Nano Today 35 (2020) 100941

Contents lists available at ScienceDirect

Nano Today

journal homepage: www.elsevier.com/locate/nanotoday

# A framework for grouping and read-across of nanomaterials-supporting innovation and risk assessment

Vicki Stone[a,*], Stefania Gottardo[b], Eric A.J. Bleeker[c], Hedwig Braakhuis[c], Susan Dekkers[c], Teresa Fernandes[a], Andrea Haase[d], Neil Hunt[e], Danail Hristozov[f], Paula Jantunen[b], Nina Jeliazkova[g], Helinor Johnston[a], Lara Lamon[f], Fiona Murphy[a], Kirsten Rasmussen[b], Hubert Rauscher[b], Araceli Sánchez Jiménez[h], Claus Svendsen[i], David Spurgeon[i], Socorro Vázquez-Campos[j], Wendel Wohlleben[k], Agnes G. Oomen[c]

[a] NanoSafety Research Group, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS, UK
[b] European Commission, Joint Research Centre (JRC), Ispra, Italy
[c] National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands
[d] German Federal Institute for Risk Assessment (BfR), Department of Chemical and Product Safety, Berlin, Germany
[e] Yordas Group, Lancaster Environment Centre, Lancaster University, Lancaster, UK
[f] Green Decisions, Venice, Italy
[g] Ideaconsult Ltd, Sofia, Bulgaria
[h] Insititute of Occupational Medicine (IOM), Research Avenue North, Edinburgh EH14 4AP, UK
[i] UK Centre for Ecology & Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, OX10 8BB, UK
[j] LEITAT Technological Center, Barcelona, Spain
[k] BASF SE, Dept. Material Physics and Dept. of Experimental Toxicology & Ecology, Ludwigshafen, Germany

ARTICLE INFO

*Article history:*
Received 20 March 2020
Received in revised form 12 June 2020
Accepted 16 July 2020
Available online 8 August 2020

*Keywords:*
Nanoform
Grouping
Read-across
Risk assessment
Regulation
Safe(r) by design
Integrated approach to testing and assessment

ABSTRACT

According to some legislation grouping can streamline data gap filling for the hazard assessment of substances. The GRACIOUS Framework aims to facilitate the application of grouping of nanomaterials or nanoforms (NFs), in a regulatory context and to support innovation. This includes using grouping to enable read-across from (a) source(s), for which data and information exist, to a similar target NF where information is lacking.

The Framework provides an initial set of hypotheses for the grouping of NFs which take into account the identity and use(s) of the NFs, as well as the purpose of grouping. Initial collection of basic information allows selection of an appropriate pre-defined grouping hypothesis and a tailored Integrated Approach to Testing and Assessment (IATA), designed to generate new evidence to support acceptance or rejection of the hypothesis. Users needing to develop their own user-defined hypothesis (and IATA) are also supported by the Framework. In addition, the IATA guides acquisition of the information needed to support read-across.

This approach gathers information to render risk assessment more efficient, affordable, as well as reducing the use of test animals.



## Introduction and aims

Manufacture and functionalisation of materials at the nanoscale leads to a range of nanomaterials or nanoforms (NFs) (see Box 1) that vary not only in chemical composition, but also in other physicochemical properties (e.g. size, morphology and surface

*Abbreviations:* ECHA, European Chemicals Agency; HARN, high aspect ratio nanomaterials; IATA, integrated approach to testing and assessment; ISO, International Organization for Standardization; JRC, Joint Research Centre; NF, nanoform; NEP, nano-enabled product; OECD, Organisation for Economic Co-operation and Development; PC, physicochemical; Prc, precautionary measures to reduce risk; REACH, Registration, Evaluation, Authorisation and Restriction of Chemicals; Reg, regulatory compliance; SbD, safe(r) by design; SNEP, solid matrix nano-enabled products; TSCA, Toxic Substances Control Act.

* Corresponding author.
*E-mail address:* v.stone@hw.ac.uk (V. Stone).

https://doi.org/10.1016/j.nantod.2020.100941

**Box 1: Nanomaterials and nanoforms (NFs)**
A 'nanomaterial' is defined only by the size of its constituent particles according to the European Commission's (EC) Recommendation 2011/696/EU [1].
The European REACH Regulation [2] has introduced the concept of 'nanoform' [3]. Annex VI to REACH states that "a nanoform is a form of a natural or manufactured substance containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm – 100 nm, including also by derogation fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm".
A substance may have one or more NFs, based e.g. on differences in their number based particle size distribution, shape, aspect ratio, crystallinity, assembly structure, specific surface area and surface functionalisation or treatment (REACH Annex VI, points 2.4.2. – 2.4.5).
Where technically and scientifically justified, grouping and read-across can be applied within a registration dossier to two or more NFs for the purposes of one or more information requirements. However, for grouping different NFs of the same substance, consideration of the molecular structural similarities alone are not sufficient to serve as a justification (REACH Annex XI) [3].
The term NF is preferred in this paper for its regulatory relevance in the EU.

characteristics). Apart from the expected benefits, a modified NF (e.g. coated) may also affect the environment and human health to a greater or lesser extent than the unmodified NF (e.g. uncoated). Risk assessment requires detailed physicochemical characterization, exposure and hazard data for each unique NF, but testing every NF would demand substantial resources. More efficient and effective ways of characterising physicochemical properties, fate, behaviour and (eco)toxicity of NFs are therefore needed, and this can be achieved by applying grouping and read-across approaches.

For chemicals in general, grouping and read-across are well-established regulatory concepts to minimise the need for specific testing (e.g. REACH [4], TSCA [5]), and for which guidance is available [6,7]. These approaches are built on well-established hypotheses that describe how specific hazards can be predicted based on a (limited) set of known physicochemical properties and toxicity testing. This includes gathering the information needed for a certain hazard endpoint by using data from the most hazardous substance(s) in the group, to cover the rest of the group. A clear relationship between chemical properties and hazard endpoints needs to be established in order to allow prediction of missing data for some members of the group by interpolation to other members.

In order to streamline the process of implementing grouping and read-across for NFs (e.g. within one REACH substance identity), and to provide more support for stakeholders such as industry, policy makers, regulators and academics, the European Union Horizon 2020 project GRACIOUS ('Grouping, read-across and classification framework for regulatory risk assessment of manufactured nanomaterials and safe(r) design (SbD) of nano-enabled products (NEPs)'; https://www.h2020gracious.eu/) has developed a dedicated framework addressing different purposes for performing risk assessment, such as filling in regulatory data gaps, or incorporating safety into the design of new NFs or NEPs, and is relevant to a variety of end users.

The following manuscript illustrates how the GRACIOUS project has taken existing information, methods and regulatory provisions (described in the Methods section), and used them to identify what is required to support grouping and read-across for NFs. The GRACIOUS Framework aims to:

- Support practical and evidence-based grouping of NFs for facilitating data gathering for hazard and risk assessment, risk management and related decision making, thereby meeting the needs of various global stakeholders, particularly regulators and industry.
- Develop a number of robust scientific arguments (so called predefined hypotheses) that justify grouping and read-across of NFs and facilitate the development of new (so called user-defined) hypotheses.
- Consider not only intrinsic physicochemical properties and (eco)toxicological effects, but also extrinsic (system-dependent) descriptors of exposure, toxicokinetics and environmental fate.
- Provide guidance on how the outputs can subsequently be used, and aligned to the initial purpose of grouping.
- Support decision making spanning regulatory risk assessment and safe innovation/SbD of NEPs.
- Reduce, refine and replace (3Rs) animal testing for human health and environmental hazard assessment where possible, by supporting the use of grouping, read-across, modelling and *in vitro* tests.

In summary, the GRACIOUS Framework aims to provide practical guidance on how to conduct both grouping and read-across of NFs, considering the different exposure scenarios resulting from the various applications and life cycle stages of NFs in a wide array of NEPs.

## Methods

The following sections summarise the main research, information and regulatory considerations which influenced design of the GRACIOUS Framework.

### *Grouping approaches that informed the framework design*

In recent years, several scientific approaches for grouping and read-across of NFs have been developed [8,9]; these approaches have increased in complexity with time, to include an expanding array of parameters. Initially, such approaches were similar to non-NF approaches in that they focused largely on physicochemical characteristics, and their association with hazards [10–12]. Subsequent projects went beyond hazard and physicochemical properties of the pristine material, taking into account changes occurring during the life cycle of NFs [13,14]. This includes NF transformations in the environment or human body (e.g. changes in hydrophobicity, agglomeration, zeta potential). Such changes can subsequently impact upon fate, behaviour, uptake and hazard. Building on these early experiences, it was suggested that grouping should be supported by information on kinetics (e.g. uptake, distribution, biopersistence) and early and apical biological effects [15].

Development of a hypothesis to describe the behaviour of a NF [16] has also been proposed. In this context, the behaviour is usually related to a hazard endpoint (e.g. genotoxicity), and is informed by existing scientific information in a tiered approach, where data are collected at different levels of complexity. Thus data-gap filling in a regulatory setting is supported endpoint-by-endpoint, according to similarities identified from the collected information (mainly based on chemical identity, physicochemical properties and behaviour in environmental or biological media) [16].

The first real application of a grouping framework for nanomaterials [17] focused on inhalation as the route of exposure. This study defined a tiered approach to group nanomaterials (DF4NanoGrouping project), in which Tier 1 included collection of data on the intrinsic physicochemical properties and identification

of soluble nanomaterials. This latter information allowed the user to group soluble nanomaterials along with their molecular counterparts and apply read-across, as well as indicating the testing requirements of Tier 2. Tier 2 distinguished three other grouping options (passive, biopersistent fibres and active materials) by comparing system-dependent properties (e.g. agglomeration, reactivity, dissolution) against defined cut-off values derived from benchmark materials. It was proposed that each of the groups may inform read-across and waiving of testing, as well as providing the option for division into sub-groups. Toxicological information (e.g. from short term *in vivo* inhalation studies) was used in Tier 3 to corroborate the assignment of the nanomaterial to a group or subgroup. Applicability of the framework was subsequently addressed using 24 materials of different composition (carbonaceous, metal oxides and sulphates, amorphous silica, organic pigments). The materials were assigned to one of the four pre-defined groups by applying the framework and using available information; the suitability of the grouping was then assessed on the basis of No Observed Adverse Effect Concentrations from long-term studies [18].

Building on the DF4NanoGrouping, the nanoGRAVUR framework added more data requirements to enable grouping by ecological risk and by consumer risk, including aspects of lifecycle releases from composite nano-enabled products. Case studies have explored which system-dependent properties are most sensitive [14,18].

The complexity of current grouping approaches reflects our increased understanding of the sequence of events in the life cycle of NFs and the biological pathways, which can influence their potential hazard [17].

*Regulatory considerations on grouping*

It is important that any grouping framework meant to be used in a regulatory context aligns to legal requirements. In 2014 the Organisation for Economic Co-operation and Development (OECD) concluded that the application of grouping to nanomaterials was at that time premature and r̈esearch first needs to pave the way for it[6]. Subsequent expert meetings have aimed to share information and discuss categorisation, grouping and read-across of nanomaterials [19]. In the EU regulatory framework, possibilities for applying grouping and read-across approaches for the assessment of nanomaterials exist but differ in each piece of legislation [20].

In the amended REACH Annexes [3], it is specified that NFs (Box 1) should in principle be addressed separately, and that molecular structural similarities alone cannot serve as a justification for grouping. In anticipation of these changes, ECHA developed nano-specific guidance for grouping, which considers properties beyond chemical composition to support the hypothesis (e.g. aspect ratio, particle size, shape, or solubility), and highlights the importance of toxicokinetic studies in grouping, read-across, and for *in vitro* to *in vivo* extrapolation [21,22].

It is worth noting that a group of NFs identified using the GRACIOUS Framework is not the same as a 'set of similar NFs' as defined in REACH Annex VI [3,4]. REACH Annex VI specifies that "*A 'set of similar nanoforms' is a group of nanoforms characterised in accordance with section 2.4 [of this annex] where the clearly defined boundaries in the parameters . . . of the individual nanoforms within the set still allow to conclude that the hazard assessment, exposure assessment and risk assessment of these nanoforms can be performed jointly*".

*Read-across approaches that informed the Framework design*

Read-across requires development of a robust scientific explanation of similarity to support the use of data on one or more source materials to fill in a data gap of the target material [4]. A methodology for systematically quantifying similarity between NFs for human hazard assessment, based on physicochemical properties of NFs (chemical composition, crystalline form, impurities, primary particle size distribution, aggregate/ agglomerate size distribution, density and shape) has been reported [23]. This methodology is a first attempt to use current knowledge on NF property-hazard relationships to develop a series of pragmatic and systematic rules for establishing similarity scores. Similarity scores are used to select the studies that are considered adequate for read-across purposes and to fill in a data gap in a given hazard endpoint for the NF studied. Assessment of study quality and relevance are equally important when selecting that particular study for assessment of NF similarity.

*Constructing the overall GRACIOUS framework*

Based on the information above, the GRACIOUS Framework was designed to integrate the industrial (DF4NanoGrouping) [17] and regulatory [22] grouping concepts. A hypothesis-driven approach to grouping is essential in order to align with European legislation (e.g. REACH), but also to provide a scientific basis for any grouping decision [16]. The first phase of development of the GRACIOUS Framework was therefore to identify how grouping hypotheses could be formulated in a more standardised manner. The Framework description included definitions of key terms and generation of a diagram explaining how the Framework would be used.

In order to ensure that the Framework would be fit for purpose, the opinions of different stakeholders were sought through multiple approaches such as:

- One-to-one interviews with representatives from regulation/policy making, standardisation, industry and academia (e.g. ECHA, European Food Safety Authority, Health Canada, International Organization for Standardization (ISO), the National Institute for Occupational Safety and Health, US Food and Drug Administration, Harvard School of Public Health, Department of Environmental Health, Arizona State University, Nanotechnologies Industries Association)
- An open online consultation (July – September 2018) receiving over 30 responses representing Europe, Asia and North America, as well as academia, policy makers, regulators and industry.
- Inviting feedback through a stakeholder engagement platform (https://www.h2020gracious.eu/about/stakeholders).
- A stakeholder event co-organised by GRACIOUS and NanoReg2 consortia, held at the OECD in 2018. The workshop brought together an international audience of 120 participants featuring representatives from industry, policy-making, regulation, standardisation and academia.

This feedback has been analysed and edits incorporated in order to generate the GRACIOUS Grouping and Read-Across Framework described below. The Framework has been constructed to address regulatory applications of grouping and read-across. However, the purposes for which the Framework can be used will be flexible.

*Construction of hypotheses for grouping*

It was immediately clear that there was no standardised way to generate a hypothesis for grouping. A template was therefore designed to guide the user to structure the available information in a consistent manner, allowing its integration in order to generate a hypothesis suitable for grouping [24]. The template includes sections, which detail:

- The purpose and the context for using the grouping hypothesis,

- The 'life cycle' of the NF or NEP (in particular, potential exposure scenario(s), level of exposure (dose), the released form of the NF),
- Descriptions of 'What they are' (physicochemical characteristics), 'Where they go' (environmental fate and behaviour, uptake and toxicokinetics) and 'What they do' ((eco)toxicological effects),
- The 'potential implications' of accepting the hypothesis.

During development of the Framework, an in-depth systematic survey of the literature, summarised in extensive tables, along with expert judgement has allowed identification of over 35 pre-defined grouping hypotheses that are considered to be evidence-based and relatively robust (a few examples are provided in Table 1).

The pre-defined hypotheses will be further analysed and published in subsequent papers, along with extensive literature reviews that summarise their suitability for grouping. These publications will include definition of key terms and suggestions of either thresholds or similarity assessment criteria for inclusion/exclusion within a group. If grouping by thresholds, then theses are oriented on biological relevance. The thresholds assign NFs to predefined groups and thus ensure homogeneity by the "what they are", "where they go", "what they do" aspects. E.g. if the aspect ratio, inflammation potential and dissolution rate in specific media are below predefined thresholds for all NFs of the same substance, they can be grouped regarding their low acute inhalation hazard, but potential bioaccumulation can be selected. Alternatively, similarity is assessed on exactly the same descriptors from the decision nodes of the IATAs: We then compare the aspect ratio, inflammation potential and dissolution halftime of all NFs, and exclude outliers, until the remaining group members fulfil homogeneity criteria. Note that the final IATA will require more than the three descriptors used here to represent the "what they are", "where they go", "what they do" aspects. The scientific basis of each pre-defined hypothesis will therefore be accessible and transparent.

*Construction of the framework IATAs*

The IATAs are designed in line with OECD recommendations [25], providing a structured strategy for collecting the required evidence through the identification and description of the most appropriate data sources, models and test methods currently available for each endpoint. The strategy, and therefore any testing to be done, can be tiered to reflect the level of information required for different purposes and to promote implementation of the 3Rs principles. The tiers of the IATA span from basic information to the use of *in silico tools*, *in vitro* models, extrinsic physicochemical descriptors, and finally more sophisticated models such as mesocosm studies (indoor or outdoor experimental systems that study the wider environment under controlled conditions), 3D *in vitro* models (e.g. organoids, 3D models including multiple cell types, lab on a chip technologies), and animal models. Not all tiers of testing need to be completed to make a grouping decision. The details of the IATAs depend upon the route of exposure or environmental compartment and will be discussed in detail in several future publications. Combination of existing information with the IATA outputs will allow population of a data matrix that compiles the evidence base on which to base a grouping or read-across decision.

*Identification of methods to include within the IATAs*

Where possible, the methodologies recommended within the IATAs are well established, based upon peer reviewed literature and if possible standardised (e.g. OECD Test Guidelines). For example, the basic physicochemical information requirements for NFs are specified in the revised REACH Annexes [3], whereas properties that should be considered to assess similarity between NFs are recommended in regulatory guidance [22], reviews [26,27], frameworks [28], grouping approaches [29] and earlier projects (e.g. NANoREG and nanoGRAVUR [14]). Information on tools, methods and protocols to be used in the GRACIOUS IATAs to determine these physicochemical properties is provided in Appendix A.

For assessment of the most relevant form of release, the IATAs will include methods such as those exemplified in ISO/TC 229 (Nanotechnologies) ISO/TR 22293 (in preparation). The similarity of the released forms is estimated by relevant methods in a stepwise decision-making process [30] which takes into account the intended use and its characteristic stresses, the matrix of the NEP, and the specific NF.

Reference materials and benchmark materials should be used as controls, to assess whether specific tests have been performed adequately. Benchmark materials are also essential to calibrate the amplitude of the response for the target NF for a specific endpoint. For example, benchmark materials, when used as negative and positive controls, can provide an understanding of the dynamic range (maximum and minimum) of response sizes expected for a given endpoint (Appendix A). This information can then allow analysis of the relative similarity or dissimilarity between two or more forms of a substance (including the target NF). Any comparison between different NFs or between source and target will require data obtained by the same method. This is equally required by US-EPA when comparing different "discrete forms" in their terminology in the reporting of nanomaterials [31].

Analogous to properties that are well-established for molecular chemicals (e.g. the log $K_{ow}$), many of the extrinsic properties for NFs (e.g. attachment affinity or dissolution rate) are assessed on a logarithmic scale [32]. This scale may span several orders of magnitude, and a range of values that fall within an order of magnitude, may sometimes be considered similar. Such a criterion will need to be assessed for each property and each grouping purpose.

Potential source materials will be identified through interrogation of the data matrix. The source material may or may not be a reference or benchmark material. Where the benchmark materials and potential source materials are NFs, as far as possible, the GRACIOUS Framework will select them from the OECD Testing Programme of Manufactured Nanomaterials [33], available from the Joint Research Centre (JRC) Nanomaterials Repository [34, as these are generally well-characterised materials for which a large dataset is available.

*Linking the GRACIOUS framework to a database*

To allow the Framework and IATAs to function, they must link via the GRACIOUS data matrix to existing databases. An initial list of relevant sources has been derived [35,36], updated and supplemented with more recent information and expert knowledge from project developments in Europe (7th Framework Programme, Horizon 2020) and elsewhere, including the "EU US Roadmap Nanoinformatics 2030" [37]. This allowed the compilation of a contemporary picture of the database landscape, covering databases containing study data, Nano-EHS Knowledge Bases and portals. For each database, the availability status of experimental data has been noted (May 2019) in terms of:

- Existing accessible datasets including data within the eNanoMapper database (subject to appropriate permissions or Data Sharing Agreement);
- Data not yet available;
- Potential data source (subject to permissions, Data Sharing Agreement and/or upload/indexing to the eNanoMapper database).

Based on this list, the GRACIOUS Framework will be able to extract existing information and data about source NFs or non-

**Table 1**
Brief examples of pre-defined hypotheses. These hypotheses are strongly supported by published literature. Whether a NF can be included within a group is guided by an IATA associated with the hypothesis. Inclusion in a group will trigger relatively clear implications. Potential implications are related to 1) supporting activities leading to regulatory compliance (Reg), 2) steering safe innovation/safe(r)-by-design (SbD), and 3) identifying precautionary measures to reduce risk (Prc).

| Pre-defined Hypothesis (scientific rationale) | Information needed to determine if the hypothesis is applicable | Implication type | Implications of a NF being placed in this group | Key Refs |
|---|---|---|---|---|
| Instantaneous dissolution results in uptake of the ionic or molecular form. No nano-specific risks expected. | Substantiate that NFs have a high dissolution rate in relevant media. This results in only the ionic or molecular form reaching the viable cells or environmental organisms. | SbD, Prc, Reg | Assume any effects are only resulting from the dissolution products. Read-across from ionic or molecular form. | [32,50,51] |
| Non flexible NFs > 5 nm: Following dermal application will not penetrate (in their particle form) to viable layers of the skin above 1% of the applied dose. | Substantiate that NFs have low dissolution in simulated sweat, low flexibility and sizes larger than 5 nm. | SbD, Reg, Prc | If NF is in this group then dermal penetration is expected to be minimal (<1%) for healthy skin. If it is not in this group, design larger alternatives that are unlikely to penetrate skin. | [52,53,54,55,56] |
| Respirable, biopersistent, rigid HARN over a threshold length of 5μm: Following inhalation and translocation of NFs to the pleura, where mesothelioma development can occur. | Substantiate low dissolution rate, rigidity and the dimensions of all NFs in the provisional group are in the critical range known from benchmark materials. | SbD, Prc | Assess alternatives that do not fulfil this group's criteria, and thus prevent the potential of the NF to cause lung fibrosis, lung cancer and mesothelioma. | [57,58,59,60,61] |
| | | Reg | Targeted testing to assess if concerns associated with mesothelioma apply. | |
| | | Reg, Prc | Read-across from asbestos or from another rigid HARN may be possible. | |
| Very slowly dissolving NFs that form an unstable dispersion in environmentally relevant aquatic media can be grouped: Following aqueous exposure NFs in this group will settle to benthic systems and can cause lethal/sub-lethal toxicity to representative species. | Substantiate a very slow dissolution/transformation rate and instability of dispersion. | SbD, Prc | Assume NF is biopersistent and may lead to long long-term toxicity in sediment. | [32,62,63,64] |
| | Substantiate similar very slow dissolution/ transformation rate and similar instability of dispersion. | Reg | Read-across from aquatic pelagic species is not possible. Read-across from other NFs for accumulation, and toxicity in benthic species | |

NB Thresholds and similarity criteria for e.g. dissolution rate, and reactivity, to be proposed by GRACIOUS.

NFs wherever available. Each accessible dataset will have to be assessed for completeness of the (meta)data [38]. The GRACIOUS project will, where possible, fill data gaps identified in existing accessible datasets for key benchmark materials as well as for a number of GRACIOUS case study materials that will be used to test the Framework.

*Identification of terminology relevant to grouping and read-across*

In order to allow clarity and compatibility with other nanosafety initiatives, harmonised terminology is used in the Framework. Harmonised terminology relevant for nanosafety was developed in NANoREG [39] in line with existing terminology in OECD and EU legislation. Similarly, six terms have been identified as key to the GRACIOUS Framework (Appendix B), namely nanomaterial, nanoform, grouping, read-across, SbD, and representative test material/reference material. For most of the terms, the NANoREG harmonised definition, where one exists, has been used as a basis and updated in view of new developments.

*Read-Across*

For read-across, there is a need to provide scientific justification that the target material is similar to or less hazardous than the source for that specific endpoint. Information on one or multiple endpoints from one or more existing NFs can be used to predict the same endpoint(s) for a similar NF, if proposed for the same use. This can inform the need for additional studies, risk management measures, or communication on potential safety issues along the NEP value chain. Read-across is typically used in a regulatory setting, though it is also relevant for SbD. The required level of detail of information on safety increases as the *Stages* of product development progress (see SbD). Furthermore, when read-across can be applied for one or multiple endpoints, this may lead to more cost-efficient gathering of information for regulatory registration before market launch. It may be possible therefore to anticipate the regulatory application of read-across early in the development progress, so that less resources may be needed for information gathering for subsequent regulatory approval.

As indicated before, read-across requires that the target material is similar enough to or less hazardous than the to be grouped together and share data for one or more endpoints [4]. A comparison of their similarity related to one or more endpoints is performed. Similarity may be considered based on two principles. The read-across hypothesis can be based on either structurally similar NFs having similar properties, or on the principle that (bio)transformations lead to common exposure-relevant forms of the material [4].

A read-across case should consider information on:

- Physicochemical properties ("what they are"),
- Fate/toxicokinetic behaviour ("where they go") related to the endpoint (e.g. will the target site be reached in a similar way, and to a similar or lesser extent?),
- A comparison of hazard ("what they do") also related to the endpoint (e.g. will the same toxicological mechanism be triggered, and to a similar or lesser extent?).

*Assessing the quality of information*

Regarding the general criterion' Reliable and adequate source data', ECHA recommends that each study result used in a read-across case (either to justify read-across or to predict the missing data for the target member) is evaluated in terms of relevance, reliability and adequacy [40]. For chemicals, reliability is defined by the OECD and under REACH it is generally addressed by apply-

ing the Klimisch criteria [41] as implemented in the ToxRTool [42]. However, for many of the nano-specific properties, including those required by the revised REACH Annexes for NF characterisation and recommended by ECHA for grouping of NFs, there are currently no internationally accepted testing guidelines available, or the applicability of existing test guidelines is questioned. As a result Klimisch scores are currently worse by at least one digit as compared to chemicals in general. It is worth noting that progress is being made in the OECD, but for most of the guidelines, adoption will still take some years. Furthermore, in the case of data obtained from (eco)toxicological studies with NFs, it is critical that physicochemical characterisation of the material(s) in the test system is available (in addition to characterisation of the pristine material(s)) [43], which is not directly reflected in Klimisch scores. Klimisch scores also contain some subjectivity for scores higher than one. For these reasons they may not be the best option for evaluating study results on NFs [44].

Several alternative evaluation methods of data quality for nanomaterials have therefore been considered for use within the Framework. These methods make the process of assessing data quality less subjective by giving clear criteria for the quality for nanomaterial studies:

- For human health risk assessment:
  - Two-step process [43];
  - DaNa Literature Criteria Checklist [44]; and
  - GUIDEnano quality assessment approach [45].
- For environmental risk assessment:
  - NanoCRED evaluation method [46] and
  - GUIDEnano quality assessment approach [45].

*Safe(r) by design (SbD)*

Portfolio management tools such as *Stage-Gate* aim at stopping the innovation process if there is unacceptable compromise in (i) profitability, (ii) technical probability of success, (iii) commercial probability of success, and/or (iv) risks or uncertainties regarding risks [46].

The NANoREG and NanoReg 2 SbD concept proposed a screening strategy to identify and characterise risks associated with NFs, NEPs or manufacturing processes at early stages of innovation. This requires determination of 'potential for risk' at Stage 1 (i.e. lists of potential scenarios, risks and uncertainties), 'indicators for risk' at Stage 2 (theoretical risk analysis based on existing data and subjective information) and Stage 3 (experimental risk analysis), and 'demonstrators of risk' at Stage 4 and 5 (risk assessment as laid down in regulatory requirements) [39,47]. The uncertainty therefore reduces progressively along the innovation process according to the *Stage-Gate* innovation model, being large and subjective when a decision is made at Gate 1, and becoming substantially smaller (driven by legislation) at Gate 5 [39].

## Results - the GRACIOUS framework for grouping and read-across of nanoforms

*Framework overview*

This section provides a brief overview of the GRACIOUS Framework. A more detailed description is provided in the sections below.

The GRACIOUS Framework is structured according to evidence supported decision nodes (Fig. 1 – boxes with an orange border), which guide the user through generating the information needed to develop a justified grouping and/or read-across decision. Either *a single NF or a provisional group of NFs* can represent the *entry point* to the Framework.

The first step is to gather *basic information* about the NF or provisional group of NFs. This includes a selection of physicochemical properties (predicted or known), consideration of the purpose of grouping and identification of the intended use(s) of the NF(s) under investigation. The latter allows derivation of the relevant fate pathway and exposure route(s).

The basic information is used to identify a scientific hypothesis to facilitate grouping. At this point the user can *assess the applicability of pre-defined hypotheses for grouping* for the investigated NF(s). If *a pre-defined hypothesis is applicable*, the GRACIOUS Framework will provide a *tailored IATA* to substantiate the similarity concept underlying grouping. The term IATA describes the combination of different types of modelling, testing and assessment approaches, plus it is frequently used in a regulatory context (e.g. OECD). The term IATA is used as defined in OECD Guidance Document 255 [48]. Each tailored IATA is specific for a given pre-defined hypothesis and recommends a series of tests to the user to verify the applicability of the hypothesis for the material under investigation. As the validity of these tests, the proposed thresholds or similarity criteria and the implications of being in the group associated to the hypothesis have already been investigated, such an approach allows for easy application of grouping. In addition, options for developing read-across justifications are provided. For hazard testing the IATAs span acellular, *in vitro* and *in vivo* test systems of varying complexity.

If the predicted or measured information via the IATA suggests that *a pre-defined hypothesis is not applicable*, then a new *user-defined hypothesis* can be generated via use of our hypothesis template [24].

To further strengthen a grouping or read-across decision, more information may be required to demonstrate similarity between the source and target NFs or non-NFs and justify the applicability of the hypothesis (i.e. Does the NF fit in the group?). Generation of this information is guided via the *tailored IATA* and gathered in a *data matrix*. This allows methodical and efficient assessment of the evidence against *justification criteria*.

The following sections describe the Framework in more detail.

*Getting started*

- *Entry point* - Which NF(s) are the focus of the assessment?

The user can enter the Framework with either a *single NF* or a *provisional group of NFs*. These may be existing materials that are already on the market, or they may be in the design phase. In the case of a provisional group of NFs, the goal is to establish whether two or more NFs can be grouped together based on a hypothesis by assessing their similarity with respect to criteria that are of relevance for the hypothesis. Similarly, for a single NF, the goal is to see if this NF can be assigned to a pre-defined group, also based on similarity. In both cases the hypothesis needs to be verified and justified.

If the NF does not fit to a pre-defined group, the Framework can be used to establish a provisional hypothesis-based group, via searching the eNanoMapper database at search.data.enanomapper.net (see section 2.8), to identify suitable source NF(s) or non-NF(s). The GRACIOUS experimental data will be made public via the eNanoMapper database at a later date. Relevant properties will be used as search terms as indicated in the approach by GUIDEnano [23]. If these are not known, they can be identified by use of the IATA.

- *Basic information*

The next step is to collect and assess the available basic information via use of a structured matrix. This information is used to

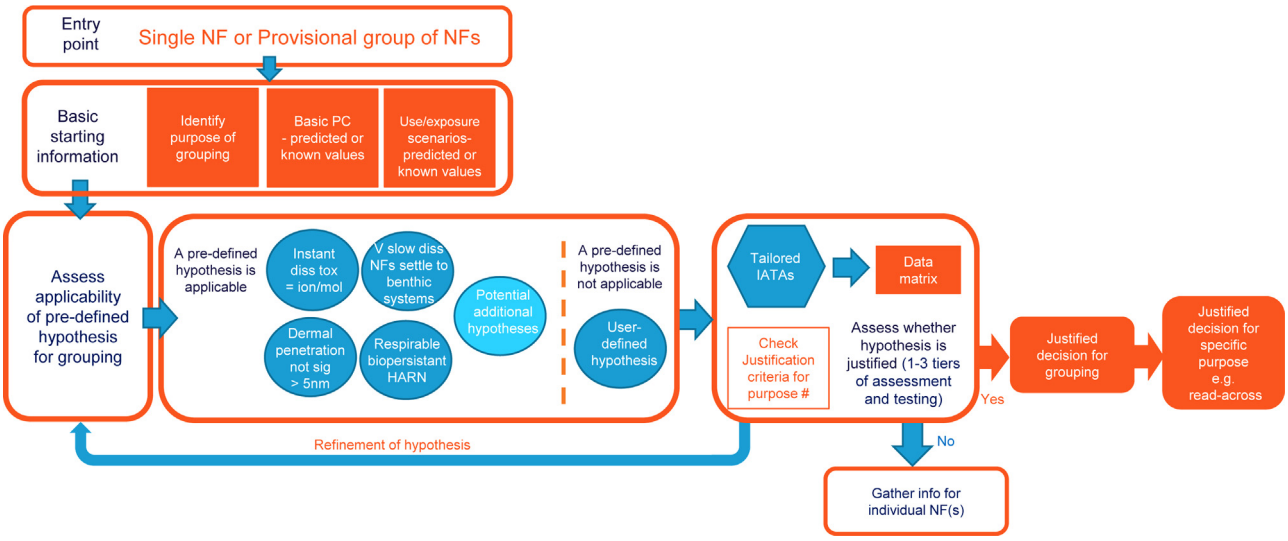


**Fig. 1.** Overview of the GRACIOUS Framework for Grouping and Read-Across of nanoforms (NFs). The Framework diagram demonstrates the key decision nodes (boxes with an orange border), and the information and activities required to support the decision making. Justification criteria could be purpose specific (i.e. less stringent for Safe(r)-by-design (SbD) than for data gaps filling in regulatory context). Abbreviations include physicochemical (PC), high aspect ratio nanomaterials (HARN), not significant (not sig), very slow dissolution (v slow diss), dissolution (diss), toxicity (tox), molecule (mol), Integrated Approach to Testing and Assessment (IATA), information (info), nanoform (NF).

identify whether a pre-defined hypotheses is appropriate, and/or to generate a user-defined, tailor-made grouping hypothesis.

o *Identify the purpose of grouping*

The GRACIOUS Framework is useful:

■ To support regulatory compliance (including read-across)

When compiling dossiers for regulatory purposes (e.g. REACH registration dossiers), grouping can be used to fill data gaps for a substance using data available on similar substances instead of conducting particular tests. This includes the possibility to apply read-across, see section 2.10. Note that the source substance could be another NF, a larger particle or molecular form (a non-particulate version of the same chemical) registered in the same dossier.

■ To facilitate more efficient hazard testing

A clear scientific hypothesis for grouping similar NFs, focused on a hazard endpoint (e.g. ability to induce inflammation), helps to target information gathering, assessment and test design. In addition, inclusion within the hypothesis of specific exposure routes (e.g. human inhalation) or environmental compartments (e.g. aquatic), further refines the choices for information gathering and testing.

■ To steer safe innovation / SbD processes

Testing for safety during the development phase of a material has to be cost-efficient. The GRACIOUS Framework therefore aligns with the *Stage-Gate* model [49] in order to ensure relevance of the Framework to business decision-making.

■ To identify precautionary measures to reduce exposure

At any time during NF/NEP development or use, the Framework can help to identify adequate risk management measures by providing information on similar materials and uses for which such measures are already established. An examples of risk management measures include reducing exposure (e.g. via enclosure of a process to prevent the release of NFs, or coating of a NF to reduce dustiness).

■ To improve scientific understanding

Grouping could help to improve the understanding of mechanistic toxicology of certain NFs. Grouping also allows identification of a NF's physicochemical descriptors, exposure contexts, transformations and mechanisms of hazard that drive risk. Construction of the IATAs also allows identification and prioritisation of relevant methods to be developed or standardised.

- Early clarification of the purpose of grouping allows the user to:
- Recognise whether grouping is suitable for the purpose proposed,
- Identify the extent and detail of information required to support grouping,

Recognise the acceptable level of uncertainty, taking into account the scientific robustness of the similarity hypothesis and the quality of the data used to justify it.

At this stage, users will need to consider if grouping will be the most effective approach in terms of saving time, costs and/or test animals. Where the substantiation of grouping would require considerable testing, it may be more appropriate to gather the required information for each NF individually. In general, for (a) large group(s) it may be more challenging to justify the grouping. In addition, it is useful to consider the implications of placing a NF inside a group, outside a group, or in multiple groups (for multiple endpoints).

o *Basic physicochemical (PC)* information – What they are

The basic physicochemical information should, as a minimum, enable characterisation of the pristine NF(s), helping the user to consider relevant hazards (what they do) and/or fate/kinetics (where they go). Physicochemical data allows a first assessment of similarity between NFs, for which information is available and which may be used either as benchmark materials for testing and/or as source materials for read-across. At this stage, the basic physicochemical information may be predicted or known. An advantage

of using predicted information is that the most relevant grouping hypotheses can be easily identified, reducing the need to assess less relevant hazard endpoints. Predicting basic information is most relevant for safe-by-design or targeted testing. The basic information reflects regulatory requirements. For regulatory application of grouping and read-across, this information should thus be known already rather than predicted.

Within the GRACIOUS project, the following physicochemical properties are required as basic information for the pristine material: composition, crystallinity, particle size, specific surface area, dustiness, water solubility (incl. dissolution rate), density, surface hydrophobicity, surface charge, shape, chemical nature of the surface (henceforth ‘surface chemistry’).

Of these properties 6 are considered priorities: composition, crystallinity, particle size, shape, surface chemistry, and specific surface area [38]. The above list consists of intrinsic properties that are considered to be essential to uniquely identify a NF, and is in line with the NF registration requirements of the revised REACH Annexes [3].

- *Release and exposure* scenarios – descriptors for the intended uses

The Framework includes decision trees which guide the user to specify:

- Intended use and physical form during use (e.g. whether the NF or NEP is used as a powder, suspension or aerosol).
- If the NF is associated with a matrix of the NEP (e.g. surface coating, matrix-embedded or mixed powder) and the characteristics of this matrix.
- Potential release pathways and exposure route(s) to humans and the environment (within relevant compartments).

The NF functionality in the intended use determines many of the NF physicochemical properties (composition, size, reactivity, persistence) that then also influence options for grouping with respect to hazard, fate, exposure. For example, pigments for transparent coatings in food contact need to have sizes around 30 nm and need to be insoluble to achieve the desired functionality, but the same properties can be the basis to establish groups with respect to hazard. These life cycle considerations (exposure scenario descriptors, predicted or known) help the user to select the hypothesis more specific considering the exposure relevant nanoform, exposure route and potential dose range of exposure. All of this information, in turn, helps to further refine the IATA to generate the most important information required to support grouping (and subsequently read-across). At this stage, the release and exposure scenarios may be predicted or known.

*Using the basic information to identify a hypothesis for grouping*

- *Assess applicability of a pre-defined hypothesis for grouping*

The hypotheses in Table 1 are ‘pre-defined’ in terms of both the scientific evidence supporting them and the understanding of the implications for risk assessment of placing a NF in each group.

For early-stage industrial SbD decision-making, or for precautionary risk management, final decisions may already be made at this node. For example, if the High Aspect Ratio Nanomaterial (HARN) grouping hypothesis applies to the NF(s) under investigation, one may recommend NF(s) substitution or procedures to manage safe use (e.g. avoid abrasive modification of the solid matrix nanoenabled product) without further testing.

Pre-defined hypotheses of this nature provide an opportunity to quickly assess whether a NF fits into such a group and directs focus to the information-gathering required to justify the grouping decision. The GRACIOUS Framework is designed to provide evidence-based thresholds indicating the cases in which pre-defined hypotheses may be applicable, based on comparison to benchmark materials that are specific to each hypothesis.

As previously indicated, if the pre-defined hypotheses are not applicable, then a ‘user-defined’, tailor-made hypothesis can be generated via use of a hypothesis template. When a NF aligns with more than one pre-defined hypothesis, the user can select the one that best serves the specific purpose. For example, for a carbon nanotube intended to be embedded in a nanocomposite, in an occupational scenario where the manufacturing processes might lead to release of free NFs, the HARN hypothesis may be more applicable. In contrast, in the finished goods, where one aims to prevent exposure of consumers to free NFs, a hypothesis relating to the relatively low release of NFs from a solid matrix may be a more relevant consumer product scenario.

Note that for release, the grouping hypotheses detail the life cycle processes (considering the intended use) that exert similar energy on the NF or NEP (mechanical, thermal, etc.), matrices with similar resilience, NFs with similar dustiness, or grouping by article category. For read-across, the hypotheses address whether the combination of process/matrix and NF leads to similar release rates and similar released forms for source and target NFs or NEPs.

Regarding exposure, grouping utilises the REACH descriptors for defining exposure scenarios (process, matrix, NF/NEP and exposure factors: exposure route, exposure controls, duration of the activity/use, amount of NF being handled). Read-across of hazard information is most appropriately conducted across similar scenarios.

*Assessing whether a hypothesis is justified*

- Use of *Tailored IATAs* to assess applicability of a group or to guide read-across

As outlined above, each hypothesis triggers development of a tailored IATA which evaluates all properties that are relevant for the hypothesis, allowing the grouping to be either accepted, rejected, or to indicate where further information is required for a specific purpose. The tailored IATAs include assessment of those descriptors (e.g. physicochemical parameters and hazard biomarkers) that are critical to decide whether the NF(s) investigated fits within the group described by the hypothesis, or whether the source and target NF(s) are sufficiently similar to apply read-across. As the validity of these tests, proposed thresholds or similarity criteria, and the implications of being in the group associated to the hypothesis have been scrutinised scientifically, the hypothesis and the information gathered according to the IATA thereby provides the grouping justification. Because the IATAs are tailored to the hypothesis, they have the added advantage that they guide the user not to generate non-essential information or data.

Not all tiers of testing outlined in the IATAs need to be completed for each application of grouping, depending on the level of similarity of the NFs under investigation. With respect to assessing release and exposure during the life cycle, the IATA developed in GRACIOUS will detail the release form and/or release rates/exposure concentration, where possible, to be used as input information for a fate and hazard-based hypothesis. Following the Use Descriptor System developed by ECHA [49], the descriptors for occupational process categories, product categories for consumer exposure and the environmental release categories are used as grouping descriptors. While these may not be optimal for input information for a hypothesis, they have the benefit of being relatively easily available.

Information generated by the IATAs will be collected into the data matrix which will be structured to provide the evidence to

reject or accept the hypothesis. In order to be effective the data matrix requires robust links to relevant databases that facilitate both acquiring existing data, as well as storage of newly acquired data for future use. For confidential data, this can be included in the data matrix without public access to these specific data.

The ability to group nanoforms or apply read-across depends on an assessment of their similarity for specific endpoints (hazard, fate/toxicokinetics, physicochemical and exposure) that relate to the decision nodes in an IATA. The Framework guides the user to higher tiers of testing to decrease uncertainty or to consider the nanoform on a case by case basis

- Check Justification criteria for purpose and quality of information for making a grouping or read-across decision

Assessment of the quality of the information used for grouping or read-across is also essential. Via the tailored IATAs the GRACIOUS Framework guides the user in evaluating:

o The adequacy of the hypothesis,

In terms of adequacy, the IATA will guide the user through production of the documentation required to justify a grouping hypothesis and application of read-across. The general criteria are summarised in Appendix C. In future case studies we will provide examples and clarification of how these justification criteria can be applied.

By providing the scientific basis of each pre-defined hypothesis and, where possible, by suggesting evidence-based cut-off values, the Framework will indicate the scenarios in which these pre-defined hypotheses may be applicable. These assessments facilitate a conclusion on whether the grouping hypothesis is sufficiently justified for the purpose.

o The quality of the information used to test the hypothesis,

Park et al. 2018 [23] provide a powerful tool for data quality evaluation of both toxicological and ecotoxicological studies performed with NFs. This approach can be combined with some aspects that are covered in other schemes, for example DaNa and NanoCRED, which provide added value by further exploring the appropriateness of the method and study setup.

*Using the GRACIOUS framework for read-across*

For grouping, the hypothesis relates to whether NFs (and non-NFs) are sufficiently similar with respect to a specific endpoint (e.g. dissolution) to be considered as members of a group. In comparison, for read-across, the hypothesis focuses on filling specific data gaps by addressing whether the target NF is likely to have a similar hazard or is less hazardous than the source NF(s) or non-NF(s). Establishing a group of two or more NFs facilitates filling data gaps for data-poor group members by read-across from data-rich ones, supporting both regulatory and innovation purposes (i.e. grouping generally precedes read-across). Such read-across can be performed, for example, when a very similar or smaller amount of the target material is observed to reach the target site, in combination with a similar or lower hazard potential than that of the source material(s) (Fig. 2).

Specific examples of read-across include the case of instantaneous dissolving NFs, for which read-across from the solute for multiple endpoints is proposed, whereas for high aspect ratio nanomaterials read-across from asbestos is possible. This section outlines how the same Framework can be used for read-across beyond the pre-defined hypotheses.

Fig. 2 starts by guiding the user to *identify potential source NFs and non-NFs*, and the development of a robust scientific justification of similarity between the source material(s) and target NF(s). As described above for grouping, the basic physicochemical information helps the user to *generate a read-across hypothesis for a specific endpoint*. Physicochemical descriptors for an exposure-relevant pristine or transformed NF may also be derived through life cycle considerations during the initial grouping, or through subsequent application of the tailored IATA for where they go. These descriptors can be used to identify source materials analogous to the exposure-relevant NF as part of a read-across assessment for a specific instance of exposure or release during the NEP life cycle. In order to *substantiate the read-across hypothesis,* the Framework allows the user to identify relevant available information on hazard (what they do) and/or fate/kinetics (where they go) via application of the tailored IATA. This process includes *assessment of* those descriptors (e.g. physicochemical parameters and hazard biomarkers) that are critical to deciding whether the source NFs or non-NFs and the target NF are sufficiently similar, or to show that the target NF has a lower risk compared to the source NF. The Framework subsequently guides the comparison of the target NF and source materials, allowing focus on the specific properties that define similarity. As for grouping, the criteria to assess whether the read-across is sufficiently justified depend on the purpose.

*Using the GRACIOUS framework for SbD*

Considerations based on similarity may be used to derive a conclusion on risk potential in the SbD process. For example, a designed NF can be assessed for similarity with its non-nanoform(s), or another NF for which information is available. This may also indicate possibilities for developing a read-across justification rather than performing general testing to comply with regulatory requirements at a later stage [47]. This can be achieved by manipulation of the stringency of the justification criteria. For example, grouping for regulatory purposes would only include NFs consisting of one substance per group, whereas for SbD multiple substances could be included within the same group.

A hypothesis at *Stage* 1 (Research/Business idea) could be formulated based on expert judgment and existing information, without the need to build a robust scientific justification and without the need to provide the full documentation. At this stage more uncertainty is often accepted and the precautionary principle is usually applied to ensure that the designed NFs or NEPs, with potential elements of high hazard or exposure, are properly managed by the design process, allowing product exclusion at early Gates (*Gate 1*) of the *Stage-Gate* process (Fig. 3).

However, high uncertainty at *Stage* 1 could also allow progression of a NF or NEP to *Stage* 2, where more extensive testing will help to reduce uncertainty and aid decision making. This progression in complexity of information requirements to reduce uncertainties corresponds with the increasing complexity of the models or assays used in the tiers of testing outlined in the IATAs (Fig. 3). The IATAs:

- Provide screening-level hazard assessments based on *in silico* modelling before the "Go to development" decision at Gate 3. Specifically, QSAR models and machine learning approaches (e.g. nonlinear Principal Component Analysis, matrix factorization, deep learning autoencoders) can help to cost-effectively identify key nano-bio/eco interactions and Modes of Action in the early stages of innovation as a basis to establish hypotheses for grouping and rules for read-across. These models can also be applied to predict hazard endpoints based on physicochemical data. Moreover, PBPK models can be employed to model the toxicokinetics/dynamics of the materials in the human body as a basis for *in vitro-in vivo* to human extrapolations. This can help

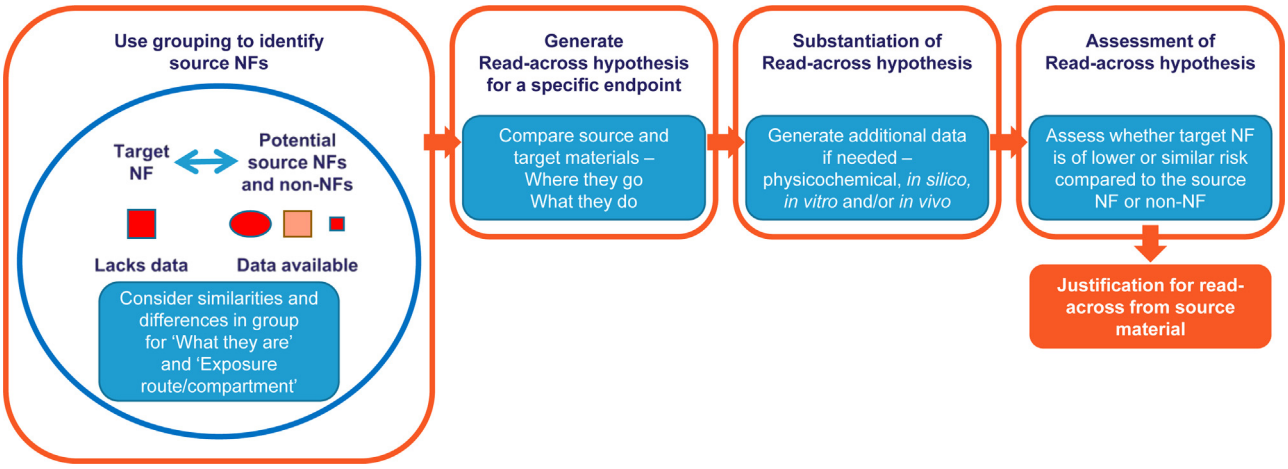


**Fig. 2.** Developing a read-across justification for one or more NFs for a specific endpoint (Figure adapted from Oomen et al., 2015). For substantiation, in vivo testing can become necessary if the evidence by physicochemical, *in silico, in vitro* methods remains inconclusive at the required level of justification criteria.

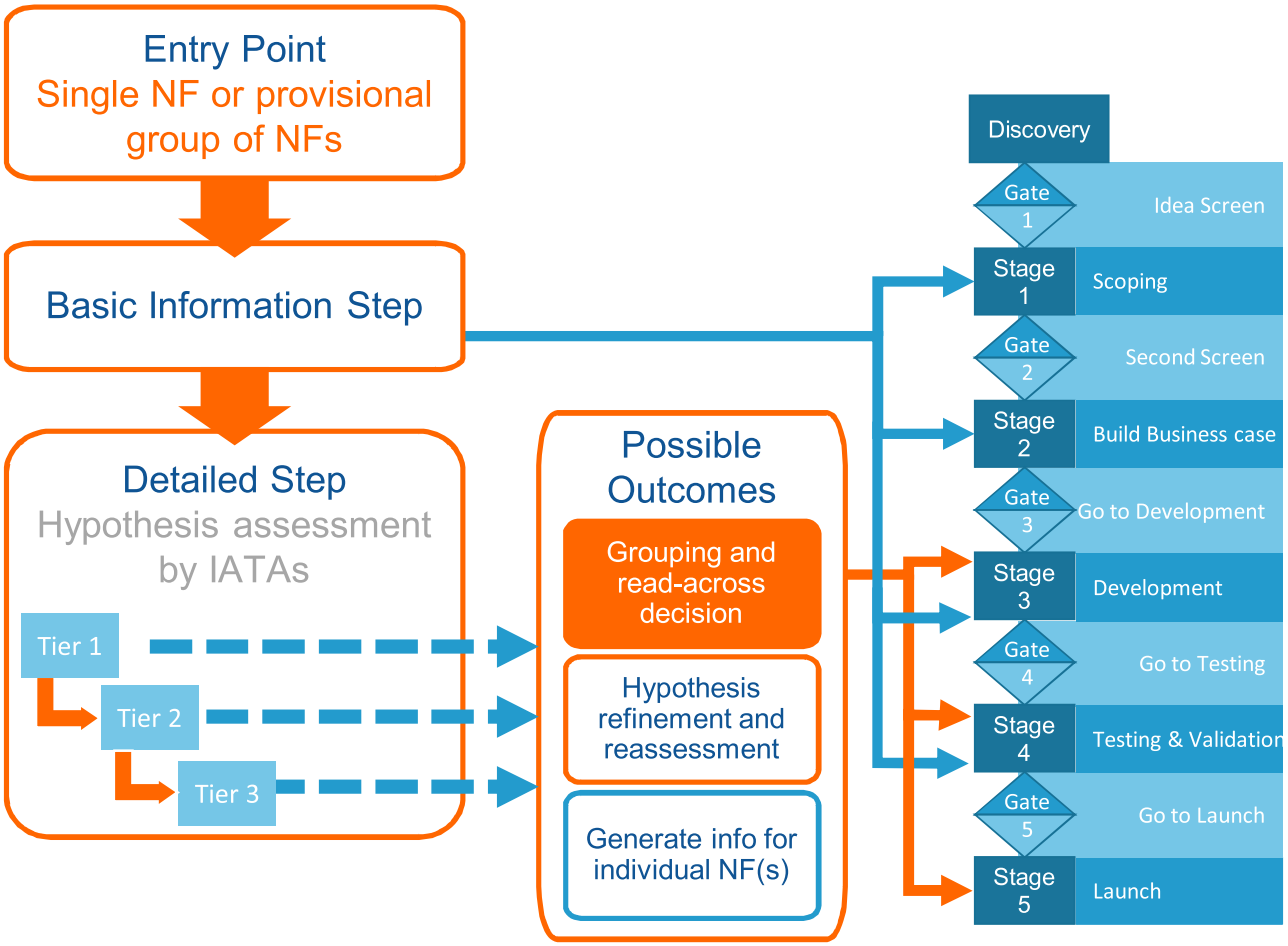


**Fig. 3.** Outline for the relationship between the GRACIOUS Framework and SbD via the Stage-Gate Idea-to-launch innovation process. The blue arrows are relevant to the initial tier of the IATAs, while the orange arrows relate to mid and higher tiers of the IATAs.

to use/read-across existing physicochemical and *in vitro* toxicity data for SbD purposes, which can substantially reduce costs and eliminate the need for animal testing before Gate 3. To enable this, we shall directly use such modelling approaches as part of the IATA. The available *in silico* tools that we have identified as relevant for the GRACIOUS framework have been thoroughly reviewed by the project and are described in [65].

- Support SbD decisions at Gate 3, by allowing an acceptable level of uncertainty. For example, it is sufficient to know that the intended use and the resilience properties of the NEP matrix are similar to those of a well-known reference NEP.
- Support SbD decisions at Gate 4, by aligning the risk assessment of the NF with that of a similar source substance. Here similarity can be indicated by the relevant descriptors of the source and

target NF being within the same (few) order(s) of magnitude. The thresholds or similarity criteria are typically derived from the dynamic range of values generated through testing of the positive and negative controls.
- Support SbD decisions at Gate 5, by feeding into the preparation of a regulatory dossier, with the aim of fulfilling the information requirements without (additional) animal testing. For hypotheses with clear implications, the data generated at early IATA tiers would generally suffice to substantiate the grouping or read-across. For user-defined hypotheses, and for data requirements at high tonnages, substantiation may require Tier 3 testing.

More robust criteria for justification of a hypothesis would therefore be required at *Stages* 4 and 5, to align the safety assessment of the selected NF or NEP with the regulatory requirements (See Appendix D), even before the registration or approval process starts. The criteria to assess the scientific adequacy of grouping and read-across justifications are provided in Appendix C. The practical applicability of the general criteria in Appendix D in a SbD context will be verified on a case-by-case basis through the GRACIOUS case studies.

*Conclusions and future outlook*

The GRACIOUS Framework builds upon and combines concepts from multiple previous and current projects, as well as guidance from regulators (notably REACH, but also product specific regulations and non-European legislation). The Framework supports the use of grouping hypotheses related to one or several endpoints. The Framework also supports demonstration of the adequacy of the hypothesis and development of a grouping or read-across justification in view of the similarity between NFs. The Framework identifies key information and data requirements to substantiate specific grouping hypotheses. It also provides the most relevant protocols to generate the data and information needed to test the hypotheses and it structures these tests via a scientifically based IATA.

As suggested by other projects [16], there is a wealth of information available about the safety of NFs that can be employed in risk-related decision making. The GRACIOUS Framework is therefore designed to use hypotheses and associated IATAs to promote the use of existing information and data, combined with modelling and generation of new data.

Both the DF4NanoGrouping and the ECHA concepts recommend that the data needed for applying grouping and read-across to NFs is obtained in a cost-effective manner. The GRACIOUS Framework tailored IATAs therefore enable the generation of adequate information to substantiate a number of pre-defined grouping hypotheses, starting relatively simple and increasing in depth and/or complexity where uncertainty needs to be minimised. The IATAs may also be used to develop and justify other hypotheses for grouping and read-across.

In order to ensure sustainability, the final Framework will be open for easy integration of new knowledge, and will be modular and sufficiently flexible to accommodate new insights, making the Framework future proof. The first iteration of the Framework has been constructed to address regulatory applications of grouping and read-across. A subsequent version will then be adapted for the purposes of SbD through reduction of the stringency of the justification criteria.

The Framework is synergistic with the 3Rs strategies. A recent review [66] has interpreted the "3Rs as a framework to support a 21 st century approach for nanosafety assessment", and proposed a tiered approach, elements of which are congruent with the elements of the GRACIOUS Framework. For example, the first "plane" of the 3Rs framework described by this review [50] includes intrinsic material properties, life cycle use and release. The GRACIOUS Framework also starts from intrinsic material properties, and positions the intended use at the interface before targeted testing, in order to focus on relevant compartments. The elements of the 3Rs-motivated testing strategy in the second "plane" [66], include uptake, biodistribution, biopersistence, biophysical interaction and cellular effects. These are highly consistent with mid-tiers of the GRACIOUS IATAs; however, the GRACIOUS Framework also seeks to incorporate assessment of environmental endpoints.

While the GRACIOUS Framework is primarily designed to work under European legislation, the Framework could also be of global relevance in risk assessment and management. Among the stakeholders engaged by GRACIOUS are regulatory bodies from the USA and Canada, and so more will be done in the future to consider the applicability of the Framework outside the EU. Wherever possible, the Framework will refer to OECD Test Guidelines and Guidance Documents in order to support mutual acceptance of data. Also references to standards, e.g. from ISO, will be included, and both ISO and OECD have global relevance.

While the Framework we present here is extensively developed, we anticipate that during construction of the individual elements (e.g. the IATAs), consultation with stakeholders and testing on case-studies, modifications may be required in order to generate the final functional version (to be launched in 2021).

In summary:

- The GRACIOUS Grouping Framework design
  - o aligns with EU chemicals legislation (in particular REACH) in order to facilitate use of grouping and read-across in a regulatory setting, which will also be globally relevant;
  - o is informed by existing approaches and recommendations on grouping and read-across;
  - o aligns with the industrial innovation process (e.g. *Stage-Gate* Idea to Launch process);
  - o is hypothesis-driven and evidence-based.
- The information and data for justifying the grouping hypotheses are generated via a tailored, scientifically-based IATA. Potential implications of grouping according to the hypotheses are indicated.
- The IATA is of increasing specificity and complexity and serves to acquire the data needed to justify grouping and read-across.
- Grouping considers not only intrinsic properties and toxicological effects, but also extrinsic (system-dependent) descriptors of exposure, toxicokinetics and environmental fate.
- The outcome of grouping is influenced by the grouping purpose (regulatory compliance including read-across, innovation/SbD, identification of precautionary measures, scientific understanding) and context (e.g. inhalation vs dermal, or human vs environment).

## Author contributions

Vicki Stone; conceptualisation, methodology, investigation, writing – original draft, writing – review & editing, visualisation, supervision, project administration, funding acquisition. Specifically Vicki drafted and led the editing of the manuscript based on stakeholder feedback and author contributions.

Stefania Gottardo, Paula Jantunen, Kirsten Rasmussen and Hubert Rauscher; conceptualisation, investigation, writing – original draft, writing – review & editing, supervision, funding acquisition. These authors led the writing of the sections pertaining to terminology, data quality and justification for grouping and read-across. They also ensured that text relating to current regulatory documents is accurate and appropriate.

Agnes G. Oomen, Eric A.J. Bleeker, Susan Dekkers; conceptualisation, methodology, investigation, writing – original draft, writing – review & editing, visualisation, supervision, funding acquisition. These authors were responsible for generating the first draft of the Framework presented to stakeholders and co-authors. They contributed to extensive editing of all sections, but in particular the Framework description, hypotheses and read-across.

Teresa Fernandes; conceptualisation, writing – review & editing, funding acquisition. Teresa led the environmental hazard activities and therefore worked to ensure environmental relevance to the Framework structure as well as hypothesis generation.

Andrea Haase and Helinor Johnston; conceptualisation, methodology, investigation, writing – review & editing, visualisation, supervision, funding acquisition. These authors led the human hazard activities and therefore worked to ensure human health relevance to the Framework structure as well as hypothesis generation.

Neil Hunt; writing – review & editing. Neil provided input on the relevance of the content to REACH and other regulations.

Nina Jeliazkova, Danail Hristozov and Lara Lamon; conceptualisation, methodology, investigation, writing – review & editing, supervision, funding acquisition. These authors led the writing of sections describing the database sections as well as modelling approaches for grouping and read-across. Danail Hristozov contributed to the discussions shaping the overall Framework.

Fiona Murphy and Hedwig Braakhuis; methodology, investigation, writing – review & editing. These authors led the construction of IATAs relating to human hazard, and how these IATAs feed into the Framework.

Claus Svendsen and David Spurgeon; conceptualisation, methodology, investigation, writing – review & editing, supervision, funding acquisition. These authors led the environmental fate and behaviour work that contributes to the hypotheses and IATAs

Socorro Vázquez-Campos and Araceli Sánchez Jiménez; conceptualisation, methodology, investigation, writing – review & editing, supervision, funding acquisition. These authors led the Framework sections pertaining to exposure and release of NFs.

Wendel Wohlleben; conceptualisation, methodology, investigation, writing – review & editing, supervision, funding acquisition. Wendel led the work responsible for characterisation strategies for NFs, as well as providing an industrial perspective in relation to regulation interpretation and innovation.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

The GRACIOUS project was supported by the European Commission Horizon 2020 programme, Grant Agreement No. 760840. The funding body played no role in the study design, data collection, analysis and interpretation, in the writing of the manuscript and in the decision to submit the article for publication.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.nantod.2020.100941.

## References

[1] Commission Recommendation (EU) 2011/696 of 18 October 2011 on the definition of nanomaterialEC, OJ No. L 275, 20.10.2011, European Union, 2011, 38-40. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011H0696&qid=1595955733547&from=EN.

[2] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/ECEC, OJ No. L396, 30.12.2006, European Union, 2006, 1-850, https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1595956878711&uri=CELEX:32006R1907.

[3] Commission Regulation (EU) 2018/1881 of 3 December 2018 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards Annexes I, III, VI, VII, VIII, IX, X, XI, and XII to address nanoforms of substancesEU, OJ L 308, 4.12.2018, European Union, 2018, 1-20. https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R1881.

[4] ECHA, Read-Across Assessment Framework (RAAF), ECHA-17-R-01-EN, European Chemicals Agency, Helsinki, 2017 https://doi.org/10.2823/619212.

[5] USEPA, Forty-Seventh Report of the TSCA Interagency Testing Committee to the Administrator, Receipt of Report and Request for Comments, 66 FR 17767, Office of the Federal Register, 2001, pp. 17768–17774 https://www.govinfo.gov/content/pkg/FR-2001-04-03/pdf/01-8133.pdf.

[6] OECD, Guidance on Grouping of Chemicals, Series on Testing and Assessment, No. 194, ENV/JM/MONO(2014)4, Second Edition, Organisation for Economic Co-operation and Development, 2014 https://www.oecd.org/publications/guidance-on-grouping-of-chemicals-second-edition-9789264274679-en.htm.

[7] ECHA, Chapter R.6: QSARs and grouping of chemicals, in: Guidance on information requirements and chemical safety assessment, European Chemicals Agency, Helsinki, 2008 https://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf.

[8] A. Worth, K. Aschberger, D. Asturiol Bofill, J. Bessems, K.B. Gerloff, R. Graepel, E. Joossens, L. Lamon, T. Palosaari, A. Richarz, Evaluation of the availability and applicability of computational approaches in the safety assessment of nanomaterials: Final report of the Nanocomput project, in: JRC Technical Reports, Publications Office of the European Union, 2017 https://doi.org/10.2760/248139.

[9] A. Giusti, R. Atluri, R. Tsekovska, A. Gajewicz, M.D. Apostolova, C.L. Battistelli, E.A.J. Bleeker, C. Bossa, J. Bouillard, M. Dusinska, P. Gomez-Fernandez, R. Grafstrom, M. Gromelski, Y. Handzhiyski, N.R. Jacobsen, P. Jantunen, K.A. Jensen, A. Mech, J.M. Navas, P. Nymark, A.G. Oomen, T. Puzyn, K. Rasmussen, C. Riebeling, I. Rodriguez-Llopis, S. Sabella, J.R. Sintes, B. Suarez-Merino, S. Tanasescu, H. Wallin, A. Haase, Nanoimpact 16 (2019), http://dx.doi.org/10.1016/j.impact.2019.100182.

[10] E.D. Kuempel, V. Castranova, C.L. Geraci, P.A. Schulte, J. Nanoparticle Res. 14 (2012), http://dx.doi.org/10.1007/s11051-012-1029-8.

[11] RCC, Nanotechnology Initiative Work Element 2, Priority Setting: Development of a Joint Nanomaterials Classification Scheme. Final Report, Regulatory Cooperation Council, 2013 https://www.ic.gc.ca/eic/site/063.nsf/vwapj/nano2_e.pdf/$file/nano2_e.pdf.

[12] RCC, Nanotechnology Initiative Work Element 3: Risk Assessment/Risk Management. Final Report, Regulatory Cooperation Council, 2013 https://www.ic.gc.ca/eic/site/063.nsf/vwapj/nano3_e.pdf/$file/nano3_e.pdf.

[13] V. Stone, S. Pozzi-Mucelli, L. Tran, K. Aschberger, S. Sabella, U. Vogel, C. Poland, D. Balharry, T. Fernandes, S. Gottardo, S. Hankin, M.G. Hartl, N. Hartmann, D. Hristozov, K. Hund-Rinke, H. Johnston, A. Marcomini, O. Panzer, D. Roncato, A.T. Saber, H. Wallin, J.J. Scott-Fordsmand, Part. Fibre Toxicol. 11 (2014) 9, http://dx.doi.org/10.1186/1743-8977-11-9.

[14] W. Wohlleben, B. Hellack, C. Nickel, M. Herrchen, K. Hund-Rinke, K. Kettler, C. Riebeling, A. Haase, B. Funk, D. Kühnelk, D. Göhler, M. Stintz, C.M. Schumacher, M. Wiemann, J.G. Keller, R. Landsiedei, D. Broßell, S. Pitzko, T. Kuhlbusch, Nanoscale (2019), http://dx.doi.org/10.1039/C9NR03306H.

[15] A.G. Oomen, E.A.J. Bleeker, P.M.J. Bos, F. van Broekhuizen, S. Gottardo, M. Groenewold, D. Hristozov, K. Hund-Rinke, M.A. Irfan, A. Marcomini, W. Peijnenburg, K. Rasmussen, A.S. Jimenez, J.J. Scott-Fordsmand, M. van Tongeren, K. Wiench, W. Wohlleben, R. Landsiedel, Int. J. Environ. Res. Public Health 12 (2015) 13415–13434, http://dx.doi.org/10.3390/ijerph121013415.

[16] K. Sellers, N.M.E. Deleebeeck, M. Messiaen, M. Jackson, E.A.J. Bleeker, D.T.H.M. Sijm, F.A. van Broekhuizen, Grouping Nanomaterials - A Strategy towards Grouping and Read-Across, National Institute for Public Health and the Environment (RIVM) & Arcadis, 2015.

[17] J.H.E. Arts, M. Hadi, M.A. Irfan, A.M. Keene, R. Kreiling, D. Lyon, M. Maier, K. Michel, T. Petry, U.G. Sauer, D. Warheit, K. Wiench, W. Wohlleben, R. Landsiedel, Regul. Toxicol. Pharmacol. 71 (2015) S1–S27, http://dx.doi.org/10.1016/j.yrtph.2015.11.020.

[18] J.H.E. Arts, M.A. Irfan, A.M. Keene, R. Kreiling, D. Lyon, M. Maier, K. Michel, N. Neubauer, T. Petry, U.G. Sauer, D. Warheit, K. Wiench, W. Wohileben, R. Landsiedel, Regul. Toxicol. Pharmacol. 76 (2016) 234–261, http://dx.doi.org/10.1016/j.yrtph.2015.11.020.

[19] OECD, Grouping and Read-across for the Hazard Assessment of Manufactured Nanomaterials. Report from the Expert Meeting. ENV/JM/MONO(2016)59 (2016), 2016 https://one.oecd.org/document/ENV/JM/MONO%282016%2959/en/pdf.

[20] A. Mech, K. Rasmussen, P. Jantunen, L. Aicher, M. Alessandrelli, U. Bernauer, E.A.J. Bleeker, J. Bouillard, P. Di Prospero Fanghella, R. Draisci, M. Dusinska, G. Encheva, G. Flament, A. Haase, Y. Handzhiyski, F. Herzberg, J. Huwyler, N.R. Jacobsen, V. Jeliazkov, N. Jeliazkova, P. Nymark, R. Grafström, A.G. Oomen, M.L. Polci, C. Riebeling, J. Sandström, B. Shivachev, S. Stateva, S. Tanasescu, R. Tsekovska, H. Wallin, M.F. Wilks, S. Zellmer, M.D. Apostolova, Nanotoxicology 13 (2018) 1–23, http://dx.doi.org/10.1080/17435390.2018.1513092.

[21] ECHA, Appendix R.6-1 for nanoforms applicable to the Guidance on QSARs and Grouping of Chemicals, in: Guidance on Information Requirements and Chemical Safety Assessment, European Chemicals Agency, Helsinki, 2019 https://echa.europa.eu/documents/10162/23036412/appendix_r6_nanomaterials_en.pdf.

[22] ECHA, JRC, RIVM, Usage of (eco)toxicological Data for Bridging Data Gaps Between and Grouping of Nanoforms of the Same Substance – Elements to Consider. (ed͡eds), European Chemicals Agency (ECHA), Joint Research Centre (JRC), Dutch National Institute for Public Health and the Environment (RIVM), 2016 http://echa.europa.eu/documents/10162/13630/eco_toxicological_for_bridging_grouping_nanoforms_en.pdf.

[23] M. Park, J. Catalan, N. Ferraz, J. Cabellos, R. Vanhauten, S. Vazquez-Campos, G. Janer, Nanotoxicology 12 (2018) 652–676, http://dx.doi.org/10.1080/17435390.2018.1465142.

[24] F. Murphy, H.J. Johnston, S. Dekkers, E.A.J. Bleeker, A.G. Oomen, T.F. Fernandes, K. Rasmussen, P. Jantunen, H. Rauscher, N. Hunt, W. Wohlleben, V. Stone, To be submitted, (2020).

[25] OECD, Integrated Approaches to Testing and Assessment (IATA) (2018), 2018 http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm.

[26] K.G. Steinhäuser, P.G. Sayre, NanoImpact 7 (2017) 66–74, http://dx.doi.org/10.1016/j.impact.2017.06.001.

[27] K. Rasmussen, H. Rauscher, A. Mech, J.M. Riego Sintes, D. Gilliland, M. Gonzalez, P. Kearns, K. Moss, M. Visser, M. Groenewold, E. Bleeker, Regul. Toxicol. Pharmacol. 92 (2018) 8–28, http://dx.doi.org/10.1016/j.yrtph.2017.10.019.

[28] A.G. Oomen, K.G. Steinhäuser, E.A.J. Bleeker, F. Van Broekhuizen, A. Sips, S. Dekkers, S.W.P. Wijnhoven, P.G. Sayre, NanoImpact 9 (2018) 1–13, http://dx.doi.org/10.1016/j.impact.2017.09.001.

[29] L. Lamon, K. Aschberger, D. Asturiol, A. Richarz, A. Worth, Nanotoxicology, Online (2018), http://dx.doi.org/10.1080/17435390.2018.1506060.

[30] W. Wohlleben, C. Kingston, J. Carter, E. Sahle-Demessie, S. Vazquez-Campos, B. Acrey, C.Y. Chen, E. Walton, H. Egenolf, P. Muller, R. Zepp, Carbon 113 (2017) 346–360, http://dx.doi.org/10.1016/j.carbon.2016.11.011.

[31] USEPA, Chemical Substances When Manufactured or Processed as Nanoscale Materials; TSCA Reporting and Recordkeeping Requirements, 82 FR 22088, Office of the Federal Register, 2017, pp. 22088–22089 https://www.govinfo.gov/app/details/FR-2017-05-12/2017-09683.

[32] J.A.J. Meesters, W.J.G.M. Peijnenburg, A.J. Hendriks, D. Van de Meenta, J.T.K. Quik, Environ. Sci. Nano 6 (2019) 2049–2060, http://dx.doi.org/10.1039/C9EN00117D.

[33] K. Rasmussen, M. Gonzalez, P. Kearns, J.M. Riego Sintes, F. Rossi, P. Sayre, Regul. Toxicol. Pharmacol. 74 (2016) 147–160, http://dx.doi.org/10.1016/j.yrtph.2015.11.004.

[34] S. Totaro, G. Cotogno, K. Rasmussen, F. Pianella, M. Roncaglia, H. Olsson, J. Riego Sintes, H. Crutzen, The JRC Nanomaterials Repository: a unique facility providing representative test materials for nanoEHS research, Regul. Toxicol. Pharm. 81 (2016) 334–340, http://dx.doi.org/10.1016/j.yrtph.2016.08.008.

[35] A.P. Mustad, B. Smeets, N. Jeliazkova, V. Jeliazkov, E. Willighagen, Summary of the Spring NSC Database Survey (2014), 2014, http://dx.doi.org/10.6084/m9.figshare.1195888.v1.

[36] P. Ritchie, I. Basinas, M. van Tongeren, H. Crutzen, S. Totaro, Landscape of Databases Useful to EHS Assessment of Nanomaterials – Gaps and Overlaps Review (2016), 2016 https://www.rivm.nl/en/About_RIVM/Mission_and_strategy/International_Affairs/International_Projects/Completed/ProSafe/ProSafe_Deliverables.

[37] A. Haase, F. Klaessig, EU US Roadmap Nanoinformatics 2030 (2017), 2017 https://www.nanosafetycluster.eu/uploads/files/pdf/Interim%20Roadmap_Complete_published_V2.pdf.

[38] D. Comandella, S. Gottardo, I.M. Rio-Echevarria, H. Rauscher, Nanoscale 12 (2020), http://dx.doi.org/10.1039/C9NR08323E.

[39] S. Gottardo, M. Alessandrelli, V. Amenta, R. Atluri, G. Barberio, C. Bekker, P. Bergonzo, E. Bleeker, A.M. Booth, T. Borges, P. Buttol, D. Carlander, S. Castelli, S. Chevillard, S. Clavaguera, S. Dekkers, C. Delpivo, P. Di Prospero Fanghella, M. Dusinska, J. Einola, E. Ekokoski, C. Fito, H. Gouveia, R. Grall, K. Hoehener, P. Jantunen, G. Johanson, P. Laux, H.C. Lehmann, R. Leinonen, A. Mech, C. Micheletti, C. Noorlander, M. Olof-Mattsson, A. Oomen, L. Quiros Pesudo, M.L. Polci, A. Prina-Mello, K. Rasmussen, H. Rauscher, A. Sanchez Jimenez, J.M. Riego Sintes, S. Scalbi, J.-A. Sergent, H. Stockmann-Juvala, M. Simko, A. Sips, B. Suarez, A. Sumrein, M. van Tongeren, S. Vázquez-Campos, N. Vital, T. Walser, S. Wijnhoven, H. Crutzen, NANoREG framework for the safety assessment of nanomaterials. JRC Science for Policy Report EUR 28550 EN, European Union, 2017 https://doi.org/10.2760/245972.

[40] ECHA, Chapter R.4: Evaluation of Available Information, Version 1.1, in: Guidance on Information Requirements and Chemical Safety Assessment, European Chemicals Agency, Helsinki, 2011 https://echa.europa.eu/documents/10162/13643/information_requirements_r4_en.pdf.

[41] H.J. Klimisch, M. Andreae, U. Tillmann, Regul. Toxicol. Pharmacol. 25 (1997) 1–5, http://dx.doi.org/10.1006/rtph.1996.1076.

[42] K. Schneider, M. Schwarz, I. Burkholder, A. Kopp-Schneider, L. Edler, A. Kinsner-Ovaskainen, T. Hartung, S. Hoffmann, Toxicol. Lett. 189 (2009) 138–144, http://dx.doi.org/10.1016/j.toxlet.2009.05.013.

[43] J.W. Card, B.A. Magnuson, Int. J. Toxicol. 29 (2010) 402–410, http://dx.doi.org/10.1177/1091581810370720.

[44] DaNa, Methodology of Selection, Acquisition and Evaluation of Toxicological Publications in the Project DaNa. DaNa 2.0, Information about Nanomaterials and Their Safety Assessment, 2016 https://www.nanopartikel.info/files/methodik/DaNa_criteria_checklist_2016tox_en.pdf.

[45] M.L. Fernandez-Cruz, D. Hernandez-Moreno, J. Catalan, R.K. Cross, H. Stockmann-Juvala, J. Cabellos, V.R. Lopes, M. Matzke, N. Ferraz, J.J. Izquierdo, J.M. Navas, M. Park, C. Svendsen, G. Janer, Environ. Sci. Nano 5 (2018) 381–397, http://dx.doi.org/10.1039/C7EN00716G.

[46] N.B. Hartmann, M. Agerstrand, H.C.H. Lutzhoft, A. Baun, Nanoimpact 6 (2017) 81–89, http://dx.doi.org/10.1016/j.impact.2017.03.004.

[47] L.G. Soeteman-Hernandez, M.D. Apostolova, C. Bekker, S. Dekkers, R.C. Grafstrom, M. Groenewold, Y. Handzhiyski, P. Herbeck-Engel, K. Hoehener, V. Karagkiozaki, S. Kelly, I. Llopis, S. Sabella, A. Sanchez Jimenez, A.J.A.M. Sips, B. Suarez-Merino, I. Tavernaro, J. van Engelen, S.W.P. Wijnhoven, C.W. Noorlander, Mater. Today Commun. 20 (2019), http://dx.doi.org/10.1016/j.mtcomm.2019.100548.

[48] OECD, Guidance Document on the Reporting of Defined Approaches to be Used Within Integrated Approaches to Testing and Assessment, OECD Series on Testing and Assessment No. 255, Organisation for Economic Cooperation and Development, 2017 https://doi.org/10.1787/9789264274822-en.

[49] R.G. Cooper, Res. Manag. 60 (2017) 48–52, http://dx.doi.org/10.1080/08956308.2017.1255057.

[50] EFSA, Guidance on Risk Assessment of the Application on Nanoscience and Nanotechnolgies in the Food and Feed Chain: Part 1, Human and Animal Health, 2018 https://www.efsa.europa.eu/en/efsajournal/pub/5327.

[51] W.J.G.M. Peijnenburg, E. Ruggiero, M. Boyles, F. Murphy, V. Stone, D.A. Elam, K. Werle, W. Wohlleben, Materials 13 (2020), http://dx.doi.org/10.3390/ma13102235.

[52] F.L. Filon, M. Crosera, G. Adami, M. Bovenzi, F. Rossi, G. Maina, Nanotoxicology 5 (2011) 493–501, http://dx.doi.org/10.3109/17435390.2010.551428.

[53] O. Lee, S.H. Jeong, W.U. Shin, G. Lee, C. Oh, S.W. Son, Ski. Res. Technol. 19 (2013) E390–E396, http://dx.doi.org/10.1111/j.1600-0846.2012.00656.x.

[54] N. Sadrieh, A.M. Wokovich, N.V. Gopee, J.W. Zheng, D. Haines, D. Parmiter, P.H. Siitonen, C.R. Cozart, A.K. Patri, S.E. McNeil, P.C. Howard, W.H. Doub, L.F. Buhse, Toxicol. Sci. 115 (2010) 156–166, http://dx.doi.org/10.1093/toxsci/kfq041.

[55] C. Miquel-Jeanjean, F. Crepel, V. Raufast, B. Payre, L. Datas, S. Bessou-Touya, H. Duplan, Photochem. Photobiol. 88 (2012) 1513–1521, http://dx.doi.org/10.1111/j.1751-1097.2012.01181.

[56] K. Adachi, N. Yamada, Y. Yoshida, O. Yamamoto, Exp. Dermatol. 22 (2013) 278–283, http://dx.doi.org/10.1111/exd.12121.

[57] F.A. Murphy, C.A. Poland, R. Duffin, K.T. Al-Jamal, H. li-Boucetta, A. Nunes, F. Byrne, A. Prina-Mello, Y. Volkov, S. Li, S.J. Mather, A. Bianco, M. Prato, W. MacNee, W.A. Wallace, K. Kostarelos, K. Donaldson, Am. J. Pathol. 178 (2011) 2587–2600, http://dx.doi.org/10.1016/j.ajpath.2011.02.040.

[58] J. Muller, M. Delos, N. Panin, V. Rabolli, F. Huaux, D. Lison, Toxicol. Sci. 128 (2012), http://dx.doi.org/10.1073/pnas.1110013108, 553-553.

[59] H. Nagai, Y. Okazaki, S.H. Chew, N. Misawa, Y. Yamashita, S. Akatsuka, T. Ishihara, K. Yamashita, Y. Yoshikawa, H. Yasui, L. Jiang, H. Ohara, T. Takahashi, G. Ichihara, K. Kostarelos, Y. Miyata, H. Shinohara, S. Toyokuni, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) E1330–E1338, http://dx.doi.org/10.1073/pnas.1110013108.

[60] Y. Sakamoto, M. Hojo, Y. Kosugi, K. Watanabe, A. Hirose, A. Inomata, T. Suzukil, D. Nakae, J. Toxicol. Sci. 43 (2018) 587–600, http://dx.doi.org/10.2131/jts.43.587.

[61] A. Schinwald, F.A. Murphy, A. Prina-Mello, C.A. Poland, F. Byrne, D. Movia, J.R. Glass, J.C. Dickerson, D.A. Schultz, C.E. Jeffree, W. MacNee, K. Donaldson, Toxicol. Sci. 128 (2012) 461–470, http://dx.doi.org/10.1093/toxsci/kfs171.

[62] A. Praetorius, E. Badetti, A. Brunelli, A. Clavier, J.A.G. Gallego-Urrea, A. Gondikas, M. Hassellov, T. Hofmann, A. Mackevica, A. Marcomini, W. Peijnenburg, J.T.K. Quik, M. Seijo, S. Stoll, N. Tepe, H. Walch, F. von der Kammer, Environ. Sci. Nano 7 (2020) 351–367, http://dx.doi.org/10.1039/C9EN01016E.

[63] J.R. Lead, G.E. Batley, P.J.J. Alvarez, M.N. Croteau, R.D. Handy, M.J. McLaughlin, J.D. Judy, K. Schirmer, Environ. Toxicol. Chem. 37 (2018) 2029–2063, http://dx.doi.org/10.1002/etc.4147.

[64] K.L. Garner, S. Suh, A.A. Keller, Environ. Sci. Technol. 51 (2017) 5541–5551, http://dx.doi.org/10.1021/acs.est.6b05279.

[65] G. Basei, D. Hristozov, L. Lamon, A. Zabeo, N. Jeliazkova, G. Tsiliki, A. Marcomini, A. Torsello, Nanoimpact 13 (2019) 76–99, http://dx.doi.org/10.1016/j.impact.2019.01.003.

[66] N. Burden, K. Aschberger, Q. Chaudhry, M.J.D. Clift, S.H. Doak, P. Fowler, H. Johnston, R. Landsiedel, J. Rowland, V. Stone, Nano Today 12 (2017) 10–13, http://dx.doi.org/10.1016/j.nantod.2016.06.007.

**Vicki Stone** is Director of the Institute of Biological Chemistry, Biophysics and Bioengineering at Heriot-Watt University, Edinburgh, UK. Vicki is a toxicologist and has published over 165 publications with a h-index of 62. Vicki is coordinator of GRACIOUS https://www.h2020gracious.eu/. She was the holder of the Royal Society of Chemistries Toxicology Award (2015–16) was recognised (2015–2018) by Clarivate Analytics (formerly Thomson Reuters) as one of the top 1% of all researchers in the world for the most cited publications in the field of Pharmacology and Toxicology.

**Dr. Stefania Gottardo** holds a Ph.D. in Environmental Sciences from University Ca' Foscari Venice (UCV, Italy). She is an expert on risk assessment of nanomaterials and associated regulatory aspects at European level (e.g. REACH). She has been involved in several EU-funded research projects dealing with nanosafety, and contributed to the inventory of nanotechnology applications in agriculture, food and feed commissioned by the European Food Safety Agency (EFSA). She has also knowledge on chemicals and nanomaterials used for hydraulic fracturing of shale gas reservoirs. She has attended several international events and published peer-reviewed papers.

**Eric A.J. Bleeker**. Senior researcher, environmental toxicologist. Work package leader, co-leader and steering board member in several European projects (e.g. Gov4nano, NanoHarmony, GRACIOUS). He also contributed to several other European projects (e.g. NanoReg, ProSafe). He is active member in ECHA's Nanomaterial Expert Group and OECD Working Party on Manufactured Nanomaterials. He continues to actively contribute to guidance and test guidelines for regulatory risk assessment of nanomaterials. His research focusses on the interface between science and policy.

**Hedwig Braakhuis**. Researcher and European Registered Toxicologist. She is WP-leader in SAbyNA that focuses on safe-by-design methodologies for nanomaterials. She also contributes to PATROLS and GRACIOUS, and participated in GUIDEnano and NanoReg2. Her research focusses on the development of innovative, animal-free methods and strategies for assessing the hazard of nanomaterials and medical devices.

**Susan Dekkers**. Senior researcher, risk assessor human toxicology. She has acted as co-WP-leader in NANoREG and contributed or is still contributing to several other projects on the risk assessment of nanomaterials, including NanoMILE, NanoReg2, caLIBRAte, Gracious, REFINE and Gov4Nano. She is involved in scientific projects and advisory work on human risk assessment of chemical substances and nanomaterials in food, consumer products and occupational settings. She is a member of the ISO Technical Committee on nanotechnology and leading the OECD project on the development of a test guideline on the toxicokinetics of nanoparticles.

**Teresa F. Fernandes** is Professor of Environmental Science and Director of the Institute of Life and Earth Sciences at Heriot-Watt University, Edinburgh, UK. She is also co-Director of the Nanosafety Research Group at Heriot-Watt. She has over 25 years' experience working on the effects of stressors in natural systems and recovery. Over the last 15 years TF's work has concentrated mostly on the effects of nanomaterials (NMs) in the environment, closely working with scientists assessing the effects of NMs in human systems. This close collaboration is unique and has paved the way to the current approach assessing risk of NMs. Funded research projects include EU projects NanoImpactNet, Marina, NanoReg, FutureNanoNeeds, NanoSolutions, SUN, Nanomicex, Biobeauty, Gracious, Biorima, Patrols, amongst others.

**Andrea Haase** studied biochemistry at the University of Tübingen, obtained a PhD from the University of Heidelberg, finished a postgraduate toxicology study at the University of Leipzig and completed her habilitation in toxicology at the Freie Universität Berlin. Since 2008 she is working at the German Federal Institute for Risk Assessment (BfR) in Berlin, where she heading the unit "Fibre- and Nanotoxicology". Since 2008 Andrea's work is focused on nanomaterials, addressing the integration of nanomaterials in different EU regulatory frameworks and including nanosafety research. Andrea has published more than 50 scientific publications and was involved in several large research projects.

**Dr Neil Hunt** has worked in the regulatory arena for Yordas Ltd for over eight years. During this time he has managed all aspects of the REACH and heads the services for nanomaterials. Neil has played an active role in a variety of EU funded projects (inc. MARINA, SUN, GRACIOUS). He has presented internationally to both industry and the research community. Neil has represented the Nanotechnology Industry Association on ECHA's Partner Expert Group for nanomaterials. Previously, he was a process development chemist and is a named inventor on a number of process patents.

**Dr. Danail Hristozov** is the Head of Research at Greendecision Srl, a spin-off company of University of Venice in Italy. There he researches the risks of nano and biomaterials used in consumer products and medicine as part of several EU H2020 projects. Danail has been a senior researcher at University of Venice, where he coordinated the large EU FP7 project SUN. Over the last decade Danail has worked also for the private sector companies Venice Research Consortium and Veneto Nanotech. In his early years, Danail worked at the Chair of Industrial Sustainability of the Brandenburg University of Technology in Germany.

**Dr. Paula Jantunen** is an environmental scientist and obtained a PhD in ecotoxicology from the University of Eastern Finland in 2011. After finishing her doctoral thesis work on the role of sorption in the ecological risk assessment of xenobiotics, she worked in chemical safety consulting in Finland until joining the Nanosafety and Regulatory Methods team at the European Commission's Joint Research Centre in 2015. Her interests include the environmental fate of nanomaterials and tools for their regulatory safety assessment.

**Nina Jeliazkova** received MSc in Computer Science from the ITMO University, St. Petersburg, Russia in 1991, and PhD in Computer Science in 2001 (joint supervision by the Laboratory for Mathematical Chemistry, Burgas, Bulgaria and Bulgarian Academy of Science in Sofia). She is founder and co-owner of Ideaconsult Ltd and is technical manager of the company since 2009. She has expertise in machine learning, cheminformatics, databases and data management for chemicals and nanomaterials, software engineering and cloud computing. She is associate editor in Journal of Cheminformatics since 2018.

**Dr Helinor Johnston** is an associate professor of toxicology at Heriot Watt University and specialises in the assessment of nanomaterial hazard to human health. Her current research focuses on investigating the suitability of employing alternative, non-rodent systems to screen nanomaterial safety (e.g. *in vitro* models and zebrafish larvae) at different target sites including the lung, liver, intestine, skin and immune system. Helinor was previously a scientific advisor at DEFRA and worked as a post-doc on desk based projects which identified knowledge gaps in nanotoxicology. Helinor's PhD evaluated the toxicity of nanomaterials to the liver in vitro (Edinburgh Napier University).

**Lara Lamon's** research activity has been focusing on pollutants emissions and fate in environmental compartments, and on their exposure and effects on human health and on ecosystems. Her field of expertise covers Chemicals environmental risk assessment, including environmental fate of chemicals and their impact on the environment and on human health. Her expertise also encompasses the field of Nanosafety, regarding the classification and grouping of nanomaterials for the purpose of hazard assessment. She is also interested in how in silico methods are implemented and accepted by regulators when assessing the impact of chemicals on the environment and on human health.

**Dr Fiona Murphy** is a Research Associate in the Nano-Safety Research Group at Heriot-Watt University, Edinburgh. Prior to joining Heriot-Watt in 2018, Fiona held the positions of Research Assistant in the Centre for Inflammation Research at the University of Edinburgh and Career Development Fellow with the MRC Toxicology Unit, Leicester. Fiona completed her PhD entitled 'The role of carbon nanotube structure in their retention and pathogenicity in the pleural cavity' at the University of Edinburgh under the supervision of Prof Ken Donaldson. Fiona has a particular interest in assessing the inhalation toxicity of high aspect ratio and fibrous (nano)materials.

**Kirsten Rasmussen**. Since 1988 Kirsten Rasmussen, MSc Chem. Eng. from the Technical University of Denmark, has worked for the European Commission's Joint Research Centre. Since 2008 she has studied the scientific/regulatory aspects of nanomaterials, e.g. in the OECD Working Party on Manufactured Nanomaterials, and nanotechnology standardisation in ISO and CEN. She was detached to the European Chemicals Agency 2007/2008 to start-up its Committee on Risk Assessment, based on her experience with chemicals legislation and risk assessment e.g. from her role as co-ordinator of the JRC support to the new (1998) Biocides Directive, defining the processes for the review and assessment of active substances, and chairing the Biocides technical committee.

**Dr. Hubert Rauscher** is a Scientific Officer and Principal Administrator at the European Commission's Joint Research Centre (JRC) and holds a PhD in Physics from the Technical University of Munich (Germany). He carried out postdoctoral research at the University of Wisconsin-Madison (USA) and worked on the physical chemistry of nanomaterials at the University of Ulm (Germany), where he was appointed Lecturer in Physical Chemistry in 1999. He joined the JRC in 2005. Hubert is an expert on the physico-chemical characterisation of nanomaterials and supports the European Commission in the implementation of nanomaterial-specific provisions in Community legislation related to the characterisation and safety of nanomaterials. He leads the JRC's activities on the review, revision and implementation of the EC's definition of nanomaterial.

**Araceli Sánchez Jiménez** is a Senior Exposure Scientist at the Institute of Occupational Medicine in the UK. Her research focuses on the understanding of the processes that chemical, biological or physical agents undergo from emission through transport and final uptake by humans. Specifically she addresses exposure markers, and develops exposure assessment methodologies and models that can be used in risk assessment and epidemiological research. She graduated in Chemistry from University Complutense in Madrid and obtained an MPhil and then a PhD in Environmental Pollution and Health from Strathclyde University in Glasgow. She also holds an MSc in Occupational Hygiene from Manchester University.

**Dr Claus Svendsen** (PhD in Ecotoxicology Reading University 2000) is Group Leader for Ecotoxicology and Chemical Risk at the UKRI-CEH. Leads projects on nanoecotoxicology, microplastics, pesticides and biological pest control, comparative environmental genomics, bioavailability, and mixture toxicity with funding from UK and EU sources and stakeholders. Cooordinated the EU NMBP projects NanoFATE and NanoFASE, WP leader in GUIDEnano, NanoCommons, GRACIOUS, and participating in ACEnano, CaLIBRAte, NanoSolveIt. Co-chairs the Exposure Working Group of the EU NanoSafety Cluster and contributes to the EU-US CoRs. Has acted as advisor to ECHA and EFSA on nano risk assessment. 120+ papers; H-index = 42; > 5100 citations.

**Prof David Spurgeon** undertakes research on the ecotoxicological effects of contaminants including industrial pollutants, pesticides, nanomaterials and microplastics. Current research interests includes the assessment, modelling and mechanistic understanding of mixture toxicity, the fate and effect of micro-organic chemicals in waters and projects on the environmental hazards of micro- and nanoplastics in soil. He has contributed extensively to EU projects investigating the exposure and risk assessment of nanomaterials including in pristine and transformed form (EU GRACIOUS, H2020 caLIBRAte & NanoFASE). He has to date published over 130 ISI papers that have collectively been cited >9000 times.

**Socorro Vázquez-Campos**. Manager of the Human & Environmental Health & Safety (HEHS) Division at LEITAT Technological Centre. Her research aims at assessing the potential impact of nanomaterials and nanotechnology-based products on human health and the environment. Research activities in her group include: Monitoring the properties of nano-enabled products along the life cycle, nanomaterial characterization in complex matrices, human & environmental exposure assessment, nano(eco)toxicology and risk assessment. In the last ten years, she has coordinated and participated in numerous European and national projects in nanosafety, leading to numerous papers, contributions to several book chapters, conference proceedings, and presentations in international conferences and workshops.

**Wendel Wohlleben** is Senior Principal Scientist at BASF, Dept. of Material Physics, second affiliation with Dept. of Experimental Toxicology and Ecology. Research projects on advanced materials development and on the safety of particles. Studied physics (minor: chemistry) at the University Heidelberg and at the Ecole Normale Supérieure in Paris. PhD in 2003 from LMU Munich with a biophysical thesis on energy harvesting in photosynthesis, performed at the Max-Planck-Institute for Quantum Optics. Postdoc at Physical Chemistry, University Marburg. Visiting scientist relations at Dept. of Materials and Interfaces, Weizmann Institute, Rehovot and at Harvard TH Chan School of Public Health, Boston.

**Agnes G. Oomen**. Senior Researcher, toxicokineticist and experienced project leader. She has been acting as (co-)WP-leader and member of the steering board in a series of European projects such as MARINA, NANoREG and GRACIOUS. She is and has been project leader of a series of projects on grouping and read-across of nanomaterials as well as projects on human health risks and risk assessment methodologies, for example on the exposure and health risks of nanomaterials in food. She is an active member of the EFSA crosscutting Working Group on Nanotechnologies. Her work takes often place at the interface between science and policy.