Guidance in a Nutshell

GRACIOUS Framework

for Grouping and Read-Across of Nanomaterials and Nanoforms

gracious

Grouping, Read-Across, CharacterIsation and classificatiOn framework for regUlatory risk assessment of manufactured nanomaterials and Safer design of nano-enabled products

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This Guidance in a Nutshell is intended to be an introduction to the concepts used in the GRACIOUS Framework and presents the main steps the user will need to address during the use of the Framework. It is intended for use by interested parties who are new or relatively inexperienced to grouping nanoforms.

INTRODUCTION

Grouping is an approach that has been used for chemicals that lack the data needed to support risk assessment. The application of grouping reduces the need to commission expensive testing, especially using vertebrates. It is explicitly discussed in the EU REACH regulations (European Parliament, 2006) as a viable approach that registrants can use to satisfy some obligatory requirements within the registration of their substance(s).

Grouping can also be used to reduce the testing needed for risk assessment of nanomaterials. A nanomaterial of a particular substance (e.g. TiO2) may be available in multiple nanoforms (NFs) that vary in characteristics such as shape, size and coating.

Recent amendments to the Annexes of the European regulation REACH, require the identification and assessment of different NFs of the same substance (European Commission, 2018). This identification and assessment apply if the total annual tonnage of the substance placed on the market by the manufacturer or importer exceeds 1 tonne. This requirement applies to the total tonnage level of the substance, regardless of the amount of the individual NF produced. ECHA has recommended the use of grouping to avoid the need for extensive animal testing of each NF.

Grouping can also be used for non-regulatory reasons, such as to assist the introduction of Safe(r)-by-Design methods into product development of NFs and nano-enabled products (NEPs), or for the identification of suitable risk management measures for industrial or professional facilities using nanomaterials.

The project GRACIOUS (Grouping, Read-Across, Characterisation and classificatiOn framework for regUlatory risk assessment of manufactured nanomaterials and Safer design of nano-enabled products) was set up to develop and promote grouping and read-across approaches for nanomaterials. The GRACIOUS Framework provides the structure for a user to make their own grouping assessment and to generate the necessary scientific justification. A user needs to be aware that every grouping exercise will be different, so they will need to apply their own knowledge and experience to the design and interpretation of their studies when using the Framework to guide the structure of their investigations.

This “Guidance in a Nutshell” will give a very brief overview of the key aspects of the GRACIOUS Framework. If a user wants to know more about the Framework, we recommend that they read:

- The full GRACIOUS Framework Guidance Document (to be available from GRACIOUS project website https://www.h2020gracious.eu/). This document follows the same structure as the Guidance in a nutshell document, but gives more detail and also presents worked examples to allow the reader to see how the concepts presented can be applied in real-life.
- Deliverable reports and published articles from the GRACIOUS project. These documents present the detailed scientific experimentation and discussion used to develop the Framework, pre-defined hypotheses and associated supporting structures. A good introduction to the Framework can be found in Stone et al. (2020). Links to these documents can be found at https://www.h2020gracious.eu/library/publications.

The GRACIOUS Framework Structure

The Framework uses a stepwise approach, from which users can exit if they believe the grouping has been justified (or conclusively rejected). When moving through the steps of the Framework, an increasing amount and complexity of data is required.

The Framework consists of two steps (Figure 1):

1. The user collects a suite of basic information required for each NF under assessment.
2. Based on this basic information, the user can identify a hypothesis relevant to their purpose for grouping and test it using an appropriate Integrated Approach to Testing and Assessment (IATA). The GRACIOUS Framework has defined a range of hypotheses, developed IATAs suitable for use with these hypotheses and executed case studies with NFs to test the utility of the Framework. We recommend that a new user investigates whether these "pre-defined" hypotheses can be used before devising and testing their own hypothesis. The hypotheses must address three main aspects of the NFs considered for grouping (Figure 2):
   a. What they are (physicochemical characteristics)
   b. Where they go (fate and behaviour in the environment and/or toxicokinetics in the body)
   c. What they do (hazard)

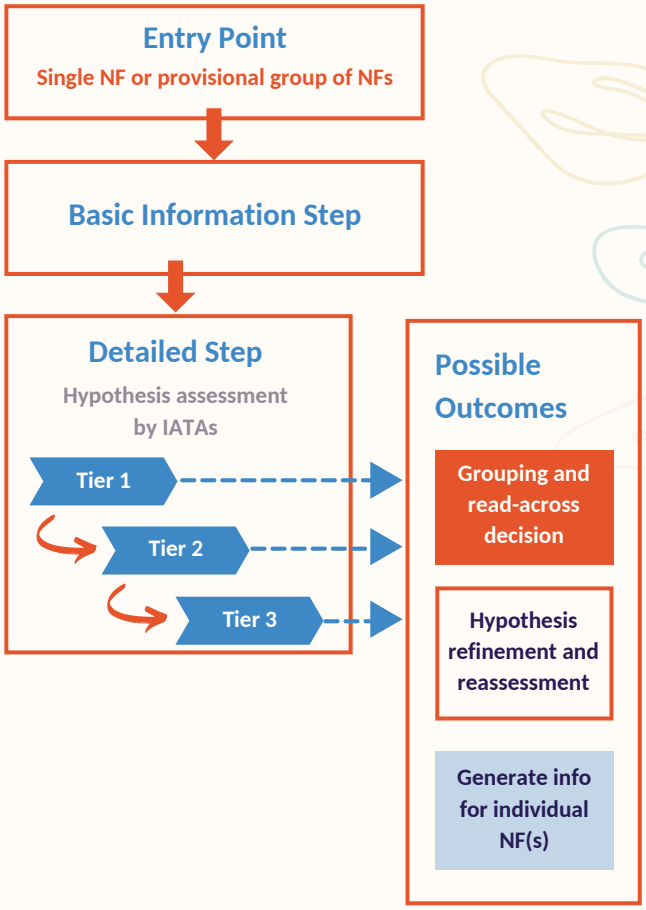
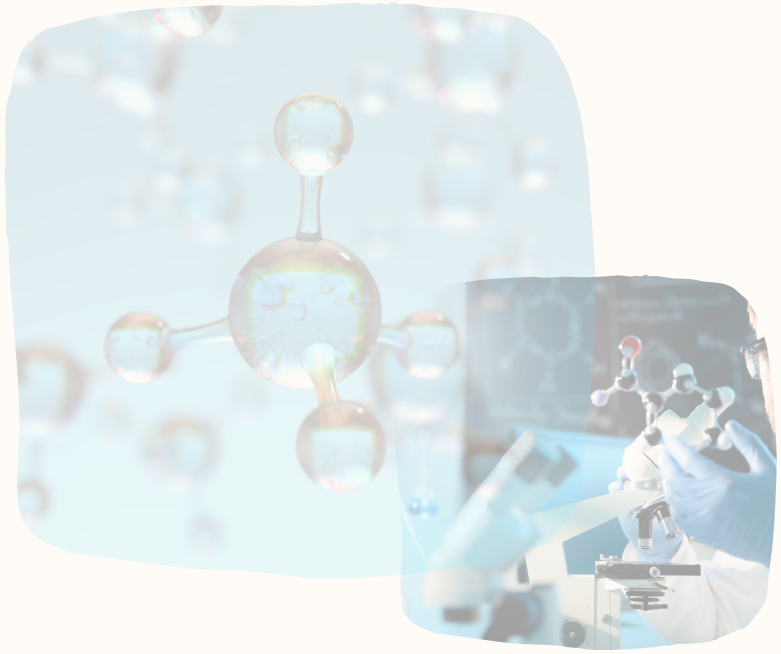


Figure 1: Basic structure of the GRACIOUS Framework.



Each IATA consists of a series of ***Decision Nodes*** that guide the user to collect the information needed via three *tiers* of information gathering.

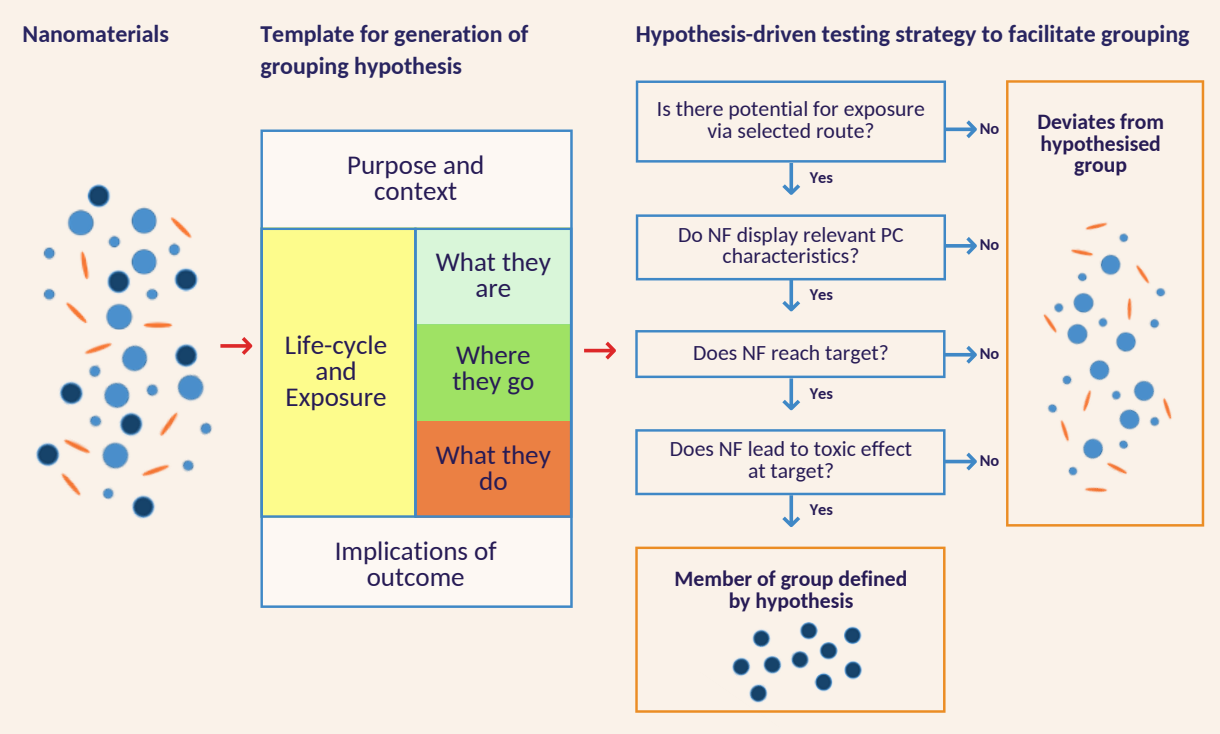


Figure 2: Diagram that shows how the hypotheses and associated IATAs are used to group NFs

The remainder of this document will introduce the detailed aspects of these steps and how the outcome of the grouping exercise can be used to address the purpose of grouping.

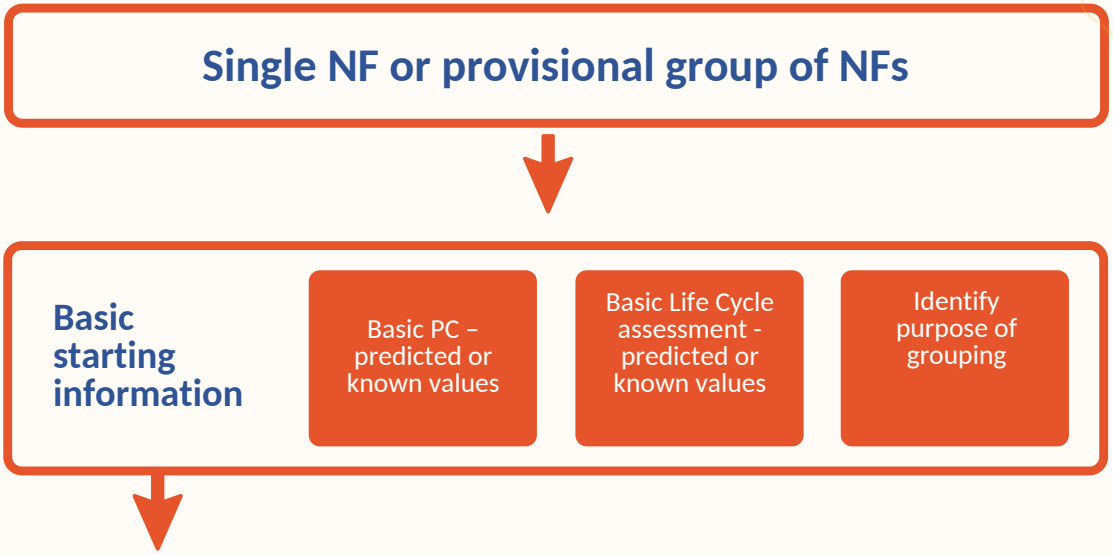
Entering the Framework: Which NF should be used?

The user needs to understand which NFs they wish to consider for grouping via the Framework. The Framework describes these NFs as ***Candidate NFs***. The choice of ***Candidate NFs*** may depend on the role of the user (e.g. industrial, regulatory authority) and the purpose of grouping. Some of the decisions that may need to be addressed at this stage could be whether:

- To include only NFs from the users' portfolio or whether other NFs should also be included;
- To use NFs of only a single substance or from different substances;
- To include only NFs or to include non-nanoforms (e.g. as sources for read-across).

The choice of NFs is not fixed at this stage, but if NFs (or non-NFs) are introduced as candidates later in the Framework, the user should be aware that they may need to retrospectively generate data for these new candidates.

Step 1: Basic Information Step



Grouping always needs a strong scientific justification, so good understanding of the Candidate NFs is essential before the next steps in the Framework can be undertaken. Many of the questions raised when progressing through an IATA will require that the identity and life cycle of each candidate NF are well-understood. If these aspects are missing or poorly defined, drawing a scientifically justified conclusion on grouping will be impossible.

Purpose of Grouping

Defining the purpose of grouping has a significant impact on how the Framework is used, so it is important that the choice of purpose is well-considered. The Framework is designed to support at least three different potential purposes:

- Regulatory Risk Assessment.
- Safe(r) by Design.
- Precautionary risk management measures.

Beyond identifying the basic purpose of grouping, the user should then elaborate their specific purpose. For example, a regulatory purpose may be further elaborated by identifying the specific hazard endpoints that grouping aims to address. The user can then start to identify how the purpose might impact on how the Framework is used, such as by understanding the level of scientific justification required for grouping and what type of studies can be used in the IATA.

Physicochemical Characterisation

A full understanding of "what they are" is essential for all aspects of hazard and risk assessment of NFs. This is a principle firmly established in the REACH Regulation (Annex VI, point 2.4), where both chemical and particle characterisation is needed for every NF registered. A set of basic physicochemical characteristics is required in the Basic Information step of the GRACIOUS Framework, irrespective of the purpose of grouping or the identity of the candidate NFs. The Framework requests the same characteristics as required in Annex VI of REACH. The following steps are recommended to ensure all necessary information is collected:

1. Identify required physicochemical parameters;
    a. Composition,
    b. Constituent particle size and size distribution,
    c. Particle shape
    d. Chemical nature of the surface,
    e. Specific surface area.
2. Identify the most appropriate technique for each property;
3. Perform the characterisation and collate results.

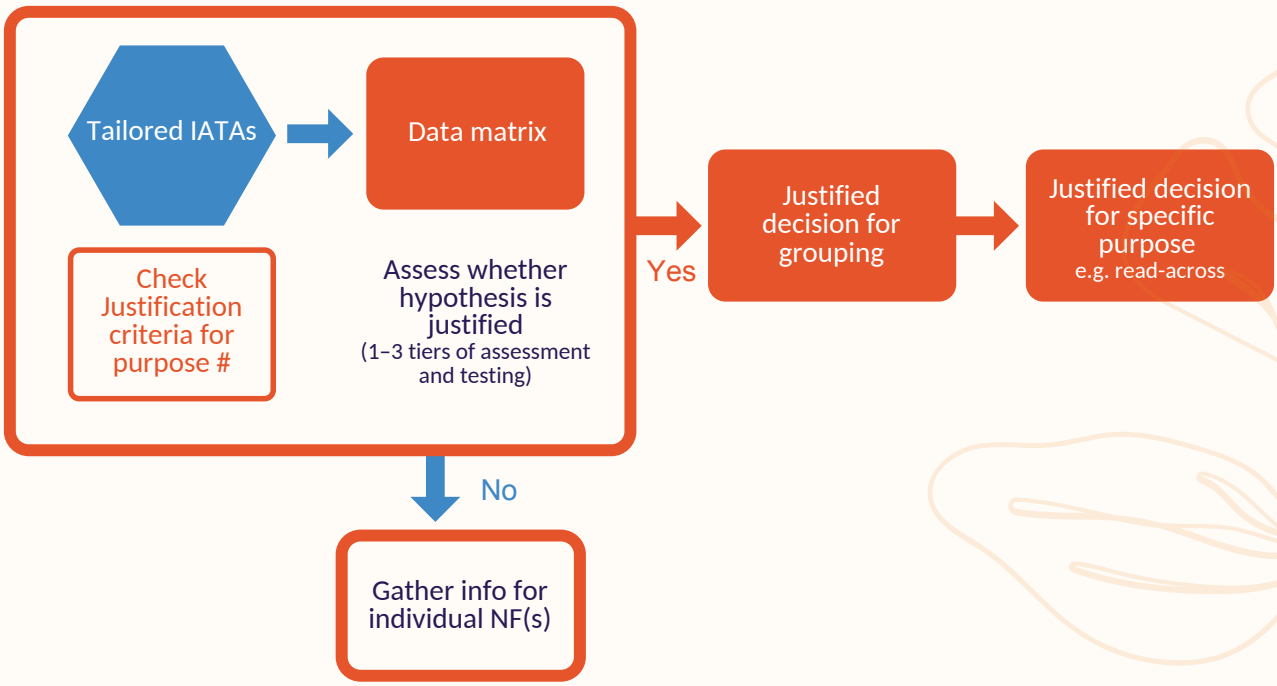
Uses and exposure scenario

Having a clear understanding of how NFs are (going to be) used, whether release may occur and how their physicochemical characteristics might change through the NF lifecycle are vital components of the basic information in the GRACIOUS Framework. They will allow the user to identify both the NFs and routes of exposure that require most urgent attention. The process can be split into two stages:

1. Description of uses, activities and exposure scenarios;
    a. Describe the life cycle of NFs and identify the target populations and the environmental compartments affected.
    b. Identify uses with similar release profiles.

2. Assess likelihood of release/exposure and the physicochemical form of NF during release/ exposure;
    a. Identify the likelihood of release and the physicochemical form of NF during release in relevant scenarios.
    b. Identify the likelihood of exposure.
    c. Identify the physicochemical form of NF during exposure.
    d. Refine exposure assessment using existing tools.

Step 2: Detailed Step - Testing the Grouping Hypothesis



Once the Basic Information for all candidate NFs is collected, it can be used to define the grouping hypothesis. The GRACIOUS project has defined 44 grouping hypotheses and developed IATAs to test each of these hypotheses. The hypotheses span across all primary routes of exposure to humans and across most of the major environmental compartments, and are referred to as pre-defined hypotheses. As much of the background work for these hypotheses has already been done (e.g. building IATAs, identification of suitable testing protocols, definition of decision nodes), the user should first investigate whether these pre-defined hypotheses apply to their NFs or whether they can modify them.

The process of identifying the most appropriate hypothesis occurs at the start of the Detailed Step of the Framework and is addressed in two parts in this Guidance Document.

Selecting a shortlist of pre-defined hypotheses

The collated Basic Information on physicochemical characteristics, along with the identified routes and types of exposure of concern allow identification of a shortlist of relevant pre-defined hypotheses. A list of all GRACIOUS pre-defined hypotheses is provided in the full GRACIOUS Framework Guidance Document

Refining the shortlist to identify the most relevant pre-defined hypothesis

In order to narrow down the selection of pre-defined hypotheses and identify a specific hypothesis to test, additional information is required. Information is gathered via IATAs that take the form of decision trees, consisting of a series of questions known as Decision Nodes. GRACIOUS lists several pre-defined hypotheses per route of exposure, or per environmental compartment. Different IATAs often have some early Decision Nodes in common, in particular when they address the same route of exposure or environmental compartment. By starting the IATA for one of the shortlisted hypotheses, it is therefore usually possible to examine the relevance of the other hypotheses in the shortlist. This can be done using the following process:

1. Identify the most probable hypothesis;
2. Start the IATA of the most probable hypothesis and use results to eliminate other hypotheses in the short-list;
3. Finalise the choice of hypothesis that will be tested (consider testing a few hypotheses in parallel to justify subgroups).

Using an IATA for a pre-defined hypothesis

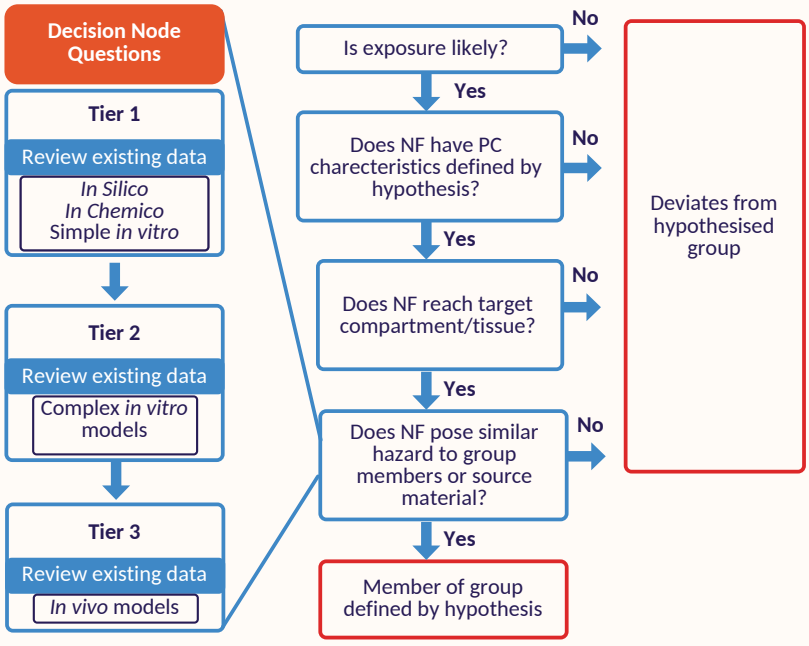


Figure 3: Use of a tiered testing strategy within the Decision Nodes of an IATA in the GRACIOUS Framework.

For each pre-defined hypothesis, the GRACIOUS project has designed an IATA as a structured way of identifying the most relevant information needed to reach a grouping conclusion with strong scientific justification. This information is gathered via the Decision Nodes that ask questions vital to assessing the grouping hypothesis. Answering 'yes' to each Decision Node question allows the user to move onto the next Decision Node and ultimately to accept the grouping hypothesis. Answering 'No' to any Decision Node leads to rejection of the hypothesis.

These questions can be answered via application of suggested experimental or in silico methods. The methods required or recommended at each Decision Node are organised into 3 tiers of increasing complexity, although not every Decision Node will have three tiers. Tier 1 generally consists of in silico, physicochemical, simple in vitro or acute invertebrate/algae/microbial studies, tier 2 uses more complex in vitro studies or longer-term invertebrate studies, and tier 3 requires in vivo or mesocosm studies (Figure 3). We recommend that the following steps are followed before starting the first Decision Node:

1. Identify the IATA associated with the chosen pre-defined hypothesis.
2. Construct the data matrix to include all data requirements of the IATA.
3. Populate the data matrix with available information and identify data gaps.
4. Identify potential source NF(s) or non-NF(s) for read-across (if required for the purpose).

At this point some Decision Nodes may have sufficient information to allow the user to answer the Decision Node question. If any answers are 'No' then the hypothesis can immediately be rejected, prompting the user to either consider a different hypothesis and IATA, or to refine the hypothesis or to take another approach other than grouping to support risk assessment.

If any Decision Node generates a 'Yes' answer, then the user proceeds to consider the remaining Decision Nodes. If any Decision Node includes data gaps the user proceeds to fill the data gaps. The user can either start at the top of the IATA with the first Decision Node, or they may choose to start with a Decision Node for which they can easily obtain data. For filling data gaps, the following steps are recommended:

1. Examine the data requirements of the Decision Node;
2. Fill data gaps by performing studies, usually starting with the lowest tier;
3. Once a tier is completed, decide whether there is sufficient quality and certainty of information to address the Decision Node or whether higher tier studies are needed.

If any Decision Node generates a 'No' answer for an NF at any time then the user may not need to complete information for all remaining Decision Nodes for that NF. Once all the Decision Nodes are complete, the user needs to make an assessment of the ***similarity*** of the candidate NFs for each Decision Node and the ***quality*** of the data used to draw a conclusion on whether the NFs can be grouped. The final conclusion of grouping can be any of the following:

- All candidate NFs can be grouped into a single group;
- Some candidate NFs can be grouped but others cannot. The ones that cannot may be moved to a different pre-defined hypothesis;
- None of the candidate NFs can be grouped.

Use of Data Quality Assessment within the GRACIOUS Framework

A scientifically justified grouping decision must be made using physicochemical and (eco)toxicological data in which there is sufficient confidence in their quality. This applies both to data extracted from external sources and for data generated to fill the data gaps. The GRACIOUS Framework recommends the use of a "traffic light" approach to assessing the overall quality of such data.

The data quality assessment approach is based on four established criteria (Basei *et al.*, 2021), namely:

1. Data completeness: which refers to the degree to which all required (meta)data in a data set is available;
2. Data reliability: which measures if a study was conducted in a reliable manner;
3. Data relevance: which measures if a study was conducted using agreed (standard) protocols/procedures;
4. Data adequacy: defining the usefulness of the data for risk assessment purposes.

Assessing Similarity in the GRACIOUS Framework

Similarity of substances is a concept commonly used both within REACH and beyond (Bender and Glen, 2004). It is used as the principal method to justify grouping in the GRACIOUS Framework. Similarity can be measured within a single Decision Node or across the whole data matrix.

Similarity (qualitative or quantitative) within a single Decision Node

1. If not recommended specifically by the pre-defined IATA, identify whether similarity is the best method to reach a conclusion for the Decision Node of interest.
2. Assess the range of values that can be measured (the dynamic range) and the range of values which are environmentally or biologically relevant (applicability range) of the property under examination to ensure the data considered falls into both ranges.
3. Consider data reduction to scalar descriptors (e.g. convert a concentration response curve to a single representative value such as the concentration effective at killing half of the cells or organisms in the experimental conditions (LC50)).
4. Understand achievable data accuracy.
5. Decide the type of similarity assessment required (quantitative or qualitative) ((Jeliazkova et al., submitted).
6. Choose the method for quantifying similarity and apply it to the data sets.
7. Identify the degree of similarity required to justify grouping and rely on provisional similarity limits in case studies (please see Full Guidance Document and NanoImpact special issue on NF similarity (2021-2022) to see how this is done).

Quantitative similarity assessment across the whole data matrix

1. Decide the type of similarity assessment required (pairwise for each DN or more multidimensional clustering approaches).
2. Choose the method for quantifying similarity and apply it to the data sets.
3. Identify the level of similarity required to define a group and rely on provisional similarity limits in case studies (NanoImpact special issue on NF similarity, 2021-2022).

Outcomes from Grouping

The outcome for grouping depends on the purpose for grouping. Although the exact purpose and outcome of a grouping exercise will be unique, it is expected that they would fall into one of the following:

Read-across

Once a group of NFs has been identified using the Framework, the user can conduct read-across from one or more source NFs or non-NFs, for which data and information exist, to a similar target NF where information is lacking. Read-across usually applies to higher tier data such as vertebrate studies. The read-across methodology includes:

1. Finalise the grouping of some (or all) of the candidate NFs;
2. Review the purpose and data matrix to identify data gaps that need to be filled by read-across.
3. Use the grouping hypothesis to generate a read-across hypothesis;
4. Check the proposed source NF (or non-NF) is part of the group and generate further data if required;
5. Assess the read-across hypothesis using the existing data matrix;
6. Fill data-gaps for target NFs if needed;
7. Document the read-across justification using the data matrix as evidence.

Safe(r) by Design

SbD approaches can apply at any stage of the development process, for example, to reduce hazard(s) of the product (e.g. by elimination of a hazardous substance and/or substitution for another one that is known to be safer). SbD approaches can also provide the information needed to reduce the release of nanomaterials from a product during its use, or from a process (e.g. coating of a NF to reduce dustiness). Grouping of NFs can be used to define the limits of certain physicochemical parameters within which NFs can be considered for further product development, e.g.because their use presents acceptable and controllable risks. Alternatively it can be used to identify which NFs should be excluded from further research and development.

Precautionary measures

The precautionary measures approach is likely to be applied in the manufacturing and downstream use of a material/product that already exists, but for which specific regulatory obligations do not apply. For example, grouping may be used to identify a single set of risk management approaches to be used across a range of NFs used on a site.

Writing a user-defined hypotheses

It is felt that the pre-defined hypotheses developed by the GRACIOUS project should cover most issues that a user could encounter in the current scientific and regulatory environment. However, it is possible that new toxicological concerns will arise in the future, so this section is intended to highlight the key principles that should be kept in mind if a user needs to write their own hypothesis and associated IATA.

Hypothesis

The hypothesis should cover:

- What they are – Relevant physicochemical characteristics;
- Where they go – Where the NF goes in the environment, how is the NF taken into, distributed around and excreted from the target organism;
- What they do – Mechanism/Mode of Action (MoA) causing the hazard;
- Use and release scenarios in order to identify exposed environmental compartments and routes of exposure for exposed organisms.

A template is available to support generation of a user-defined hypothesis.

IATA

Once the hypothesis is drafted, an IATA to test the hypothesis should be defined. It is recommended that the following issues should be addressed by the hypothesis:

1. Identify an existing IATA relevant to the route of exposure which can be edited to support the hypothesis testing;
2. Delete Decision Nodes from the existing IATA that are not relevant to the new user-defined hypothesis;
3. Identify additional Decision Nodes that are needed to investigate critical parameters (see above) of the hypothesis;
4. Identify studies that can detect or measure the critical parameters associated with each Decision Node;
5. Assign each study to a tier within a single Decision Node based on its complexity, resource intensiveness or whether it requires in vivo models;
6. Decide how a conclusion to each Decision Node will be made; this may be by using a fixed threshold or using a similarity assessment;
7. Examine which similarity method(s) can be used to draw a final conclusion.

Introduction to the tools to assist with the Framework

GRACIOUS Blueprint

The highly user-interactive nature of the GRACIOUS Framework can be supported via software. Rather than generating a stand-alone grouping software tool, GRACIOUS has generated a software blueprint that can be integrated into existing risk assessment or decision support tools. The GRACIOUS Blueprint is an object-oriented model and contains most of the generic decision logic used in the Framework described in decision tables and algorithms in a pseudo code manner. Therefore, the Blueprint is a document intended for software developers who want to implement the GRACIOUS Framework or parts of it into their software product.

GRACIOUS Wiki

The GRACIOUS Wiki was established to ensure that terminology was used in a consistent fashion across all of the GRACIOUS Framework. It is now hosted within the Terminology Harmonizer developed by GreenDecisions (https://terminology-harmonizer.greendecision.eu/), where it is joined by similar developments from other projects.

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Contacts

If you require further details on the GRACIOUS Framework then these can be obtained via the full Guidance Document or by contacting any of the following people:

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GRACIOUS FRAMEWORK

GUIDANCE IN A NUTSHELL

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