative estimations of exposure and hazard levels lead to the calculation of the Risk Characterization Ratio (RCR). The RCR needs to be calculated for each identified ES and for each relevant combination of human populations, environmental compartments, exposure routes, time scales, and (eco)toxicological endpoints. The RCR is calculated by comparing the measured or estimated exposure levels (i.e. Predicted Environmental Concentrations (PECs), sub-section 3.5) and the Predicted No Effect Concentrations (PNECs) for the environment or Derived No Effect Levels (DNELs) for human health (sub-section 3.4). If it is not possible to derive a DNEL (e.g. for non-threshold endpoints such as mutagenesis and carcinogenicity), a Derived Minimal Effect Level (DMEL) can be obtained. If no quantitative information is available to calculate the RCR, then the risk characterisation can be performed in a qualitative way to estimate the likelihood that effects are avoided in that specific ES. When both quantitative and qualitative information for different endpoints (not for the same endpoint) for the same substance are available, both a (semi-)quantitative and qualitative assessment of the risks has to be made, for the respective endpoints.
To demonstrate that the risk associated with a certain ES is adequately controlled and therefore the use is safe, the RCR for that ES has to be below 1, or, for semi-quantitative or qualitative RC, the high likelihood that effects are avoided in that specific ES has to be demonstrated.

According to ECHA guidance on adaptation of information requirements (ECHA 2011), it is possible to waive the derivation of PNEC/DNEL/DMEL and the risk characterization only when no exposure is expected with a high level of certainty for a certain life cycle stage of the substance

and the related targets (sub-section 3.3). When exposure is low, or considered to be unlikely to happen (but not excluded), the risk characterisation has to be performed anyway and the consequent negligible or absent risk has to be demonstrated.

It is important to highlight that the risk characterisation heavily relies on expert judgement; therefore, all assessments need to be transparent and traceable.

If a substance is assessed to have hazardous properties, the risk characterization has to be performed taking into account the physicochemical properties of the substance, the exposure factors (e.g. storage, on site transfer), and the likelihood and severity of the exposure. The scope is to estimate the magnitude of risks in different conditions, verify if risks are controlled, and identify where Risk Management Measures (RMMs) are needed. RMMs for occupational exposure are addressed in sub-section 3.5.

The outcome of the risk characterization, being it a quantitative or semi-quantitative one, needs to be qualitatively discussed by identifying the uncertainties and any other aspect that was not addressed in the assessment.

Qualitative risk characterisation is defined as *"the likelihood that effects are avoided when implementing the exposure scenario"* (ECHA 2016a). It aims at reducing or avoiding the contact of the targets (i.e. human beings or environmental species) with the substance; therefore, the implementation of RMMs is highly important in this context, and the strictness of the required RMMs is linked to the hazard classification of the substance according to the CLP Regulation (sub-section 3.4). According to ECHA (2016a), the human health-related information requirements for which a qualitative risk characterisation may be necessary are: irritation/corrosion, sensitisation, acute toxicity, carcinogenicity and mutagenicity (sub-section 3.2). For the environment, a qualitative risk characterisation is recommended when a PNEC cannot be calculated but also in another case, i.e. when the calculated short-term PNECs show no risks but a long-term effect is suspected or possible according to inherent properties of the substance, such as K_{ow} and K_d partitioning coefficients (ECHA 2016a).

'Combined exposure' is defined in ECHA guidance for consumers' exposure assessment (ECHA 2016b) as exposure to the same substance through multiple exposure routes (e.g. inhalation and ingestion), while 'aggregated exposure' is intended as exposure to the same substance through one exposure route but from multiple sources. Both combined and aggregated exposures are sometimes relevant for chemicals, when the human population and the environmental targets are

exposed to the same substance through a variety of exposure routes and products. In case of combined exposure, the overall risk is obtained by adding up the RCRs of the substance per contributing ES. In case of aggregated exposure, risks resulting from exposure to the substance via simultaneous use of different products may be derived by summing up the RCRs for systemic effects across ESs.

3.6.2. Uncertainty analysis

Uncertainty analysis under REACH is well described in ECHA guidance (ECHA 2012).

Key elements of uncertainty analysis, which are relevant to NMs as well, include:

- A tiered approach should be applied, with the level of detail proportionate to the level of uncertainty and impact of the risk characterization;
- It is necessary to distinguish between 'uncertainty', which can be reduced, and 'variability', which is inherent to the system, and address both;
- There are three categories of uncertainty, i.e. scenario, model and parameter uncertainty: 'scenario uncertainty' is linked to the uses of the substance; 'model uncertainty' is linked to use of extrapolation, parametrisation, and correlation between parameters; and, finally, 'parameter uncertainty' is linked to the measurement of the parameter, sampling error, choice of dose descriptors, and extrapolation factors.

When is uncertainty analysis necessary and to which degree

Uncertainty analysis is not always necessary. ECHA suggests that uncertainty analysis is used in CSA when: i) the RCR is close to 1; ii) more insight about the robustness of the risk characterisation is needed; iii) non-standard regulatory methods are used to derive exposure and/or hazard; or iv) the registrant sees a specific need.

Uncertainty analysis is organized into three levels:

- Level 1: qualitative uncertainty analysis to refine the exposure estimate and provide an indicative range of unquantifiable uncertainties;
- Level 2: derivation of a range of point estimates by means of a deterministic approach to describe the extent of uncertainty;
- Level 3: use of probability distributions to provide statistical information about the likelihood that the RCR is exceeded under specific circumstances and according to the parameterisation used.

Qualitative uncertainty analysis (Level 1) is basically a process where as many sources of uncertainty are identified and described through a stepwise process. This process involves a great

deal of expert judgement. Uncertainties regarding exposure and hazard are firstly identified and secondly categorized, e.g. as model uncertainty or data variability. As a third step, the direction (i.e. underestimation or overestimation of risk) of each uncertainty item is identified and, as a fourth step, the magnitude of each uncertainty item is qualitatively estimated. No numerical integration of qualitative uncertainties is performed. The final outcome describes the main sources of uncertainty, the ways to reduce it, and the overall effect of uncertainty on risk estimation.

This kind of uncertainty analysis always needs to be performed. A checklist of uncertainty sources for each category (i.e. model, parameter, and scenario uncertainty) is provided by ECHA (2012).

If Level 1 (qualitative uncertainty analysis) shows that uncertainty can affect the risk estimation, it is necessary to proceed to the deterministic analysis (Level 2) based on the creation of alternative scenarios – by varying selected parameters – such as reasonable worst case and average case. A tiered process to carry out the Level 2 analysis is also described by ECHA (2012). The last step (Level 3), the probabilistic analysis, is undertaken only for substances of high risk and when a large amount of data is available.

What must be considered in uncertainty analysis

The ECHA guidance provides the registrant checklists to be followed to ensure that as many sources of uncertainty as possible in both effect and exposure assessment are considered. A short list of sources is reported below (for a full dissertation consult the ECHA guidance (ECHA 2012)). The sources of uncertainty are generic and therefore relevant for all chemicals including NMs. Of course, given the specific physicochemical properties and the state-of-the-art of the scientific knowledge on exposure and effects of NMs, different sources of uncertainty may have higher or lower weight than for other chemicals. Nanospecific considerations are reported in the next sub-section 3.6.3.

Effects

- Model uncertainty: oversimplification, use of out-of-domain models, dependency errors
- Parameter uncertainty: measurement errors, sample size, dose descriptor, AF adequacy, extrapolation uncertainty

Exposure

- Scenario uncertainty: disregarding sources and pathways/routes, target population/community, environment of exposure, spatial and temporal settings, use scenario
- Model uncertainty: oversimplification, use of out-of-domain models, dependency errors

- Parameter and data uncertainty: see effects, conservativeness in emission scenarios, exposure concentration choice, environmental variability, behaviour variability

3.6.3. Nanospecific considerations

The risk characterisation under REACH may present challenges when it comes to NMs. Such nanospecific considerations are illustrated in this sub-section. The currently available tools (e.g. guidance documents, models, protocols, decision trees), including those developed under NANoREG, for addressing the nanospecific considerations discussed in this sub-section are listed in the NANoREG Toolbox (Deliverable D1.12, see worksheet "3.6 REACH Risk characterisation").

Risk characterization for NMs is, as for all chemicals, a combination of exposure and hazard information and the discussion of the related uncertainties. There are few nanospecific aspects directly linked to risk characterisation and those are discussed in this sub-section. Nanospecific issues concerning hazard and exposure assessment are discussed in sub-sections 3.4 and 3.5, respectively.

The current framework for safety assessment under REACH is acknowledged to be applicable to NMs but needs to take some nanospecific aspects into account (ECHA 2014).
The ECHA GAARN highlighted that there were not enough nanospecific exposure information in the submitted REACH registration dossiers that were assessed at that time (ECHA 2014). In order to quantitatively or qualitatively characterize the risks, identification and estimation of exposure is indeed essential. In addition, NMs may be subjected to change in exposure parameters over time since they can assume a different nature along their life cycle e.g. going from pristine powder to be dispersed in liquid, functionalised, incorporated into polymer, elaborated by intermediate user, and finally recycled, all in one value chain. This may dramatically change the release potential and likelihood of reaching a given target. The same issue can be envisioned for different value chains (i.e. different uses of the same NM), in different environmental media after release (e.g. water, air, soil), and over time (i.e. aging, interaction with the environment). Hence, it is necessary to consider NM transformation along its life cycle and to quantify exposure accordingly. To simplify the assessment, each life cycle stage should be considered in a dedicated Exposure Scenario (ES) linked, for example, to a specific production process, usage, and environmental release. When a specific target is not exposed to the NM under evaluation in any ES, risk characterisation can be waived. However, the absence of exposure to the NM has to be demonstrated. At the same time, the formation of aggregates and agglomerates is not grounds for waiving the risk characterisation of the NM. Aggregates and agglomerates can release constituent nanoparticles in various

conditions, so it needs to be demonstrated through experimental data that aggregates and agglomerates do not release nanoparticles in relevant exposure conditions.

The use of models to estimate the exposure to NMs, both primary particles and aggregates/agglomerates, is also often not feasible due to unsuitable assumptions and parametrisation as well as lack of model validation for NMs (sub-section 3.5). Therefore, field measurements combined with laboratory observations (e.g. TEM-EDX to evaluate the size and composition of metallic nanoparticles) are preferred. Conversely, field measurements are not easy to perform, and there is the issue of background concentrations of natural/accidental nanoparticles generated by processes not related to the NM under investigation, which is usually generated by a specific production process (sub-section 3.5). In the worst case, the combined fractions of background (natural/accidental) nanoparticles and engineered NM need to be considered in the risk characterisation. However, this approach may result in overestimated risk.

Combined and aggregated exposure, even if relevant, is a complex element of risk assessment for all chemicals including NMs. Calculating combined and aggregated exposure means considering all potential (and relevant) exposure routes and sources. Therefore, combined/aggregated exposure is impossible at this time to be considered both for an individual NM and a group of 'similar' NMs. On the other hand, aside from a few high-volume NMs that are applied in several industrial sectors, most NMs have a low penetration into the market and their use is limited to specific technological applications and commercial products.

Another nanospecific issue is metrics, which represents one of the regulatory questions directly addressed by NANoREG. The work carried out in NANoREG suggests that the most appropriate metric to express the biologically effective dose largely depends on the exposure pattern and on the type of NM (NANoREG Deliverable D1.9). The ECHA GAARN states that (obviously) the same metric must be used in order to compare exposure and hazard and that hazard results should be reported using the metric that correlates best with the measured effect, which can be particle size, surface area, number of particles, etc. (ECHA 2014). Based on the work conducted within NANoREG, mass can also be used, and there is no scientific reason to consider mass not suitable for assessing the dose-response curve in regulatory toxicology. However, relationships for converting mass into other metrics need to be developed, expressing hazard information in different ways. This is also a recommendation of the ECHA GAARN, which, if properly addressed, may allow a proper RCR calculation. The main issue is linked to the possibility to carry out a complete NM physicochemical characterization in relevant testing media. Technical considerations, such as measurement techniques, available toxicological assays, and available exposure measurement methods, thus influence the selection of a specific metric. Based on NANoREG Deliverable D1.9, some specific considerations for groups of NMs have been formulated:

- Rigid biopersistent fibrous materials (WHO 1997): fibre number concentration is the adequate dose metric with additional parameters that are used by models such as median particle size, geometric standard deviation, and the density of a NM as it occurs in the exposure media (air, fluids)
- Biopersistent HARN: number concentration is probably the most appropriate dose metric, also because all Occupational Exposure Limits (OELs) for all types of fibres (not only NMs) are expressed in this way. More work is required to link surface area to mass and particle number to provide conversion factors
- Granular biopersistent NMs: particle agglomerate volume seems to be the best applicable metric to describe long-term toxicity of particles with varying size or different chemical identity
- Granular NMs (low aspect ratio): different metrics can be calculated following a proper physicochemical characterization that should include robust measurements, in terms of particles and aggregates, of morphology, particle and aggregate size distribution, surface area as well as particle and aggregate density
- Not-rigid and not biopersistent HARN: the proper metric is not fully understood yet

The difficulties in deriving reliable long-term DNELs and PNECs for NMs reduce the ability to perform a quantitative risk characterisation (sub-section 3.4). For conventional chemicals, read-across and modelling approaches (on the basis of experimental data and physicochemical properties) can be applied to identify long-term effect concentrations. However, long-term studies on NMs are in general scarce for both humans and the environment, thus making read-across and modelling more difficult. Moreover, long-term studies are often performed with well-known NMs, therefore the possibility of applying read-across for NMs used in low volumes or for specific NM applications with modified physicochemical properties is limited. Technical difficulties in detecting and measuring NM transformation, persistence and environmental fate, the lack of standardized methods, and the high costs of carrying out a long-term study are strong limiting factors.

In environmental risk characterization, it is important to consider indirect effects in the PNEC assessment. In case of NMs, their particulate nature can lead to effects other than the specific endpoint measured in the single toxicity assay, involving other organisms and ecological functions of the investigated compartment (e.g. aquatic or benthic). In addition, qualitative risk characterization for the environment is necessary if long-term effects are expected. The potential of a NM to cause long-term effect should be assessed by using partitioning constants, as indicated in the guidance (ECHA 2016a). However, traditional parameters such as K_d or K_{ow} turned out not to be meaningful for those NMs that do not dissolve but disperse as particles. Therefore, for those

NMs the need for long-term tests has to be inferred by other means (see sub-sections 3.2 and 3.4 for more information). Direct observation of accumulation of a NM in organisms over long periods, the calculation of uptake and clearance kinetics, or the evaluation of NM behaviour in complex systems such as wastewater treatment plants or mesocosms can support such reasoning. However, the implementation of such experimental setups is clearly costly and complicated.

Risk Management Measures (RMMs), as an integrated part of the qualitative risk characterisation, are extremely relevant for both occupational and environmental exposure. The ECHA GAARN mentions some documents (i.e. ISO 2008, ISO 2012, Vogel et al. 2012), which detail the effectiveness of RMMs for NMs and can be used to discuss risk characterisation results, especially in occupational settings. Some nanospecific considerations concerning RMMs are reported also in sub-section 3.5.

Concerning uncertainty assessment, the uncertainty categories, levels and approaches discussed in sub-section 3.6.2 are valid also for NMs. Nanospecific issues can arise from the type of information that is lacking and the way the lack of information is addressed. In other words, where are the main exposure and hazard data-generation bottlenecks and how is this related to the qualitative and quantitative risk characterisation? Model uncertainty is always relevant and the application of models must always be properly validated and justified. However, given the need to fill in data gaps and at the same time the lack of validation of available models for NMs, model uncertainty can be considered an even more important issue with NMs than with other chemicals. Several available models are not applicable to NMs, starting from physicochemical properties estimation procedures to more complex hazard, fate and behaviour models. There are some efforts to develop models for the estimation of partitioning coefficients for specific NMs (e.g. carbon nanotubes) on basis of colloidal theories (Bouchard et al. 2015). There are also efforts to develop (Q)SAR (and of the sort) models on basis of experimental measurements. More of such models are going to be available in time with more quality data available. However, since adequate knowledge of the basics of NM behaviour and NM properties affecting such behaviour is missing, great care should be taken in using models to perform risk characterisation for NMs and relying on nanospecific experimental data is recommended. When a model is used, attention should be paid to clearly addressing the applicability domain and the uncertainties related to model parametrisation.

When experimental data are used to characterise the risk those uncertainties that are more relevant for NMs and need to be considered are:

- Suitability of exposure measurement methods (e.g. specific adaptations)

- Suitability of assays
- Relevance of data on other forms (bulk forms or nanoforms) and specific endpoints for the hazard assessment of the investigated NM
- Extrapolation correctness

In the hazard assessment phase, major sources of uncertainty appear to be physicochemical and hazard information. Physicochemical information is essential for proper interpretation of the results of physicochemical hazard and (eco)toxicological testing, both *in vitro* and *in vivo*: i.e. what NM is being tested, exactly? However, most of the protocols for physicochemical properties are not standardized or are simply not available, e.g. protocols for measuring physicochemical properties in complex matrices such as environmental matrices, which increases the uncertainty of the obtained experimental value and of the models built upon the obtained experimental data.

Concerning the *in vitro* and *in vivo* hazard data, standardized testing methods for chemicals are not always considered standardized for NMs, and while the work of OECD WPMN is starting to give answers and guidelines there is still a lot of work to do to reach the point where validated methods with known uncertainty limits and for all toxicity endpoints are available. Therefore, when using the result of a toxicological test, this should be done with caution taking into account all possible uncertainties related to the type of test used (e.g. NM interference, test not specific for the endpoint, test not applicable for NMs, mode of action not relevant for NMs).
In hazard assessment, there are also uncertainties linked to difficulties in identifying the lead health effect. Lacking an accurate measurement of the adverse effects, the lead health effect can be misidentified. This uncertainty needs to be accounted for in the discussion of the outcomes of the risk characterisation.

In exposure assessment, main uncertainties are related to the available models, methods and instruments for exposure measurement and data. In general, scenario uncertainties are likely to be very similar for NMs as for conventional chemicals. Models that are currently available for NMs tend to be qualitative/semi-quantitative and not to have gone through a complete model evaluation or validation. In occupational settings, even if research work is ongoing and some progress is shown, there is only little personal monitoring equipment that is suitable for NMs and can measure the appropriate metrics. Unless samples can be analysed off-line for the NM in question, it is often difficult to obtain specific estimates of NM exposure (i.e. differentiated from background nanoparticles concentrations). Consequently, available measurement data come from stationary equipment results, which may not be representative of personal exposure. In addition, due to difficulties in and costs of the measurements, available data sets are often relatively small (in terms

of days covered by the measurement, individuals or tasks measured, not in terms of real-time data sets), which limits the possibilities of meaningful statistical analysis (ECHA 2012) and raises questions in terms of how representative the measurements are.

3.6.4. References

Bouchard D, Chang X, Chowdhury I, 2015. Heteroaggregation of multiwalled carbon nanotubes with sediments. Environmental Nanotechnology, Monitoring & Management, Vol. 4: 42-50

ECHA 2011. Guidance on information requirements and chemical safety assessment. Chapter R.5: Adaptation of information requirements. Version 2.1, December 2011. European Chemicals Agency, Helsinki

ECHA 2012. Guidance on information requirements and chemical safety assessment. Chapter R19: Uncertainty analysis. November 2012. European Chemicals Agency, Helsinki

ECHA 2014. Human health and environmental exposure assessment and risk characterisation of nanomaterials. Best practice for REACH registrants. Third GAARN meeting, Helsinki, 30 September 2013. European Chemicals Agency, Helsinki

ECHA 2016a. Guidance on information requirements and chemical safety assessment. Part E: Risk Characterisation. Version 3.0, May 2016. European Chemicals Agency, Helsinki

ECHA 2016b. Guidance on information requirements and chemical safety assessment. Chapter R.15: Consumer exposure assessment. Version 3.0, July 2016. European Chemicals Agency, Helsinki

ISO 2012. Nanotechnologies — Occupational risk management applied to engineered nanomaterials — Part 1: Principles and approaches. ISO TS/12901-1:2012

ISO 2008. Nanotechnologies — Health and safety practices in occupational settings relevant to nanotechnologies. ISO 2008 TR/12885

NANoREG Deliverable D1.9. Deliverable report for NANoREG. Grant Agreement Number 310584. NANoREG final report with answers to selected issues/questions

Vogel U, Savolainen K, Wu Q, van Tongeren M, Brouwer D H, Berges M. 2014. Handbook of Nanosafety. Measurement, Exposure and Toxicology. Elsevier Inc

WHO 1997. Determination of airborne fibre number concentrations: a recommended method, by phase- contrast optical microscopy (membrane filter method)

PART II – FORWARD-LOOKING STRATEGIES FOR THE SAFETY ASSESSMENT OF NANOMATERIALS

This part describes from a scientist's point of view three forward-looking strategies seeking to facilitate/accelerate the implementation of REACH for NMs, while discussing their benefits and potential limitations. Those three schemes include: i) the use of a nanospecific approach for prioritisation and risk assessment of NMs (NanoRA) (section 4); ii) the development and implementation of the NANoREG Safe-by-Design (SbD) concept (section 5); and iii) the integration of Life Cycle Assessment (LCA) and risk assessment in the case of NMs (section 6).

4. A NEW APPROACH TOWARDS NANOSPECIFIC PRIORITISATION AND RISK ASSESSMENT

In this section, a new approach for nanospecific prioritisation and risk assessment developed within NANoREG is illustrated. The currently available tools (e.g. guidance documents, models, protocols, decision trees) for addressing the nanospecific considerations discussed in this section are listed in the NANoREG Toolbox (Deliverable D1.12, see worksheet "4 Nanospecific prioritisation and risk assessment").

4.1. Introduction

Sections 2 and 3 of this document outline considerations on how to address NMs in the current legislative context, with focus on REACH (European Parliament and Council 2006). For the forward-looking strategies in Part II a different approach was chosen, which initially looked beyond the current legislative context to enable a more open view on how to tackle the challenges in the safety assessment of nanomaterials (NMs). This resulted in a proposal for a prioritisation and risk assessment strategy for NMs[35] that may not be implemented directly in the current legislative context. Nevertheless, the proposed approach herein may still provide valuable insights for risk assessment within REACH, in particular when adaptation of the standard testing regime (sub-section 3.3) is considered. Where links between the proposed approach and REACH are identified, this is indicated in the text and summarised in annex IV of this document.

NMs of the same chemical composition can have many different physicochemical properties (e.g. size, shape, charge), which results in a much larger variation of nanoforms (sub-section 3.1) compared to non-NMs (Maynard et al. 2006). As it may not be feasible for each individual nanoform to obtain the necessary physicochemical, exposure and hazard data for all relevant exposure scenarios and endpoints, many initiatives have been taken to explore ways that enable the risk assessment of NMs with a smaller data set. Important initiatives include amending tools like (Q)SAR, grouping, read-across and high-throughput screening/testing for NMs. For successful applicability of such new approaches it is crucial that sufficient good quality nanospecific information becomes available to form a sound scientific basis.

[35] NANoREG was not meant to cover next generation nanomaterials, therefore they are not explicitly considered in the approach illustrated in this section. However, this does not exclude that the approach may be applicable to next generation nanomaterials to some extent.

In this section, a new risk assessment strategy for NMs is described, which builds upon previous project outcomes. It has been developed within Task 5.7 of NANoREG and published by Dekkers et al. (2016). In the proposed strategy, approaches for (Q)SAR, grouping and read-across are integrated and expanded to give direction to where and how a more efficient risk assessment of NMs (e.g. across multiple nanoforms) can be performed and what type of information could be used for scientific justification. The proposed strategy uses the current scientific insights in the specific properties that are crucial in the behaviour and toxicity of NMs. It facilitates further development of a more efficient risk assessment for NMs in the future and accelerates the rate at which information needed for risk assessment can be generated. The main objectives of the proposed approach are:

a. To prioritise those applications of NMs that have the highest potential to cause human health effects (due to high exposure and/or toxicity)
b. To identify those aspects of exposure, kinetics or hazard that are most important to address in the human health risk assessment of NMs
c. To identify those situations where the use of nanospecific grouping, read-across and (Q)SAR is likely to become feasible and regulatory acceptable in the near future, and
d. To identify the type of information needed for the regulatory acceptance.

4.2. Proposed approach

The proposed approach consists of different phases. The first objective (prioritisation of NMs) is addressed in the first phase while the other three objectives (identification of information) are mainly addressed in the second and further phases.

From the first phase, one should be able to get a rough idea on the potential of a specific NM to cause adverse health effects by identifying:

a) Materials that have the highest potential to be hazardous (flagged red)
b) Materials for which the conventional (non-NM) risk assessment approach can be performed (flagged green), and
c) Materials that need further evaluation (flagged orange).

It is expected that only a few of the NMs that are currently on the market fall into the 'red' or 'green' category, because manufacturers tend to avoid the use of NMs which may be hazardous or quickly lose their functionality by falling apart into their ionic or molecular form. Therefore, the 'orange' category will probably be the largest group, for which further ranking is needed to indicate a relatively 'high', 'medium' or 'low' potential to cause harmful effects.

The proposed approach should be suitable for different uses by policy makers, regulators and industry. Policy makers and regulators can predominantly benefit from running the first phase of the approach to prioritise those applications that need to be addressed most urgently. Industry can use the first phase to get an initial impression on the suitability of the NM in a specific product based on the potential of that NM to cause adverse health effects across the different life stages of the product. The second and further phases can be used by regulators and industry to identify the most important information needs to address the nanospecific issues and/or investigate the possibilities for grouping or read-across[36].

The proposed approach is developed to be applicable to NMs that are already on the market and relates to the existing practice within REACH as described in section 2 and 3 of this document. Table IV.1 in annex IV of this document links the different phases and aspects of the proposed approach to the content of sections 2 and 3 of the NANoREG framework and related toolbox (NANoREG Deliverable D1.12).

The current regulatory frameworks on the safe use of chemicals, including REACH, are generally considered suitable to address the risks of NMs (EC 2012, OECD 2013). Within REACH, NMs fall under the substance identity of the chemical component of which the NM is made and are therefore subject to the same obligations as for any substance (see sub-section 3.1 for a more detailed discussion about substance identification of NMs under REACH). ECHA guidance is being modified or developed to provide registrants with recommendations on this aspect while there is also a call to adapt the legal text of REACH, especially with regard to the information requirements on physicochemical properties (DG GROWTH 2016, Roberts 2016) (see sub-section 3.2 for a more detailed discussion about the information requirements for NMs under REACH). The proposed approach gives direction where and how in REACH a more efficient risk assessment (e.g. across multiple nanoforms) can be performed and what type of information could be used for scientific justification. Dekkers et al. (2016) provide an overview of the relevant information and the tools that can be used to generate this information (reported in Table SI-1 of the supplementary information).

[36] Irrespective of the most important information needs identified within the proposed approach, information requirements set by regulatory frameworks, e.g. REACH, should be met.

It is to be noted that the focus is on human health. The potential risks for environment are also of importance, though beyond the scope of NANoREG Task 5.7, and therefore remain to be further investigated in the future.

4.2.1. Elements

The proposed prioritisation and risk assessment strategy is based on six elements, describing the most important nanospecific determinants in the process. Below, the six elements are briefly explained and a short argumentation for the selection of the element is given. The flow chart in Figure 4.1 gives an overview of the different phases of the proposed approach with the relevant elements indicated for each phase. Each of the six elements has its own colour. Next to the elements, specific aspects (e.g. properties, assays) are depicted in the same colour(s) as the elements they relate to.

More details on how these elements are incorporated within the proposed approach and considered in each phase are given in sub-sections 4.3 (on phase I) and 4.4 (on phase II).

1. Exposure potential

Exposure potential is included early in the present approach because exposure assessment is, in addition to hazard assessment, essential for performing risk assessment. Although some of the determinants for exposure (e.g. transformation) are also addressed in the other elements, these other elements mainly focus on the toxicokinetics and toxicodynamics of the NMs in relation to human health effects. The element exposure potential also includes the other determinants (e.g. routes of exposure, amount of NM used) that are important in identifying the 'hot spots' for exposure throughout the entire life cycle of the NM under investigation.

2. Dissolution

Dissolution is the key element to identify whether a NM is stable enough to exert nanospecific behaviour. It is very important to know if a NM dissolves into its molecular or ionic form and how fast (i.e. dissolution rate), where and under which circumstances this takes place. If a NM fully dissolves into its molecular or ionic form before it reaches its target, it may not exert any nanospecific behaviour and it is suggested to perform the conventional (non-NM) risk assessment approach. If a NM does not fully dissolve before reaching the target, the nanospecific behaviour and related effects should be further investigated. The NM may distribute to specific sites, where release of ions or molecules may cause acute effects. No or very slow dissolution may relate to accumulation in case of repeated exposure, and thereby increase the likelihood of nanospecific effects after long-term exposure. How fast dissolution occurs can indeed have a huge impact on

the exposure potential, behaviour and effects of a NM in humans (including absorption, translocation to secondary organs and accumulation in tissues)[37].

3. Transformation

This element is important since NMs may be transformed during their life cycle. The stability of their original appearance during manufacturing and the subsequent transformations (including the coating, corona, agglomeration, aggregation and disintegration to smaller units, dissolution, precipitation, adsorption and desorption, combustion, abrasion, oxidation and reduction) is very important for their behaviour and effects in humans and the environment.

4. Accumulation

The ability of NMs to accumulate in the human body may increase the likelihood for effects after long-term exposure. Some NMs have been shown to accumulate in the human body. Although it is not always known if this accumulation results in toxic effects or not, accumulation is a serious reason for concern in risk assessment and therefore needs to be included as one of the fundamental elements in the proposed approach.

5. Genotoxicity

This element is an important mechanism of toxicity, also for NMs, since genotoxicity is one of the possible mechanisms that may lead to cancer and, if germ cells are affected, also to developmental and reproductive effects. It is known that NMs can induce genotoxicity by directly or indirectly damaging or interacting with a DNA molecule (Louro et al. 2015).

6. Immunotoxicity

Another important mechanism of toxicity of NMs is the onset or triggering of an immune response, causing for example inflammation, immune stimulation or immunosuppression. In its chronic form, inflammation may lead to several health effects such as fibrosis, cirrhosis, lung cancer, cardiovascular diseases, neurological diseases, etc. There are different pathways by which NMs can trigger an immune response but not all cellular immune responses lead to notable inflammation.

[37] This issue is matter for discussion at a regulatory level.

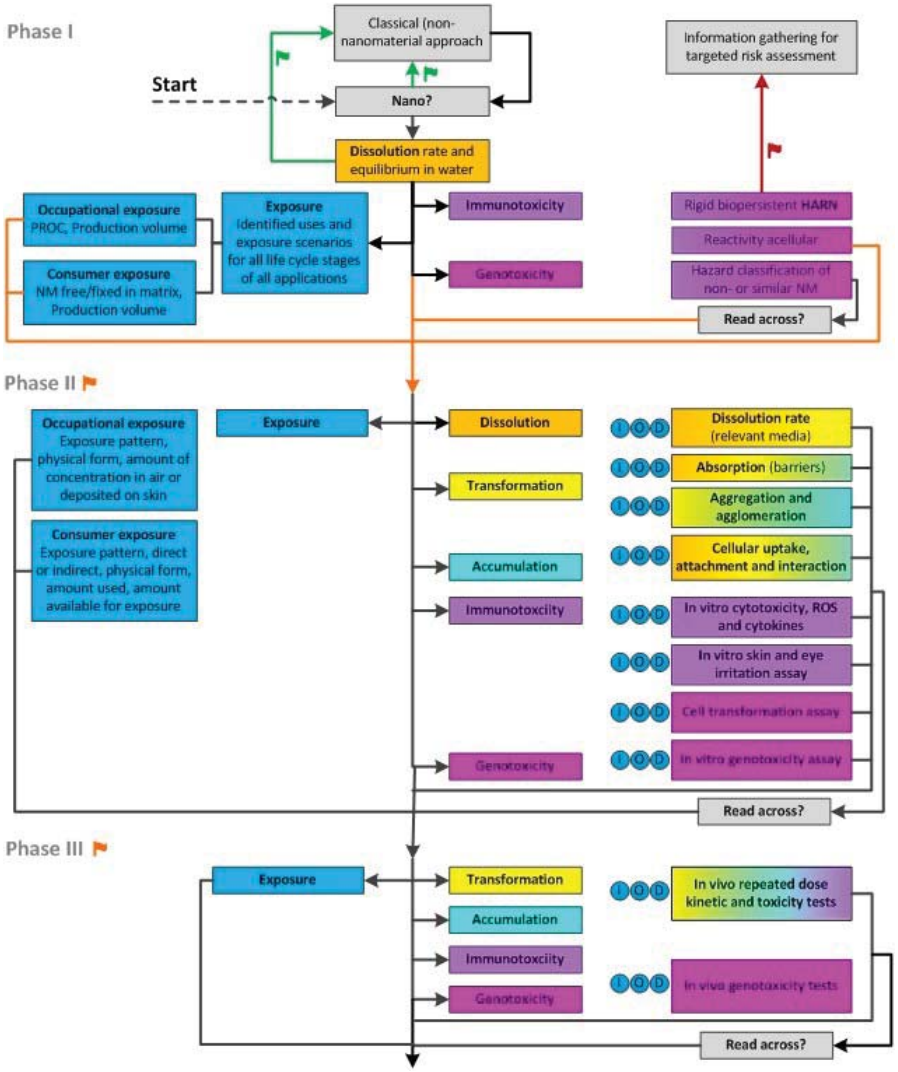

Figure 4.1: Flow chart showing the different phases of the proposed approach towards nanospecific prioritisation and risk assessment (source: Dekkers et al. 2016). Black arrows: evaluation of the nanomaterial (NM) following the elements related to kinetics, toxicity and exposure in phase I, II, III and further. Green arrows: the material is not a NM or has such a high dissolution rate in water that it dissolves into its molecular or ionic form before it reaches its target → the classical (non-NM) risk assessment can be performed. Red arrows: the material is a "rigid and biopersistent High Aspect Ratio Nanomaterial (HARN)" → substitution or information gathering for targeted risk assessment to evaluate the potential to cause mesothelioma is needed. Orange arrows: the material does not meet the criteria for classical (non-NM) risk assessment or targeted risk assessment to evaluate the potential to cause mesothelioma → use the information from phase I for prioritisation and/or further evaluation following the elements related to kinetics, toxicity and exposure in phase II, III and further. D = Dermal route of exposure; I = Inhalation route of exposure; NM = nanomaterial; O = Oral route of exposure; PROC = Process Category; ROS = Reactive Oxygen Species.

4.3. Description of phase I

4.3.1. Input (phase I)

In the following sub-section, the reader is guided through phase I of the proposed approach. Going through the flow chart (Figure 4.1), suitable information should be gathered or generated within each of the boxes. The flow chart starts in phase I on the upper left side of Figure 4.1, where the dashed black arrow "Start" points to the grey box "Nano?". To determine whether the investigated material is a NM, information is needed on physicochemical characteristics such as size and/or surface area. Other information needed in other boxes of phase I is the aspect ratio (shape and size), rigidity, biopersistence, dissolution and reactivity of the NM. For exposure, possible applications as well as production volumes and process and operational conditions are important. A more detailed description can be found in the paragraphs underneath. The information needed in phase I is often available from manufacturers or can be obtained through analytical or acellular assays. An overview of the relevant information for going through the entire flow chart is given in Table SI-1 of the supplementary information to the manuscript published by Dekkers et al. (2016). The way to move forward in phase I of the flow chart is described below.

4.3.2. Physicochemical characteristics (phase I)

4.3.2.1. Nano?

Phase I starts with determining if the material indeed is a NM (Figure 4.1: see the dotted line from the left). There has been a lot of discussion on the definition of a NM and multiple definitions are used in various international organisations, committees and jurisdictions all over the world. Within NANoREG the European Commission's Recommendation 2011/696/EU on the definition of the term 'nanomaterial' (EC Definition) is used (see section 2 for more information). The analytical methods to determine whether a material meets the criteria of the EC Definition have been evaluated by JRC and within NANoREG work package 2 (JRC 2012, De Temmerman et al. 2014). If the material does not meet the criteria of the EC Definition, it can be evaluated using the information on the chemical composition of the non-NM (i.e. Figure 4.1: follow the green arrow), effectively leaving the proposed approach. If the material does meet the criteria of the EC Definition, the black arrow in Figure 4.1 should be followed and dissolution in water should be evaluated.

4.3.2.2. Dissolution rate and equilibrium in water

The water solubility is conventionally measured using the OECD Test Guideline (TG) 105, which defines the water solubility of a substance as the saturation mass concentration of the substance in water at a given temperature and proposes two methods to measure it for conventional substances (the column elution method and the flask method). The OECD TG 105 is already used for aggregated and agglomerated NMs but it needs to be revised and refined especially for NMs that disperse into small primary nanoparticles (OECD 2014). Several approaches for risk assessment of NMs propose to use the outcomes of these types of tests to distinguish soluble from non-soluble NMs (BAuA 2013, Arts et al. 2015). However, as no equilibrium is reached in many situations relevant for human health risk assessment, the water solubility does not provide sufficient insight in the possibility of uptake of NMs as physiologically relevant time frames are not considered (Oomen et al. 2015). It might be more informative to use the dissolution rate, because the information on whether a NM dissolves into its molecular or ionic form and at what rate before (or after) it reaches its potential target is far more relevant. OECD guidance document n° 29 describes how the dissolution rate of metals and metal compounds in aqueous media can be measured. However, there are no nanospecific guidelines for such tests and also no proposed cut-off values to distinguish soluble from non-soluble NMs are proposed (Tantra et al. 2015). Therefore, a comparison of the dissolution rate of the NM to that of the chemical components of which is it composed might give an indication on the possibility to use the data of the non-NM (read-across). If a NM has a very fast dissolution rate (i.e. close to instantly dissolved), the NM can be evaluated using the information on the chemical composition of the non-NM (i.e. Figure 4.1: follow the green arrow towards the box "Classical (non-NM) approach"). If a NM does not have a very fast dissolution rate or a slower dissolution rate than its non-NM counterpart, the black arrow down in Figure 4.1 should be followed and the nanospecific behaviour and effects should be further evaluated both for kinetics/hazard (Figure 4.1: right side) and exposure (Figure 4.1: left side).

4.3.3. Exposure (phase I)

4.3.3.1. Exposure: identified uses and exposure scenarios for all life cycle stages and applications

The first indication of exposure for workers and consumers is based on a similar (qualitative) approach for identifying priorities in exposure scenarios as described in NANoREG Deliverable 3.1. In this report, Exposure Scenarios (ESs) of the highest potential occupational (and environmental) exposure along the life cycle of the currently marketed NMs have been prioritised. The approach starts with the identification of the main applications in which the NM is used. After this step, the life cycle for the NM and each of its main applications is mapped, followed by the identification of

identified uses[38] and ESs[39] for each life cycle stage of each application, as required by REACH (see section 3.5 for more information).

4.3.3.2. Occupational exposure: PROC and production volume

In order to get a more specific understanding of the occupational exposure, the information gathered on identified uses is coupled to the Contributing Exposure Scenarios (CESs) for each life cycle stage of each application. Under REACH, a CES represents a set of specific exposure conditions that describe a single worker's or consumer's activity. A CES can be directly linked to the Process Categories (PROCs) for occupational exposure, for which ranking values have been determined within ECETOC Targeted Risk Assessment (TRA) (ECETOC 2012). The ranking of the PROCs within ECETOC TRA is mainly based on the process and operating conditions, including dustiness, energy in the process, enclosure level of the process, concentration in the preparation, duration of the activity, ventilation and the use of personal protection (ECETOC 2012). A first ranking of the occupational exposure can be obtained by combining the ranking values of the PROCs with the estimated production volume of the NM in a certain application (see Figure 4.1 and Table 4.1). The relevant route(s) of exposure are also important for determining the strategy in phase II of the proposed approach.

Table 4.1: Ranking of the occupational exposure potential in phase I based on production volume and PROC of the most important occupational scenarios within the life cycle of each nanomaterial application. PROC = Process Category.

Production Volume → PROC ↓	high	medium	low
high	high	high	high
medium	high	medium	medium
low	medium	low	low

[38] REACH definition of Identified Use (IU): a use of a substance on its own or in a mixture, which is intended by an actor in the supply chain, including his own use, or a use that is made known to him in writing by an immediate downstream user.

[39] Exposure Scenarios (ES) should address the manufacture and identified uses. According to REACH Annex I, registrants who are required to carry out a Chemical Safety Assessment (CSA) with exposure assessment have to address all stages of the life cycle of the substance including those resulting from the manufacture and identified uses if they happen in the EU territory (e.g. the use of substances in articles).

4.3.3.3. Consumer exposure: NM fixed in matrix/free and production volume

For consumer exposure, no ranking of the ESs was performed in NANoREG Deliverable 3.1, because of the absence of information on the main determinant of consumer exposure (i.e. the transfer factor[40]). However, when the main applications in which the NM is used are known, the most important exposure aspects for phase I of the proposed approach can be selected based on information from the following sources: Wijnhoven et al. (2009), NANoREG Deliverable D3.1, and RIVM (2015). For consumer exposure, the first ranking is based on the production volume of the NM in the application in combination with the way the NM is incorporated in the consumer product (fixed within a matrix or freely available) (Table 4.2). Products containing freely available nanoparticles suspended in liquids or airborne aerosols (e.g. spray applications) are expected to cause a higher consumer exposure than products in which the NMs are fixed or incorporated into a solid matrix (e.g. a bicycle frame). However, it is not always clear if and how the nanoparticles are fixed in the matrix of the product and if they stay fixed or migrate, evaporate, washout, wear off, etc. during the use of the product. In addition, the most important route(s) of exposure are important for further determination of the strategy in the next phase of the approach (i.e. phase II).

Table 4.2: Ranking of the consumer exposure potential in phase I based on production volume and way of incorporation in the exposure matrix (fixed/free) for the most important consumer exposure scenarios within the life cycle of each nanomaterial application

Production Volume → Fixed in matrix / Free ↓	high	medium	low
fixed in matrix	medium	low	low
free	high	high	high

4.3.4. Kinetic and hazard aspects (phase I)

4.3.4.1. Rigid biopersistent HARN

One of the established mechanisms of toxicity of NMs is the potential of rigid and biopersistent High Aspect Ratio (fibre-like) NMs (HARN) to cause "frustrated phagocytosis" by macrophages after inhalation. This may lead to mesothelioma, a specific form of cancer also known from exposure to asbestos (Donaldson et al. 2010). Information needed for determining whether a NM is

[40] Transfer factor is the fraction (0 to 1) of the substance transferred from the product to the skin and represents a realistic worst-case dose.

a potential "rigid biopersistent HARN" includes the aspect ratio, rigidity as well as the biopersistence of the NM under investigation. Rigid biopersistent HARN materials with a length (L) ≥ 5 µm, a diameter (D) < 3 µm and a L/D ratio > 3 should either be substituted by an alternative substance or evaluated for their potential to cause this specific type of health effect. This means avoiding most elements in phase II and III of the proposed approach and instead target information gathering on the potential to cause mesothelioma (i.e. Figure 4.1: follow red arrow to the box "Information gathering for targeted risk assessment").

4.3.4.2. Acellular reactivity

One of the most important hypotheses of nanospecific toxicity is the increased surface reactivity of NMs due to their relatively large surface-to-volume ratio and sometimes also surface modification. Due to the relatively large surface-to-volume ratio and specific functionalization of NMs, the reactivity of NMs can be enhanced compared to non-NMs. This reactivity may trigger the generation of reactive oxygen species (ROS), leading to oxidative stress and subsequent inflammation in biological tissues.

For metal and metal oxide nanoparticles, the surface reactivity can for example be predicted using the conduction band energy levels in combination with the solubility (Zhang et al. 2012). Based on this publication, metal and metal oxide particles can be ranked for their potential to cause oxidative stress *in vitro* as well as acute inflammation after inhalation *in vivo*. For other NMs and other exposure routes, a first indication on the reactivity of NMs can be obtained by using acellular assays, for example by measuring ROS formation or biological oxidant damage in serum (e.g. with the FRAS (Ferric Reducing Ability of Serum) assay) (Nel et al. 2014, Arts et al. 2015). It should be noted that these assays only provide a first indication of the oxidative properties, since the local environment, i.e. cell culture media *in vitro* or body fluids *in vivo*, can influence the reactivity of the NMs in various ways, for example by containing antioxidants or by altering the nanoparticles surface due to biomolecule corona formation. The results in the band gap analysis or acellular reactivity assays are used to define further hazard ranking or subgroups (Table 4.3). In addition, the results can give direction to further investigation of the reactivity in cellular environments in phase II.

Table 4.3: Further hazard ranking in phase I based on classification and reactivity

Classification → Reactivity ↓	high	medium	low
high	high	high	medium
low	high	medium	low

4.3.4.3. Hazard classification of non- or similar NM

Another important indication on the toxicodynamics of a NM can be obtained by looking at the hazard classification of the correspondent non-NM (i.e. bulk form) or a similar NM according to the CLP Regulation (European Parliament and Council 2008) (see section 3.4 for more information on CLP Regulation). It can be expected that NMs having chemical components classified, for instance, as genotoxic or sensitizer also have genotoxic and/or sensitizing properties. Although there may be differences with respect to the critical dose levels and target organs, the possibilities to use read-across and grouping for these specific endpoints based on the hazard classification of the non-NM or similar NMs might be considered (i.e. Figure 4.1: follow the arrow towards the box "Read-across?").

Guidance on grouping and read-across for chemicals has been published by ECHA (2013) and OECD (2014). The use of read-across and grouping primarily based on the hazard classification of non-NMs or similar NMs is probably only feasible and regulatory acceptable if this leads to a (worst-case) classification of the NM into the highest category, which requires risk reducing measurements that prevent or minimise human exposure in all life cycle stages of the NM. If the chemical components of a NM or a similar NM are not classified or if no chemical counterpart can be identified, this does not mean that the NMs do not cause these specific health effects. Further evaluation of the other elements related to kinetics, toxicity and exposure in phase II is then recommended by following the orange arrow down in Figure 4.1.

4.3.5. Output phase I: prioritisation and ranking

Within the orange category, prioritisation can be obtained by combining further exposure and hazard ranking to indicate a high, medium or low potential to cause harmful effects within the life cycle of each NM application. The kinetic behaviour is explicitly included in this further ranking but is already implicitly taken into account by only including NMs that do not have a fast dissolution in water in the orange category.

Further exposure ranking for occupational and consumer exposure is described in previous sub-sections 4.3.3.2 and 4.3.3.3 and Tables 4.1 and 4.2.

Further hazard ranking is based on classification according to CLP Regulation and reactivity:

- If one of the chemical components of a NM or a similar NM is classified as carcinogenic, mutagenic or reprotoxic (CMR, all categories), this NM is ranked 'high' with respect to its hazard. The 'medium' classification category describes NMs that are not classified as CMR

but as (respiratory) sensitizers or irritating substances, whereas a material with only acute toxicity or no classification for toxicity is ranked 'low'.

- If a NM has a high reactivity as predicted by the band gap analysis for metal and metal oxide nanoparticles or acellular ROS or FRAS assays for non-metal NMs, it is also ranked 'high' with respect to its hazard. At the moment, the criteria for high reactivity are not precisely defined for each of the assays.
- If the chemical components or similar NMs are not classified and the NM under investigation does not have a high reactivity, the NM is ranked 'low' with respect to its hazard. The total hazard ranking results in the 'medium' category when the classification is medium with a low reactivity or when the classification is low with a high reactivity (Table 4.3). If no information on the hazard classification or reactivity is available, the material is ranked 'high' with respect to its hazard.

Combining the exposure ranking with the hazard ranking of the most important occupational and consumer ESs within the life cycle of each NM application gives a further ranking in three subgroups to indicate a high, medium or low potential to cause harmful effects (Table 4.4).

Table 4.4: Combined ranking of potential exposure and hazard in phase I of the most important occupational and consumer exposure scenarios within the life cycle of each NM application

Hazard → Exposure ↓	high	medium	low
high	high	high	medium
medium	high	medium	medium
low	medium	low	low

Please note that 'low' in Table 4.4 does not mean that further action for risk assessment is not needed. Information in line with regulatory requirements needs to be complete. However, this ranking (high, medium, low) can be used for prioritisation as indicated in sub-section 4.1.

4.3.6. Output phase I: information used in phase II

The materials that are flagged 'green' or 'red' do not enter phase II of this flow chart. The materials that are flagged 'green' need to be evaluated according to the classical non-NM risk assessment. The materials that are flagged 'red' can skip most elements in phase I, II and maybe also III, to enable targeted information gathering on the potential to cause mesothelioma.

The group of materials that are flagged 'orange' do enter phase II. The rankings as described in the previous sub-sections are not used in phase II as such. However, the information on which these rankings are based is used to give direction to those elements that should be addressed in phase II by preference. The information indicating which elements are most important is different for each exposure situation and should be evaluated on a case-by-case basis. Although it is not possible to describe this for each situation, an attempt to a general description of which information of phase I indicates which type of information is most relevant to obtain in each of the boxes in phase II is given in the sub-sections underneath.

4.4. Description of phase II

As depicted with the blue circles in Figure 4.1, the most important route(s) of exposure is (are) key in determining which type of information is relevant to obtain in each of the boxes in phase II. The route(s) of exposure also indicate(s) the relevant media for testing the dissolution rate and also the specific *in vitro* models (cell types and endpoints) to be investigated. In addition, information on the hazard classification based on CLP Regulation of the chemical components of the NMs may also point towards relevant cell types and endpoints to be investigated.

Relevant cell types for *in vitro* assays on the cytotoxicity, immunotoxicity and genotoxicity can be selected based on the information on the dissolution rate in relevant media and *in vitro* absorption together with the limited amount of knowledge available on the absorption, distribution and translocation of nanomaterials (in general).

Below a more detailed description of the type of information and possible methods to generate this information is given for each of the boxes in Figure 4.1. Most information needed in phase II can be obtained through analytical and *in vitro* assays. An overview of the relevant information for going through the entire flow chart is given in Table SI-1 of the supplementary information to the manuscript published by Dekkers et al. (2016). Table IV.1 in annex IV of this document links the different aspects of the flow chart (Figure 4.1) to sections 2 and 3 of the NANoREG framework and related toolbox (NANoREG Deliverable D1.12).

4.4.1. Exposure (phase II)

4.4.1.1. Occupational exposure: exposure pattern, physical form and amount or concentration

In phase II, it is proposed to extend the information obtained on the occupational exposure in the first phase with information on the exposure pattern (frequency and duration), physical form and concentration (in air) or amount (deposited on skin). These determinants were selected because they have the largest influence on the final ranking score illustrated in NANoREG Deliverable D3.1 and information on these determinants is generally available for most exposure scenarios. Using

this additional information further ranking of the most important occupational ESs as described in NANoREG Deliverable D3.1 can be performed.

4.4.1.2. Consumer exposure: exposure pattern, direct or indirect, physical form and amount

For consumer exposure, it is proposed to obtain additional information on the exposure pattern (including direct or indirect exposure, frequency and duration), physical form, amount used and/or amount available for exposure in phase II. This selection was based on ECETOC TRA (ECETOC 2012), ConsExpo Nano (**Error! Hyperlink reference not valid.**RIVM 2015) and Wijnhoven et al. (2009). The amount available for exposure is based on the release out of the matrix of a product, which is often very difficult to measure. However, based on the product description, an indication of the potential release of the NM out of the matrix of the product can be obtained. In general, the potential release from solid consumer products is expected to be less than the release from liquid or powdered products. In addition, incorporation of the NM into the solid matrix of the consumer product itself (e.g. incorporation of silver NMs into textile fibres) probably leads to less release of the NM than applying a coating to the surface of a solid consumer product (e.g. spraying a coating containing silver nanoparticles onto the textile product). If no information is available, the assumption that all material is released may be considered as worst-case assumption. Using this additional information, further ranking of the most important consumer ESs similar to the ranking described in Wijnhoven et al. (2009) can be performed.

4.4.2. Kinetic and hazard aspects (phase II)

4.4.2.1. Dissolution rate (relevant media)

Recently, the different analytical methods available to measure solubility of NMs have been described (Tantra et al. 2015). Although a wide variety of techniques are available with the capability to measure total dissolved species or free ions, but not both, only a limited number of them are suitable for measurement in biological media. Electrochemical and colorimetric based detection schemes are able to measure the latter whilst atomic spectrometry based techniques are able to measure the former if combined with separation techniques such as ultrafiltration or ultracentrifugation.

In general, the exposure route determines what the relevant medium is. For the inhalation route of exposure, dissolution in lung airway epithelial lining fluid and (macrophage) phagolysosomal simulant fluid is relevant. The oral route can be covered by measuring dissolution of NMs in food matrices, gastrointestinal tract simulation fluid and macrophage phagolysosomal fluid. For dermal conditions, the dissolution rate in artificial sweat could be used.

In general, the dissolution rate in relevant media can provide information on the forms or speciation (coated or uncoated nanoparticle, agglomerate, aggregate, ionic and molecular form) of the NM when it comes into contact with the relevant areas in the human body, when it is absorbed and when it is distributed and translocated into specific organs and/or cellular compartments. This information is very important because the extent and rate in which the NM transforms into these different forms of the material (including the extent to which it is dissolved) greatly influences its kinetic behaviour and toxicity. For some NMs, the toxicity is mainly determined by the extent and rate in which it releases ions, while the toxicity of other NMs is mainly determined by the particulate properties that induce an inflammatory response (Cho et al. 2012). It should be noted that more complex NMs cannot be seen as homogeneous objects when evaluating the dissolution rate.

4.4.2.2. Absorption (barriers)

In vitro test methods simulating pulmonary (MucilAir™) or gastrointestinal barriers (Caco2) have been developed within NANoREG based on existing protocols (ECVAM 2013) but these still need to be validated. Other physiological barrier models based on cell cultures and *ex vivo* tissues have also been used within NANoREG to simulate the blood brain barrier (Dominguez et al. 2014) and the oral mucosa barrier. To investigate uptake through the skin, an accepted *in vitro* test method is available (i.e. the *in vitro* skin absorption method in accordance to OECD TG 428) but it still needs to be validated for NMs.

For the inhalation route, generally only a very small percentage of insoluble NMs is translocated or accumulated in extra-pulmonary organs. Studies with partially soluble NMs typically show a larger percentage of particle translocation to extra-pulmonary organs as compared to the insoluble particles. However, it should be noted that with the current analytical tools it is difficult to determine whether either the particles themselves or another form of the material (e.g. molecular or ionic) are translocated (Powell et al. 2010). Therefore, aggregation and agglomeration state of the NM influences its bioavailability. The rate of NM agglomeration in different vehicles is affected by the pH level. The different pH conditions in the GT and the presence of digestion enzymes might influence the behaviour (i.e. ion release, dissolution) of some NMs. It has been suggested that positively charged materials exhibit poor bioavailability due to electrostatic repulsion and mucus entrapment (Kermanizadeh et al. 2015). For NMs, dissolution rates in physiologically relevant media like the gastrointestinal simulated fluid have been suggested to be the decisive factor determining oral uptake.

NM size appears to be highly significant for dermal penetration. Materials larger than 100 nm in one or more dimensions do not seem to penetrate through the stratum corneum. Aggregation and agglomeration state is crucial in the degree of penetration and potential translocation (Kermanizadeh et al. 2015).

Information on absorption into the body provides information on the need to consider only local or also systemic effects. However, it is often difficult to distinguish between complete absence and little transport in *in vitro* barrier systems. For NMs even very little uptake may result in relevant internal levels due to low elimination and accumulation in time. This should be considered when data from *in vitro* barrier models is used. Currently, the scientific knowledge on the behaviour of NMs within the human body is not sufficiently developed to predict the distribution and translocation of NMs throughout the human body after inhalation, dermal or oral exposure. Without specific modifications, most poorly soluble NMs that reach the systemic circulation are mainly distributed to tissues that are rich in reticuloendothelial cells, such as liver and spleen. However, the nanospecific Physiologically Based Pharmacokinetic (PBPK) models developed to date mostly concern models in rats and mice for a specific type of NM (Bachler et al. 2013, Lin et al. 2015, Lin et al. 2016). Development of more general PBPK models and extrapolation of these models to humans should, in the near future, make it possible to predict the distribution and translocation of several types of NMs in the human body. In the meantime, one may use absorption rates in combination with intravenous kinetic models developed for specific NMs to estimate internal dose levels, taking into account the physicochemical properties of the NM and the NM on which the kinetic model is based (e.g. Van Kesteren et al. 2014). Based on these estimated internal dose levels relevant internal barrier models and relevant cell types for *in vitro* assays can be selected. When, for example, a NM is likely to reach the systemic circulation, *in vitro* blood-brain or placental barrier models might be relevant, though it should be noticed that such *in vitro* models cannot distinguish between low and no translocation. For NMs that are likely to be distributed to the liver, hepatic cell lines should be considered for *in vitro* genotoxicity testing.

4.4.2.3. Aggregation and agglomeration

Some of the analytical methods used to determine if a material meets the criteria of the EC Definition (JRC 2012, De Temmerman 2015) (see section 2) can also be used to determine the aggregation and agglomeration. The most suitable methods should be selected taking the environment or matrix surrounding the nanomaterial into account. If inhalation is one of the most important routes of exposure, information on the aggregation and agglomeration as estimated by the size distribution of the aerodynamic diameter of the aerosol is very important to determine the deposition in the respiratory tract and subsequent translocation from the lungs to the blood stream, which are largely dependent on the diameter of the aggregated or agglomerated NMs.

The largest level of deposition is at the smaller sub-micron size range (< 0.1µm), with particles able to penetrate the trachea-bronchial and alveolar regions. Deposition of particles in the range > 0.5 µm is related to their aerodynamic diameter whilst for particles < 0.5 µm deposition is related to their diffusion equivalent diameter (Schulz et al. 2000).

The Average Agglomeration Number (AAN) has been proposed to assess the dispersibility of NMs (Arts et al. 2015). NMs that remain dispersed as constituent particles (with AAN < 3) are defined as 'mobile', since they may potentially move between body compartments.

Information on the aggregation and agglomeration of a NM can be used to predict the ability of absorption, translocation and distribution in the body, which can be used in the selection of relevant internal barrier models and relevant cell types for *in vitro* assays.

4.4.2.4. Cellular uptake, attachment and interaction

Information on the cellular uptake, attachment and interaction of NMs can be studied using flow-cytometry, microscopy and inductively coupled plasma mass spectrometry (ICP-MS). Flow-cytometry and ICP-MS can measure quantitatively, but cannot distinguish between externally attached and fully internalised NMs. Furthermore, ICP-MS cannot distinguish between dissolved ions and nanoparticles and can only be used for electron-dense material and not for detecting liposomes, polymers, or dendrimers. Confocal microscopy gives qualitative insight into the subcellular localisation and three-dimensional structure of particles. Transmission electron microscopy (TEM) can be used to confirm subcellular particle localisation and tree-dimensional structure with high resolution. This method allows semi-quantitative assessments but the procedure is time-consuming. Combining TEM with energy-dispersive X-ray spectroscopy (EDX) makes it possible to confirm the elemental composition of the nanoparticles (Kettiger et al. 2013).

Several *in vitro* assays have been tested and further developed within the NanoREG project based on standard toxicity protocols developed for pharmaceutical products but these still need to be validated for NMs. One may also consider studying the cellular uptake, attachment and interaction in the same *in vitro* assay(s) used to investigate the cytotoxicity and cytokine induction.

Information on cellular uptake, attachment and interaction gives a first indication on the possible mechanisms of toxicity, such as damaging different cellular targets through the release of ions, the generation of ROS or the binding and interaction with intracellular proteins (Nel et al. 2009). For example, direct interaction of a NM with DNA can only occur if the NM is taken up by the cell and is able to reach the DNA within the nucleus.

4.4.2.5. In vitro cytotoxicity, ROS and cytokines

There are many *in vitro* assays based on a range of cell types and endpoints to investigate cytotoxicity, ROS generation and cytokine induction. Several *in vitro* assays have been tested and further developed within NANoREG. These include standard protocols for MTS assay, the neutral red assay (adapted from OECD TG 432), cellular impedance (Paget et al. 2014), micronucleus assay (OECD TG 487), mammalian gene mutation test (OECD TG 490), colony forming efficacy

(OECD TG 476), the comet assay (Collins et al. 2004), ROS detection by dichlorofluorescin diacetate (DCFDA), and interleukin expression upon exposure of NMs to cells, but these still need to be validated for NMs since in several cases the nature of the NMs may interfere with detection methodologies (OECD 2013).

Some of these assays allow studying the cellular uptake, attachment, interaction, cytotoxicity, ROS generation and/or cytokine induction in the same *in vitro* system. The appropriate assay should be selected taking into account that NMs often show major interference with the *in vitro* assay or read-out system. Furthermore, it is essential to test for endotoxin contamination before studying the immunotoxicity of NMs *in vitro* (Dobrovolskaia et al. 2009).

As for non-NMs, the results of these *in vitro* assays cannot be used to predict human limit values. However, they give insight in the possible mechanisms of toxicity. Measuring the levels of pro-inflammatory cytokines and other inflammatory mediators may give insight into the mechanisms of the immunomodulating effects of a NM *in vitro*, such as inflammasome activation or dendritic cell maturation (Elsabahy and Wooley 2013). In addition, *in vitro* assays may give a first indication on the ability of the NMs to cause immunotoxic effects *in vivo*. Cellular ROS assays provide information on the ability of NMs to generate ROS within a cellular environment. Measuring the cytotoxicity is important for a good interpretation of the results of the *in vitro* cytokine and genotoxicity assays. In addition*, in vitro* cytotoxicity assays may give insight into the mechanisms of cytotoxicity, including damaging the plasma membrane, mitochondria, lysosomes or DNA through the release of ions, the generation of ROS or the binding and interaction with intracellular proteins (Nel et al. 2009).

4.4.2.6. In vitro skin and eye irritation tests

Several *in vitro* skin and eye irritation tests are available, including the rat skin transcutaneous electrical resistance (TER) test (OECD TG 431), the reconstructed human epidermis (RHE) skin irritation test (OECD TG 439), the Bovine Cornea Opacity Permeability (BCOP) test (OECD TG 437), the Isolated Chicken Eye (ICE) test (OECD TG 438), and an *in vitro* cell assay (OECD TG 460). These assays were developed for the evaluation of skin and eye irritation of chemical substances but not all of them have been validated for chemical substances yet and none of them have been validated for NMs (SCENIHR 2015).

Information on *in vitro* skin and eye irritation gives an indication on the ability of the NMs to cause these effects *in vivo*.

4.4.2.7. Cell transformation assay

Several *in vitro* cell transformation assays (CTAs) are available to assess initiation and tumour promotion potentials but none of them have been validated for chemical substances or NMs (OECD 2016). CTAs measure induction of phenotypic alterations characteristic of tumourigenic cells. CTAs mimic some key stages of *in vivo* multistep carcinogenesis and have been shown to have a good concordance with rodent bioassay results, detecting both genotoxic and non-genotoxic carcinogens (Creton et al. 2011).

Information on *in vitro* cell transformation ability gives an indication on the possible mechanisms of carcinogenicity and an indication on the ability of the NMs to cause these types of effects *in vivo*.

4.4.2.8. In vitro genotoxicity

The strategy for *in vitro* genotoxicity testing of NMs needs to include the detection of the most relevant events for the multistep process of malignancy (gene mutations, clastogenicity and aneugenicity). At each stage of the testing strategy, expert judgment is necessary to decide on the relevance of a result considering the existing weight of evidence.

Tests for gene mutation in mammalian cells can be used, e.g. the mouse lymphoma TK gene mutation assay (MLA) (OECD TG 490), which uses the autosomal thymidine kinase (Tk) gene as a reporter of mutations in the L5178Y/Tk+/− mouse lymphoma cell line or the hypoxanthine guanine phosphoribosyltransferase (Hprt) gene forward mutation assay in Chinese Hamster Ovary (CHO) cells (OECD TG 476). In addition, the chromosomal aberration (OECD TG 473) and the cytokinesis-blocked micronucleus (CBMN) tests (OECD TG 487) are sensitive and reliable assays for the analysis of chromosome damage in mammalian cells. The former is used for detection of structural chromosome aberrations, i.e., chromatid- and chromosome-type breaks and rearrangements in cultured mammalian cells (OECD TG 473). The CBMN test allows the detection of micronuclei in the cytoplasm of interphase cells (Fenech 2000) containing whole chromosomes (aneugenic events) or chromosome fragments (clastogenic events) during cell division (OECD TG 487). On the other hand, a genotoxicity assay that has been strongly recommended for regulatory purposes is the alkaline single cell electrophoresis or comet assay (EFSA 2012). The comet assay is a sensitive and cost-effective method for the identification of DNA strand breaks and oxidative DNA lesions having the added advantage of requiring a very low quantity of substance or material for analysis compared to other *in vitro* genotoxicity assays. Alternatively, DNA double strand breaks could be assessed by analysing the phosphorymation of the H2AX histone using specific antibody. Several *in vitro* genotoxicity assays have been tested and further developed within NANoREG (adapted from OECD TG 487, 476 and 490) (Collins et al. 2016).

Information on *in vitro* genotoxic mechanisms gives an indication on the possible genotoxicity and the ability of the NMs to cause cancer. Positive results indicate that these genotoxic endpoints

might need to be investigated *in vivo* (or read-across to *in vivo* studies with similar materials should be considered). Before performing *in vivo* tests kinetic information is needed to assess which target tissues might be reached (including germ cells for potential reproductive effects). Negative results might in the future be sufficient to rule out these genotoxic effects, provided the most relevant test methods, cell types, and dose levels have been tested according to high quality standards to gather enough weight of evidence. In the future, when more scientific knowledge becomes available, it might also be possible to use *in silico* methods (e.g. validated (Q)SAR for NMs) to build stronger predictions and support the weight of evidence.

4.4.3. Output phase II

In contrast to the output of phase I, the information obtained in phase II does not lead to a ranking of NM applications. However, the output gives direction to the information that needs to be obtained in phase III.

4.5. Description of phase III and further

In phase III, additional information on other determinants or exposure measurements may be obtained to give further insight into the risks associated to critical ESs. Guided by information obtained on the kinetics and hazard in phase II, *in vivo* studies to confirm the potential absorption, irritation, immunotoxicity and genotoxicity indicated by the *in vitro* studies might be needed. What information from phase II may trigger the type of information to be gathered in phase III (and further) is different for each NM application and exposure situation. Although it is not possible to describe this for each situation, a general description of which information of phase II may trigger the need to generate which type of information in phase III is given in the paragraphs underneath. Table SI-1 of the supplementary information to the manuscript published by Dekkers et al. (2016) provides an overview of the relevant information and tools, which can be used to generate this information.

Positive results of *in vitro* absorption assays may trigger further investigation of the ability of a NM to become systemically available (and possibly cause systemic effects) in an *in vivo* repeated dose kinetic and toxicity test. Negative results of *in vitro* absorption assays should be interpreted with care because it is often difficult to distinguish between complete absence and little transport in *in vitro* barrier systems. Therefore, negative results may indicate the need for more information on the dissolution, transformation and systemic toxicity of the NMs under investigation. Together with information on the size, aggregation, agglomeration as well as information on the lack of

absorption, systemic distribution and toxicity of similar NM or non-NMs, the possibility of read-across might be considered.

The results of *in vitro* assays investigating cellular uptake, attachment, interaction, cytotoxicity, ROS generation and/or cytokine induction give insight in the possible mechanisms of toxicity, which may trigger the measurement of specific parameters (cytokines, oxidative stress markers) in *in vivo* studies. Eventually, this may also highlight the relevance of specific endpoints to be considered.

Positive results of *in vitro* genotoxic assays may trigger further investigation of genotoxicity by *in vivo* genotoxicity testing (or read-across to *in vivo* studies with similar materials should be considered). Before *in vivo* genotoxicity tests are performed, information on the kinetics of the NM is needed to enable the selection of the relevant tissues.

Positive results of *in vitro* cell transformation and *in vivo* genotoxicity studies together with observed systemic availability, expected accumulation and toxicity (e.g. inflammatory effects) from *in vivo* repeated dose toxicity tests may trigger long-term repeated dose kinetic and toxicity testing to rule out accumulation and long-term effects, including carcinogenic, cardiovascular and adverse reproductive effects.

4.6. Discussion and conclusions

Performing risk assessment for each individual nanoform on a case-by-case basis may require a lot of experimental animals as well as time, effort and money. The proposed approach, based on six elements, provides alternative ways to address the risk assessment of NMs, by prioritising those applications with the highest potential health risks and identifying the most important information to address the nanospecific issues or perform risk assessment across different nanoforms (e.g. using (Q)SAR, grouping or read-across).

The prioritisation is just a first indication on the potential health risk of a nano-enabled application. Because it should only be used for prioritisation, applications within the 'low' risk category should not be disregarded for further evaluation. Potential health risks of all categories ('low', 'medium' and 'high') still need to be verified and refined. Possibly, in phase I, not all exposure situations have been identified or unexpected toxicokinetic or toxicodynamic effects have not been identified.

The proposal suggests specific steps to gather certain pieces of key information. It should be noted that these selected pieces of information might not always be easy to obtain or generate. Within

REACH, industry is responsible for providing sufficient information to ensure safe use of the application of the NM. These information requirements can be met in different ways including the use of read-across and grouping. The methods proposed to obtain the selected pieces of information should be seen as suggestions. In case similar information can be obtained with other methods or tests, which might for example appear (scientifically) more suitable for specific cases, these can also be used. Clearly, the completeness, quality and uncertainty of the information are of utmost importance but this is not always possible to verify. Without good quality data, and the ability to assess the quality of the data, the information obtained or generated might be inadequate for risk assessment.

It is also widely accepted that the scientific knowledge on NMs is not yet sufficient for defining all benchmarks or cut-off values needed within this approach or for broad application of nanospecific (Q)SAR, grouping and read-across tools. With the current approach it is possible to identify those situations where defining such benchmarks or cut-off values is likely to become feasible in the near future as well as which type of data needs to be generated for scientific justification. Some of the benchmarks and cut-off values are rather general and applicable to many different situations while others are more specific for the NM application and exposure situation. In general, systematic sets of high quality data are needed to identify, verify and validate which NM characteristics influence which aspect of the exposure, kinetics or toxicity. In the near future, only interpolation within these tested data sets and not extrapolation outside the tested range seems possible.

The proposed approach, including the type of information linked to the various elements and endpoints, is based on the current state of knowledge and is flexible enough to accommodate future insights and knowledge of all types of NMs (1^{st}, 2^{nd}, 3^{rd}, and 4^{th} generation). Further elaboration and refinement of especially phase III (and further) is needed based on experience with case studies. Although the current approach focuses only on the human health risk assessment of NMs, the approach can be expanded to environmental risk assessment in the future.

To conclude, the proposed risk assessment strategy, which is based on six elements, can be used to prioritise those NM applications that may lead to high risks for human health. The different phases of the flow chart guide the user to the most important information needed to address the nanospecific issues within the risk assessment, depending on the specific NM application, life cycle stage and exposure situation. Furthermore, the approach can also be used to identify those situations where the use of nanospecific grouping, read-across and (Q)SAR tools is likely to become feasible in the future and to point towards the generation of the type of data that is needed

for scientific justification, which may lead to regulatory acceptance of the nanospecific applications of these tools.

4.7. References

Arts JH, Hadi M, Irfan MA, 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). Regul Toxicol Pharmacol, 71(2 Suppl):S1-27. doi: 10.1016/j.yrtph.2015.03.007

Bachler G, von Goetz N, Hungerbühler K. 2013. A physiologically based pharmacokinetic model for ionic silver and silver nanoparticles. Int J Nanomedicine, 8: 3365-82. doi: 10.2147/IJN.S46624

BAuA 2013. Announcements on Hazardous Substances- Manufactured Nanomaterials Announcement 527. Joint Ministerial Gazette (GMBl). May 2013. N° 25. 498-511

Cho WS, Duffin R, Thielbeer F, Bradley M, Megson IL, Macnee W, Poland CA, Tran CL, Donaldson K. 2012. Zeta potential and solubility to toxic ions as mechanisms of lung inflammation caused by metal/metal oxide nanoparticles. Toxicol Sci, 126(2): 469-77

Collins AR, Annangi B, Rubio L, Marcos R, Dorn M, Merker C, Estrela-Lopis I, Roxana Cimpan M, Ibrahim M, Cimpan E, Ostermann M, Sauter A, El Yamani N, Shaposhnikov S, Chevillard S, Paget V, Grall R, Delic J, Goñi- de-Cerio F, Suarez-Merino B, Fessard V, Hogeveen KN, Fjellsbø LM, Runden Pran E, Brzicova T, Topinka J, Silva MJ, Leite PE, Ribeiro AR, Granjeiro JM, Grafström R, Prina-Mello A, Dusinska M. 2016. High throughput toxicity screening and intracellular detection of nanomaterials. Nanomedicine and Nanobiotechnology, Published Online: Jun 07 2016. doi: 10.1002/wnan.1413

Creton S, Aardema MJ, Carmichael PL, Harvey JS, Martin FL, Newbold RF, O'Donovan MR, Pant K, Poth A, Sakai A, Sasaki K, Scott AD, Schechtman LM, Shen RR, Tanaka N, Yasaei H. 2012. Cell transformation assays for prediction of carcinogenic potential: state of the science and future research needs. Mutagenesis, 27(1): 93-101. doi: 10.1093/mutage/ger053

Dekkers S, Oomen AG, Bleeker EA, Vandebriel RJ, Micheletti C, Cabellos J, Janer G, Fuentes N, Vázquez-Campos S, Borges T, Silva MJ, Prina-Mello A, Movia D, Nesslany F, Ribeiro AR, Leite PE, Groenewold M, Cassee FR, Sips AJ, Dijkzeul A, van Teunenbroek T, Wijnhoven SW. 2016. Towards a nanospecific approach for risk assessment. Regul Toxicol Pharmacol, 80: 46-59

De Temmerman P, Verleysen E, Lammertyn J, Mast J. 2014. Semi-automatic size measurement of primary particles in aggregated nanomaterials by transmission electron microscopy. Powder Technology, 261: 191-200

DG GROWTH 2016.
http://ec.europa.eu/growth/sectors/chemicals/reach/nanomaterials/index_en.htm

Dobrovolskaia MA, Germolec DR, Weaver JL. 2009. Evaluation of nanoparticle immunotoxicity. Nat Nanotechnol, 4: 411-414

Domínguez A, Suarez-Merino B, Go~ni-de-Cerio F. 2014. Nanoparticles and bloodbrain barrier: the key to central nervous system diseases. J. Nanosci. Nanotechnol., 14: 766-779

Donaldson K, Murphy FA, Duffin R, Poland CA. 2010. Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. Part. Fibre Toxicol., 7: 5

ECETOC 2012. ECETOC TRA version 3: Background and Rationale for the Improvements. Technical Report No. 114. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium

ECHA 2012. Guidance on information requirements and chemical safety assessment – Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7a Endpoint specific guidance. Guidance for the implementation of REACH. European Chemicals Agency, Helsinki

ECHA 2013. Grouping of substances and read-across approach. Part 1: Introductory note and Part 2: Read-across illustrative example (Analogue approach: similarity based on breakdown products)

ECHA/RIVM/JRC 2016. Usage of (eco)toxicological data for bridging data gaps between and grouping of nanoforms of the same substance. Elements to consider. European Chemicals Agency, Dutch National Institute for Public Health and the Environment, Joint Research Centre

ECVAM 2013. Permeability Assay on Caco-2 Cells. Protocol N° 142. ECVAM. http://ecvam-dbalm.jrc.ec.europa.eu/

EFSA 2011. Scientific opinion on guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain. EFSA Journal, 9: 2140

EFSA 2012. Minimum Criteria for the acceptance of *in vivo* alkaline Comet Assay Reports. European Food Safety Authority, Parma, Italy

Elsabahy M, Wooley KL. 2013. Cytokines as biomarkers of nanoparticle immunotoxicity. Chem Soc Rev., 42(12): 5552–5576. doi: 10.1039/c3cs60064e

European Commission 2011. Commission Recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU). Official Journal of the European Union. L275 (pp. 38-40)

European Commission 2012. Second Regulatory Review on Nanomaterials. COM(2012) 572 final. Brussel, 2-10-2012

European Parliament and Council 2006. Regulation (EC) N° 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) N° 793/93 and Commission Regulation (EC) N° 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. 30.12.2006. Official Journal of the European Union, L 396/1

European Parliament and Council 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. Official Journal of the European Union, L 353. 31.12.2008

JRC 2012. Requirements on measurements for the implementation of the European Commission definition of the term nanomaterial. Joint Research Centre, Ispra, Italy

Kermanizadeh A, Balharry D, Wallin H, Loft S, Møller P. 2015. Nanomaterial translocation--the biokinetics, tissue accumulation, toxicity and fate of materials in secondary organs--a review. Crit Rev Toxicol, 45(10): 837-72. doi: 10.3109/10408444.2015.1058747

Kettiger H, Schipanski A, Wick P, Huwyler J. 2013. Engineered nanomaterial uptake and tissue distribution: from cell to organism. International journal of nanomedicine, 8: 3255-3269

Lin Z, Monteiro-Riviere NA, Riviere JE. 2015. Pharmacokinetics of metallic nanoparticles. Wiley Interdiscip Rev Nanomed Nanobiotechnol, 7(2): 189-217. doi: 10.1002/wnan.1304

Lin Z, Monteiro-Riviere NA, Riviere JE. 2016. A physiologically based pharmacokinetic model for polyethylene glycol-coated gold nanoparticles of different sizes in adult mice. Nanotoxicology, 10(2): 162-72. doi: 10.3109/17435390.2015.1027314

Louro H, Bettencourt A, Gonçalves LM, Almeida AJ, Silva MJ. 2015. Role of nanogenotoxicology studies in safety evaluation of nanomaterials. In: Sabu Thomas, Yves Grohens, Neethu Ninan (eds). Nanotechnology applications in tissue engineering. Elsevier Inc., Oxford, UK (pp.263-288)

Maynard AD, Aitken RJ, Butz T, Colvin V, Donaldson K, Oberdörster G, Philbert MA, Ryan J, Seaton A, Stone V, Tinkle SS, Tran L, Walker NJ, Warheit DB. 2006. Safe handling of nanotechnology. Nature, 16;444(7117): 267-9

NANoREG Deliverable D3.1. Deliverable report for NANoREG. Grant Agreement Number 310584. Gap analysis report, identifying the critical exposure scenarios within the key value chains. http://www.nanoreg.eu/images/2015_05_20_NANoREG_Factsheet_D3.1.pdf

NANoREG Deliverable D1.12. Deliverable report for NANoREG. Grant Agreement Number 310584. Toolbox

Nel AE, Mädler L, Velegol D, Xia T, Hoek EM, Somasundaran P, Klaessig F, Castranova V, Thompson M. 2009. Understanding biophysicochemical interactions at the nano-bio interface. Nat Mater, 8(7): 543-57. doi: 10.1038/nmat2442

Nel AE, Xia T, Meng H, Wang X, Lin, S, Ji Z, Zhang H. 2014. Nanomaterial toxicity testing in the 21st century: Use of a predictive toxicological approach and high-throughput screening. Accounts Chem. Res., 46: 607-621

OECD 2013. Recommendation of the Council on the Safety Testing and Assessment of Manufactured Nanomaterials. 19 September 2013 - C(2013)107

OECD 2014. Report of the OECD expert meeting on the physical chemical properties of manufactured nanomaterials and test guidelines. OECD Series on the Safety of Manufactured Nanomaterials N° 41. ENV/JM/MONO(2014)15

OECD 2016. Guidance document on the in vitro Bhas 42 cell transformation assay. Series on Testing & Assessment N° 231 (approved January 8, 2016)

Oomen AG, Bleeker EA, Bos PM, van Broekhuizen F, Gottardo S, Groenewold M, Hristozov D, Hund-Rinke K, Irfan MA, Marcomini A, Peijnenburg WJ, Rasmussen K, Jiménez AS, Scott-Fordsmand JJ, van Tongeren M, Wiench K, Wohlleben W, Landsiedel R. 2015. Grouping and Read-Across Approaches for Risk Assessment of Nanomaterials. Int J Environ Res Public Health,12(10): 13415-34. doi: 10.3390/ijerph121013415

Paget V, Sergent JA, Grall R, Altmeyer-Morel S, Girard HA, Petit T, Gesset C, Mermoux M, Bergonzo P, Arnault JC, Chevillard S. 2014. Carboxylated nanodiamonds are neither cytotoxic nor genotoxic on liver, kidney, intestine and lung human cell lines. Nanotoxicology, 1: 46-56. doi: 10.3109/17435390.2013.855828

Powell JJ, Faria N, Thomas-McKay E, Pele LC. 2010. Origin and fate of dietary nanoparticles and microparticles in the gastrointestinal tract. J Autoimmun, 34(3): J226-33. doi: 10.1016/j.jaut.2009.11.006

RIVM 2015. ConsExpo Nano. (https://www.consexponano.nl/)

Roberts G. 2016. Commission rejects idea of EU nano register: Officials also confirm changes to REACH annexes won't apply before 2018 deadline. Chemical Watch: Global risk and regulation news, 16 March 2016. Europe, Nanomaterials

Schulz H, Brand P, Heyder J. 2000. Particle-lung interactions: Chapter 5: Particle Deposition in the Respiratory Tract. Edited by Gehr and Heyder. Marcel Dekker Inc., New York – Basel

SCENIHR 2009. Risk assessment of products of nanotechnologies. Scientific Committee on Emerging and Newly Identified Health Risks, European Commission

SCENHIR 2015. Guidance on the Determination of Potential Health Effects of Nanomaterials Used in Medical Devices. Scientific Committee on Emerging and Newly Identified Health Risks, European Commission

Sips AJAM, Noorlander CW, Lehman HC, Hoehener C. 2015. NANoREG Safe-by-Design (SbD) concept. http://www.nanoreg.eu/images/20150530_SbD_Working_Draft_EU_US_and_ProSafe.pdf

Tantra R, Bouwmeester H, Bolea E, Rey-Castro C, David CA, Dogné JM, Jarman J, Laborda F, Laloy J, Robinson KN, Undas AK, van der Zande M. 2015. Suitability of analytical methods to measure solubility for the purpose of nanoregulation. Nanotoxicology, 22: 1-12

van Kesteren PC, Cubadda F, Bouwmeester H, van Eijkeren JC, Dekkers S, de Jong WH, Oomen AG. 2015. Novel insights into the risk assessment of the nanomaterial synthetic amorphous silica, additive E551, in food. Nanotoxicology, 9(4): 442-52. doi: 10.3109/17435390.2014.940408

Wijnhoven SWP, Dekkers S, Hagens WI, De Jong WH. 2009. Exposure to nanomaterials in consumer products. RIVM letter report 340370001/2009

Zhang H, Ji Z, Xia T, Meng H, Low-Kam C, Liu R, Pokhrel S, Lin S, Wang X, Liao YP, Wang M, Li L, Rallo R, Damoiseaux R, Telesca D, Mädler L, Cohen Y, Zink JI, Nel AE. 2013. Use of metal oxide nanoparticle band gap to develop a predictive paradigm for oxidative stress and acute pulmonary inflammation. ACS Nano, 6(5): 4349-68. doi: 10.1021/nn3010087

5. SAFE-BY-DESIGN

In this section, the NAnoREG Safe-by-Design concept is illustrated.

The currently available tools (e.g. guidance documents, models, protocols, decision trees), including those developed under NANoREG, for addressing the nanospecific considerations discussed in this section are listed in the NANoREG Toolbox (Deliverable D1.12, see worksheet "5 Safe-by-Design").

5.1. Scope

One of the long-term goals of NANoREG is to develop new testing strategies for nanomaterials (NMs), which account for innovation requirements and help both regulatory authorities and industry with 'keeping pace with innovation'. In this context, safety of NMs and related products is turned into a building block of innovation rather than a hurdle, without compromising on the level of safety itself. Innovation requirements indicate that something has to change although industry already takes safety into account and although regulators already have defined requirements. Nevertheless, several stakeholders have advocated a change of paradigm in the safety assessment of NMs.

In this context, a forward-looking strategy called the 'NANoREG Safe-by-Design (SbD) concept' has been developed (see sections 5.3 and 5.4 for more details). This concept has to be regarded as a first outline. Ongoing projects as ProSafe[41] and NanoReg2[42] are carrying on with filling in this outline, making it implementable by industry and useful for regulators. The concept envisages including safety into innovation from early development of a new NM or nano-enabled product onwards.

5.2. Why Safe-by-Design

Emerging technologies increasingly seem to give rise to questions about the safety of their products. Some believe this is due to the convergence of various technologies, resulting into products not sufficiently covered by present regulations (Tourney 2012). Others (Owen et al. 2009) regard it as an issue of timing, a temporal discrepancy between the market readiness of these new technologies and the questioning of potential new risk issues that come along with these

[41] http://www.h2020-prosafe.eu/
[42] http://www.nanoreg2.eu/

technologies and their products. When insights in (new) safety aspects are lagging behind the development, appropriate legislation cannot be developed timely. Present discussions about the potential health risks of NMs form perfect illustration of this discrepancy in timing.
Owen et al. (2009) mention that society regards state-led regulations as pivotal, providing confidence for both investors and the public that innovations are safe. When innovative products hit the market, the public relies on the state that safety for human and environmental health is warranted. There is, however, a real bottleneck in this approach. Various innovations and innovative products require amendment or development of existing regulation(s) but the stimulus to come to such adaptions is lagging behind considerably. The stimulus requires evidence for undesirable social, health or environmental consequences. However, the lack of such evidence in combination with products on market results in so-called 'uncertain risks'. The report of the European Environment Agency (EEA) "Early warnings, late lessons" demonstrates that uncertainty about safety has led to early warnings, but too often these warnings were not or could not be translated into the required actions (EEA 2013).

For nanotechnology, and more specific for NMs, there is awareness that information requirements as laid down in regulations and related guidance may not fully cover the information needs to characterise the human health and environmental risks of certain NMs. This discrepancy between information requirements and information needs may lead to uncertain estimations of the risks.

One of the solutions to escape from this situation is foreseen in the concept of Safe-by-Design (SbD). SbD aims at an integrated and iterative process, where safety information on a certain material, substance or product is integrated from early research and development (R&D) phases onwards and iterations occur in order to search for the best achievable safety conditions. This concept seems by nature plausible for many stakeholders. In the end, it is thought to reduce the necessity for risk management actions, which can be beneficial both for industry and authorities. On the other hand, it might require larger investments in R&D. SbD can only be an acceptable concept in the innovation chain if it does not come at the cost of competitiveness.

5.3. NANoREG Safe-by-Design concept

Safety by Design or Safe(r)-by-Design is originally a concept that was developed and utilized by engineers, particularly those working within the construction industry. The basic idea is that in the design and development of products, it is important to consider and incorporate safety considerations. Within the SbD concept, the functionality of a material and its toxicity are considered in an integrated way. SbD has traditionally been about incorporating safety

considerations into the design, construction and maintenance of engineered products and workplaces.

SbD in NANoREG has the following features:

- It forms an exemplary platform for the early stage application of the precautionary principle in R&D projects in industrial innovation processes
- It includes precautionary measures and tools for the timely identification of uncertainties and potential risks as well as timely actions to reduce or eliminate these uncertainties and, if possible, the respective risks at the earliest possible and/or feasible stage of development. The basis is a modular approach considering the various stages of innovation and allowing for different information requirements in line with the stage of innovation
- The NANoREG SbD concept is modularly designed based on commonly applied innovation models in order to be ready to be implemented in existing industrial R&D and innovation processes

The NANoREG SbD concept aims at:

1. Identification and reduction of uncertainty about health risks, products and processes
2. Management of (potential) health risks of innovative materials, products and processes

Innovators are encouraged to incorporate considerations on potential health (workers and envisaged users) and environmental safety into the R&D phase of an innovation process and, where necessary, adapt the process and/or product design so as to create safer outcomes.

5.4. The NANoREG SbD approach

In industry, structured innovation management processes for R&D projects to develop products, processes, technologies are the *de facto* standard today. Consequently, one of the most common structured innovation processes, the "stage gate innovation model" (Cooper 2016) (Figure 5.1), is used as the backbone of the NANoREG SbD concept.

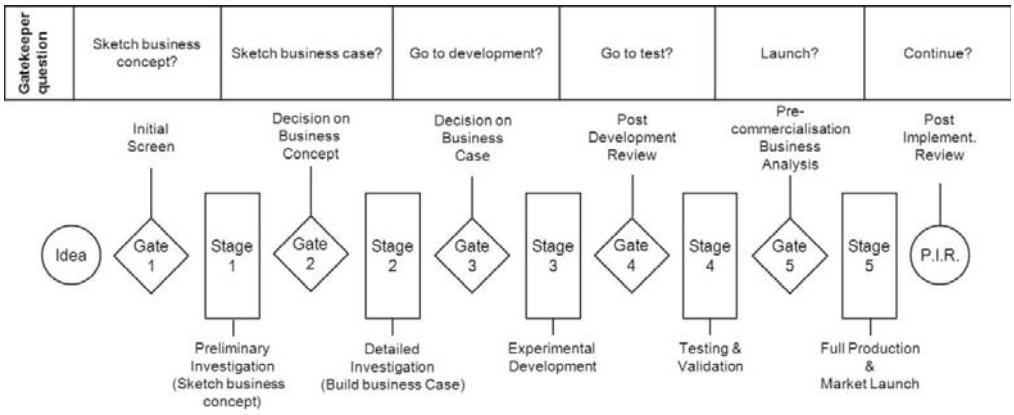

Figure 5.1: The stage gate innovation model (Cooper 2016). PIR = Post Implementation Review

During the "stages", the proper work is carried out: ideation, development, tests, up-scaling etc. Moreover, information is gathered about technology readiness, market perspectives, and required investments. To some extent, information about safety as required by legislation is taken into account. In each "gate", so-called "gatekeepers" decide on the fate of an innovation project: proceed, alter (proceed through gate but with minor alterations in the next phase), recycle (repeat the stage with major alterations), on-hold (wait for other projects, technologies, licenses, regulations, etc.), and terminate. The decision is always based on balancing (expected) risks, costs and benefits.

Whether, how, and to which extent a stage gate process is run depends on the scope of the R&D or innovation project and the way a specific industry has organized its innovation processes. For smaller projects, stages 1 and 2 and/or stages 3 and 4 (Figure 5.1) can be merged; with only one idea for a smaller project, gate 1 may be merged with gates 2 and 3 (Figure 5.1). The stage gate process can be run in two or more sequences and these can also occur in parallel. For instance, during the first stage gate of a process/innovation project a technology is developed; during the second one, a product platform using this technology is developed (there might be other platforms and products developed in yet other stage gate processes); and, in the third one, every geographical business unit develops a product for its market requirements (i.e. several daughter projects run parallel). The stage gate process can even contain built-in loops within a stage, e.g. if certain criteria are failed.

There are different generalised types of stages which can be combined *ad libitum*:

- Idea phases (to generate ideas)
- First conceptual phases (to find technical solutions for one idea, screening phases)
- Second conceptual phases (development planning for one technical solution)
- Different development stages (research, technology development, system development)

- Market testing phases
- Initial market phases (up until the Post Implementation Review (PIR)).

The NANoREG SbD concept addresses nanospecific safety issues along the stages of innovation. It encourages innovators to think how to address the following questions at each stage of innovation:

- Is the material/product safe?
- How to handle waste safely?
- How to use the material/product safely?

Regulations appear to lag behind in addressing these questions for innovations. Nevertheless, this does not mean that existing regulations may not form a good basis. The current situation for NMs and nano-enabled products, however, makes clear that insight in potential hazards or risks is needed during the early stages of innovation. In order to make it an implementable approach, performing safety testing should be in line with the level of technology readiness. When innovations progress towards market application, information needs to be lined up in the direction of regulatory requirements. The NANoREG SbD approach, therefore, includes for the first stages of innovation the identification of the *potential* for risks, followed by *indicators* for risk at mid stages of innovation, and by *demonstrators* for risks in the final stages of innovation, as laid down in regulatory requirements (Figure 5.2). This approach aims at improved insights into risks, both environmental as human, before marketing of innovative NMs or nano-enabled products. Moreover, it supports decisions at the various stages of development e.g. for further investments, thereby supporting to build a strong business case.

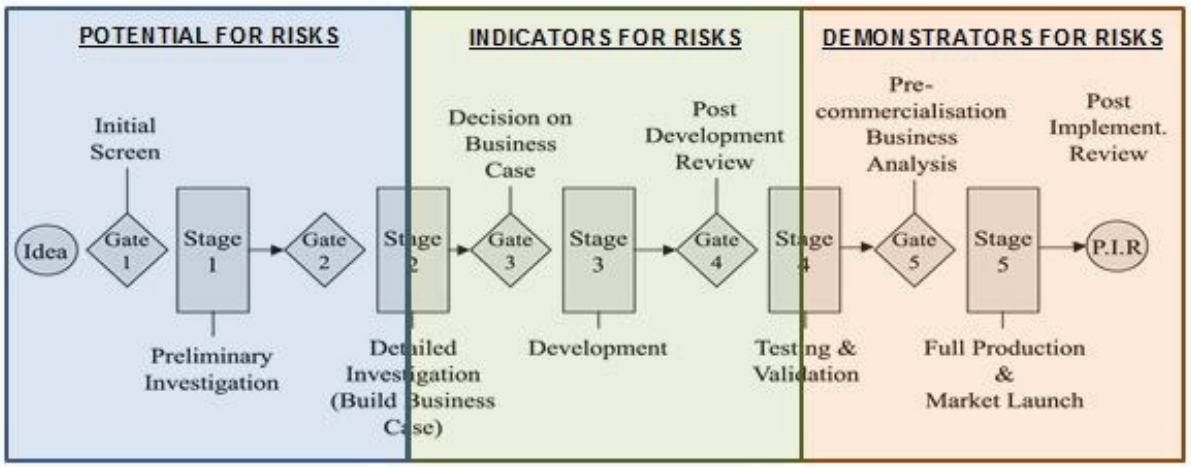

Figure 5.2: NANoREG Safe-by-Design (SbD) approach: stage-gate innovation process including risk terminology (i.e. potential, indicators and demonstrators). PIR = Post Implementation Review

The NANoREG SbD approach comprises:

- An innovation-stage dependent approach
- A strategy to identify nanospecific risks
- Safe use, safe products/materials, safe waste handling

Whereas the concept can be applied to many different products, companies and industries – albeit with slightly different industrial management processes – the data is case specific, i.e. for every product a new data set is needed and needs to be structured in accordance. Figure 5.3 gives an overview of the coherent innovation model.

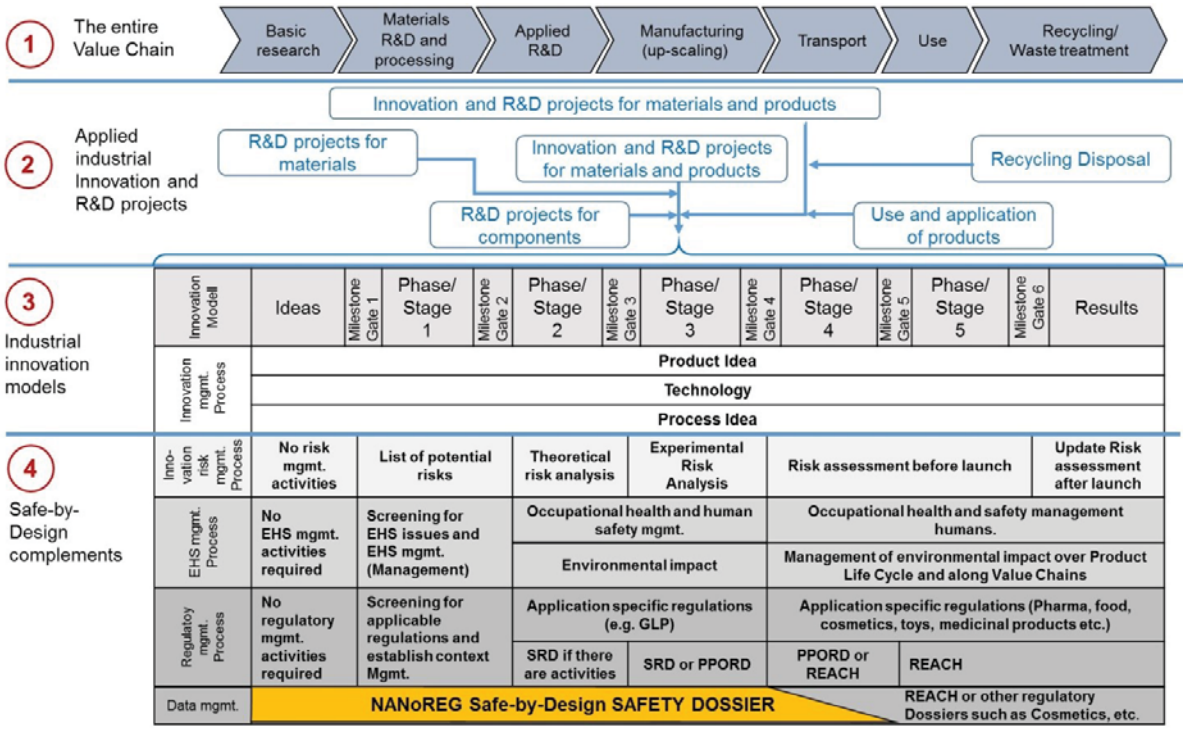

Figure 5.3: The NANoREG Safe-by-Design (SbD) concept as part of a coherent innovation model. The Safety Dossier is under development in ProSafe. GLP = Good Laboratory Practice. EHS = Environmental Health and Safety. PPORD = Product and Process Orientated Research and Development. R&D = Research and Development

The four elements visualised in Figure 5.3 are:

1. Exemplary illustration of an entire value chain as a basis for the arrangement of the various innovation and R&D projects along this chain
2. Illustration of the arrangement of different types of applied industrial innovation and R&D projects along the entire value chains of a material or product
3. Exemplary illustration of an industrial innovation model with the different phases/stages and the corresponding milestones/gates in between
4. Representation of the various sub-processes within the NANoREG SbD concept such as:

innovation risk management process, environmental health and safety (EHS) management process, pre-regulatory and regulatory management process

Within the NANoREG SbD concept, the focus is on risk assessment under data constraints in the early stages of product development. For this purpose, control banding tools (such as the Swiss Precautionary Matrix[43]), safety screening strategies, risk potentials, decision trees (such as the one discussed in section 4), exposure scenarios, life cycle maps and the safety dossiers could be used, amongst others.

Elaboration towards a full-fledged SbD approach is foreseen to be delivered by ProSafe[44] and NanoReg2[45].

5.5. Managing Uncertainties and Risks

5.5.1. Managing uncertainties

According to the ISO standards, a risk is the "*negative, positive, or deviation from the expected effect of uncertainty on objectives*". Hence, risks are the consequences of uncertainties and uncertainties are a cause of risks. Risk is also often described by an event, a change in circumstances or a consequence.

Alternatively, a risk can be split into the probability of occurrence of an event (e.g. the exposure of the human population to a chemical) and the magnitude/impact of the event (e.g. the inherent toxicity of that chemical) once it occurs, i.e.:

- Uncertainty about the occurrence of an event expressed as a probability distribution (if a single probability is stated without a safety/error margin and without a caveat or assumption, then the uncertainty must be 0%)
- Uncertainty about the magnitude/impact of the event once it occurs

Because ambiguous, missing, or faulty information/data causes uncertainties, they also cause increase of risks. Hence, to reduce uncertainties and risks, more or more reliable/objective information/data is needed.

Within the NANoREG SbD concept, an important task is to identify and reduce uncertainties. Figure 5.4 illustrates exemplarily the process of identification and evaluation of uncertainties within each stage of innovation.

[43] http://www.bag.admin.ch/nanotechnologie/12171/12174/index.html?lang=en
[44] http://www.h2020-prosafe.eu/
[45] http://www.nanoreg2.eu/

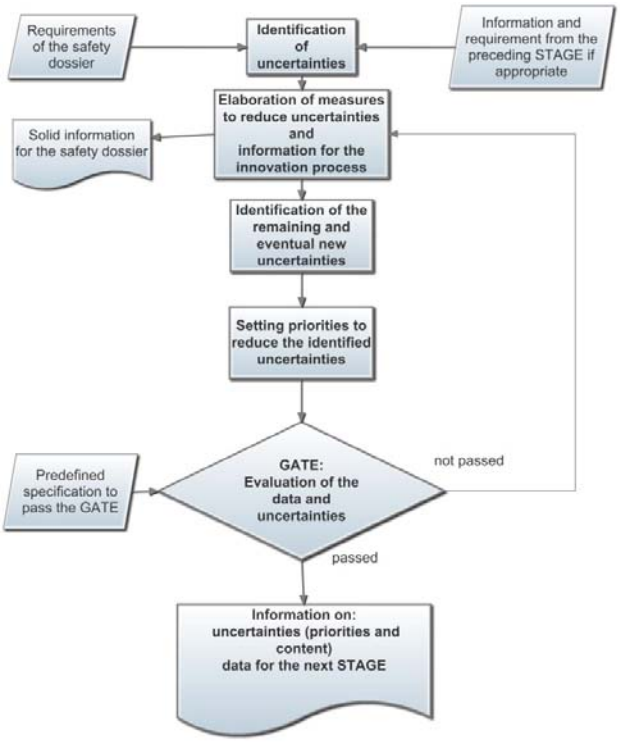

Figure 5.4: Overall workflow of the NANoREG Safe-by-Design (SbD) concept for dealing with identification and evaluation of uncertainties.

5.5.2. Managing risks

In the NANoREG SbD concept, the ISO 31000:2009 standard for risk management[46] can be used. Risk management is split into risk assessment (including risk identification and formulation, analysis, evaluation) and risk treatment. ISO also designed the ISO 21500 "Guidance on Project Management" standard[47] to align with ISO 31000:2009. This is an important remark, because proper project management is a prerequisite and necessity for a successful innovation project.

Innovators are, however, also encouraged to actively seek for products or NMs with the best achievable inherent safety. This might mean redesigning instead of going over to the next stage of innovation.

[46] http://www.iso.org/iso/home/standards/iso31000.htm
[47] http://www.iso.org/iso/catalogue_detail?csnumber=50003

5.5.3. *Risks and their general risk treatment options*

Once risks have been identified, analysed and evaluated in risk assessment according to ISO 31000:2009 standard, all techniques for risk treatment fall into one or more categories ranked from the one aimed to address the highest risk (i.e. "Risk Avoidance") to the one aimed to address the lowest risk (i.e. "Risk Retention") (see Table 5.1).

Table 5.1: Risk types and their treatment options according to ISO 31000:2009 standard

Risk type	**High impact and High probability**	**Low impact and High probability**	**High impact and Low probability**	**Low impact and Low probability**
Risk treatment option	Risk Avoidance	Risk Reduction	Risk Sharing	Risk Retention
What to do with the risk?	Eliminate Withdraw from Avoid involvement	Optimise Mitigate (impact) Reduce probability	Transfer Outsource Insure and budget	Accept and budget

The NANoREG SbD approach allows a selection of the best risk treatment options per stage of innovation. The followings aspects need to be looked at in any case in the course of this SbD approach.

Risks with low occurrence/probability but high impact are often overestimated (e.g. the public discussion tends to focus on these risks with nuclear energy being the prime example for this). These risks tend to be perceived as catastrophes. With respect to hazard of chemicals and NMs, these types of risks should be thoroughly examined for both acute and chronic toxicity.

In contrast, risks with low impact but high occurrence/probability are underestimated because of inurement, i.e. in case of chemicals and NMs, people tend to neglect the significant contribution of exposure and tend to focus on the low impact, be it consciously or unconsciously. With respect to hazard of chemicals and NMs, these types of risks should be thoroughly examined for chronic toxicity, with acute toxicity usually being low.

A high exposure or a high hazard in itself does not exclude a NM *a priori*; instead, it should be thoroughly examined and proper risk treatment options should be developed, e.g. constrict applications of high hazard NMs to those with controlled and/or very low exposure; prescribe the usage of personal protective equipment to reduce the exposure so that the NM is only slightly risky despite is inherent hazard.

In the context of chemicals, it should be noted that some functionalities like high reactivity may inherently lead to high hazardousness. This may be a challenge for finding the most optimal balance between functionality and safety.

5.5.4. *Risk analysis and costs*

Costs of measures to reduce a risk have a direct impact on the remaining risk: the higher the costs, the lower the remaining risk. However, the costs of risk reduction have to be balanced with the costs of the remaining risk to find the most efficient solutions (e.g. a reduction of a risk to zero is usually inefficient because of exponentially increasing costs).

As it can be seen from Table 5.2, the earlier a potential risk is addressed, the smaller the necessary costs for a given risk-reduction or for a given remaining risk is.

Table 5.2: Costs of risk reduction based on timing

Timing	Risk reduction investment	Benefit of investment	Remaining risk	Remark
Early	small	large	small	Small investments have large benefits
In time	medium	medium	medium	-
Late	large	small	large	Large investments have small benefits

5.5.5. *Uncertainty identification and risk assessment in the stages*

Uncertainty is also reduced in an innovation-stage dependent way:

- Potentials for risks: During early stages uncertainties are identified and information is gathered to reduce the uncertainties. Potential risk situations and scenarios are formulated as well as risks identified and listed for the next stage(s).

- Indicators for risks: Uncertainties are further reduced and new uncertainties identified. A theoretical (i.e. only using subjective and existing objective data) risk assessment is carried out and risk treatment options are prepared. This may be an iterative process until the results of market testing and scaling up can be included.

- Demonstrators for risks: Addressing uncertainties is guided by regulatory requirements and at a later phase also by from the market introduction, i.e. the post launch review.

5.6. Identification of nanospecific risks along stages of innovation

An innovative screening strategy is proposed to identify potential nanospecific risks along the innovation process (Figure 5.5). Subsequently, appropriate methods need to be described to measure the required parameters. At this moment, the development of a kind of manual rather than defining benchmark values is preferred. The latter is still a source of debate and may distract

attention from working towards safe designs. Within NANoREG, only the first part of this strategy is elaborated. The next steps are worked out in NanoReg2.

To identify potential nanospecific risks during the first stages of innovation requires a screening strategy. In NANoREG, a screening strategy was developed based on by six topics: solubility/dissolution rate, stability of coating, accumulation, genotoxicity, inflammation, and environmental toxicity (Figure 5.5). These topics resemble the ones discussed in section 4 but are addressed from another perspective as during the first steps of innovation there is much less known about the NM and or the nano-enabled product.

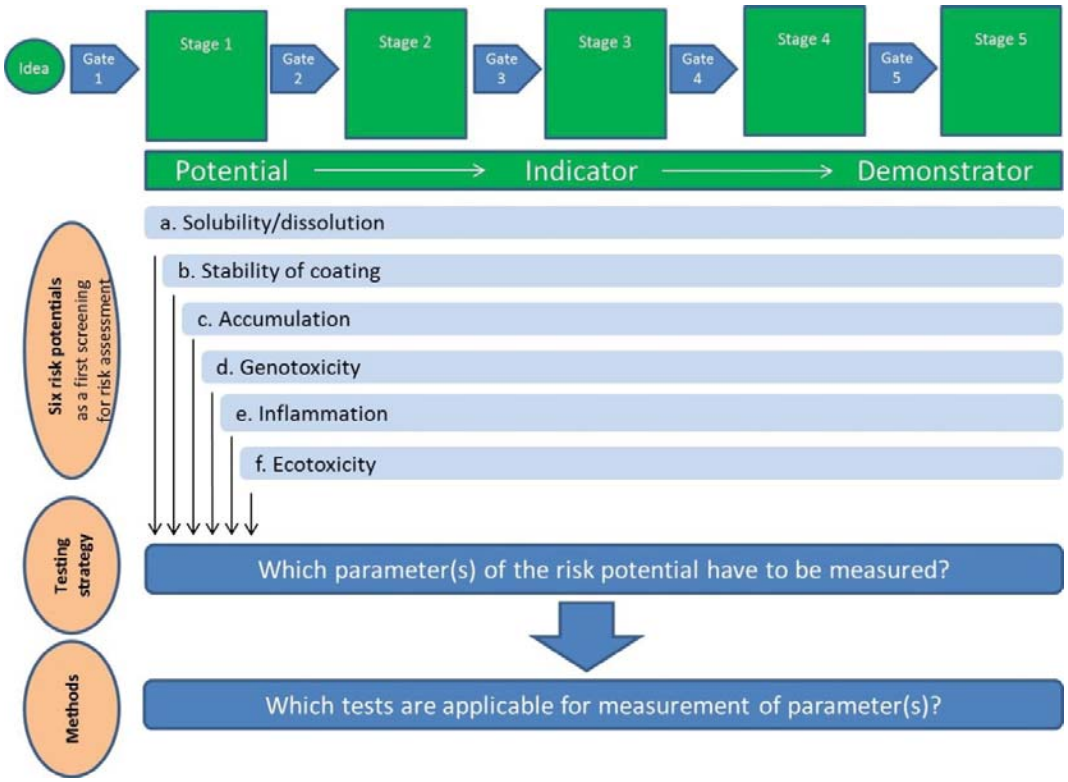

Figure 5.5: Schematic view of the screening strategy for risk assessment of NM in relation to the stages of innovation, the testing strategy and the test methods

Parameters to be measured and applicable tests to measure these parameters are described in NANoREG Deliverables D6.3 "Comparison on toxicity testing in drug development and in present MNMs safety testing**"** and D6.4 "Inventory of existing regulatory accepted toxicity tests applicable for safety screening of MNMs" and can be found in the NANoREG Toolbox under the correspondent section 5 on Safe-by-Design (NANoREG Deliverable D1.12).

5.7. References

Cooper RG. 2016. The stage gate innovation model. http://www.bobcooper.ca/about-dr-cooper

EEA 2013. Late lessons from early warnings: science, precaution, innovation. EEA report, N° 1/2013, ISBN 978-92-9213-349-8. European Environment Agency, Copenhagen. Publications Office of the European Union, Luxembourg

NANoREG Deliverable D1.12. Deliverable report for NANoREG. Grant Agreement Number 310584. Toolbox

Owen R, Baxter D, Maynard T, Depledge M. 2009. Beyond regulation: risk pricing and responsible innovation. Environ. Sci. Technol., 43: 6902–6906

Slob and Staman 2012. Policy and the evidence beast. A Dutch study of the expectations and practices in the area of evidence-based policy. Rathenau Instituut, Den Haag. http://www.rathenau.nl/uploads/tx_tferathenau/Beleid_en_het_bewijsbeest-Rathenau.pdf (visited: September 1st, 2016)

Tourney C. 2012. Lessons from before and after nanotech. Nature NanoTechnology, Vol. 7: 611-612

6. LIFE CYCLE ASSESSMENT

In this section, the Life Cycle Assessment (LCA) approach and its application to nanomaterials (NMs) in the REACH context are illustrated. Supporting information on this subject is reported in annex V of this document.

The currently available tools (e.g. guidance documents, models, protocols, decision trees), including those developed under NANoREG, for addressing the nanospecific considerations discussed in this section are listed in the NANoREG Toolbox (Deliverable D1.12, see worksheet "6 Life Cycle Assessment").

6.1. Introduction

Life Cycle Assessment (LCA) is a standardized method for the compilation and evaluation of the inputs, outputs and related potential environmental impacts of a product system throughout its life cycle, from raw material acquisition through production, use, end-of-life treatment, recycling and final disposal (i.e. cradle-to-grave) (ISO 2006a, 2006b)[48]. LCA considers the overall impacts of a product system on both human health and the environment (i.e. depletion of resources and emissions in air, water and soil). It is an important tool in evaluating the negative and positive environmental implications of a product, process, and technology, which can also be employed for the sustainability assessment of nanomaterials (NMs) (OECD 2013). Such a method permits the identification of the environmental issues, the definition of the hotspots of a product, process and technological system, the analysis of alternative solutions to improve the environmental performance of them and the comparison of different scenarios. It therefore represents a powerful tool for supporting decision-making and policy development processes on nanotechnology, NMs and nano-enabled products.

According to ISO (2006a, 2006b), there are four phases in an LCA study (see also supporting information in annex V of this document). In "Goal and scope definition" the objective of the study is defined, a description of the product system is provided in terms of the functionality and functional unit, the system boundaries, allocation and the target audience of the study are defined. The functional unit is a quantitative measure of the functions that the products (or service) provide. In accordance with the functional unit, the reference flow shall be defined, i.e. the flow to which all

[48] CEN/TC 352 (Nanotechnologies) is currently (February 2017) adapting this ISO standard to nanomaterials.

other input and output flows are quantitatively related. Comparisons between systems are made on the basis of the same function(s), quantified by the same functional unit(s). In "Inventory analysis" procedures for data collection and calculation in order to quantify from cradle-to-grave the relevant inputs (e.g. material inputs) and outputs (e.g. emissions to air) of the product/technological system are defined. The result of this phase is the Life Cycle Inventory (LCI), which is a compilation of the inputs (resources) and the outputs (emissions) from the product over its life-cycle in relation to the functional unit and to the system boundary defined. In "Life Cycle Impact Assessment (LCIA)" the inputs and outputs that have been collected and reported in the inventory are evaluated to understand the magnitude and significance of the potential environmental impacts for a product/technological system throughout its life cycle. The data are translated into a set of indicators for each environmental impact category (e.g. global warming potential as an indicator for climate change). In "Interpretation" the findings from both LCI and LCIA are used to draw conclusions and recommendations.

LCA can be a comprehensive and powerful tool for environmental sustainability assessment of emerging technologies such as nanotechnologies and related nano-enabled products and their comparison with conventional technologies/products. The "Report on the European Commission's Public Online Consultation towards a Strategic Nanotechnology Action Plan (SNAP) 2010-2015"[49] recommends that NMs safety throughout their life cycle is ensured and suggests that sustainability of nanotechnologies and related nano-enabled products may be evaluated using tools such as LCA and Social Impact Assessment. However, the new functions and properties of nano-enabled products along with the lack of available information on their life cycle and releases into the environment are crucial limitations to the use of LCA in this field; this is discussed in section 6.2.

The application of LCA to NMs was also proposed and encouraged by the OECD Working Party on Manufactured Nanomaterials (WPMN). During a workshop on the "Environmentally Sustainable Use of Manufactured Nanomaterials" held in Rome (Italy) on the 14th of September 2011 (OECD 2013), experts agreed on two main conclusions: 1) LCA is an important tool to evaluate the negative and positive environmental implications of a product, process, or technology and it is a suitable for NMs; and 2) establishing linkages between LCA and Risk Assessment (RA) is a key aspect since LCA practitioners need RA information in the LCIA. As a follow-up on the conclusions of this workshop, the Guidance Manual "Towards the Integration of Risk Assessment into Life Cycle Assessment of Nano-Enabled Applications" was prepared, which proposes a complementary use of RA and LCA for a more complete evaluation of nano-enabled products

[49] https://ec.europa.eu/research/consultations/snap/report_en.pdf

(OECD 2015). The Guidance Manual also takes on board the experience accumulated by several authors in the recent years, who compared the environmental performances of emerging nanotechnologies and nano-enabled products with conventional ones.

Furthermore, integration of LCA and RA for NMs was discussed in the context of the EU NanoSafety Cluster[50] (Savolainen et al. 2013). Finally, as highlighted by Askham (2012), implementation of REACH and the associated guidance can provide sources of data to fill data gaps in LCIA and also strengthen the use of LCA methodology. Both these arguments are discussed in section 6.3.

For more information on international initiatives concerning LCA consult annex V of this document.

6.2. LCA and nanomaterials

The ISO 14040/44 framework for LCA (ISO 2006a, 2006b) is considered as a suitable, harmonised, user-friendly and validated framework applicable to NMs (OECD 2015).

In the literature, the number of scientific papers with LCA studies on NMs and nanotechnologies/nano-enabled products has been increasing in the last three years. To date, about 40 studies have been published. These studies, carried out in agreement with ISO (2006a), have different goals and focused on different applications such as:

- To support the eco-design of a new nanotechnology, define the hotspots in the life cycle of a product from the early phase (Joshi 2008; Pizza et al. 2014), and suggest improvement options in the production phase, for example the use of different technologies (Li et al. 2014, Pini et al. 2014) or new materials (Dahlben et al. 2009, Scalbi et al. 2014);
- To compare the environmental performance of new technologies with traditional ones, with the goal to evaluate if the new nano-enabled products/nanotechnologies reach better environmental performance (Sengül et al. 2011; Meyer et al. 2011; Walser et al. 2011);
- To compare different nanotechnologies in order to choose the most environmentally-friendly one (Barberio et al. 2014); and
- To examine the implications of life cycle thinking on nanotechnologies and related market products (Bauer et al. 2008).

From these studies it emerges that there are several issues to be dealt with when applying LCA to nano-enabled products.

When carrying out comparative assessments, different products with the same function need to be considered. However, due to the fact that nano-enabled products have quite new functions, it may

[50] http://www.nanosafetycluster.eu

be difficult to identify alternative products and define the functional unit (Klöpffer et al. 2006; OECD 2015). It is therefore often necessary to expand the system boundary to include additional functions.

A critical issue in LCA is considering the entire life cycle of the investigated applications. Indeed, the amount of available information is scarce for new applications such as nanotechnologies: 1) there are not commercial or public LCA databases that include specific processes on NMs (Hischier 2014); 2) the literature data on LCA of NMs often do not cover the use and end-of-life stages of the life cycle; 3) data on the production stage are generally obtained at laboratory or pilot scale, and therefore may lack reliability and robustness when transferred at macro scale (Hischer and Walser 2012, Gavankar et al. 2012, Li 2014); 4) there is no consolidated method to calculate the release into the environment of NMs along life cycle stages and predict the physicochemical modifications that these NMs undergo in the environment (Subramanian et al. 2015; Hischier 2014); and 5) it is necessary to identify a specific elementary flow for NMs, which considers chemical composition, size, crystalline structure and any other parameter that influence the toxicity of these materials (Hischier 2014).

All these aspects affect the reliability of the results of LCA when applied to NMs. Solutions have been proposed in the scientific literature to overcome these problems. For example, the use and end-of-life stages as well as the scale-up of production can be assumed from literature data and background data of proxy materials (Roses et al. 2007, Bauer et al. 2007, Walser et al. 2012, Pizza et al. 2014, Hischier et al. 2015). Hischier (2014) proposed a framework for modelling the release scenarios of NMs along their life cycle and the characteristics of the elementary flow of NMs.

To assess the environmental profile of NMs there are no particular difficulties in the application of 'traditional' LCA-impact categories (e.g. global warming, acidification, eutrophication, abiotic resource depletion). The application of toxic impacts category (human and ecological toxicity), instead, deserves special attention as the Characterisation Factors (CFs) of NMs for the toxicity-related impact categories are not available. Some attempts have been done to incorporate toxicity effect of NMs in LCIA. Some authors such as Eckelman et al. (2012), Rodriguez-Garcia et al. (2014) and Salieri et al. (2014) proposed ecotoxicity CFs for carbon nanotubes, MWCNTs and SWCNTs, and for nanotitania emitted into freshwater, respectively. Moreover Rodriguez-Garcia et al. (2014) developed human toxicity CFs for MWCNTs and SWCNTs. Another approach was proposed by Pini et al. (2014) for $NanoTiO_2$ with the use of two new impact categories called *"$NanoTiO_2$ ecotoxicity in freshwater"* and *"$NanoTiO_2$ carcinogens in fresh water"* in the frame of the IMPACT 2002+ method. Hishier et al. (2015) in the LCA study on coatings containing nano-TiO_2 used the CF of nanotitania and showed that the release of nano-TiO_2 in the freshwater ecotoxicity is not negligible and depends on the magnitude of the impact factor. A detailed discussion on CFs for NMs is reported in annex V of this document.

In this context, it is particularly effective to carry out an uncertainty analysis related both to the process and assessment data in terms of elementary flows and CFs (i.e. stochastic uncertainty) and to the methodological choices such as system boundary setting, cut-off criteria, selection of impact assessment, assumption, etc. (i.e. sensitivity analysis). Some examples are available in the scientific literature: Walser et al. (2011), Pizza et al. (2014), Kanna et al. (2008), and Barberio et al. (2014). However, further studies should be developed to define harmonized approaches, to produce robust and reliable data and therefore calculate scientifically sound CFs for toxicity-related impact categories (Scalbi et al. 2015).

More details on LCA applied to NMs, nano-enabled products and nanotechnologies are reported in annex V of this document.

6.3. LCA and Risk Assessment

Sustainability assessment of NMs, nano-enabled products and nanotechnologies should take into consideration the impacts on the environment, health and safety (EHS). This integration requires the combined use of different methods such as risk assessment and LCA.

Risk assessment evaluates the possible risk to workers, the public health and the environment due to exposure to chemical substances, whilst LCA quantifies the potential environmental impacts of the whole life cycle of a product. Indeed, the risk assessment focuses on the toxic impacts, while LCA provides a more comprehensive overview of the potential environmental impacts of a chemical product, including all other substances used during the entire life cycle of that product. Furthermore, there is a difference in how the 'life cycle' is perceived in risk assessment and LCA. Finally, LCA allows the environmental assessment at a global/regional scale, but the assessment of local impacts relevant for human health and ecological receptors is critical. For these evaluations, risk assessment is a better tool as it allows the identification at a local scale of situations that are critical (i.e. above a defined threshold) at an early stage for a specific substance release.

According to the scientific literature, risk assessment and LCA can be applied in different ways in the sustainability assessment of NMs:

1. Combined use of risk assessment and LCA (section 6.3.1): the two methods are applied separately and the results of both are discussed to improve the performance of NMs/nano-enabled products/nanotechnologies;
2. Risk assessment is applied from a life cycle perspective (section 6.3.2);
3. Risk assessment under REACH and LCA (section 6.3.3).

6.3.1. Combined use of risk assessment and LCA

OEDC (2015) proposes the complementary use of risk assessment and LCA for a more comprehensive evaluation of nano-enabled products. The main aim of the OECD Manual is to provide guidance on how to combine risk assessment and LCA for NMs/nano-enabled products and how to improve the applicability and quality of LCA studies for decision-making. The OECD Manual presents a case study on carbon nanotubes (CNTs) in semiconductors packaging.

Another interesting case study was performed by OECD (2014), which gives an overview on applications of nanotechnology in tyres, selects the key drivers for innovation, evaluates socio-economic impacts and environmental impacts in the context of LCA, and promotes EHS of NMs throughout a risk management framework as well as the transfer of knowledge and best practices.

Furthermore, Grieger et al. (2012) provide recommendations for combined or separate use of LCA and risk assessment for NMs, addressing the specific needs of risk assessors and decision-makers throughout a literature review of LCA and risk assessment for chemicals and NMs.

Starting from the consideration that a more tailored risk assessment can be performed by addressing the hot spots highlighted by LCA through the entire life-cycle, Barberio et al. (2014) proposed to assess the EHS of two types of production of nanofluid alumina by the application of both LCA and a qualitative risk assessment of workplace[51]. The authors highlighted conflicting results between LCA, which identified the production having the best environmental performance, and risk assessment, which identified the production having the highest risk for workers. The need emerged for the optimisation of the future industrialization phase, by using strategies of risk management or by improving the efficiency in the use of resources. The authors concluded that the combined use of both tools allowed a more detailed analysis of the systems to better support a Safe-by-Design (SbD) approach and the decision-making process.

Walser et al. (2015) developed a framework for indoor emissions of synthetic nanoparticles. The goal of the framework is to implement occupational exposure as impacts category in LCA studies on synthetic NMs. Indeed, the authors highlight the importance of the health of workers during the production of NMs and propose to include this aspect in the LCA studies, thus developing a new impact category that considers the human toxicity of indoor emissions, using data from workplace exposure obtained from risk assessment.

[51] Risk assessment was assessed by using Stoffenmanager Nano tool, which allows the qualitative assessment of occupational health risks from inhalation exposure to manufactured nano-objects

6.3.2. *Risk assessment applied from a life cycle perspective*

For NM risk assessment purposes, it is very important that the material´s life cycle is comprehensively mapped, and put in the context of the value chain. As it can be seen in figure 6.1, the life cycle contains several stages. Measurements of the released amounts, as well as description of the NM forms, are necessary for each of these stages. The release of a NM, irrespective of its form, possibly occurs during production of the NM-containing product. Such a release is likely of primary concern for the worker, although environmental exposure cannot be excluded *per se*. The consumer can possibly experience exposure to one or more of the NM forms during use and maintenance of the product. A major part of the release to the environment is expected to occur at the end-of-life, where recycling, reuse of material, and various disposal activities are taking place. Occupational exposure can possibly also take place in connection with these processes.

A large knowledge gap exists regarding almost all NM-containing products and their respective life cycles. What form the specific NM takes at different stages is often unknown, and there are question marks regarding the possible NM release. This lack of knowledge precludes a relevant exposure assessment, and thus one of the corner stones for risk assessment.

Most studies so far related to specific safety aspects of a given NM have used the pristine form of the NM for at least hazard identification studies. Such pure materials are useful for studies of mechanisms and mode of actions, but are not necessarily the best choices for understanding the toxicological potential of the NM in a specific, 'real world' setting. For that purpose, LCA provides information about which form(s) a NM is taking at different stages of its life cycle, which can be used for further analysis in risk assessment.

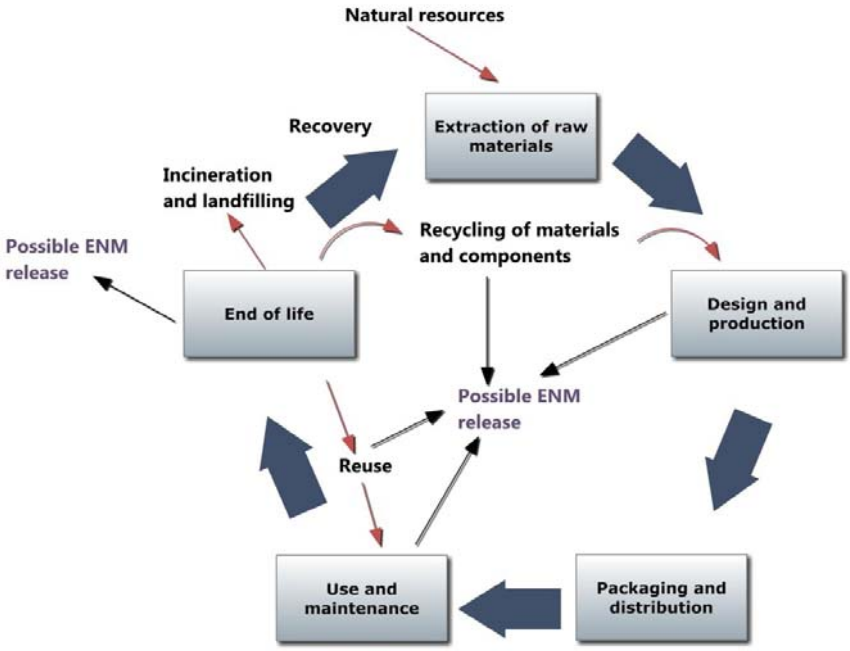

Figure 6.1: The generic life cycle of an engineered nanomaterial (ENM) that is integrated into a product. Thick arrows indicate the general progression of the life cycle. Thin red arrows symbolize additional fates for the ENM. Thin black arrows show where ENM release can occur.

The big challenge regarding consequences for risk assessment deals with the exposure assessment aspects related to the different stages of a life cycle. Thus, it is necessary to establish:

- If there is any release of NM at specific stages of the life cycle,
- The specific physical form that the NM is taking if there is any release,
- The actual quantity (taking the most appropriate metric(s) into consideration) of the NM,
- The interaction between the NM and its surrounding environmental matrix (water, soil, sediment), and
- Any background level of similar but naturally occurring materials.

These data then guide the work related to other aspects of risk assessment.

There are only a few published studies where a life cycle perspective has been applied to the risk assessment of NMs. In a recent review, Mitrano et al. (2015) concluded that few studies have been able to establish what changes a given NM undergoes when it is incorporated into and released from products.

Specific case studies covering substantial parts of the life cycle are nevertheless available (see e.g. Sotiriou et al. 2015, Bekker et al. 2015, Pirela et al. 2015). A conclusion from these studies is that very variable exposure situations develop depending on the species of NM, and also on the life cycle stage and handling method.

6.3.3. Linking risk assessment under REACH and LCA

Differences between how risk assessment is applied under REACH and LCA have been analysed in Askham et al. (2012), but also possibilities of linking can be explored. Askham et al. (2012) suggest that the good availability of toxicity data in REACH can improve LCA toxicity assessments and methods for elaborating new nanospecific CFs. On the other hand, the authors highlight that LCA has an iterative approach that gives a holistic vision on the life cycle of products and can be useful to design optimisation and to compare different options. This approach can be usefully taken into account when the REACH principles are implemented in companies not just for complying with REACH information requirements but to assess other scenarios/options with improved environmental performance and exploit the potential for innovative solutions (i.e. substitution of harmful chemicals with safer alternatives).

Following a 'substance-based' approach, REACH aims to evaluate the risk assessment at different stages of its life cycle. Indeed, the knowledge of exposure is of regulatory relevance throughout the different life cycle stages: production process of the substance itself, releases during the production process of products in which substances are used, waste treatment, consumer articles. Following a 'product-based' approach, LCA aims to evaluate and quantify the environmental impacts of products and services considering their whole life cycle from cradle to grave: in this context, it may deal with the substance described in REACH or its application or a product containing it. The linking of these approaches could lead to define common system boundaries of the scenarios investigated in REACH and LCA: in this case the object under investigation is the same, though there is a difference concerning the environmental indicators as REACH considers the (eco)toxicity at local scale and LCA allows the environmental assessment at a global/regional scale. To date, REACH requires that risks associated with chemicals are expressed as risk-phrases (R-phrases) in line with the international hazard labelling standards[52]. Data collected for REACH implementation could be included in LCA, but presently these data are not suitable for a direct insertion in a LCA study, as they are, and need further elaboration; indeed, LCA data include

[52] It should be noted that R-phrases have been replaced by a new system defined in the CLP Regulation, which entered into force for pure substances on 1 December 2010 and for mixtures on 1 December 2015 (European Parliament and Council 2008). CLP uses hazard statements (H statements), rather than R-phrases, introducing the new EU system for classifying and labelling chemicals, based on the United Nations' Globally Harmonised System (UN GHS 2005). Many provisions of CLP are closely linked to REACH, so hazard labels required for CLP are also considered a link to REACH. The responsibility to assess risks and hazards of substances is given to industry in REACH *("the natural or legal persons that manufacture or import substances"*, European Parliament and Council 2006); risk and hazard information forms the scientific basis for labelling to be included in safety data sheets for substances and mixtures.

processes and amount of input and output while data from REACH concern substance properties. Some authors (Askham et al. 2012, 2013) suggest a holistic evaluation for linking R-phrases and LCA and including chemical hazard information in LCA with the aim of obtaining product development options and reducing potential risk of chemical hazard. The authors developed the REACH/LCA Screening Tree Tool in close collaboration with an enterprise, in order to ensure relevance to and usefulness in their product development process. The Screening Tree Tool represents an integration among the "only above threshold" (risk assessment-based) and "less is better" approaches (LCA-based). The tool was developed in a current software LCA tool (Pre consultant 2011) and is based on an impact assessment method already existing, with further improvement for considering the R-Phrases, threshold limit values and the calculation of hazard indicators and exposure pathway indicators. The Screening Tree Tool provides information in a visual overview and facilitates the link to a life cycle perspective, useful to communicate the importance of such issues to suppliers, suggesting the level of impurity that is acceptable (also calculable using this tool) or identifying which chemicals are important in specific raw materials from known suppliers. Often, the stimulation of a broader awareness of stakeholder across the supply chain could lead to better business decisions. The Screening Tree Tool is a good starting point for combining LCA and REACH in the product development process. Useful information in the priorities of product development is given but further work is needed to achieve full function-based life cycle hazard assessment.

6.4. References

Askham C. 2012. REACH and LCA methodological approaches and challenges, Int J Life Cycle Assess, 17: 43-57

Askham C, Gade AL, Hanssen OJ. 2012. Combining REACH, environmental and economic performance indicators for strategic sustainable product development. Journal of Cleaner Production, Vol. 35: 71-78

Askham C, Gade AL, Hanssen OJ. 2013. Linking chemical risk information with life cycle assessment in product development. Journal of Cleaner Production, Vol. 51:196-204

Barberio G, Scalbi S, Buttol P, Masoni P, Righi S. 2014. Combining life cycle assessment and qualitative risk assessment: The case study of alumina nanofluid production. Science of the Total Environment, Vol. 496: 122-131

Bekker C, Kuijpers E, Brouwer DH, Vermeulen R, Fransman W. 2015. Occupational Exposure to Nano-Objects and Their Agglomerates and Aggregates Across Various Life Cycle Stages; A Broad-Scale Exposure Study. Ann Occup Hyg, 59(6): 681-704

Eckelman MJ, Mauter MS, Isaacs JA, Elimelech M. 2012. New perspectives on nanomaterial aquatic ecotoxicity: production impacts exceed direct exposure impacts for carbon nanotoubes. Environ Sci Technol, 46(5): 2902-10. doi: 10.1021/es203409a

Grieger KD, Laurent A, Miseljic M, Christensen F, Baun A, Olsen SI. 2012. Analysis of current research addressing complementary use of life-cycle assessment and risk assessment for engineered nanomaterials: have lessons been learned from previous experience with chemicals? J Nanopart Res, 14: 958. doi 10.1007/s11051-012-0958-6

Hischier R, Nowack B, Gottschalk F, Hincapie I, Steinfeldt M, Som C. 2015. Life cycle assessment of facade coating systems containing manufactured nanomaterials. J Nanopart Res, 17: 68. doi 10.1007/s11051-015-2881-0

Hischier R. 2014. Framework for LCI modelling of releases of manufactured nanomaterials along their life cycle. Int J Life Cycle Assess, 19: 838–849. doi: 10.1007/s11367-013-0688-8

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

ISO 2006a. ISO (UNI EN) 14040. Environmental Management - Life Cycle Assessment (LCA) - Principles and Framework. International Organisation for Standardisation, Geneve, Switzerland

ISO 2006b. ISO (UNI EN) 14044. Environmental Management – Life Cycle Assessment (LCA) - Requirements and Guidelines. International Organisation for Standardisation, Geneve, Switzerland

Li B, Gao X, Li J, Yuan C. 2014. Life cycle environmental impact of high-capacity lithium ion battery with silicon nanowires anode for electric vehicles. Environmental Science and Technology, Vol. 48(5): 3047-3055

OECD 2013. Environmentally Sustainable Use of Manufactured Nanomaterials - Workshop held on 14 September 2011 in Rome, Italy (2013). Series on the Safety of Manufactured Nanomaterials N° 39. ENV/JM/MONO(2013)17, 06-Aug-2013. Organisation for Economic Co-operation and Development, Paris

OECD 2014. Nanotechnology and Tyres Greening Industry and Transport. OECD publishing. ISBN 978-92-64-20914-5

OECD 2015. Guidance manual towards the integration of risk assessment into life cycle assessment of nano-enabled applications. Series on the Safety of Manufactured Nanomaterials N° 57. OECD Environment, Health and Safety Publications, Organisation for Economic Co-operation and Development, Paris

Pirela SV,Sotiriou GA, Bello D, Shafer M, Bunker KL, Castranova V, Thomas T, Demokritou P. 2015. Consumer exposures to laser printer-emitted engineered nanoparticles: A case study of life-cycle implications from nano-enabled products. Nanotoxicology, 9(6): 760-8

Pini M, Rosa R, Neri P, Bondioli F, Ferrari AM. 2014. Environmental assessment of a bottom-up hydrolytic synthesis of TiO_2 nanoparticles. Green Chemistry. doi: 10.1039/c4gc00919c

Pizza A, Metz R, Hassanzadeh M, Bantignies JL. 2014. Life cycle assessment of nanocomposites made of thermally conductive graphite nanoplatelets. International Journal Life Cycle Assessment, Vol. 19: 1226-1237

Rodriguez-Garcia G, Zimmermann B, Weil M. 2014. Nanotoxicity and Life Cycle Assessment: First attempt towards the determination of characterization factors for carbon nanotubes. IOP Conf. Series: Materials Science and Engineering, 64. doi:10.1088/1757-899X/64/1/012029

Salieri B, Righi S, Pasteris A, Olsen S. 2015. Freshwater ecotoxicity characterisation factor for metal oxide nanoparticles: a case study on titanium dioxide nanoparticle. Sci Total Environ, 505: 494-502. doi: 10.1016/j.scitotenv.2014.09.107

Savolainen K, Backman U, Brouwer D, Fadeel B, Fernandes T, Kuhlbusch T, Landsiedel R, Lynch I, Pylkkänen L. 2013. Nanosafety in Europe 2015-2025: Towards Safe and Sustainable Nanomaterials and Nanotechnology Innovations. Finnish Institute of Occupational Health, ISBN 978-952-261-310-3 (Book), ISBN 978-952-261-311-0 (PDF)

Scalbi S, Fantin V, Barberio G, Brunori C. 2015. Life Cycle Assessment as a tool for environmental sustainability of nanomaterials and nanotechnologies. Proceeding NanotechItaly 2015, Bologna 25-27 Novembre 2015

Som C, Berges M, Chaudhry Q, Dusinska M, Fernandes TF, Nowack B, Olsen SI. 2010. The importance of life cycle concepts for the development of safe nanoproducts. Toxicology, 269: 160-169

Sotiriou GA, Singh D, Zhang F, Wohlleben W, Chalbot MG, Kavouras IG, Demokritou P. 2015. An integrated methodology for the assessment of environmental health implications during thermal decomposition of nano-enabled products. Environ Sci Nano, 2(3): 262-272

Walser T, Meyer D, Frasman W, Buist H, Kuijpers E, Brouwer D. 2015. Life-cycle assessment framework for indoor emissions of synthetic nanoparticles. Journal Nanoparticles Researcher, Vol. 17: 245. doi: 10.1007/s11051-015-3053-y

7. TAKE-HOME MESSAGES AND FINAL CONSIDERATIONS

Considering the diversity of issues that are addressed by the NANoREG framework, the reader is provided with a list of take-home messages for both Part I (section 7.1) and Part II (section 7.2) of the document. In section 7.3, final considerations are given.

7.1. Take-home messages on Part I

Part I of the NANoREG framework aims to: i) illustrate step-by-step how REACH applies to NMs, ii) identify where the issues still reside, and iii) 'show the path' to solutions based on available guidance and work presented in peer-reviewed scientific literature. The main outcomes from gathering and consolidating existing information are summarised below, section by section as take-home messages.

Take-home messages on EC Definition of 'nanomaterial' (from section 2)

- Currently, the EC Definition is not included in the REACH legal text (and REACH does not define 'nanomaterial'), but the EC Definition is referred to in the ECHA guidance for implementation of REACH, in the appendices which contain recommendations for NMs.
- The European Commission's policy services are in the process of deciding whether and how the EC Definition should be revised. The decision takes into account options provided by the JRC in 2015. The options included, for instance, suggestions to improve the clarity and implementability of the definition, analysis of the consequences of varying thresholds for the particle number fraction, and a discussion on the role of VSSA.
- No single technique, but rather a range of measurement methods is needed to test whether a material meets the EC Definition.
- There thus appears to be a need to develop a detailed guidance on the implementation of the EC Definition to indicate i) which measurement methods are more appropriate and for which NMs, ii) the sequence of the measurements and iii) how to address or harmonise as many factors as possible that can influence each technique (e.g. SOPs for sample preparation). This guidance has to be in line with the future revised EC Definition.

Take-home messages on REACH Substance Identification (from section 3.1)

- REACH addressed substances regardless of their size, shape and physical state. NMs are therefore covered by the definition of 'substance' under REACH and are subject to the same obligations as any other substance.

- REACH currently does not explicitly address NMs in its legal text. A process of revision of the REACH Annexes VII-X is ongoing, and explicit obligations for NMs are planned in the near future.
- A new appendix to the "Guidance on Registration" including recommendations for NMs has been developed by ECHA (still under consultation in February 2017) in order to provide advice to registrants who prepare their NM registration dossiers. This Guidance may need to be updated when explicit obligations for NMs are introduced (see previous bullet).
- The term 'nanoform' refers to a particular form of a substance that meets the criteria of the EC Definition of nanomaterial, as opposed to the 'bulk form(s)' of the same substance, i.e. (the) form(s) of the substance not meeting the criteria of the EC Definition. Several nanoforms of the same chemical composition may exist.
- Registrants can register all the nanoforms of a substance under a same registration and together with the corresponding non-nanoform. The hazard information submitted to demonstrate the safe use of the registered substance must cover all forms of the substance proposed in the registration and explicitly cover the different nanoforms.
- The information included in registration dossiers shall contain sufficient characterization of surface-treated NMs. Potential hazard difference(s) between surface-treated and untreated nanoforms should not be underestimated.
- IUCLID 6 includes new "conditionally active" fields to describe composition-related information on NMs (particle number size distribution, shape and aspect ratio, specific surface area and surface treatment), therefore providing the opportunity for registrants to improve clarity when presenting information on nanoforms within their registration dossiers.

Take-home messages on REACH Information Requirements (from section 3.2)

- REACH Standard Information Requirements (Annexes VII-X) apply to all forms of the substance addressed in the registration dossier, e.g. bulk (non-nanoforms) and nanoform(s) of a substance. While preparing a registration dossier, the registrant must ensure that the data provided are representative of all the specified form(s).
- In 2012, ECHA published a series of appendices to the guidance for implementation of REACH, containing recommendations for NMs in relation to the Standard Information Requirements. These appendices are currently under revision and may need to be updated when explicit obligations for NMs are introduced in REACH.
- Concerning several physicochemical properties, no nanospecific recommendations are provided in ECHA guidance for several of them: State of the substance at 20 °C and 101.3 kPa, Melting / freezing point, Boiling point, Relative density, Vapour pressure, Surface tension, Flash point, Flammability, Explosive properties, Self-ignition temperature, Oxidizing

properties, Stability in organic solvents and identity of relevant degradation products, Dissociation constant and Viscosity. For the endpoints having an OECD TG associated[53], the available OECD TGs are either considered to be applicable to NMs without adjustments or in need of further evaluation before decisions about adjustments are made.

- Literature suggests that current methods for Water solubility assessment developed for bulk materials could in principle be used for NMs. However, OECD TG 105 is not applicable to NMs and is currently under review. Furthermore, specific nano-tailored protocols and guidelines are under development in the scientific literature. Both ECHA and OECD highlight that it is important to distinguish between 'solubility' and 'dispersibility' in the case of a NM.
- Measurement of the Water-octanol partition coefficient for NMs has turned out not to be meaningful for those NMs that do not dissolve, but rather disperse as particles. When a NM dissolves sufficiently that the Water-octanol partition coefficient is meaningful, the evaluation can then follow the bulk material evaluation.
- Under REACH, in ECHA guidance Granulometry is defined as the determination of particle size distribution. When a group of particles covers a size range, it may be described by a particle size distribution. In the case of NMs, additional information on shape, specific surface area and dustiness should also be provided, as they are key parameters that describe Granulometry.
- Concerning toxicological properties, for Skin irritation or Skin corrosion, Eye irritation, Skin sensitization and Reproductive toxicity, standard test methods are deemed valid for NMs.
- For Mutagenicity, the majority of the test methods are applicable to NMs. However, the bacterial reverse mutation test (Ames test) is not considered reliable and should not be used for mutagenicity testing on NMs.
- When testing Acute toxicity and Repeated dose toxicity, inhalation may be the most likely route of exposure for NMs. It is important to include aspects on lung overload in the interpretation of the study results.
- Regarding Toxicokinetics, it is suggested to pay attention to potential modifications of the NM occurring in the test system. The evaluation of toxicokinetic data is encouraged for grouping and read-across, as well as for extrapolation of information from *in vitro* to *in vivo*.

[53] http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-1-physical-chemical-properties_20745753

- Concerning ecotoxicological properties, when testing NMs for Acute Toxicity to fish, it is recommended to collect data on: fish ventilation rate, gill pathologies, mucus secretion, brain pathology, animal behaviour and activity levels of enzymes.

  Regarding algal tests, OECD recommends that the assay selected to be used is tested in advance for interference arising from particle presence, as this has been reported to confound the measurement of algal cell counts.
- For Degradation, ECHA has clarified that a majority of OECD TGs on biodegradability are applicable to NMs of organic nature.
- For Adsorption/Desorption screening, K_d has to be based on actual testing, since estimations from K_{oc} and K_{ow} have questionable or no merit for NMs. OECD TG 106 is not appropriate for testing the adsorption/desorption of NMs and a new test should be developed.
- It is not possible to estimate Bioaccumulation from K_{ow}, since NMs are usually dispersed rather than in solution. Hence, OECD TG 305 on bioaccumulation in fish needs to be adjusted for NMs.
- For Effects on terrestrial and sediment organisms, guidance on detection techniques for NMs in soil and sediment is needed, and understanding the state of the NM in soils and sediments is considered critical for interpreting results.
- For all properties, non-testing approaches (e.g. (Q)SAR, grouping and read-across) are applicable on a case-by-case basis only and require detailed scientific justification.

Take-home messages on Adaptations of REACH Information Requirements (from section 3.3)

- An appendix to ECHA guidance on "Grouping of chemicals" with recommendations on how to apply grouping and read-across for NMs is under development (in February 2017). The general approach is in principle applicable to NMs. However, similarity cannot be exclusively based on structural or chemical composition. Indeed, grouping and read-across for NMs largely involve different nanoforms of the same chemical composition.
- Several (Q)SAR methods for NMs are under development, but their usefulness as alternative methods in a regulatory context still needs to be demonstrated.

Take-home messages on REACH Hazard Assessment (from section 3.4)

- The CLP Regulation provides the framework for the classification of NMs as it applies to substances and mixtures in all physical states and forms. It is likely that there may be a need to classify a NM in a different way than the same substance in a bulk form or in a slightly different nanoform. In IUCLID, it is already possible to specify the classification and labelling of the nanoform.

- When deriving DNELs for NMs, the choice of metrics is of critical importance, since it is not possible to establish a single metric that is applicable to all cases.
- When using OEL in place of DNEL for NMs, it is critical to consider whether the route and duration of exposure, as well as the physicochemical attributes that may affect the toxicity, are the same as for the substance for which the OEL has been set.
- In the case of NMs, it is unwise to extrapolate from one exposure route to another.
- The thermodynamic equilibrium generally does not apply to NMs in the environment and, thus, care must be taken when applying the equilibrium partitioning method to NMs and interpreting the results. Indeed, as NMs may spread in the environment as particles, the equilibrium partitioning method may underestimate the exposure of soil and sediment environments and overestimate the exposure in water.
- AFs and SSDs do not take nanospecific processes, such as aggregation, into account during the testing of NMs and the tests may thus not always be representative of natural conditions. Three other methods are suggested in literature: the probabilistic species sensitivity distribution (PSSD), the dissolved metal ion and the indicative no effect concentration (INEC).

Take-home messages on REACH Exposure Assessment (from section 3.5)

- There are currently no agreed standardised and validated methods for measuring personal exposure to NMs.
- There are currently no validated models providing quantitative estimates of human (worker and consumer) or environmental exposure to NMs.
- A number of control banding tools and semi-quantitative exposure assessment tools are being developed that can be used to determine if exposure to NMs needs to be controlled.
- A number of instruments that are able to generate measurement data on particle size, number and/or surface area are available. However, these instruments are unable to distinguish between engineered nanoparticles and any background/ambient nano-sized particles.
- NMs are released from consumer products in forms that are different from the primary particles handled at the manufacturing stage. Further transformations in the environment affect what exactly humans or the environment are exposed to. This is currently difficult to address in exposure or risk assessment.

Take-home messages on REACH Risk Characterisation (from section 3.6)

- The same metric must be used in order to compare exposure and hazard. The work carried out in NANoREG suggests that the most appropriate metric to express the biologically effective dose largely depends on the exposure pattern and on the type of NM.
- Relationships for converting mass into other metrics need to be developed.
- Given the need to fill in data gaps and, at the same time, the lack of validation of available models for NMs, model uncertainty can be considered an even more important issue with NMs than with other chemicals.

7.2. Take-home messages on Part II

Part II of the NANoREG framework examines from the scientist's point of view three forward-looking strategies that are expected to facilitate and accelerate the implementation of REACH, discussing their benefits and potential limitations. The main outcomes from these analyses are summarised below, section by section as take-home messages.

Take-home messages on Nanospecific Risk Assessment (from section 4)

- The approach aims to i) prioritise the assessment of the NM applications that have the highest potential to cause adverse human health effects, and ii) to identify the information that should be collected or generated in order to address the nanospecific issues in risk assessment, for instance under REACH.
- The approach can also be used to identify situations where the use of grouping, read-across and (Q)SAR for NMs is likely to become feasible in the future, and to point towards the generation of the types of data needed to scientifically justify the use of those tools.
- The most important nanospecific elements to be considered in human health risk assessment of NMs are: Exposure potential, Dissolution, Transformation, Accumulation, Genotoxicity and Immunotoxicity.

Take-home messages on Safe-by-Design (from section 5)

- SbD aims at providing an integrated and iterative process where safety information on a certain material, substance or product is integrated within the innovation process from early R&D phases onwards. Iterations occur in order to search for the best achievable safety conditions. Safety hence becomes a component of the innovation process, not a hurdle.
- The NANoREG SbD concept includes the identification of the "potential for risk" in the first stages of innovation, followed by "indicators for risk" at the middle stages of innovation and, eventually, by "demonstrators for risk" in the final stages of innovation, as laid down in regulatory requirements such as REACH.

- The identification of "potential for nanospecific risk" during the first stages of innovation requires a screening strategy. The NANoREG screening strategy is based on six questions: solubility/dissolution rate, stability of coating, accumulation, genotoxicity, inflammation and environmental toxicity.
- The NANoREG SbD concept also supports the selection of the best "risk treatment options" per stage of innovation.
- Elaboration towards a fully-fledged SbD approach is foreseen by the EU-funded H2020 CSA ProSafe (for industry) and the H2020 NanoReg2 (for regulators) projects.

Take-home messages on Life Cycle Assessment (from section 6)

- The ISO 14040/44 standard for LCA[54] is considered as a suitable, harmonised, user-friendly and validated procedure applicable to NMs and nano-enabled products.
- However, due to the fact that nano-enabled products have novel types of functions, it may be difficult to identify alternative products and to define the functional unit in an LCA study.
- The amount of available information to define the entire life cycle of a nano-enabled product is scarce, especially for the end-of-life stages.
- The application of "human toxicity" and "ecological toxicity" impact categories deserves special attention, as the respective characterisation factors (CFs) are not yet available for NMs (although some attempts are illustrated in the scientific literature).
- Combined use of risk assessment and LCA for NMs is encouraged.
- The availability of toxicity data in REACH may help with the derivation of nanospecific CFs. However, these data are presently not suitable for a direct insertion into an LCA study, but need further elaboration. Indeed, LCA data include 'processes' and amounts of 'input and output', while data from REACH concern substance properties.
- On the other hand, LCA gives a holistic vision on the life cycle of products, which can be taken into account when REACH principles are implemented by companies, for instance to assess other scenarios/options with improved environmental performance, i.e. with substitution of harmful chemicals by safer alternatives.

[54] CEN/TC 352 (Nanotechnologies) is currently (February 2017) adapting this ISO standard to nanomaterials.

7.3. Final considerations

The NANoREG framework is the result of a collective effort of experts from 20+ project partners from well-recognised organisations. A large amount of information on EHS of NMs from diverse fields has been generated, gathered and linked together in a single document.

The document was conceived as a manual that both regulators and industry could consult to understand the state-of-the-art in performing safety assessment of NMs under REACH, including closed and open issues. It also provides them with forward-looking strategies to be further developed from a scientific perspective, which could pave the way for a more efficient and practical implementation of REACH principles for NMs.

The three forward-looking strategies identified by NANoREG partners are: the use of NanoRA as a screening tool; the application of the SbD process to NMs; the combined use of risk assessment and LCA for NMs. Those strategies are at present at conceptual stage of development. NanoRA has the form of a comprehensive flow chart aimed to enhance the efficiency of the risk assessment process for NMs. The next step towards practical implementation is to verify and, if necessary, refine its assumptions through dedicated case studies.

SbD is a well-known concept in industry. Its application to NMs has been first proposed by NANoREG. This NM SbD concept is maturing in ProSafe and NanoReg2, with the development of operational tools to support both industry and regulators expected in the short term.

LCA is also a well-established procedure, but its application to NMs is presently hampered by methodological uncertainties and lack of data, which still need scientific work to overcome.

In spite of the hurdles in the development and practical application of the forward-looking strategies, NANoREG partners consider NanoRA, SbD and LCA as valuable paths worth exploring by scientists, industry and regulators to achieve a more efficient implementation of REACH principles for NMs in the near future. However, as shown in figure 1.1 (Introduction, section 1), the three strategies need to be further developed, tested and debated before a decision can be made on how far they are actually relevant for assessment methodology under REACH and, if so, how they can be properly implemented at both industrial and regulatory level.

ANNEX I – TERMINOLOGY

JRC has published the harmonised terminology for environmental health and safety of nanomaterials as NANoREG output here:
http://publications.jrc.ec.europa.eu/repository/bitstream/JRC100906/jrc%20technical%20report-nanoreg%20terminology%20ehs%20assessement%20nms.pdf

The document is also reported on the NANoREG website:
http://www.nanoreg.eu/media-and-downloads/publications/267-nanoreg-harmonised-terminology-for-environmental-health-and-safety-assessment-of-nanomaterials

ANNEX II – SUPPORTING INFORMATION ON NANOMATERIALS FOR SECTION 3.2 REACH INFORMATION REQUIREMENTS

Additional information on physicochemical, toxicological and ecotoxicological properties of nanomaterials (NMs) to be considered in order to address REACH Standard Information Requirements specified in Annex VII-X are discussed below. Information comes from different sources including ECHA guidance, IUCLID 6 database, OECD documents and peer-reviewed scientific literature.

Physicochemical properties

Shape

In ECHA guidance (ECHA 2012), it is recommended to provide information on the shape of NMs as a part of the standard information requirement on "Granulometry", except in cases where the substance is marketed or used in a non-solid or non-granular form. ECHA defines 'shape' as: *"qualitative or, at best, semi qualitative geometrical description or dimension-less term(s) of the extremities of the particle or collections of particles, their agglomerates or aggregates, that make up the material under investigation (adopted from OECD 2009)"*. ECHA provides information on mesodescriptors and descriptors for shape, criteria for shape classification as well as available qualitative and semi-quantitative techniques for particle shape and morphology characterization. The reported data should contain information on: sample preparation method and analysis method used, a microscopy image, suspending medium, pH and temperature as well as a qualitative or semi-quantitative geometrical description of the particle and/or its aggregates or agglomerates. For details and methods, please consult ECHA (2012).

Surface area

It is recommended to present data on specific surface area of NMs (ECHA 2012). The requirement does not apply to substances marketed or used in a non-solid or non-granular form. The data should include information on the specific surface area (m^2/kg) and where appropriate also the calculated volume specific surface area (m^2/cm^3). In addition, information on the sample preparation, test method and test conditions, temperature, purity of the sample tested, mass of degassed sample, adsorption isotherm, evaluation parameters, physical state and reference substance used (if any) should be included. For details and methods, see ECHA (2012). ECHA also highlights that surface area is an important parameter in the characterisation of NMs, with emerging evidence of quantitative value as a dose metric or descriptor for hazard assessment. The

total surface area should not be confused with the specific surface area, which is the ratio between total surface area and mass (mass specific surface area) or volume (volume specific surface area, VSSA) of the analysed material.

Adsorption/desorption

In the case of NMs, the distribution coefficient K_d cannot be derived from the organic carbon-water coefficient (K_{oc}) or from the octanol-water coefficient (K_{ow}). Instead, K_d must be based on actual testing (ECHA 2012). Methods for the measurement of adsorption are summarized in ECHA guidance (ECHA 2015) (e.g. OECD TG 106 and 121).

The assessment of existing TGs performed by OECD concluded that OECD TG 106 "Adsorption-desorption" and OECD TG 121 "Adsorption to soil or sediment" might be applicable under some circumstances or to some classes of manufactured NMs only. However, it is not known how the test results might be impacted by the presence of a colloidal suspension, which might be present if the sample manufactured NM does not completely dissolve. Hence, further work is required to determine this and to modify the TGs, if necessary (OECD 2009). In the latest document, OECD further confirms that OECD TG 106 is not applicable to NMs (OECD 2014).

Furthermore, ECHA (2015) states that the methods may not be suitable for: i) substances that react with the column; ii) solvent or other test system components; iii) surface active substances; iv) substances that interact in a specific way with inorganic soil components such as clay minerals; v) inorganic compounds; and vi) moderate to strong acids and bases. NMs may be close in their properties to clay minerals, surface active substances and inorganic compounds. All methods require a quantitative analytical method for the substance, reliable over the range of test concentrations. This presents an issue for many NMs, if identification both by chemical nature and physical structure is to be performed. A practical solution for most cases is elemental analysis, e.g. by ICP-MS of fractions, since most NMs contain inorganic elements (Hankin et al. 2011).

IUCLID 6 fields for physicochemical endpoints

When preparing the registration dossier, information on the physicochemical characterisation of the substance is foreseen to be reported under sections 4.1-4.36 of IUCLID 5.6. The registrant should note the following endpoints, which are of particular relevance for NMs: Agglomeration/aggregation, Crystalline phase, Crystallite and grain size, Aspect ratio/shape, Specific surface area, Zeta potential, Surface chemistry, Dustiness, Porosity, Pour density, Photocatalytic properties, Radical formation potential, and Catalytic activity (IUCLID 5.6 sections 4.24-4.36). Although these endpoints are not explicitly mentioned in REACH Annexes VII-X on Standard Information Requirements, they are considered as parameters that provide relevant information on REACH Standard Information Requirements. For example: Granulometry is

characterised by size distribution, crystallite grain and size, shape/aspect ratio, dustiness, specific surface area and porosity; Water solubility is characterised by dispersibility and agglomeration/aggregation.

Future developments in IUCLID may include additional nanospecific fields to enable better reporting on NMs.

OECD identity and physicochemical characterisation endpoints

In 2006, OECD launched the Working Party on Manufactured Nanomaterials (WPMN) to provide a global forum for discussion on environmental, health and safety issues concerning manufactured NMs. The WPMN set up an exploratory test programme (the "OECD WPMN Testing Programme") to examine information needs and testing methods for manufactured NMs and a "Guidance Manual for Sponsors" (OECD 2010) was drafted to help the sponsors. A list of NMs to test was published in 2008 as well as a list of endpoints thought to be relevant for the safety assessment of NMs (OECD 2010), including endpoints describing the identity and physicochemical characterisation (table II.1). Later, the WPMN published an evaluation of the methods used for physicochemical characterisation (OECD 2016a, 2016b). The Programme was finalised in 2013 and the dossiers containing the raw data obtained from testing activities were published during 2015[55]. An overview of the WPMN work and outcomes of the testing programme is given by Rasmussen et al. (2016). The assessment of the methods (OECD 2009) and the initial considerations of the test results (SCENIHR 2009) concluded that not all proposed endpoints were relevant for all NMs.

Table II.1: Modified OECD list of endpoints regarding chemical identity and physicochemical properties for nanomaterials to be tested during the Working Party on Manufactured Nanomaterials Testing Programme (modified after OECD 2010). The endpoints numbered 1 to 26 are listed in OECD 2010; the un-numbered endpoints are added in the current publication.

N°	Nanomaterial Information / Identification
1	Nanomaterial name
2	CAS number
3	Structural formula / Molecular structure
4	Composition of the nanomaterial being tested (incl. degree of purity, known impurities or additives)
5	Basic morphology

[55] http://www.oecd.org/chemicalsafety/nanosafety/dossiers-and-endpoints-testing-programme-manufactured-nanomaterials.htm

6	Description of surface chemistry (e.g. coating or modification)
7	Major commercial uses
8	Known catalytic activity
9	Method of production (e.g. precipitation, gas phase)
	Quantity
10	Agglomeration / Aggregation
11	Water solubility
12	Crystalline phase
13	Dustiness
14	Crystallite size
15	Representative TEM picture(s)
16	Particle size distribution
17	Specific surface area
18	Zeta potential (surface charge)
19	Surface chemistry (where appropriate)
20	Photo-catalytic activity
21	Pour density
22	Porosity
23	Octanol-water partition coefficient, where relevant
24	Redox potential
25	Radical formation
26	Other relevant information (where available)
	Fat solubility/oleophilicity
	Melting point
	Boiling point
	Relative density
	Vapour pressure
	Dissociation constants

Toxicological properties

Respiratory tract corrosion and irritation

These endpoints are not required as standard information in REACH and at the moment there are no EU or OECD test methods for these effects; however, as reported in the ECHA guidance (ECHA 2015), the inhalation toxicity of some fibre-like NMs may cause persistent pulmonary inflammation leading to the destruction of the respiratory tract mucosa and the development of a granulomatous disease (Harkema et al. 2013). In order to assess inflammatory and pro-fibrogenic effects, some authors suggested a series of non-standardized assays *in vivo*, such as cytokine production profile, specific cell count and protein analysis in the bronchoalveolar lavage fluid (BALF) and *in vitro* with pulmonary, endothelial and immune cell lines (Hankin et al. 2011, Morimoto et al. 2012).

In vitro testing

The RIP-oN 2 report found a number of studies considering the inclusion of other non-standardised *in vitro* tests relevant for human toxicity assessment of NMs: cell viability (e.g. cell morphology, lung cell damage, or cell metabolic activity), oxidative stress (e.g. ROS production, glutathione status, NO generation), and pro-inflammatory effects (e.g. cytokine production). These endpoints have been linked with genotoxicity, potentially followed by carcinogenicity or cell death (Hankin et al. 2011, Kermanizadeh et al. 2013). Certain *in vitro* studies, while not required under REACH or validated, may provide supporting information in a Weight of Evidence approach or other adaptations to the Standard Information Requirements (see section 3.3).

Assay inhibition / enhancement

NMs may interfere with a number of assays utilised to determine their cellular or toxic effects. For details, see ECHA (2012). Several authors advise as a general precaution to use more than one assay to assess the endpoint or effect in question (Hankin et al. 2011, Kroll et al. 2012, Guadagnini et al. 2015).

Inhalation toxicity testing

OECD (2012) suggested specific changes to adapt the actual Test Guidelines (TGs) for assessing inhalation toxicity of NMs. Aerosol preparation and characterisation, dosimetry, application of biokinetics and detailed pathology of the brain and nervous systems are some of the requirements discussed by OECD (2012). Most experts also agreed on the inclusion of the analysis of the bronchoalveolar fluid as a mandatory test to assess the inhalation toxicity of NMs. OECD published a study on dosimetry in 2015: *"Concerning toxicological test special attention needs to be given to measuring, dosing, delivery, tracking of nanomaterials in the test system. Concerning toxicological endpoints it is also important to consider the physicochemical characteristics of the nanomaterial including in the dosing vehicle. There is need for guidance on sample preparation and in situ characterization for the toxicological assessment of nanomaterials. For toxicological tests, adequate characterization of tested nanomaterial should have consideration of actual exposure of the test system (possible agglomeration and disagglomeration) appropriate dose metric should be given".* Dosimetry and sample preparation of NMs has also been a top priority for OECD, and the organization has developed a specific guidance on this topic (OECD 2012).

Cell viability

The most non-standard tests used are: cell morphology, *in vivo* BALF (bronchoalveolar lavage fluid cell counts) and protein analysis, cell metabolic analysis; cellular membrane integrity (LDH release); lung cell damage (gamma glutamyl transferase assay); TransEpithelial Electricals Resistance; apoptosis and necrosis.

Oxidative stress

It refers to: ROS production, gluthatione status, nitric oxide (NO) generation.

Inflammatory and pro-fibrogenic effects

It refers to: TNF-alpha, Trypan Blue assay, cytokine production and analysis of signalling pathway. In particular, pulmonary inflammation and genotoxicity studies within pulmonary, endothelial and immune cell lines should be performed.

Short-Term Inhalation Study (STIS)

OECD has prepared a document (draft) reporting the discussion on the *"feasibility to include lung burden and BALF analysis measurements as part of study supporting amendments to OECD subacute and subchronic inhalation test guidelines for testing of nanomaterials"*. The document also discusses the purpose of the Short-Term Inhalation Study (STIS) as pre-screening tool for grouping and read-across or to establish the testing dose of NMs for a later subsequent sub-chronic inhalation study. The study is based on the determination of three key elements that indicate inhalation toxicity: inflammation potency of the respiratory tract tested through the cytokine profile analysis in the BALF; potential reversibility; or progression. For details, see OECD (2015).

Ecotoxicological properties

OECD (2014) identified dissolution, dispersibility, agglomeration, degradation and transformation as important pieces of information to be known before further fate tests in water compartments are conducted, and stated that a decision tree or tiered approach, to be added to the specific TG, should be established for NMs as prior testing before further ecotoxicity or environmental fate tests are conducted. Such a decision tree or tiered approach should also be developed as prior testing before soil or sediment toxicity testing. OECD (2014) also called for the development of guidance for pre-treatment scenarios which include the most probable transformation processes, in order to harmonize the handling of aging and transformation processes. The following issues were discussed in the "Conclusions and Recommendations" chapter (OECD 2014):

- For a better comparability of results from ecotoxicity and fate tests, the same test conditions should be used.

- Environmental tests should also be conducted with aged NMs. The aged NM should reflect the most likely transformation processes after its introduction into the environment compartments. Pre-treatment scenarios must therefore be identified and harmonized.
- During the environmental tests, a loss of the applied NM is expected. An adjustment should be made for the acceptance of loss of NM during the test.
- The importance of natural organic material (NOM) for the fate and transport of NMs in the environment is recognized. The type of NOM used for testing or which is already available in the system must be specified.
- Depending on the environmental behaviour of a NM, zebrafish may not be the right target organism and other organisms should be tested. The applicability of the OECD TG 305 bioaccumulation test for other organisms, e.g. mussels or daphnids, should be tested in further studies.

Recommendations for the future needs in regulatory environmental testing include method development for detection, identification and quantification of NMs in both environmental and test media, the characterization and understanding of particle behaviour in these media, data gaps in toxicity testing and category approached to group NMs (OECD 2014).

References

ECHA 2012. Guidance on information requirements and chemical safety assessment. Appendix R7-1 Recommendations for nanomaterials applicable to Chapter R7a Endpoint specific guidance. April 2012. European Chemicals Agency, Helsinki

ECHA 2015. Guidance on information requirements and chemical safety assessment. R7a Endpoint specific guidance. Version 4.1, October 2015. European Chemicals Agency, Helsinki

Guadagnini R, Halamoda Kenzaoui B, Walker L, Pojana G, Magdolenova Z, Bilanicova D, Saunders M, Juillerat-Jeanneret L, Marcomini A, Huk A, Dusinska M, Fjellsbø LM, Marano F, Boland S. 2015. Toxicity screenings of nanomaterials: challenges due to interference with assay processes and components of classic in vitro tests. Nanotoxicology, 9 sup1: 13-24

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.

Harkema JR, Nikula KJ, Haschek WM. 2013. Chapter 51: Respiratory System. In: Haschek and Rousseaux's Handbook of Toxicologic Pathology. 3rd Edition. Volume III. Haschek WM, Rousseaux CG, Wallig MA Eds. Elsevier/Academic Press, Amsterdam, The Netherlands, pp.1935-2003

Kermanizadeh A et al. 2013. An in vitro assessment of panel of engineered nanomaterials using a human renal cell line: cytotoxicity, pro-inflammatory response, oxidative stress and genotoxicity. BMC nephrology, 14(1)

Kroll A et al. 2012. Interference of engineered nanoparticles with in vitro toxicity assays. Archives of toxicology, 86(7): 1123-1136

Morimoto Y et al. 2012. Inhalation toxicity assessment of carbon-based nanoparticles. Accounts of chemical research, 46.3: 770-781

OECD 2009. Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials. ENV/JM/MONO(2009)21. Organisation for Economic Co-operation and Development, Paris

OECD 2010. Guidance Manual for the Testing of Manufactured Nanomaterials: OECD's Sponsorship Programme; first revision. ENV/JM/MONO(2009)20/REV. Organisation for Economic Co-operation and Development, Paris

OECD 2012. Guidance on sample preparation and dosimetry for the safety testing of manufactured nanomaterials. OECD Environment, Health and Safety Publications. Series on the Safety of Manufactured Nanomaterials N° 36. ENV/JM/MONO(2012)40. Organisation for Economic Co-operation and Development, Paris.

OECD 2014. Ecotoxicology and Environmental Fate of Manufactured Nanomaterials: Test Guidelines. Expert Meeting Report. OECD Environment, Health and Safety Publications. Series on the Safety of Manufactured Nanomaterials N° 40. ENV/JM/MONO(2014)1. Organization for Economic Co-operation and Development, Paris

OECD 2015. Feasibility study supporting amedments to OECD subacute and subchronic inhalation test guidelines for testing of nanomaterials. Draft Guidance and Review Documents/Monographs. http://www.oecd.org/env/ehs/testing/OECD%20Feasibility%20Study-Final%2013%20Oct%202015.pdf

OECD 2016a. Physical-chemical parameters: Measurements and methods relevant for the regulation of nanomaterials. OECD Environment, Health and Safety Publications. Series on the Safety of Manufactured Nanomaterials N° 63. ENV/JM/MONO(2016)2. Organisation for Economic Co-operation and Development, Paris

OECD 2016b. Physical-chemical properties of nanomaterials: Evaluation of methods applied in the OECD-WPMN testing programme. OECD Environment, Health and Safety Publications. Series on the Safety of Manufactured Nanomaterials N° 65. ENV/JM/MONO(2016)7. Organisation for Economic Co-operation and Development, Paris

Rasmussen K, González M, Kearns P, Riego Sintes J, Rossi F, 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, Vol. 74: 147-160

SCENIHR 2009. Risk Assessment of Products of Nanotechnologies. Scientific Committee on Emerging and Newly Identified Health Risks

ANNEX III – SUPPORTING INFORMATION ON NANOMATERIALS FOR SECTION 3.4 REACH HAZARD ASSESSMENT

In addition to the nanospecific considerations concerning how to carry out the hazard assessment required by REACH in the case of nanomaterials (NMs) (see section 3.4.1), there are additional nanospecific issues that merit mentioning since they can influence the interpretation or the execution of the hazard assessment.

Nanospecific intracellular pathways and effects

A large number of *in vitro* studies using either primary cultures from different origins or established cell lines have been performed and many types of NMs and endpoints have been studied. It has been shown that the NM size can determine the pathway of cell entry (Rejman et al. 2004, Jana 2011). The studies show that after NMs enter the cell they interact with the cytoplasm and subcellular organelles. Depending on the cell type and also on the material type, they can accumulate for longer periods of time and induce specific cellular effects (e.g. Oberdorster et al. 2005, Simko et al. 2015). The intracellular localization of the NM defines its further fate. Thus, if there is a release of ions, such as in the case of soluble NMs in lysosomes, oxidative stress, apoptosis and also cell cycle disturbances can be induced (figure III.1). The observed effects are dose dependent. Furthermore, these effects are not unique to nano-sized particles since they can appear as consequences of exposure to the bulk material as well. Thus, there are no nanospecific biological effects known so far, but known cellular and toxic reactions that are caused by NMs.

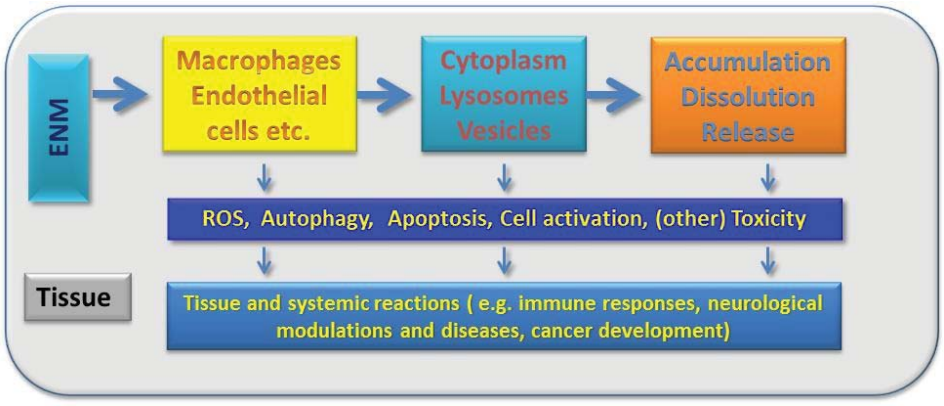

Figure III.1: Intracellular localization, cellular process activation, and tissue and systemic reactions of cellule-internalized nanomaterials. ENM = Engineered Nanomaterial. ROS = Reactive Oxygen Species

Nanospecific developed protocols

Limitations of traditional approaches to toxicity testing range from ethical issues linked to the high number of animals required in *in vivo* studies to practical aspects regarding high throughput methodologies and accuracy of the predicted toxicities. The multitude of possible formulations of NMs to be assessed for relevant toxic properties makes *in vitro* approaches highly attractive. Within NANoREG and other European projects, e.g. NanoValid[56], Nanogenotox[57], standard toxicological protocols have been adapted to NMs toxicity assessment. Assays cover viability (MTS, Alamar blue, neutral red, colony forming efficacy), genotoxicity (Comet assay, micronucleus, *in vitro* mammalian cell gene mutation test), immunotoxicity (interleukin expression), and Cell Transformation Assay (CTA). Some of the aforementioned protocols have been subject to inter-laboratory comparisons to assess the reproducibility of the assay e.g. the round robin exercises performed within NANoREG.

Exposure methodologies are also highly relevant for risk assessment. In this context, conventional submerged cultures exposed to NM in a liquid form do not represent the ideal exposure scenarios. Different efforts to solve these issues have been made in different initiatives (e.g. NanoDevice[58], NANoREG). Generally, it is currently accepted that cellular systems grown in air-liquid interphase (ALI) and exposure to NM in aerosol (or similar) better represent the occupational and consumer settings when dealing with the inhalation route. In this context, 3D reconstructed cellular models representing the epithelial airway systems (even as disease states) become a potential option for hazard studies focusing on the inhalation route. As the complexity of cellular models and exposure systems increases, it becomes more challenging to harmonize and validate procedures. The costs of such systems become relevant when considering the large number of NMs that may require toxicological assessment. In this context, NANoREG has considered both simple, monolayer cultures and complex systems; results can pave the way for a future compromise between low-throughput expensive but accurate systems and high-throughput screening methodologies.

Carcinogenicity *in vitro*

There are promising *in vitro* approaches to recognise genotoxic as well as non-genotoxic carcinogens such as the CTA. These assays measure cell transformation that is one step in the multistep cancer process and can detect both genotoxic and non-genotoxic carcinogens. Several of them such as Bhas42 CTA have been used to detect *in vitro* transformation of NMs (Sasaki et

[56] http://www.nanovalid.eu/
[57] http://www.nanogenotox.eu/
[58] http://www.nano-device.eu/

al. 2014). Two guidance documents on cell transformation assays have been drafted under the OECD umbrella to allow the scientific and regulatory communities to use them as part of a weight of evidence approach in the testing of substances for carcinogenic potential. These are the "*In vitro* Syrian hamster embryo cell transformation assay", adopted in 2015 (OECD 2015), and the "*In vitro* Bhas 42 cell transformation assay", adopted in 2016 (OECD 2016). There are also new *in vitro* toxicogenomics tools that may be potentially used to detect both genotoxic and non-genotoxic carcinogens by using global gene expression profiling via microarray technology (Doktorova et al. 2012).

References

Doktorova TY, Pauwels M, Vinken M, Vanhaecke T, Rogiers V. 2012. Opportunities for an alternative integrating testing strategy for carcinogen hazard assessment? Critical Reviews in Toxicology, 42: 91-106

Jana NR. 2011. Design and development of quantum dots and other nanoparticles based cellular imaging probe. Phys Chem Chem Phys, 13(2): 385-396

Oberdörster G, Oberdörster E, Oberdörster J. 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect, 113(7): 823-839

OECD 2015. Guidance document on the in vitro Syrian Hamster Embryo (SHE) cell transformation assay. Series on Testing & Assessment No. 214. ENV/JM/MONO(2015)18. 22-May-2105. Organisation for Economic Co-operation and Development, Paris.

OECD 2016. Guidance document on the *in vitro* Bhas 42 cell transformation assay. OECD Series on Testing and Assessment N° 231. ENV/JM/MONO(2016)1. 08-Jan-2016. Organisation for Economic Co-operation and Development, Paris.

Rejman J, Oberle V, Zuhorn IS, Hoekstra D. 2004. Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis. Biochem. J., 377: 159-169

Sasaki K, Huk A, El Yamani N, Tanaka N, Dusinska M. 2014. Bhas 42 cell transformation assay for genotoxic and non-genotoxic carcinogens. In: Genotoxicity and Repair. A Practical Approach, Eds. Sierra LM, Gavaivago I. Series: Methods in Pharmacology and Toxicology, Springer Protocols, Humana Press, Springer New York, Heidelberg Dordrecht London, 2014, XII, 483 p. 70 illus., 42 illus. in color. ISSN 1557-2153, 343-362

Simkó M, Tischler S, Mattsson MO. 2015. Pooling and Analysis of Published in Vitro Data: A Proof of Concept Study for the Grouping of Nanoparticles. Int J Mol Sci, 16(11): 26211-26236

ANNEX IV – SUPPORTING INFORMATION ON NANOMATERIALS FOR SECTION 4 A NEW APPROACH TOWARDS NANOSPECIFIC PRIORITISATION AND RISK ASSESSMENT

Table IV.1: Links between phase I/II of the flow chart illustrated in figure 4.1 and sections 2 (focused on European Commission's definition of nanomaterial) and 3 (focused on REACH) of the NANoREG Framework and Toolbox (NANoREG Deliverable D1.12)

Phase I

Phase I [flow chart in figure 4.1]	Link with sections 2 and 3 of the NANoREG Framework	Link with sections 2 and 3 of the NANoREG Toolbox
Nano?	2.1: EC Definition of nanomaterial 3.1: REACH Substance identification	2: EC Nano Definition • Measuring Particle Number Size Distribution • Measuring Volume Specific Surface Area (VSSA) 3.1: REACH Substance ID
Dissolution in water	3.2: REACH Information requirements [3.2.1.2: Physicochemical properties (water solubility)	3.2: REACH Information requirements • Water solubility
Exposure	3.3: REACH Adaptation rules [3.3.1: Substance-tailored exposure-driven testing] 3.5: REACH Exposure assessment	3.3: REACH Adaptation rules 3.5: REACH Exposure assessment • Exposure ranking methods in Control Banding Tools
HARN	 3.2: REACH Information requirements [3.2.1.2: Physicochemical properties (size, shape, rigidity and biopersistence)]	2: EC Nano Definition • Measuring Particle Number Size Distribution 3.1: REACH Substance identification • Shape (e.g. by TEM) 3.2: REACH Information requirements • Rigidity • Biopersistence
Hazard classification	3.3: REACH Adaptation rules [3.3.1: Grouping of substances and read-across approaches] 3.4: REACH Hazard assessment [Step 2 Classification and Labelling]	3.3: REACH Adaptation rules • Grouping of substances and read-across approaches 3.4: REACH Hazard assessment • Hazard classification
Reactivity	3.2: REACH Information requirements [3.2.1.2: Physicochemical properties (reactivity)] 3.3: REACH Adaptation rules [3.3.1: (Q)SAR and Grouping of substances and read-across approaches]	3.2: REACH Information requirements • Acellular reactivity assay (e.g. ROS or FRAS assay) 3.3: REACH Adaptation rules • (Q)SAR (e.g. conductivity band gap Zhang et al.) • Grouping of substances and read-across approaches

Phase II

Phase II [flow chart in figure 4.1]	Link with sections 2 and 3 of the NANoREG Framework	Link with sections 2 and 3 of the NANoREG Toolbox
Dissolution in relevant media	3.2: REACH Information requirements [3.2.1.2: Physicochemical properties (water solubility)]	3.2: REACH Information requirements • Solubility or dissolution rate and transformation in relevant media
Absorption	3.2: REACH Information requirements [3.2.1.3 Toxicological properties (toxicokinetics, *in vitro* testing)] 3.3: REACH Adaptation rules [3.3.1 *In vitro* methods] 3.4: REACH Hazard assessment [*in vitro* assays]	3.2: REACH Information requirements 3.3: REACH Adaptation rules 3.4: REACH Hazard assessment • *In vitro* absorption and barrier models
Aggregation and agglomeration	3.2: REACH Information requirements [3.2.1.2: Physicochemical properties (aggregation and agglomeration)]	3.2: REACH Information requirements • Aggregation and agglomeration
Cellular uptake and interaction	3.3: REACH Adaptation rules [3.3.1 *In vitro* methods] 3.4: REACH Hazard assessment [*in vitro* assays]	3.3: REACH Adaptation rules 3.4: REACH Hazard assessment • Cellular uptake, attachment and interaction
Cytotoxicity, ROS and cytokines	3.3: REACH Adaptation rules [3.3.1 *In vitro* methods] 3.4: REACH Hazard assessment [*in vitro* assays]	3.3: REACH Adaptation rules 3.4: REACH Hazard assessment • *In vitro* cytotoxicity, ROS and cytokine induction assays
In vitro skin and eye irritation	3.2: REACH Information requirements [3.2.1.3 Toxicological properties (skin irritation, skin corrosion and eye irritation)]	3.2: REACH Information requirements • *In vitro* skin and eye irritation tests 3.3: REACH Adaptation rules • Grouping of substances and read-across approaches
Cell transformation	3.3: REACH Adaptation rules [3.3.1 *In vitro* methods] 3.4: REACH Hazard assessment [*in vitro* assays]	3.3: REACH Adaptation rules 3.4: REACH Hazard assessment • Cell transformation assays
Genotoxicity	3.2: REACH Information requirements [3.2.1.3 Toxicological properties (mutagenicity)] 3.3: REACH Adaptation rules [3.3.1 (Q)SAR, Grouping of substances and read-across approaches] 3.4: REACH Hazard assessment [Step 2 Classification and Labelling Mode of action]	3.2: REACH Information requirements 3.3: REACH Adaptation rules 3.4: REACH Hazard assessment • *In vitro* genotoxicity tests

ANNEX V – SUPPORTING INFORMATION ON NANOMATERIALS FOR SECTION 6 LIFE CYCLE ASSESSMENT

Introduction

Life Cycle Assessment (LCA) is a tool for the analysis of potential environmental impacts associated with products (goods and services) over their whole life cycle (i.e. product systems): supply, use and end-of-life stages are taken into account.

According to ISO (2006a), there are four phases in an LCA study as shown in figure V.1.

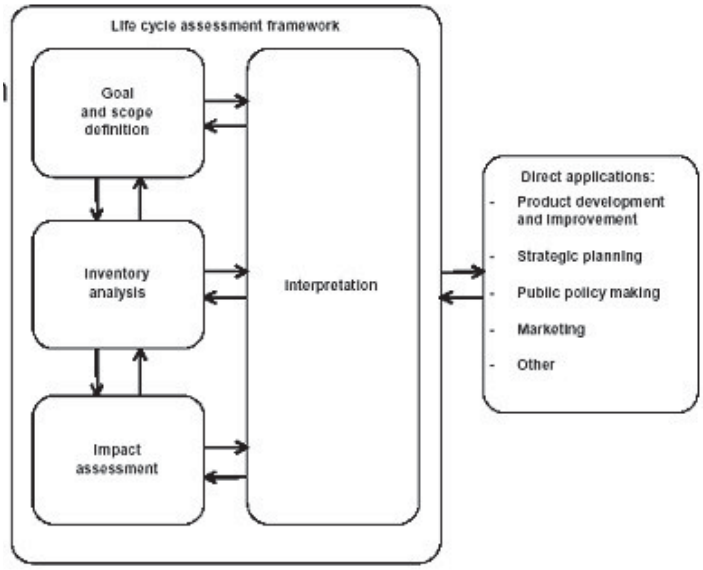

Figure V.1: Life Cycle Assessment (LCA) phases (copyright: ISO 2006a)

In "Goal and scope definition", the objective of the study is defined, a description of the product system is provided in terms of functionality and functional unit, system boundaries, allocation and target audience. The functional unit is a quantitative measure of the functions that the products (or services) provide. In accordance with the functional unit, the reference flow shall be defined, i.e. the flow to which all other input and output flows are quantitatively related.

Comparison between systems is made on the basis of the same function(s), quantified by the same functional unit(s) in the form of their reference flows.

The system boundary defines the processes of production system, included in the study. Therefore, the system boundary must be clearly defined for the product system to be evaluated and shall be consistent with the goal of the study. LCA suggests considering the entire life cycle of the investigated applications from the extraction of raw materials to waste treatment *("from cradle to grave"*). However, simplifications can be made in order to reduce the complexity of the product

system to a manageable size. Moreover, the allocation criteria used in establishing the system boundary shall be identified and explained (ISO 2006b).

The "Life Cycle Inventory analysis" phase (LCI) defines data collection and calculation procedures in order to quantify from cradle-to-grave the relevant inputs (e.g. material inputs) and outputs (e.g. emissions to air) of the product system. The result of the LCI is a compilation of the inputs (resources) and the outputs (emissions) from the product over its life cycle in relation to the functional unit and to the system boundary defined. A large number of data must be collected for each unit process included within the system boundary. Some data, “primary data”, are collected directly on site, while “secondary data” is derived from literature and databases. Several databases were developed and are still under development. These include public national or regional databases, industry databases, and consultants’ databases that are often offered in combination with LCA software tools (Finnveden 2009). Moreover, the collected data need to be referenced along with details about the relevant data collection process, the time when data have been collected, administrative information, the method used to measure, calculate or estimate (ISO 2006b).

The "Life Cycle Impact Assessment" phase (LCIA) aggregates the results from the LCI analysis to evaluate the significance of the product’s potential environmental impacts. Moreover, the inventory data are connected with specific environmental impact categories and the respective category indicators, such as global warming potential as an indicator for climate change.

The ISO 14044 standard defines the LCIA as the phase of LCA aimed at understanding and evaluating the magnitude and significance of the potential environmental impacts for a product system throughout the life cycle of the product (ISO 2006b).

In LCIA, the inputs and outputs of elementary flows that have been collected and reported in the inventory are translated into impact indicator results, related to human health, natural environment, and resource depletion (JRC 2010). According to the ISO standard on LCA (ISO 2006a, 2006b), LCIA consists of mandatory elements as selection of impact categories and classification. The phase includes: selection of impact category in accordance with goal and scope; definition of indicators for each impact category; assignment of the inventory data to the chosen impact categories (classification); and calculation of impact category indicators using Characterisation Factors (CFs). Several methods have been developed including different impact categories (JRC 2010).

The LCIA includes two optional phases: "Normalization" and "Grouping or Weighting". The first one relates the results of characterisation to reference values (e.g. a whole country or an average citizen). The latter requires the use of weighting factors, which indicate the different relevance that the impact categories may have (Finnveden 2009, JRC 2010).

LCIA methods can be grouped into two families: classical methods determining impact category indicators at an intermediate position (midpoint level) of the impact pathways (e.g. climate change, human toxicity, eco-toxicity and acidification) and damage-oriented methods aiming at more easily interpretable results in the form of damage indicators at the level of the ultimate societal concern (e.g. human health, ecosystem health and resource depletion). In figure V.2 an example of LCIA model is reported.

The "Interpretation" phase considers the findings from both LCI and LCIA and provides conclusions and recommendations.

The LCA is an iterative process, in each phase more information becomes available; this aspect permits to improve the system and promotes an iterative loops of goal and scope definition, inventory data collection and modelling (LCI), impact assessment (LCIA), and with completeness, sensitivity and consistency checks (evaluation). In this phase the aim is to implement robust conclusions and recommendations from the analysis, for example suggesting environmental improvements. Robustness can be achieved by developing: completeness checks of process coverage in the inventory analysis; sensitivity checks to assess if the results are affected by specific methodological choices; consistency checks of assumptions, methods, and data quality. The recommendations are referred to as "hot spot" or "weak point" analysis and identify environmental improvement potentials associated with specific management interventions.

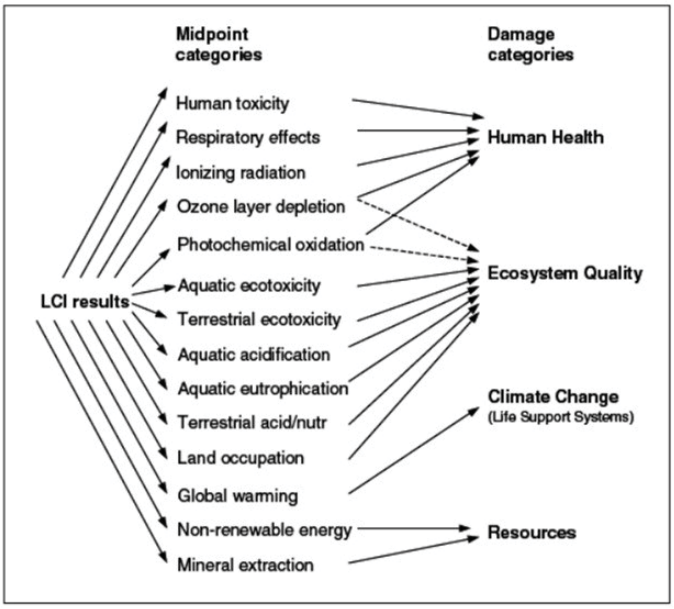

Figure V.2: Overall scheme of the IMPACT 2002+ framework, linking Life Cycle Inventory (LCI) results via the "Midpoint categories" to "Damage categories" (based on Jolliet et al. 2003)

International initiative of LCA framework harmonization

LCA is becoming more and more a policy support method, thanks to its characteristics, and, in particular, its standardisation has been one key aspect of its diffusion and acceptance. However, for a full exploitation of its potentiality in policy, additional efforts are needed, in particular in the field of harmonization to make the LCA results more reproducible and comparable.

In Europe, in its conclusion on the "Sustainable materials management and sustainable production and consumption: key contribution to a resource-efficient Europe – Draft Council conclusions" (December 2010), the European Council invited the Commission to *"develop a common methodology on the quantitative assessment of environmental impacts of products, throughout their life-cycle, in order to support the assessment and labelling of products"*[59]. This has led to the publication of the Communication from the European Commission on "Building the Single Market for Green Products"[60] and of the "Commission Recommendation on the use of common methods to measure and communicate the life cycle environmental performance of products and organisations"[61] (April 2013). A three-year test of the two methods to measure environmental performance throughout the lifecycle named Product Environmental Footprint (PEF) and Organisation Environmental Footprint (OEF) is now in progress. Both methodologies are ISO

[59] http://register.consilium.europa.eu/doc/srv?l=EN&f=ST%2017495%202010%20INIT
[60] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52013DC0196&from=EN
[61] http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013H0179&from=EN

14040 and ISO 14044 compliant (ISO 2006a, 2006b), with further specifications and recommendations. Moreover, the two methodologies adopt the concept of Product Category Rules (named PEFCR and OEFSR, respectively), in order to minimize the degree of freedom of the practitioner and simplify his work. The work in progress[62] is developing several guidelines for addressing "horizontal issues", which are aspects common to many or all the PEF/OEF studies. In addition, the European Commission is also developing a database for its use in the background processes of PEF and OEF studies, reducing the variability of the results due not to real differences in the life cycle of product with the same function but to differences in the background data.

Furthermore, the work of the UNEP – SETAC Life Cycle Initiative, started in the 2002 with the aim of fostering the Life Cycle Thinking globally, is now in its third phase, with the objectives to:

- Enhance the global consensus and relevance of existing and emerging life cycle methodologies and data management;
- Expand capability worldwide to apply and to improve life cycle approaches; making them operational for organisations;
- Communicate current life cycle knowledge and be the global voice of the Life Cycle community to influence and partner with stakeholders[63].

Besides a strong effort in "Capacity Building" and in "Communication & Stakeholder Outreach", the technical work is focussed on: improving environmental life cycle impact assessment indicators, promoting the LCA of organisations, and developing global principles and practices for hot spot analysis and data management.

On this last issue, the International Forum on LCA Cooperation, a global governmental initiative that aims to provide a space for discussion among governmental programs supporting LCA, launched the Global Network of Interoperable LCA Databases, with *"an aspirational objective that by 2017, an electronic system and protocol should be available - based as much as possible on existing structures - to enable access by users to the majority of the LCA databases and other relevant sustainability data, meaning that the LCA datasets and other data therein can be easily accessed in an exchange format that allows using them seamlessly in LCA software, with sufficient documentation of metadata that allows for defining "fitness for purpose" by any end user".*[64] Indeed, access to data of well described quality is key for the wide spread of LCA, but the use of

[62] https://webgate.ec.europa.eu/fpfis/wikis/display/EUENVFP/EU+Environmental+Footprint+Pilot+Phase
[63] http://www.lifecycleinitiative.org/activities/phase-iii/
[64] Report of the 4th Meeting of the International Forum on Life Cycle Assessment (LCA) Cooperation Including the Launch of the Global Network of Interoperable LCA Databases. Shangrila Hotel, Putrajaya and SIRIM, Shah Alam, Malaysia. 10-12 March 2015

different formats, nomenclature and metadata among the existing databases is a major barrier, making difficult the combined use of data from different databases.

LCA and nanomaterials

Several LCA studies on nanotechnologies and nanomaterials (NMs) have been published in the recent years, with different goals and focused on different products and applications. The main issues with regard to the application of LCA to NMs are discussed in section 6. Suggestions on how to address those issues including examples from recent scientific publications are also reported in section 6. In this annex, more attention is given to the nanospecific issues in the impact assessment phase, as human and ecological toxicity categories have not been completely defined for NMs. In fact, scientific consensus on these topics is currently under development and CFs of emissions of NMs into the environment (fresh and marine water, soil and air) for human and ecological toxicity categories are not available under conventional impact categories methods. Some efforts to incorporate toxicity effects of NMs in LCIA have been made and tentative approaches have been proposed. Some relevant ones are listed here below:

- Eckelman et al. (2012) proposed ecotoxicity CFs for carbon nanotubes emissions into freshwater,
- Rodriguez-Garcia et al. (2014) developed human toxicity CFs and freshwater ecotoxicity CFs for carbon nanotubes,
- Salieri et al. (2014) developed an ecotoxicity CF for nanotitania emitted into freshwater,
- Pini et al. (2014) proposed the use of two new impact categories (*"NanoTiO$_2$ ecotoxicity in freshwater"* and *"NanoTiO$_2$ carcinogens in fresh water")* in the frame of the IMPACT 2002+ framework,
- Walaser et al. (2014) developed a framework for indoor emissions of synthetic nanoparticles,
- Barberio et al. (2014) proposed a combined approach between LCA and RA where the LCA identifies the processes having the best environmental performance, and RA identifies the scenarios having the highest risk for workers.

Ecotoxicity CFs calculated with USEtox model

In USEtox^{TM} (Rosenbaum et al. 2008), the ecotoxicological CF of chemicals is calculated in agreement with equation (1):

$$CF = EF \times FF \times XF \quad (1)$$

Where: EF (m^3 kg^{-1}) is the Effect Factor and represents the ecotoxicity of the substance expressed in terms of Potentially Affected Fraction (PAF) of species; FF (day) is the Fate Factor expressing

the residence time of the substance in a particular environmental compartment (such as freshwater); and XF (dimensionless) is the eXposure Factor.

CFs are reported for freshwater aquatic ecotoxicological effects.

By applying the USEtox™ model, Eckelman et al. (2012) and Rodriguez-Garcia et al. (2014) calculated ecotoxicity CFs for carbon nanotubes and Salieri et al. (2014) for nanotitania.

In agreement with USEtox™, EF is defined by equation (2):

$$EF = \frac{PAF}{HC50} = \frac{0.5}{HC50} \quad (2)$$

EF reflects the relationship between the PAF of aquatic organisms and the hazardous concentration of a pollutant that causes effects on the 50% of aquatic organisms (HC50). The numerator is defined as the slope of the concentration-response relationship up to the point when the PAF reaches 50% (0.5) and the denominator is the geometric mean of species-specific EC50 data. EC50 is the effective concentration of a pollutant at which 50% of a single species population experiences a response, and values for EC50 found in the literature were used by the authors.

As regards the FF factors, starting from USEtox formulation, which provides a nested-multimedia mass balance model, the papers make some distinctions in their calculations.

Eckelman et al. (2012) modelled two scenarios of calculation of the FF by using physicochemical properties of the substance: a worst (unrealistic) case that maximizes the exposure of aquatic microorganisms to carbon nanotubes and a "realistic scenario" estimated by using literature data. Also Rodriguez-Garcia et al. (2014) used physicochemical properties retrieved from ECHA public database on registered substances and made some assumptions in case of lack of data.

Salieri et al. (2014) used colloidal science to develop the fate model, because, in agreement with some other authors, they assumed that substance-specific input parameters required for the fate calculation in USEtox™ are suitable for organics but not applicable to NMs, due to their different chemical and physical properties.

In USEtox™, the environmental exposure factor for freshwater ecotoxicity is the fraction of a chemical dissolved in freshwater and can be estimated via equation (3):

$$XF = \frac{1}{\left(1 + \left(K_p \times SS + K_{DOC} \times DOC + BAF \times BIO_{mass}\right) \times 10^{-6}\right)} \quad (3)$$

Where K_p is the partition coefficient between water and suspended solids (l/kg), SS is the suspended matter concentration in freshwater (= 15 mg/l in USEtox™), K_{DOC} is the partitioning coefficient between dissolved organic carbon and water, DOC is the dissolved organic carbon concentration in freshwater (= 5 mg/l in USEtox™), BAF is the bioconcentration factor in fish (l/kg) and BIO_{mass} is the concentration of biota in water (= 1 mg/l in USEtox™).

Eckelman et al. (2012) and Rodriguez-Garcia et al. (2014) applied equation (3) while Salieri et al. (2014) decided to not use it but to consider the different nature of the chemical investigated, which

is insoluble. As bioavailability and bioaccumulation factors, which could replace the XF factor, were not available in literature Salieri et al. (2014) assumed a precautionary approach by setting XF equal to 1 without weighing the final results of the model on the basis of the exposure factor.

Table V.1 shows the CF for carbon nanotubes (CNTs), single walled carbon nanotubes (SWCNTs), multi walled carbon nanotubes (MWCNTs) and nanotitania proposed by Eckelman et al. (2012), Rodriguez-Garcia et al. (2014) and Salieri et al. (2014).

Table V.1: Characterisation factors (CFs) for carbon nanotubes (CNTs), single walled carbon nanotubes (SWCNTs), multi walled carbon nanotubes (MWCNTs) and nanotitania ($nanoTiO_2$) proposed by Eckelman et al. (2012), Rodriguez-Garcia et al. (2014) and Salieri et al. (2014). EF = Effect Factor. FF = Fate Factor. XF = Exposure Factor. PAF = Potentially Affected Fraction.

Substance	EF [(PAF x m^3)/kg]	FF [day]	XF	CF [(PAF x m^3 x day)/$kg_{emitted}$)]
CNTs "worst case"[65]	200	143	1	29000
CNTs "realistic case"	200	18.5	1	3700
SWCNTs[66]	650	92	6.5E-06	0.125
MWCNTs	8	29	1	740
Nano TiO_2 (species level)[67]	31.1	10^{-2}	1	0.31
Nano TiO_2 (trophic level)	28.1	10^{-2}	1	0.28

Eckelman et al. (2012) highlighted that significant uncertainty exists in the estimates of CNTs release-based ecotoxicity, so Montecarlo analysis was performed within the USEtox model. Also, Rodriguez-Garcia et al. (2014) agreed that the calculated CFs should be considered only as interim due to the assumptions that were needed for parameters calculations. Salieri et al. (2014) highlighted high variability of toxic data on nano-TiO_2 and difficulties of calculating FF, because it is site-specific and time related, while in LCA emissions are global, considering both time and place. However, the authors do not present uncertainty analysis.

Moreover, some other critical issues stood out from these papers, in particular concerning the following aspects:

- The fate model, which should consider the semi colloidal behaviour of materials like CNTs affected by both molecular and physical forces (kinetics of aggregation, filtration, and deposition);
- The huge gap existing between the current body of research and the number of toxicity studies necessary to make a robust, specific assessment of ecotoxicological risks;

[65] Eckelman et al. 2012
[66] Rodriguez-Garcia et al. 2014
[67] Salieri et al. 2014

- The need for continuous updating of the CFs calculated to follow progress in the ecotoxicity research;
- The importance of a robust statistical analysis to improve the reliability of the toxicity impacts results.

Human toxicity characterization factors calculated with USEtox^{TM} model

In USEtox^{TM} (Rosenbaum et al. 2008), the human toxicity CF of chemicals is calculated in agreement with equation (1). The FF and EF are combined to reflect the intake Fraction (iF), which is the fraction of the emitted mass that enters the human population, mainly considering intake through inhalation and ingestion. The EF reflects the change in disease probability due to change in life time intake of a chemical (cases/kg_{intake}). Rodriguez-Garcia et al. (2014) proposed the calculation of human toxicity (non-cancer) CF for SWCNTs and MWCNTs emitted in different environmental compartments. The potential carcinogenicity of CNTs was unknown at the date of publication, so no carcinogenic effects were assessed.

The need for further investigations was highlighted by the authors, in particular data to assess chronic effects of the ingestion and empirical data for the estimation of iF.

Toxicity of TiO_2 nanoparticle released into water

Pini et al. (2014) proposed two new impact categories in the IMPACT 2002+ method:

- NanoTiO_2 ecotoxicity in freshwater, where *"Particulates, <100 nm, in freshwater"* has been introduced as a representative substance of the damage on freshwater ecosystem, with a CF calculated by Salieri et al. (2014),
- NanoTiO_2 carcinogens in freshwater, where *"NanoTiO_2 human toxicity, in freshwater"* has been introduced as a representative substance of a local damage on human health (considering an area of Emilia Romagna region in the north of Italy), with a calculated damage assessment factor determined by the Eco-indicator 99 (2001) calculation method for carcinogenic substances.

The aim was to quantify the contribution to the total damage of TiO_2 nanoparticles released into water with an approach of local damage on human health. This approach allows a preliminary screening of the relevance of this NM emission in freshwater, which is particularly interesting for the application of ecodesign principles to chemical processes. However, the published documentation is not detailed enough to evaluate reliability, consistency and uncertainty of the assessment.

Life Cycle Interpretation

Life cycle interpretation of NMs and nano-enabled products does not seem to be different from that of standard products. Uncertainty and sensitivity analyses are indispensable in the case of products with incomplete and uncertain production characteristics and impacts. Indeed, LCA studies on NMs often are based on data at laboratory and pilot line scale or even completely lack data for the use and end-of-life stages as well as lack information on emissions of NMs in the environment and on the toxicity impact categories that characterise these emissions.

To calculate the uncertainty on the accumulated LCI data, Walser et al. (2011) and Pizza et al. (2014) include information on stochastic variable as variance, mean, and probability distribution of background data, Walser et al. (2011) on primary data too, and perform a Monte Carlo analysis. Furthermore, to improve the robustness of data other authors perform a sensitivity analysis about methodological choices (e.g. system boundaries setting, cut-off criteria, selection of impact assessment methods, assumptions). Indeed, nanotechnology applications are quite new and many assumptions are often done in the LCA study. Kanna et al. (2008) developed a LCA on environmental impact of carbon nanofibers synthesis and applied a sensitivity analysis to study the effect on the life cycle energy consumption of varying 1) cycle times of production and 2) feedstock and carrier gas recycle rates. Walser et al. (2011), in comparative analysis among nanosilver T-shirts and conventional T-shirts with and without biocidal treatment, analyse several scenarios changing single parameter values, such as biocidal concentrations, different precursor production technologies, and altering assumptions of consumer behaviour, within realistic value ranges. Li et al. (2014) in LCA of a high-capacity LIB pack using SiNW consider the effects of multiple factors, including the cathode material, the service life of battery pack, the electricity mix for battery charging, and the operating geographic region of the EVs. Pizza et al. (2014), in LCA on epoxy-based composites filled with graphite nanoplatelets (GnP), verify the robustness of results considering scenarios with different electricity mix in the GnP production process and composite production. Barberio et al. (2014), in LCA of production of alumina nanofluid with two processes, check the assumption made in the precursor material used to produce the nanofluid comparing several scenarios with different precursor materials (OECD 2015).

Conclusions

LCA is an effective tool to evaluate the environmental sustainability of NMs, nano-enabled products and nanotechnologies throughout the life cycle and to highlight their environmental hot spots, which can help to identify more environmentally friendly design solutions. LCA quantifies the potential environmental impacts of the whole life cycle of the product and provides a comprehensive overview of the potential environmental impacts of nano-enabled products in several impacts categories, including all other substances used over the life cycle of that products.

However, further studies should be developed to define harmonized approaches and robust and reliable data and scientifically approved CFs for the human and ecotoxicity impact categories.

References

Barberio G, Scalbi S, Buttol P, Masoni P, Righi S. 2014. Combining life cycle assessment and qualitative risk assessment: The case study of alumina nanofluid production. Science of the Total Environment, 496: 122-131

Bauer C, Buchgeister J, Hischier R, Poganietz WR, Schebek L, Warsen J. 2008. Towards a framework for life cycle thinking in the assessment of nanotechnology. Journal of Cleaner Production, 16: 910-926

Dahlben LJ, Isaacs JA. 2009. Environmental assessment of manufacturing with carbon nanotubes. Ceramic Transactions, 207: 243-253

Eckelman MJ, Mauter MS, Isaacs JA, Elimelech M. 2012. New perspectives on nanomaterial aquatic ecotoxicity: production impacts exceed direct exposure impacts for carbon nanotoubes. Environ Sci Technol, 46(5): 2902-10. doi: 10.1021/es203409a

European Commission 2015. Data requirements in Product Environmental Footprint Category Rules (PEFCRs). Version 1.0, May 2015

Finnveden G, Hauschild MZ, Ekvall T, Guinée J, Heijungs R, Hellweg S, Koehler A, Pennington D, Suh S. 2009. Recent developments in Life Cycle Assessment. J. of Environmental Management, 91: 1-21

Hischier R, Nowack B, Gottschalk F, Hincapie I, Steinfeldt M, Som C. 2015. Life cycle assessment of facade coating systems containing manufactured nanomaterials. J Nanopart Res, 17: 68. Doi: 10.1007/s11051-015-2881-0

Hischier R. 2014. Framework for LCI modelling of releases of manufactured nanomaterials along their life cycle. Int J Life Cycle Assess, 19: 838-849. doi: 10.1007/s11367-013-0688-8

Hischier R, Walser T. 2012. Life cycle assessment of engineered nanomaterials: state of the art and strategies to overcome existing gaps. Sci Total Environ, 425:271-82. doi: 10.1016/j.scitotenv.2012.03.001.

JRC 2010. ILCD handbook. International Reference Life Cycle Data System. General guide for Life Cycle Assessment - Detailed guidance. First edition. European Commission, Joint Research Centre, Ispra (Italy)

ISO 2006a. ISO (UNI EN) 14040. Environmental management – Life cycle assessment – Principles and framework. International Organisation for Standardisation, Geneve, Switzerland

ISO 2006b. ISO (UNI EN) 14044. Environmental Management – Life Cycle Assessment (LCA) - Requirements and Guidelines. International Organisation for Standardisation, Geneve, Switzerland

Jolliet O, Margni M, Charles R, Humbert S, Payet J, Rebitzer G, Rosen R. 2003. IMPACT 2002+A New Life Cycle Impact Assessment Methodology. Int J LCA, 6: 324-330

Joshi S. 2008. Can nanotechnology improve the sustainability of biobased products? — The case of layered silicate biopolymer nanocomposites. J Ind Ecol, 12: 474-89

Vikas K, Bakshi BR, Lee LJ. 2008. Carbon Nanofiber Production Life Cycle Energy Consumption and Environmental Impact. Journal of Industrial Ecology, 12(3): 394-410

Klöpffer W, Curran MA, Frankl P, Heijungs R, Köhler A, Olsen SI. 2007. Nanotechnology and Life Cycle Assessment Synthesis of Results Obtained at a Workshop Washington, DC, 2–3 October 2006. Nanotechnology and Life Cycle Assessment, Washington DC, 2-3 October 2006 Organized

by Barbara Karn (US Environmental Protection Agency/Woodrow Wilson International Center for Scholars) and Pilar Aguar (European Commission)

Li B, Gao X, Li J, Yuan C. 2014. Life cycle environmental impact of high-capacity lithium ion battery with silicon nanowires anode for electric vehicles. Environmental Science and Technology, 48 (5): 3047-3055

Pini M, Neri P, Ferrari AM. 2013. Determination of the potential damage and benefits of TiO_2 nanoparticles by Life Cycle Assessment methodology. Internal Technology Report RT-07, University of Modena and Reggio Emilia

Meyer DE, Curran MA, Gonzalez MA. 2011. An examination of silver nanoparticles in socks using screening-level life cycle assessment. J Nanopart Res, 13: 147-156. doi: 10.1007/s11051-010-0013-4

OECD 2015. Guidance manual towards the integration of risk assessment into life cycle assessment of nano-enabled applications. OECD Environment, Health and Safety Publications, Series on the Safety of Manufactured Nanomaterials N° 57. Organisation for Economic Co-operation and Development, Paris

Pini M, Rosa R, Neri P, Bondioli F, Ferrari AM. 2014. Environmental assessment of a bottom-up hydrolytic synthesis of TiO_2 nanoparticles. Green Chemistry. doi: 10.1039/c4gc00919c

Pizza A, Metz R, Hassanzadeh M, Bantignies JL. 2014. Life cycle assessment of nanocomposites made of thermally conductive graphite nanoplatelets. International Journal Life Cycle Assessment, 19: 1226-1237

Roes AL, Marsili E, Nieuwlaar E, Patel MK. 2007. Environmental and Cost Assessment of a Polypropylene Nanocomposite. J Polym Environ, 15: 212-226. doi: 10.1007/s10924-007-0064-5

Rodriguez-Garcia G, Zimmermann B, Weil M. 2015. Nanotoxicity and Life Cycle Assessment: First attempt towards the determination of characterization factors for carbon nanotubes. IOP Conf. Series: Materials Science and Engineering, 64. doi:10.1088/1757-899X/64/1/012029

Rosenbaum RK ,Till M, Bachmann L, Swirsky G, Huijbregts MAJ, Jolliet O, Juraske R, Koehler A, Larsen HF, MacLeod M, Margni M, McKone TE, Payet J, Schuhmacher M, van de Meent D, Hauschild MZ. 2008. USEtox—the UNEP-SETAC toxicity model: recommended characterisation factors for human toxicity and freshwater ecotoxicity in life cycle impact assessment. The International Journal of Life Cycle Assessment, 13: 532. doi:10.1007/s11367-008-0038-4

Salieri B, Righi S, Pasteris A, Olsen SI. 2015. Freshwater ecotoxicity characterisation factor for metal oxide nanoparticles: a case study on titanium dioxide nanoparticle. Sci Total Environ, 1, 505: 494-502. doi: 10.1016/j.scitotenv.2014.09.107

Scalbi S, Fantin V, Antolini F. 2014. Valutazione dell'ecososteni bilità di nuove tecnologie: produzione di QDsLED con stampaggio tramite “direct laser micropatterning”. Proceeding of VIII workshop of LCA Italian Network 19-20 giugno 2014. ISBN 978-88-8286-306-7, pp. 313-322

Sengül H, Theis TL. 2011. An environmental impact assessment of quantum dot photovoltaics (QDPV) from raw material acquisition through use. Journal of Cleaner Production, Vol. 19: 21-31

Subramanian KS, Manikadan A, Thirunavukkara M, Rahale CS. 2015. Nanofertilizers for balance crop nutrition. Nanotecnologies in food and agriculture, pp. 69-80. doi: 10.1007/978-3-319-14024-7_3

Walser T, Demou E, Lang DJ, Hellweg S. 2011. Prospective Environmental Life Cycle Assessment of Nanosilver T-Shirts. Environ Sci Technol, 45(10): 4570-4578

Walser T, Meyer D, Fransman W, Buist H, Kuijpers E, Brouwer D. 2015. Life-cycle assessment framework for indoor emissions of synthetic nanoparticles. I. Nanopart Res, 17: 245. doi: 10.1007/s11051-015-3053-y