



Particles and Health 2021

How can Grouping and Read-Across support Safe(r)-by-design and regulation of Nanomaterials and Advanced Materials?

Vicki Stone

v.stone@hw.ac.uk

www.h2020gracious.eu



Introduction

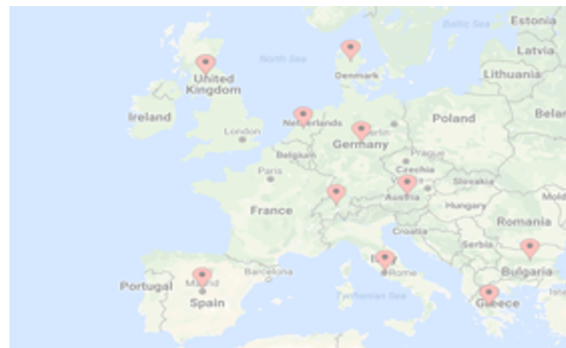


GRACIOUS H2020 Project

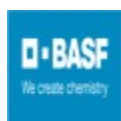
Aim

Generate a **Framework** to enable practical application of **grouping**, and subsequent **read-across** of nanomaterials (NMs)/nanoforms (NFs).

Aligns with EU legislation and needs of industry.



- Started January 2018
- Finish end Sept 2021
- 23 partners



National Research Centre
for the Working Environment

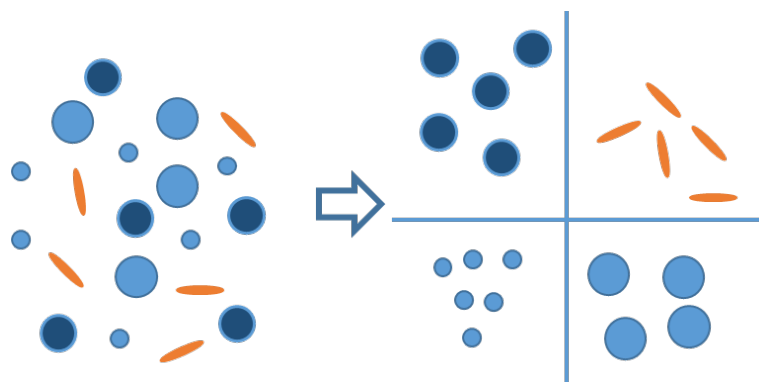


An alternative approach

Conventional risk assessment vs Grouping

- Conventional approach requires consideration of each substance on a case by case basis

- Expensive
- Time consuming
- Uses large numbers of animals



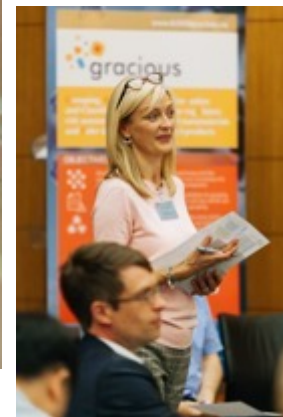
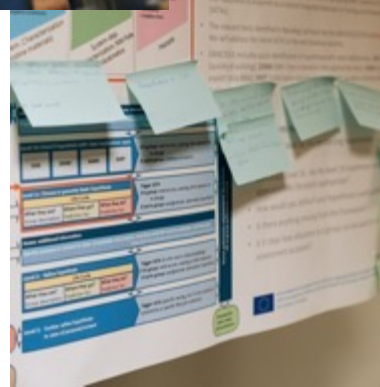
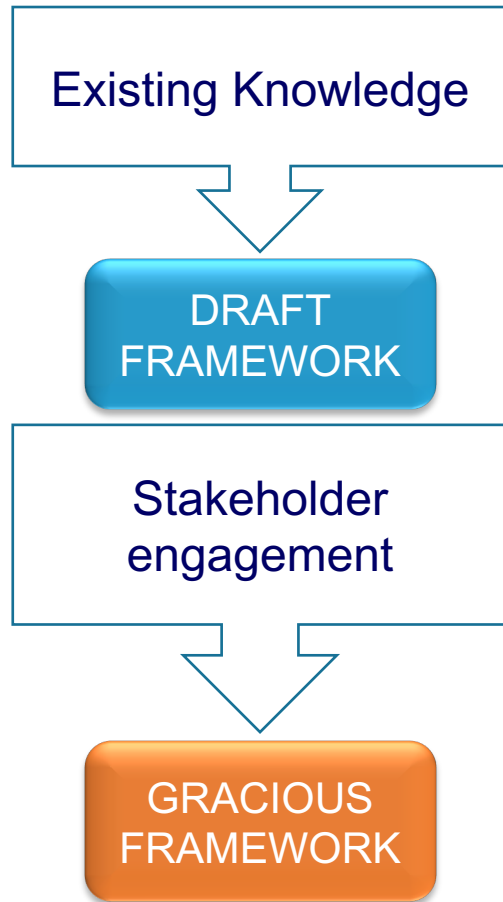
- Grouping provides intelligent methods of streamlining information gathering for risk assessment

"Substances whose physicochemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group" (REACH, Annex XI, 1.5).

GRACIOUS goes beyond qualitative similarity assessments by generating a range of protocols for quantitative similarity assessment that can support grouping in a regulatory dossier.

Framework design

Stakeholder Engagement



- EU policy makers
 - E.g. EC
- EU regulatory bodies
 - E.g. ECHA, EFSA, JRC
- European national government bodies
 - E.g. RIVM, NRCWE, BfR
- Non-EU regulatory bodies
 - E.g. US EPA, Health Canada
- Industry bodies
 - E.g. NIA, ECETOC and BIAC
- Industry
 - E.g. BASF, Black Diamond
- Consultants
 - E.g. Yordas, Blue Frog

Simple Form

GRACIOUS Framework



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 Jeliakzova^{g,} Helinor Johnston^{a,} Lara Lamon^{h,} Fiona Murphy^{a,} Kirsten Rasmussen^{b,} Hubert Rauscher^{b,} Araceli Sánchez Jiménez^{h,} Claus Svendsen^{i,} David Spurgeon^{l,} 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 Ideaconslult Ltd, Sofia, Bulgaria

^h Institute 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

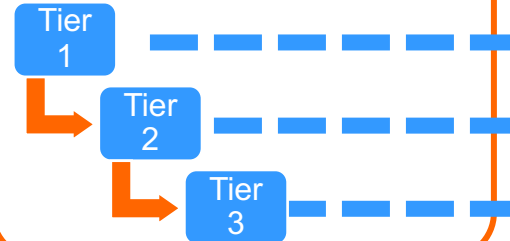
^j LEITAT Technological Center, Barcelona, Spain

^k BASF SE, Dept. Material Physics and Dept. of Experimental Toxicology & Ecology, Ludwigshafen, Germany

Entry Point
Single NF or provisional
group of NFs

Basic Information Step

Detailed Step
Hypothesis
assessment by IATAs



Applications

- Regulatory dossiers
- Support Innovation (SbD)
- Refine testing
- Precautionary measures

Possible
Outcomes

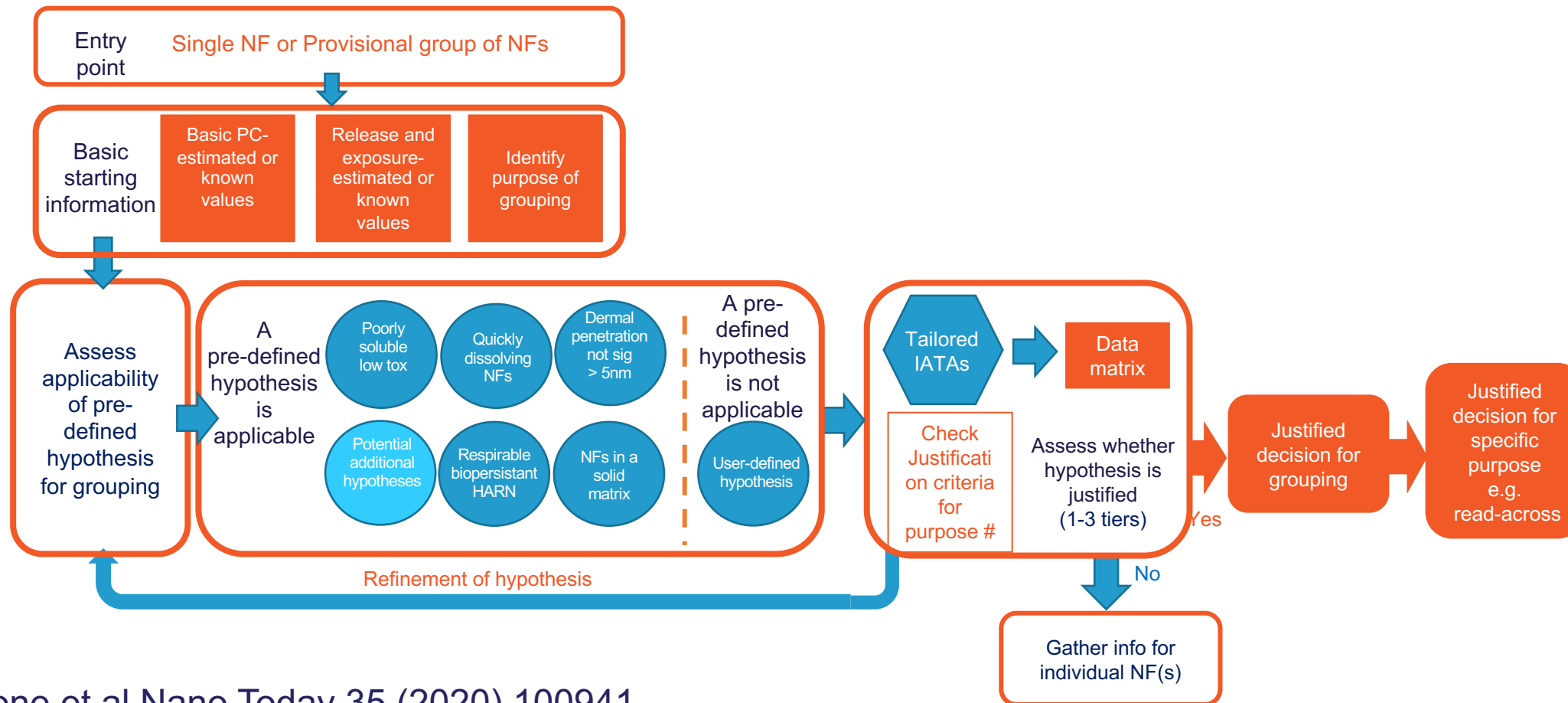
Grouping and
read-across
decision

Hypothesis
refinement and
reassessment

Generate info
for individual
NF(s)

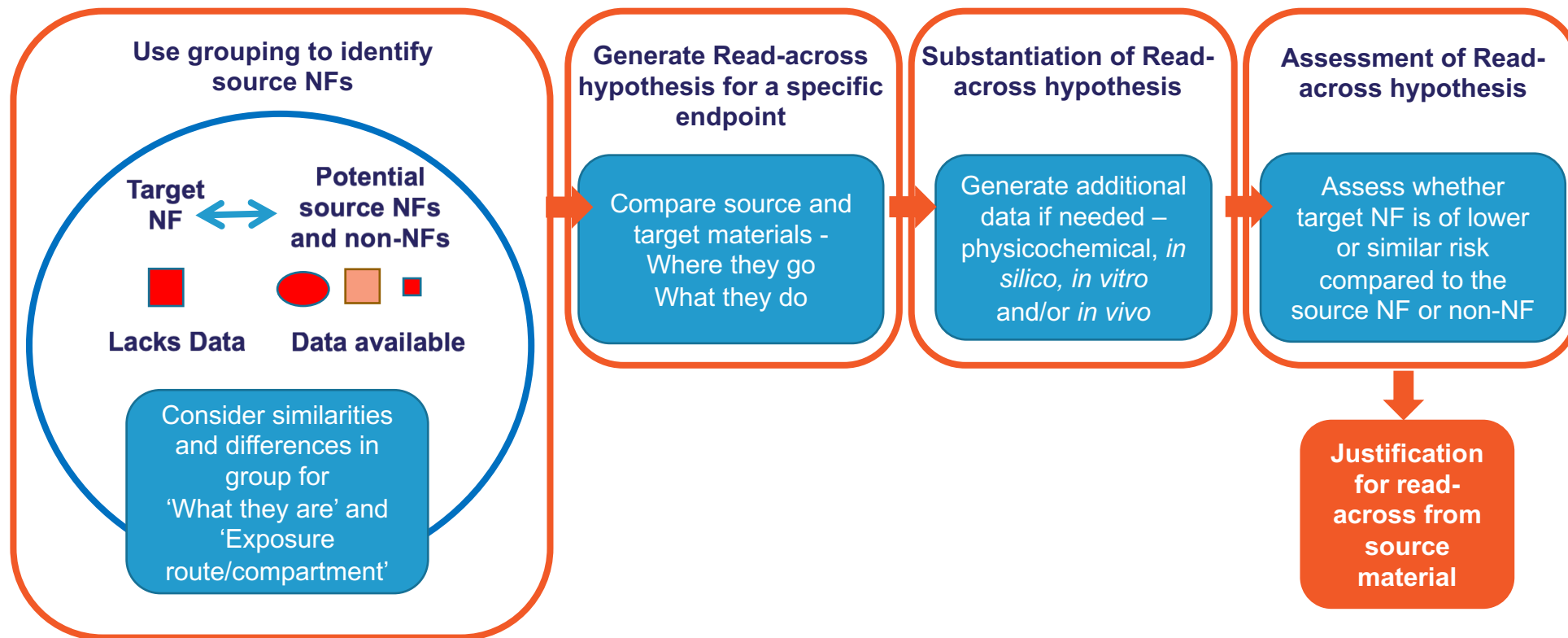
Detailed form

GRACIOUS Framework



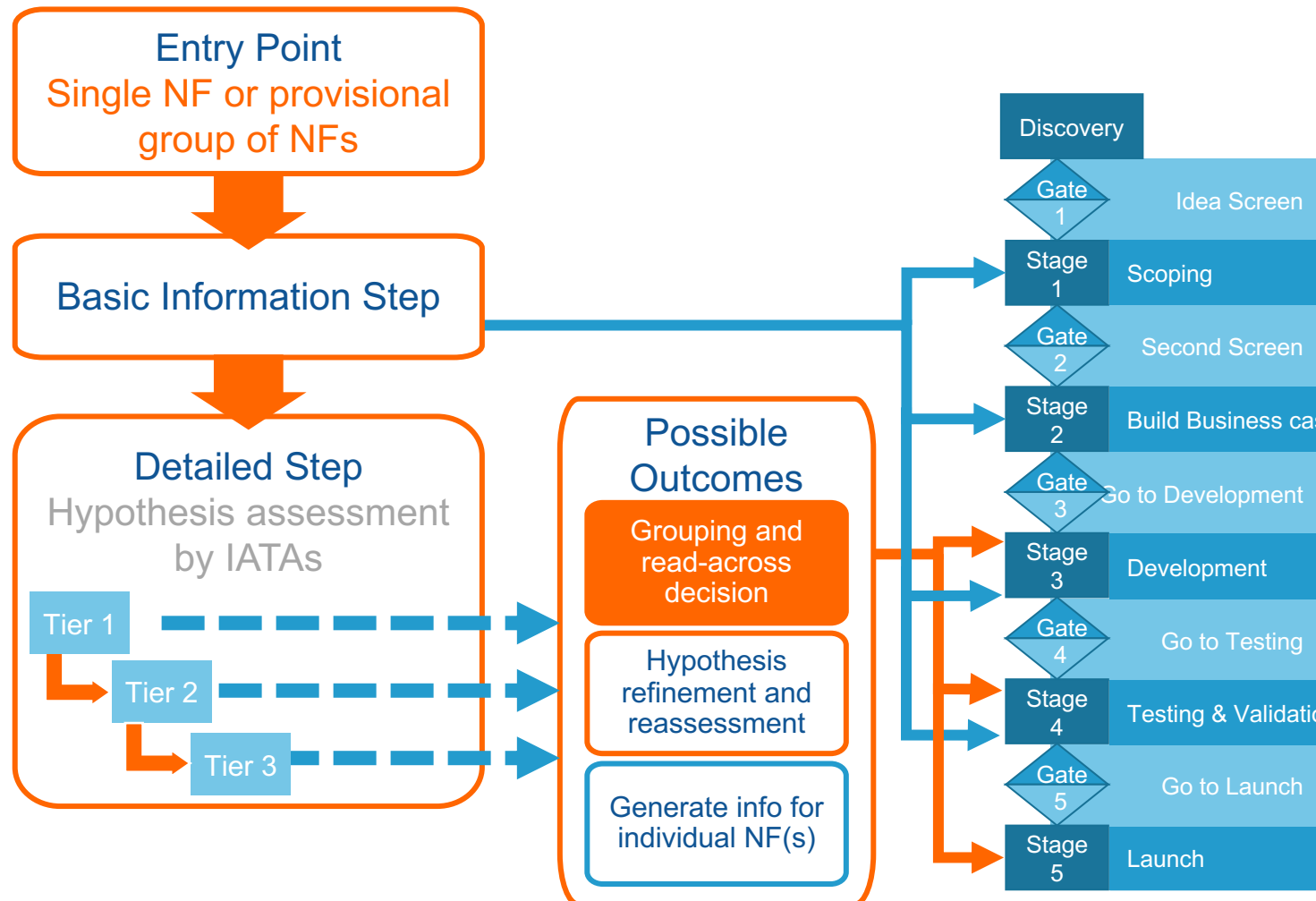
Applying the Framework

Using the Framework for Read-Across



Applying the Framework

Using the Framework for Safe(r) by Design



- During innovation, **safe(r)-by-design** approaches help to avoid expensive, time consuming, unexpected problems with new **nano-enabled products**
- Grouping and Read-across can be used during the innovation process
 - E.g. aid prioritization of lower hazard candidate NFs while ensuring product functionality

Grouping is Hypothesis Driven

Hypothesis Template

- There are many ways to word and formulate a hypothesis
- To provide guidance to the user GRACIOUS has developed a Hypothesis Template

Purpose and context	
Life Cycle	What they are?
	Where they go?
	What they do?

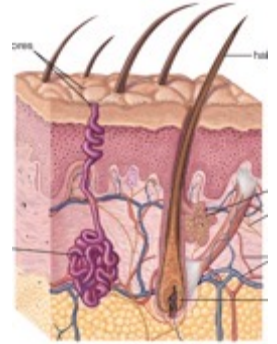
The Framework includes approx. 40 **pre-defined** hypotheses

- Based upon the literature and available data
 - 17 for human hazard
 - 23 for environmental hazard

Grouping

Hypothesis examples

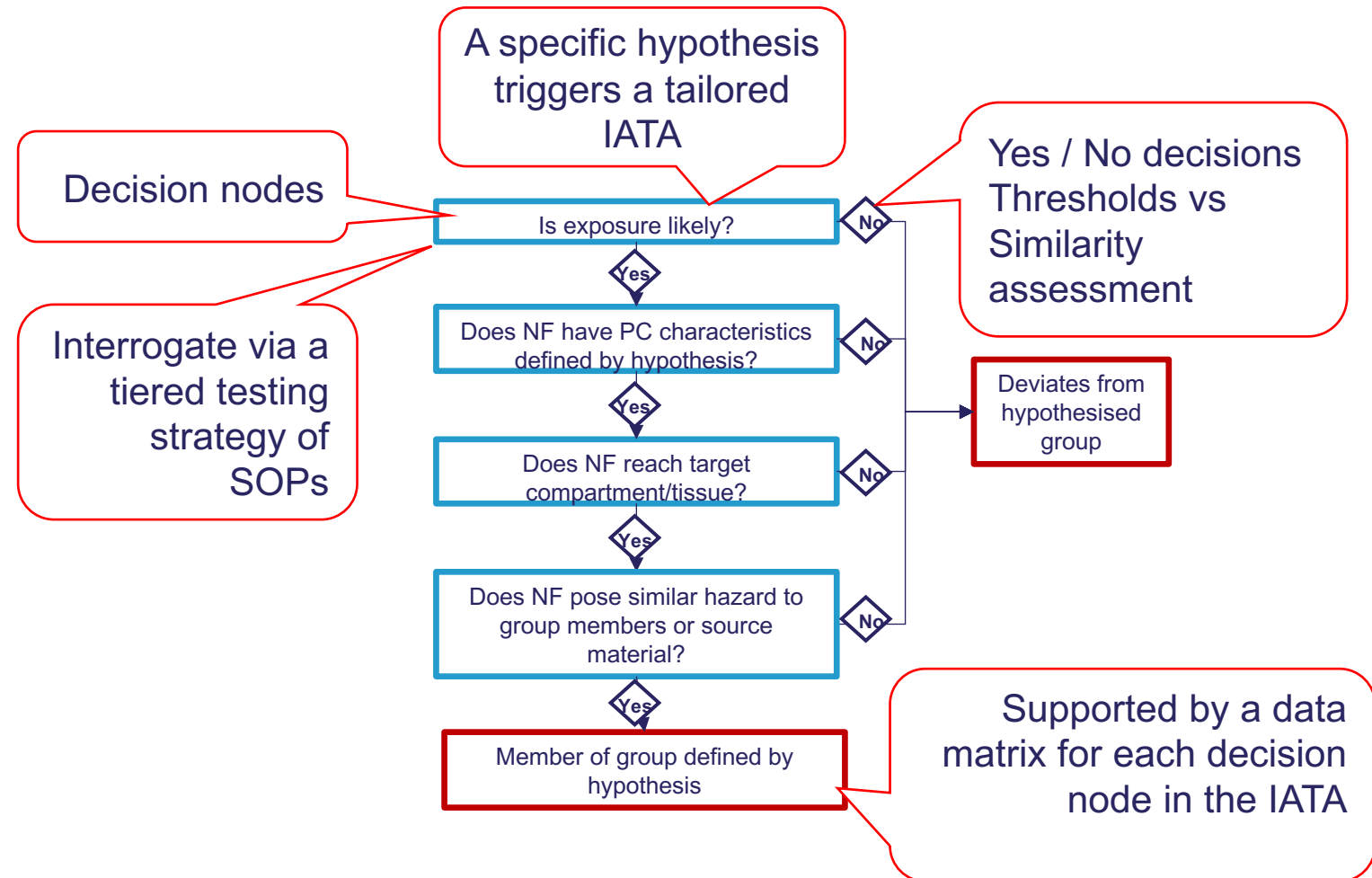
- **Respirable NFs showing quick dissolution:** Following **inhalation** both NFs and constituent ions or molecules may contribute to toxicity, but there is no concern for accumulation. Toxicity (also) depends on the location of the ionic or molecular release.
- NFs with **a chemical coating that is lost** from the NF surface following exposure in **soil** compartment can be grouped: Fate and toxicity of the exposure relevant NF can be considered similar to a non-coated analogous NF in soil compartment



Testing the Hypothesis

Integrated Approaches to Testing and Assessment

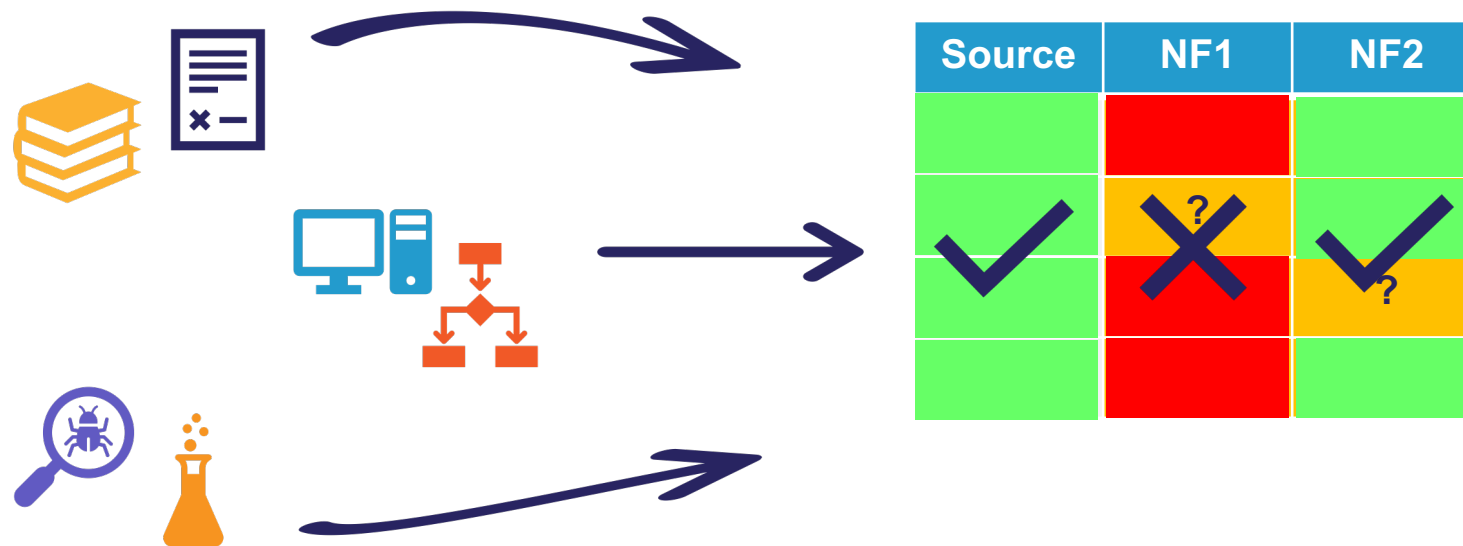
- In order to determine if NF(s) fit into a specific group, evidence is needed
- Scientific evidence is obtained via application of IATAs that are tailored to each hypothesis
- IATAs follow the format suggested by OECD
- IATAs are used to gather the information needed to test a specific hypothesis



IATAs

Data matrix

IATAs combine and integrates all relevant existing evidence and new data into a data matrix in order to support evidence-based grouping.



Oral route of exposure

Hypotheses relevant to grouping

Dissolution in
GIT fluids:

Instantaneous

Quick

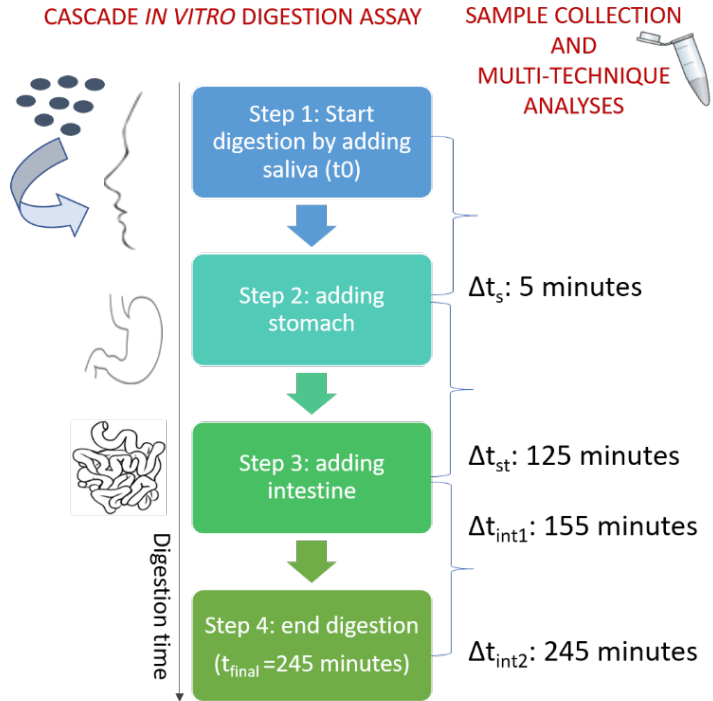
Partial

Very slow

Human oral hypotheses	
<u>H-O-I</u>	NFs with an instantaneous dissolution: Following oral exposure, the toxicity is driven by and is therefore similar to that of the constituent ions or molecules.
<u>H-O-Q1</u>	NFs with a quick dissolution: Following oral exposure both NFs and constituent ions or molecules may contribute to local inflammation in the OGI tract, but there is no concern for NF accumulation.
<u>H-O-Q3</u>	NFs with a quick dissolution: Following oral exposure both NFs and constituent ions or molecules may drive antimicrobial impacts (e.g. reducing microbial content and diversity within the OGI tract), but there is no concern for NF accumulation.
<u>H-O-P1</u>	NFs showing partial dissolution: Following oral exposure both NFs and constituent ions or molecules may lead to local inflammation in the OGI tract.
<u>H-O-P2</u>	NFs showing partial dissolution: Following oral exposure both NFs and constituent ions or molecules may translocate to secondary target organs and may lead to systemic toxicity in secondary organs.
<u>H-O-P3</u>	NFs showing partial dissolution: Following oral exposure both NFs and constituent ions or molecules may drive antimicrobial impacts, such as reducing microbial content and diversity within the OGI tract
<u>H-O-S1</u>	NFs with a very slow dissolution rate: Following oral exposure NFs will maintain nanospecific activity that may lead to local inflammation within the OGI tract.
<u>H-O-S2</u>	NFs with a very slow dissolution rate: Following oral exposure NFs will maintain nanospecific activity that may drive translocation across the intestinal wall, subsequent biopersistence in the body and systemic toxicity in secondary organs.
<u>H-O-S3</u>	NFs with a very slow dissolution rate: Following oral exposure NFs will maintain nanospecific activity that will drive antimicrobial impacts, such as reducing microbial content and diversity within the OGI tract

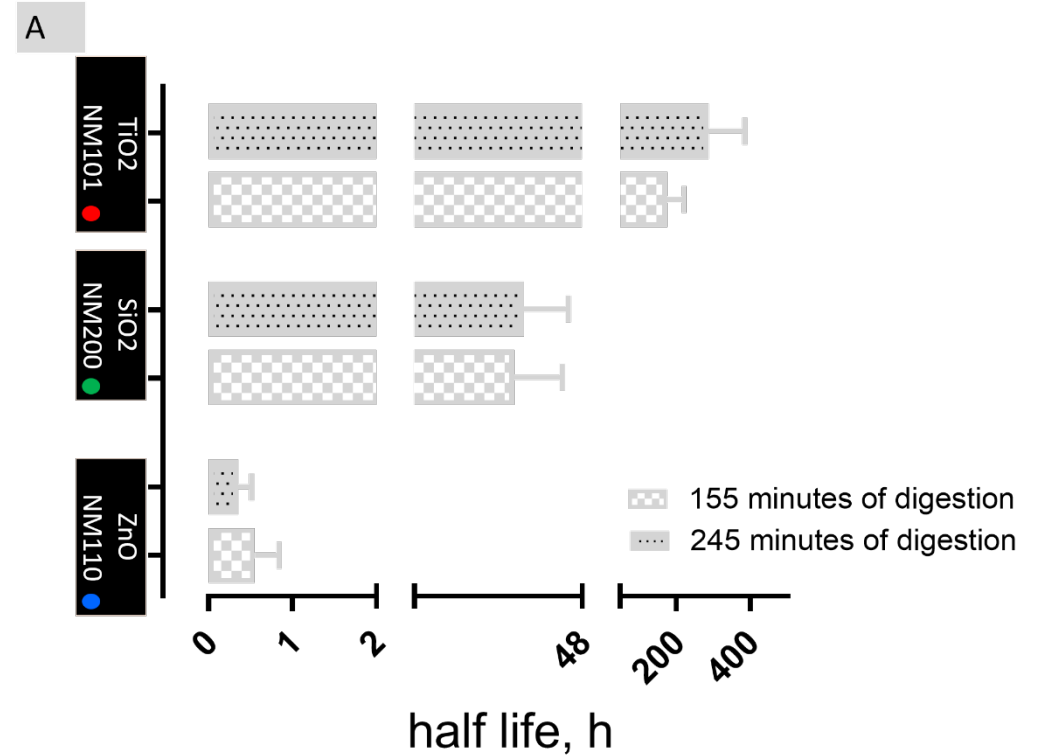
Oral grouping hypotheses

Assessment of dissolution



Simulated physiological media and cascade in vitro digestion assay

- NANoREG D2.08 SOP 06
- Bove et al. Nanoscale 2017
- Guarnieri et al. Small 2018

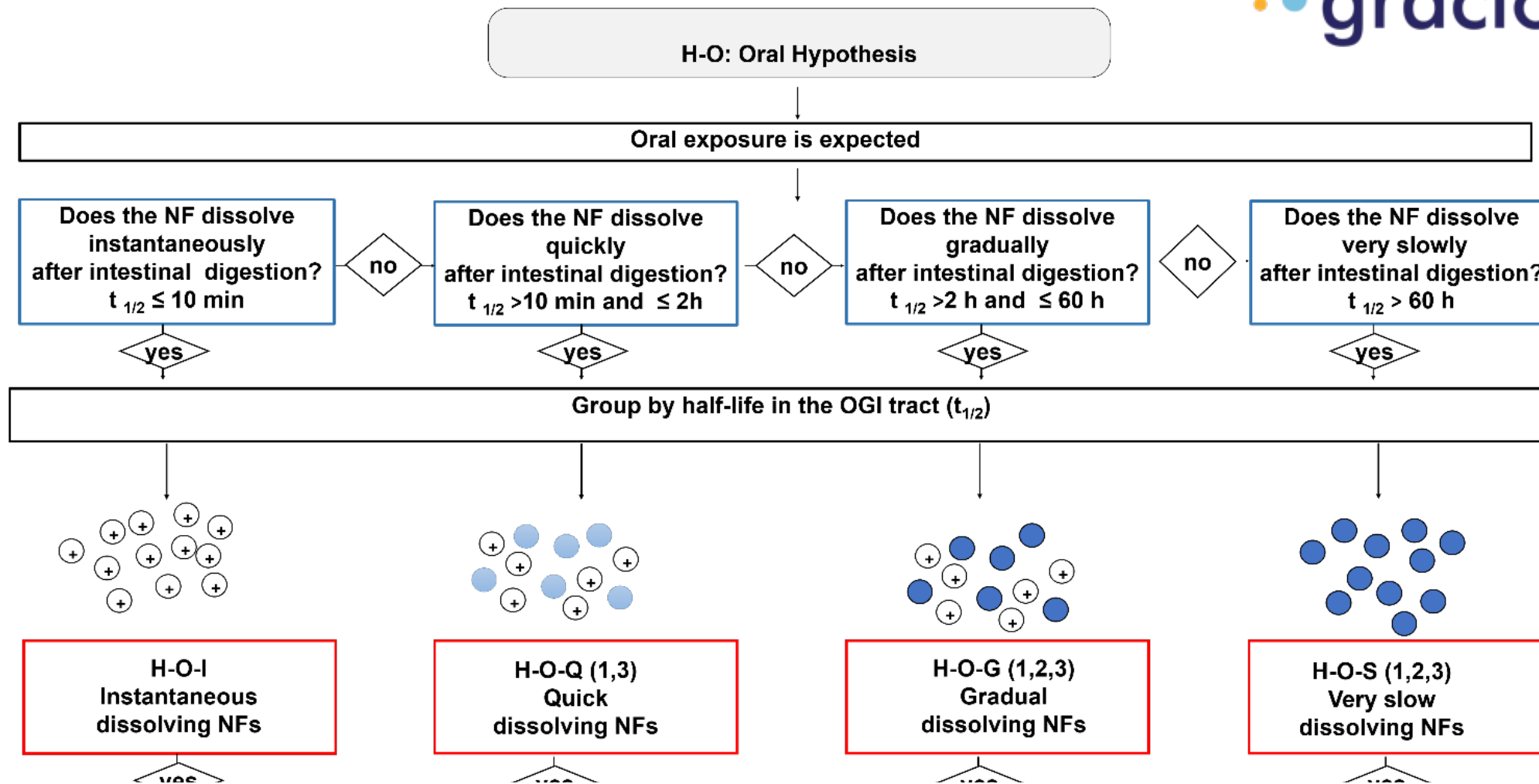


Half-life values from benchmarks align well with the cut-offs in the oral IATAs

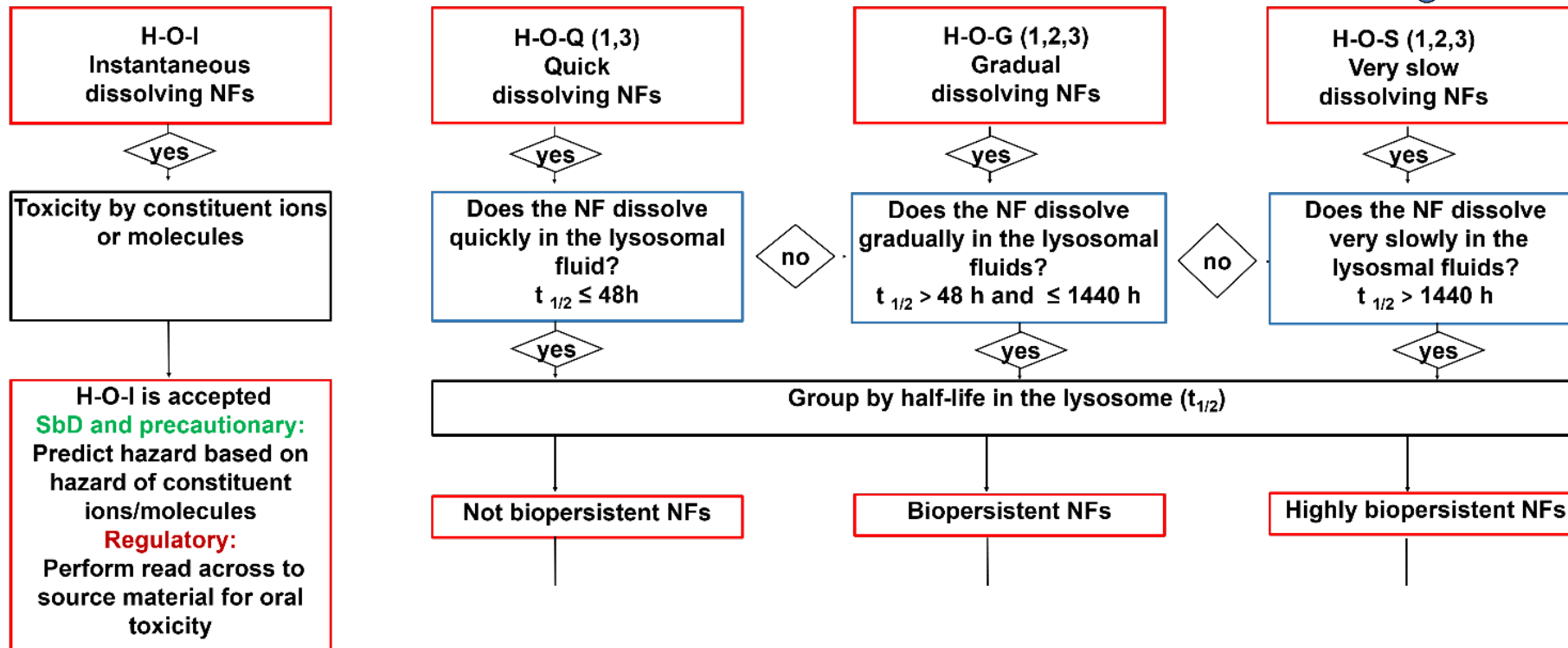
Dissolution in GIT fluids



Where they go

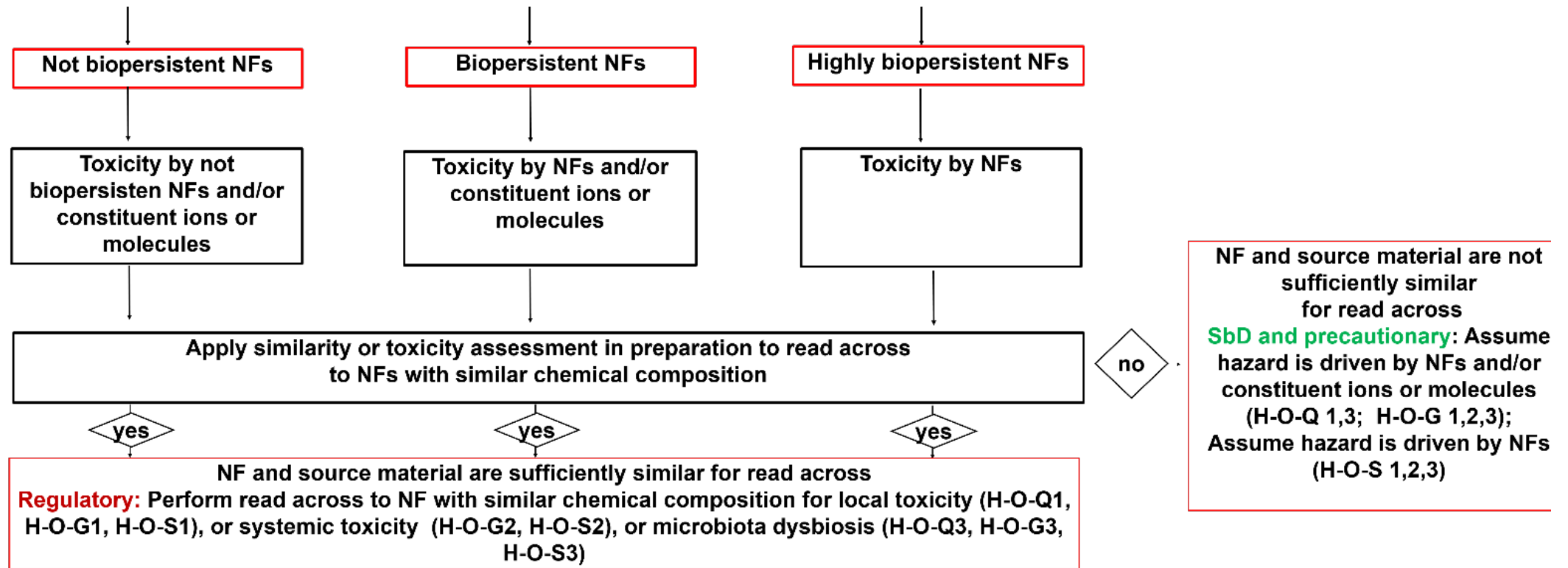


Dissolution in cells



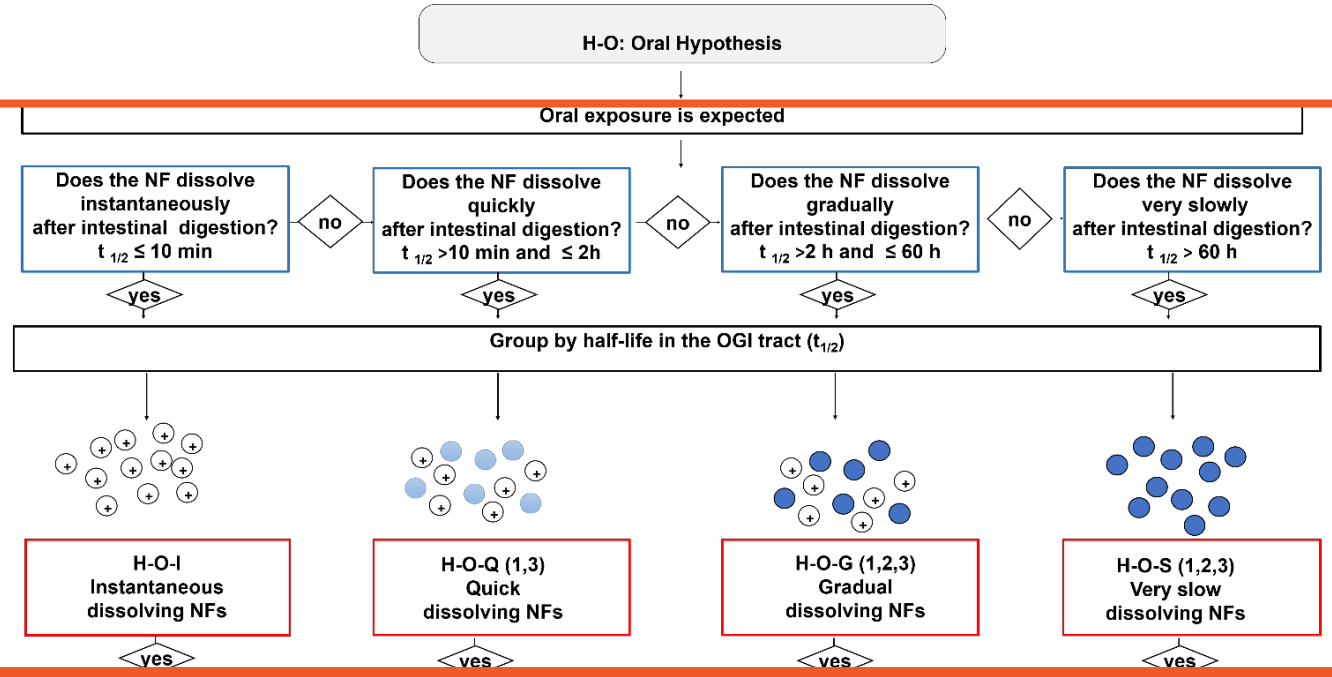
What they do

Oral IATA Hazard

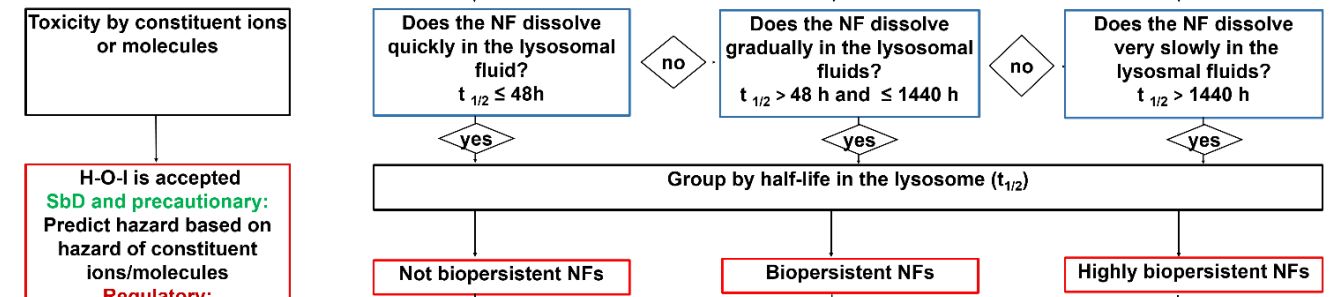


Dissolution in GIT fluids

Where they go

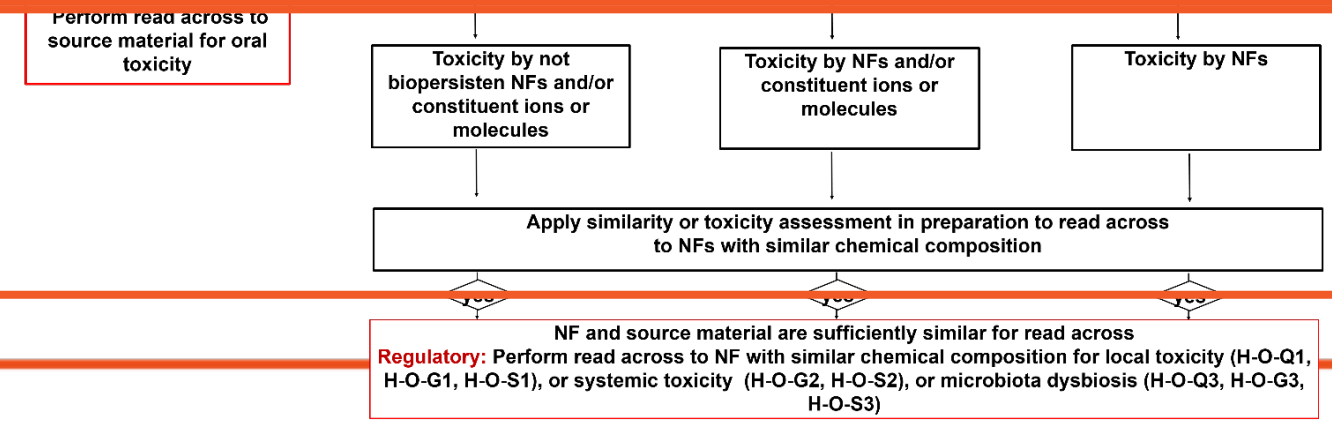


Dissolution in cells



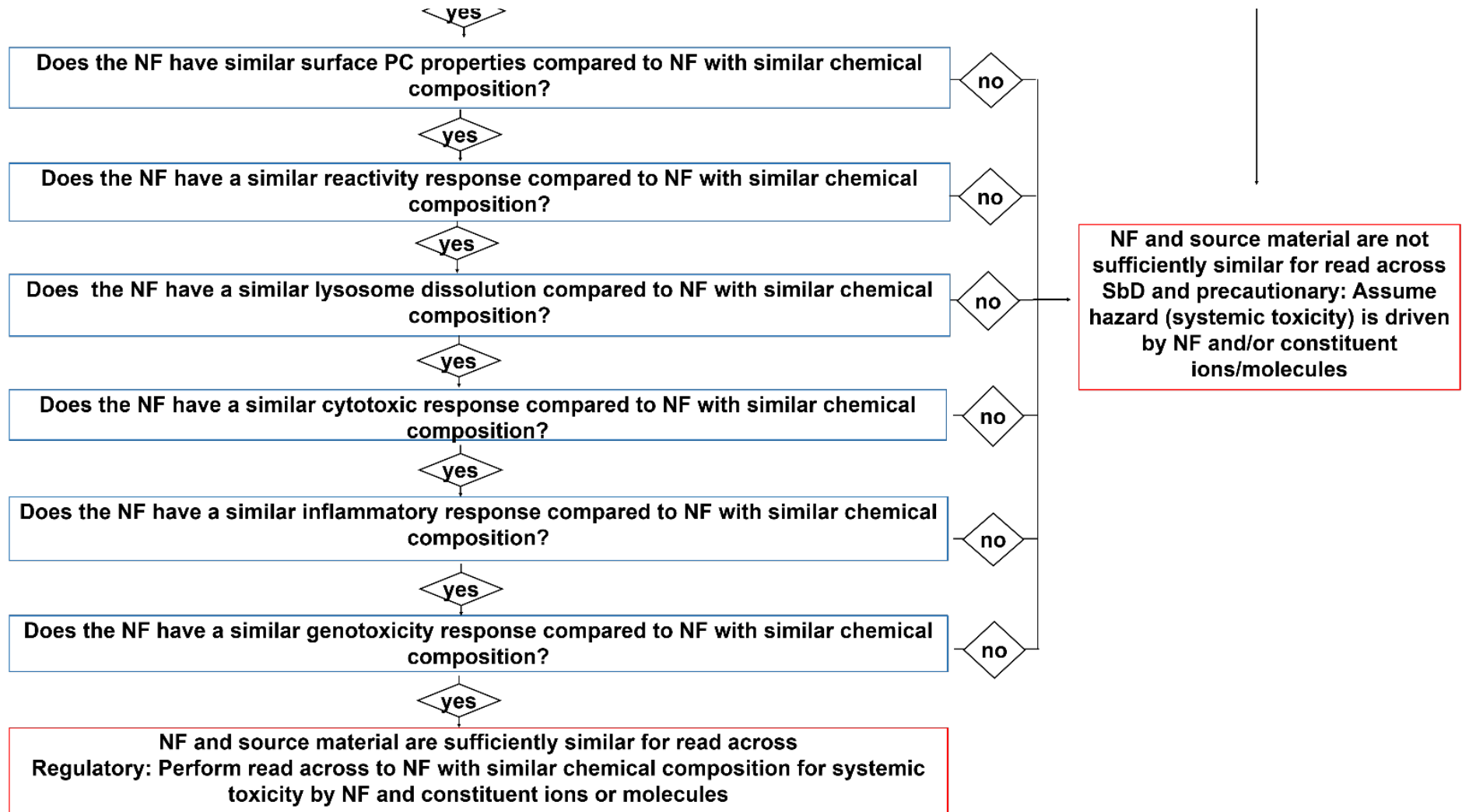
Hazard

What they do

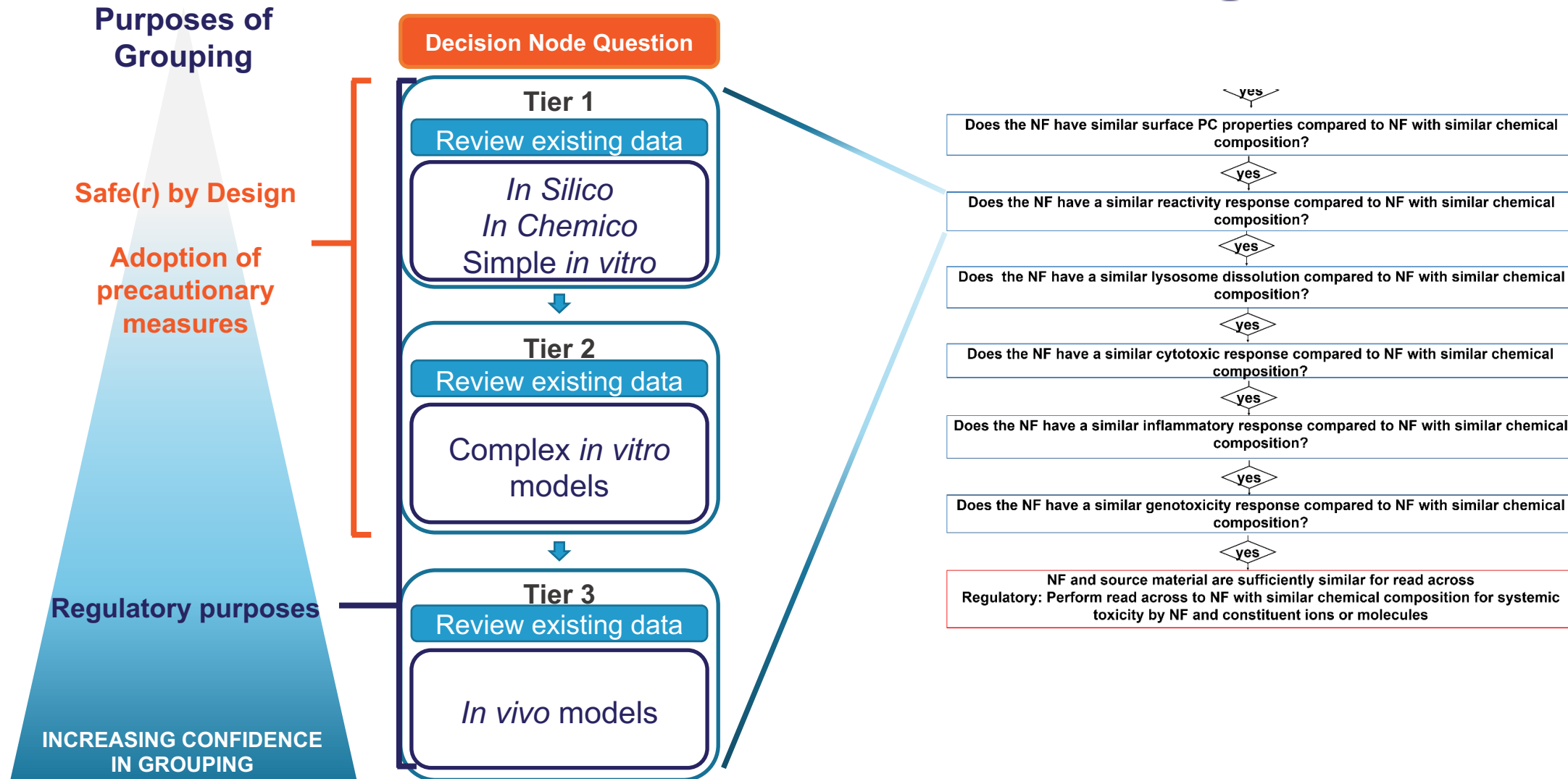


NF and source material are not sufficiently similar for read across
SbD and precautionary: Assume hazard is driven by NFs and/or constituent ions or molecules (H-O-Q 1,3; H-O-G 1,2,3); Assume hazard is driven by NFs (H-O-S 1,2,3)

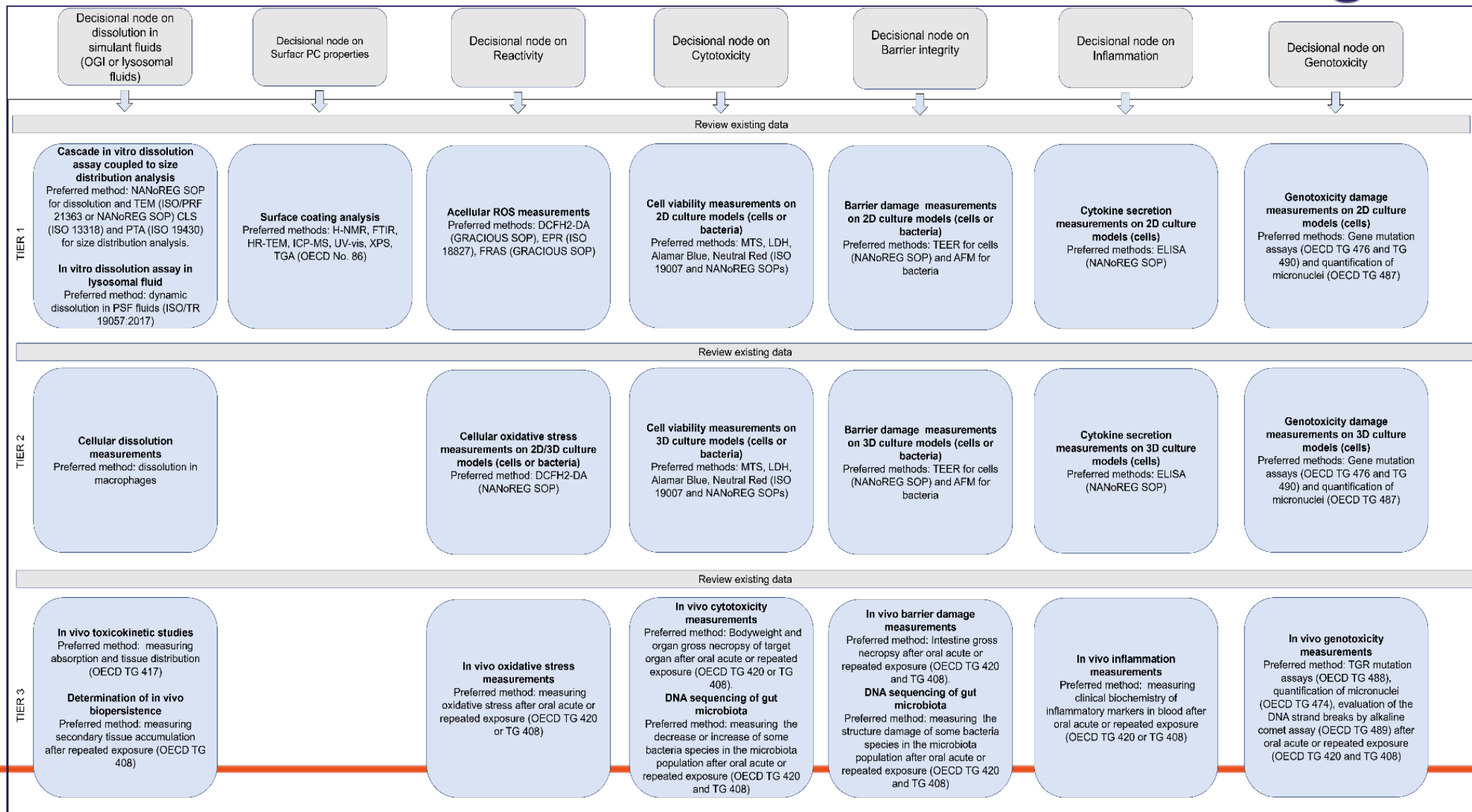
Hazard decision nodes



Tiered testing strategy Purpose



Tiered Testing Strategy



Similarity assessment

Method development



"Substances whose **physicochemical, toxicological and ecotoxicological** properties are likely to be similar or follow a regular pattern as a result of structural **similarity** may be considered as a **group**" (REACH, Annex XI, 1.5).

23 mentions of "similar" in the ECHA guidance on NFs, but no guidance on algorithms or implementation
(Appendix R.6-1 for nanoforms applicable to the Guidance on QSARs and Grouping of Chemicals, 2019)

How can we justify hazard assessment by grouping instead of animal testing? Concepts and usable tools to quantify the similarity of nanoforms

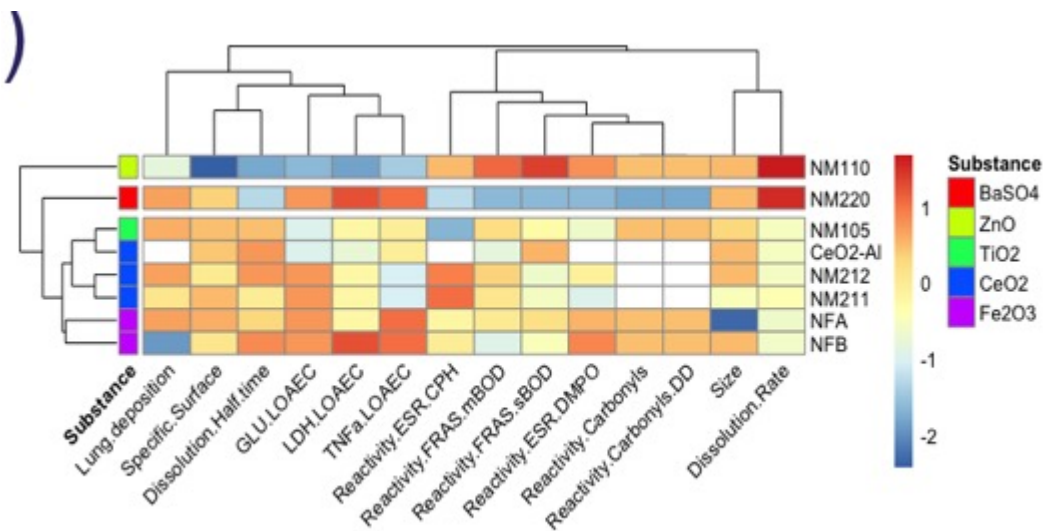
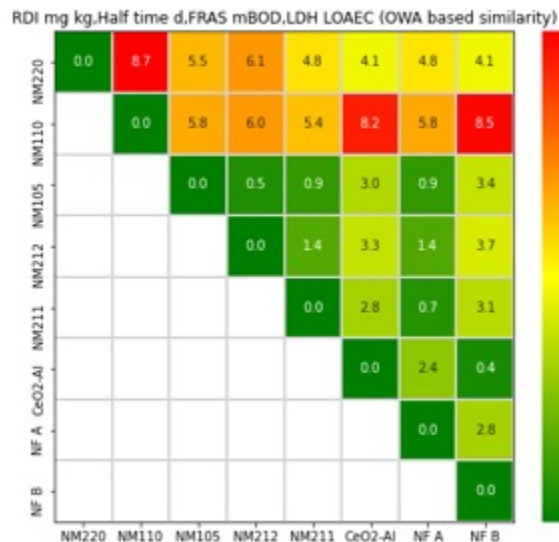
Nina Jeliaskova^a, Eric Bleeker^b, Richard Cross^c, Andrea Haase^d, Gemma Janer^e, Willie Peijnenburg^b, Mario Pink^d, Hubert Rauscher^f, Claus Svendsen^c, Georgia Tsiliki^g, Alex Zabeo^h, Danail Hristozov^h, Vicki Stoneⁱ, Wendel Wohlleben^{j*}

NanoImpact
special issue
To be submitted
June 2021

Multidimensional similarity assessment

Algorithms

- Multidimensional distances
- And/or unsupervised statistical methods (e.g. cluster analysis)
 - Very useful to explore patterns
 - Previously applied to combine multiple assays (Bahl et al 2020) and to toxicogenomics (Nikota et al. 2015, Tsiliki et al. 2017)

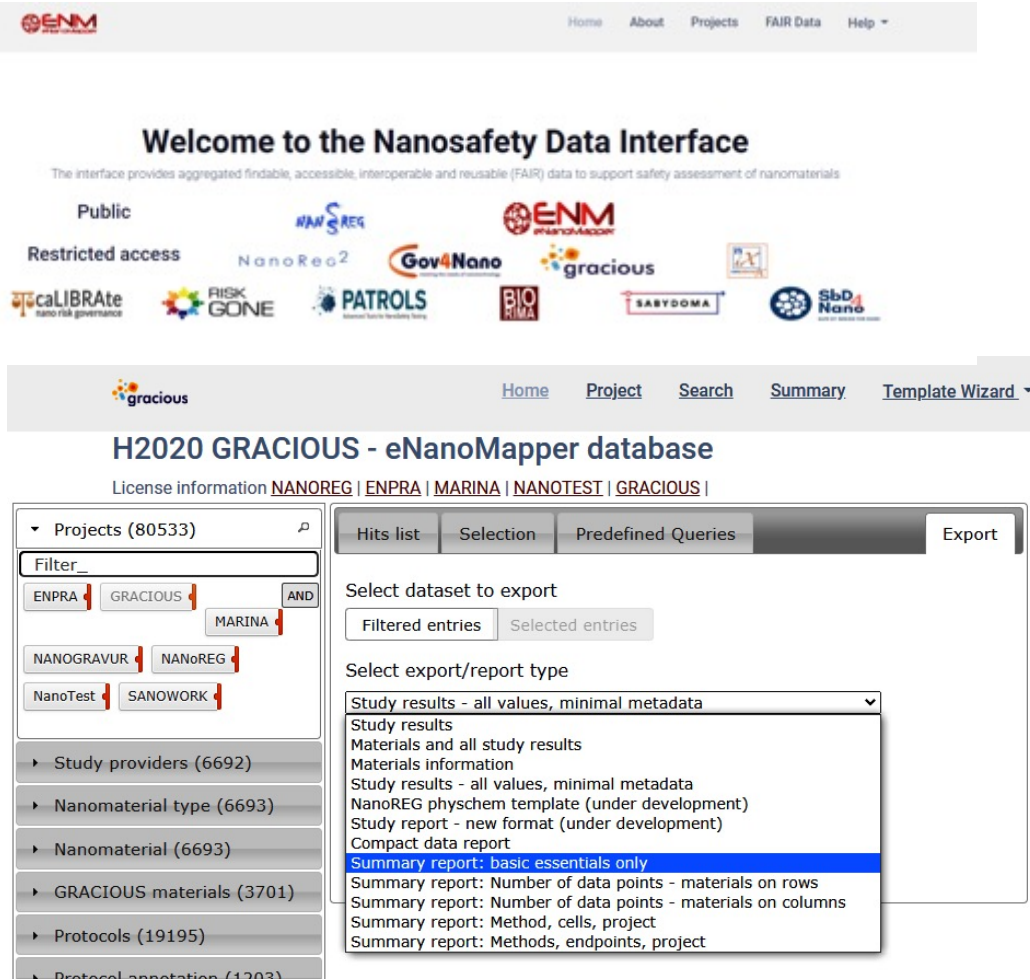


Results: Not robust between different algorithms.

Conclusion: Multidimensional approaches are considered to be tools of discovery rather than regulatory methods.

GRACIOUS links to eNanoMapper

Using existing and new data



The screenshot shows the eNanoMapper interface. At the top, there is a navigation bar with links for Home, About, Projects, FAIR Data, and Help. Below this is a welcome message: "Welcome to the Nanosafety Data Interface" with a subtext: "The interface provides aggregated findable, accessible, interoperable and reusable (FAIR) data to support safety assessment of nanomaterials".

There are two rows of logos representing different data sources. The first row includes Public access sources like ENM and NanoREG. The second row includes Restricted access sources like NanoReo2, Gov4Nano, gracious, caLIBRAte, RISK GONE, PATROLS, BIO RIMA, SABYDOMA, and Sbd Nano.

The main content area shows the "H2020 GRACIOUS - eNanoMapper database" with a search filter set to "Projects (80533)". The filter includes ENPRA, GRACIOUS, MARINA, NANOGRAVUR, NANOREG, NanoTest, and SANOWORK. On the right, there is an "Export" button and a dropdown menu for "Select export/report type". The dropdown menu is open, showing options like "Study results - all values, minimal metadata", "Materials and all study results", "Materials information", "Study results - all values, minimal metadata", "NanoREG physchem template (under development)", "Study report - new format (under development)", "Compact data report", "Summary report: basic essentials only" (which is highlighted), "Summary report: Number of data points - materials on rows", "Summary report: Number of data points - materials on columns", "Summary report: Method, cells, project", and "Summary report: Methods, endpoints, project".

Data to support GRACIOUS Framework

- Phys-chem
- Cell viability, oxidative stress, reactivity
- Harmonized templates and terminology
 - Hazard, Exposure, PC
 - Quality score added
- Data is fed into matrix to support a similarity assessment
- Blueprint test environment links to eNanoMapper to allow data transfer

New and existing data

Data Quality and Completeness

Quality and completeness

The methodology takes into account the following criteria:

- **data completeness;**
- **data reliability;**
- **data relevance;**
- **data adequacy.**
- Scores are calculated for each of these criteria and those are aggregated into a **quality score** and a **completeness score**.



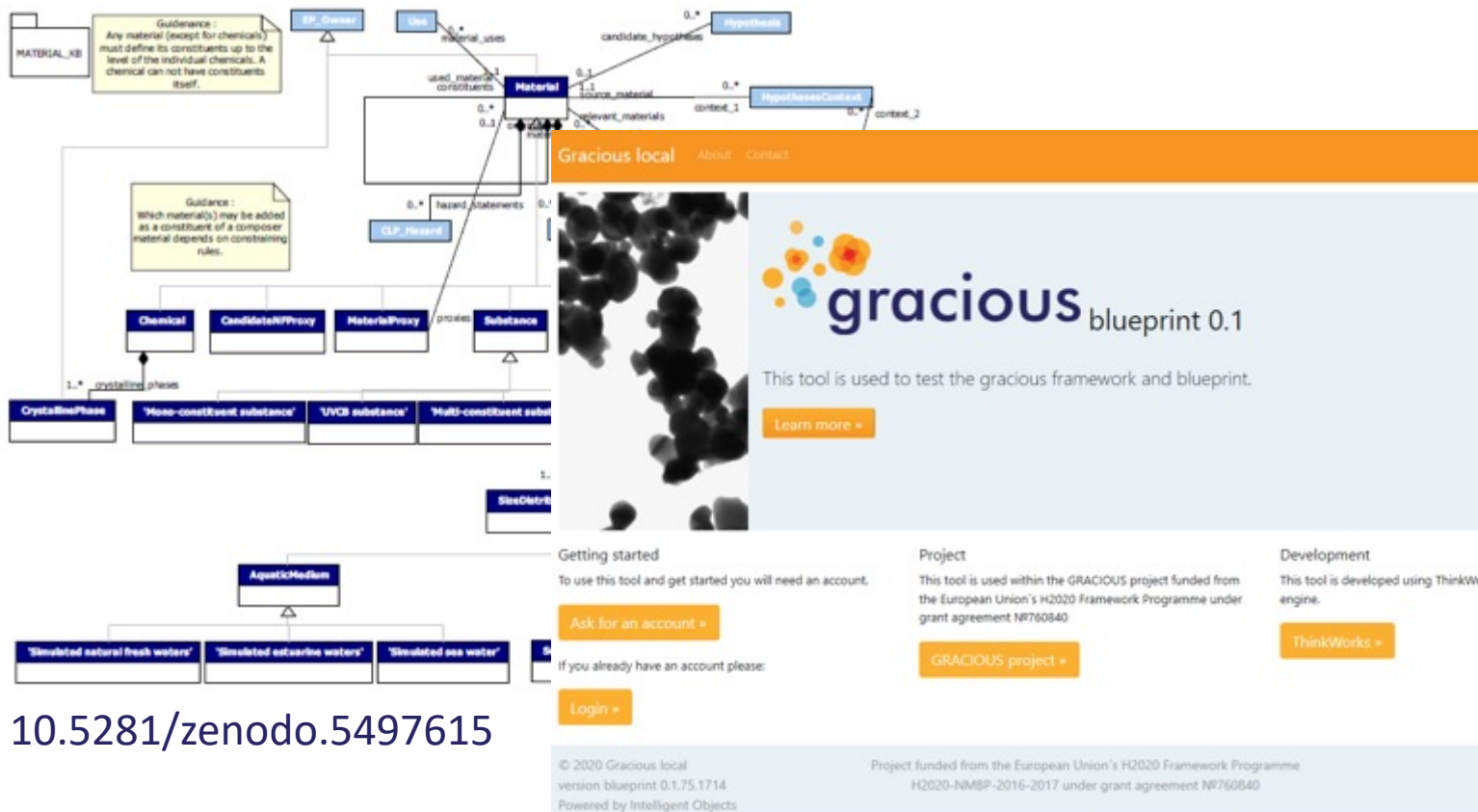
Red – data is of insufficient quality.

Yellow – data is sufficient quality, but needs further consideration to be used for a specific task.

Green – data high quality.

GRACIOUS Framework

Blueprint and Guidance Document

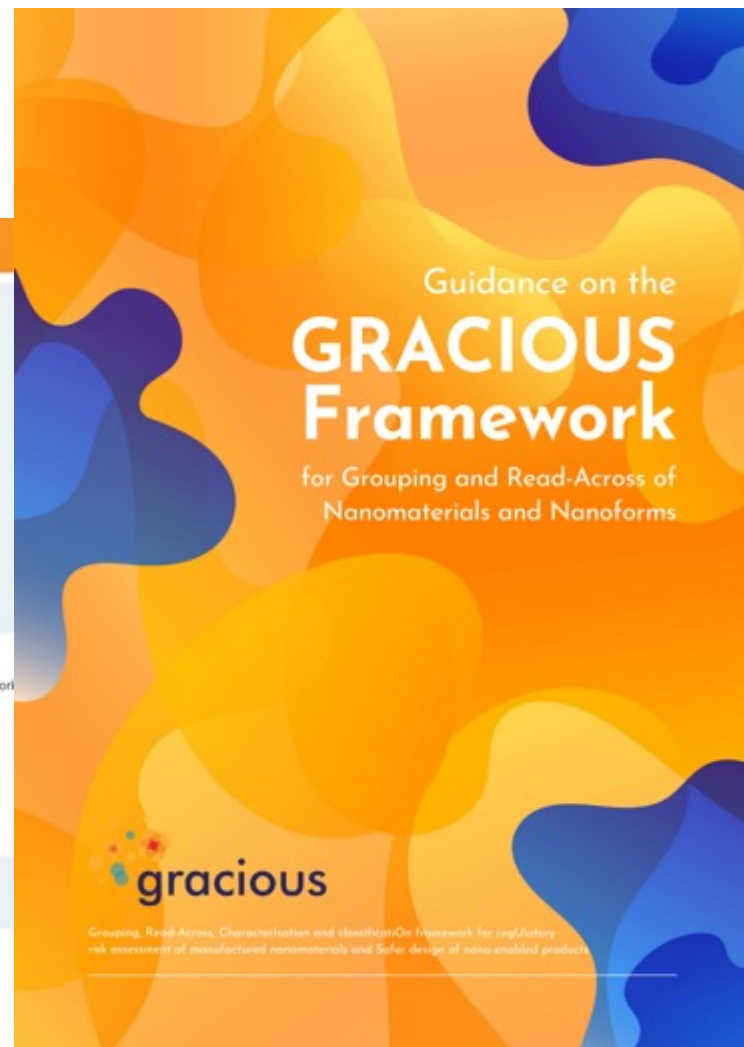


The screenshot displays the 'Gracious local' website interface. On the left, a complex blueprint diagram shows relationships between entities like 'Material', 'Chemical', 'CandidateProxy', 'MaterialProxy', 'Substance', 'CrystalPhase', 'Mono-constituent substance', 'UVCS substance', 'Multi-constituent substance', 'AqueousMedium', 'Simulated natural fresh waters', 'Simulated estuarine waters', and 'Simulated sea water'. It includes various relationships such as 'material_uses', 'used_material_constituents', 'candidate_hypotheses', 'relevant_materials', 'context_1', 'context_2', 'taxon_statements', 'proxies', 'crystalline_phases', and 'size_distributions'. Guidance notes are present, such as: 'Any material (except for chemicals) must define its constituents up to the level of the individual chemicals. A chemical can not have constituents itself.' and 'Which material(s) may be added as a constituent of a composer material depends on constraining rules.'

The website content includes:

- Header:** Gracious local | About | Contact
- Hero Section:** gracious blueprint 0.1. This tool is used to test the gracious framework and blueprint. [Learn more >](#)
- Getting started:** To use this tool and get started you will need an account. [Ask for an account >](#) If you already have an account please: [Login >](#)
- Project:** This tool is used within the GRACIOUS project funded from the European Union's H2020 Framework Programme under grant agreement N°760840. [GRACIOUS project >](#)
- Development:** This tool is developed using ThinkWorks engine. [ThinkWorks >](#)
- Footer:** © 2020 Gracious local version blueprint 0.1.75.1714 Powered by Intelligent Objects. Project funded from the European Union's H2020 Framework Programme H2020-NMBP-2016-2017 under grant agreement N°760840.

10.5281/zenodo.5497615



The cover features a vibrant background of overlapping orange and blue organic shapes. The text reads:

Guidance on the
GRACIOUS Framework
 for Grouping and Read-Across of
 Nanomaterials and Nanoforms

At the bottom, the 'gracious' logo is displayed above the text: 'Grouping, Read-Across, Characterisation and classification framework for regulatory risk assessment of manufactured nanomaterials and Safer design of nano-enabled products.'

Testing the Framework

Case studies

Research

Consultants
industry

Regulators &
Policy makers



Outputs

- ✓ **Hypotheses**
 - Pre-defined list complete
 - User-defined template complete

- ✓ **IATAs**
 - Human complete
 - Environment complete

- ✓ **Blueprint of software**
 - Machine readable and open access
 - ✓ Integrates hypotheses, IATAs and data sources
 - ✓ Integrate similarity assessment and data matrix

- ✓ **Similarity methodology**

- Multicomponent
- Pairwise property-by-property

- ✓ **Quality criteria incorporation**

- ✓ **Blueprint and Framework tested**

- 17 case studies conducted
 - Inhalation – 6 internal, 5 external
 - Oral – 1 internal, 1 external
 - Environment – 2 internal, 2 external

- ✓ **Guidance document**

- ✓ **Guidance in a nutshell**

For more information

<https://www.h2020gracious.eu/library>



A composite screenshot of three overlapping browser windows from the GRACIOUS project website. The leftmost window shows a "Draft GRACIOUS Guide" with a cover image titled "Guidance on the GRACIOUS Framework for Grouping and Read-Across of Nanomaterials and Nanoforms". The middle window displays the "PUBLICATIONS" section, listing three articles: "Quality of Physicochemical Data on Nanomaterials: An Assessment of Data Completeness and Variability", "A Method to Assess the Relevance of Nanomaterial Dissolution during Reactivity Testing", and "The effect of capping agents on the toxicity of silver nanoparticles to Danio rerio embryos". The rightmost window shows the "DISSEMINATION MATERIALS" section, featuring a "Downloads" list with items like "GRACIOUS Logo", "GRACIOUS Brochure", and "GRACIOUS Poster", along with a "RELATED LINKS" sidebar and a "SIGN UP FOR OUR NEWSLETTER" form. The Windows taskbar is visible at the bottom of the windows.



Thank you!



Vicki Stone

v.stone@hw.ac.uk

info@h2020gracious.eu

