# Particles and Health 2021

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

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www.h2020gracious.eu

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Project no. 760840

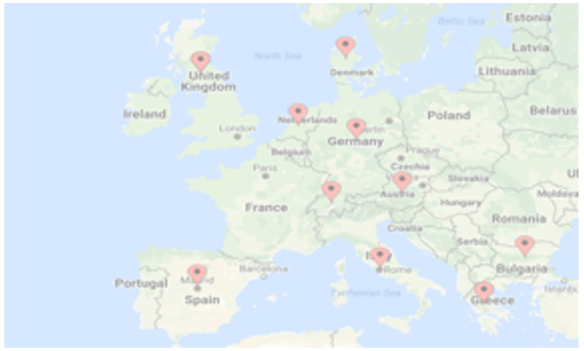
Introduction

# 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.

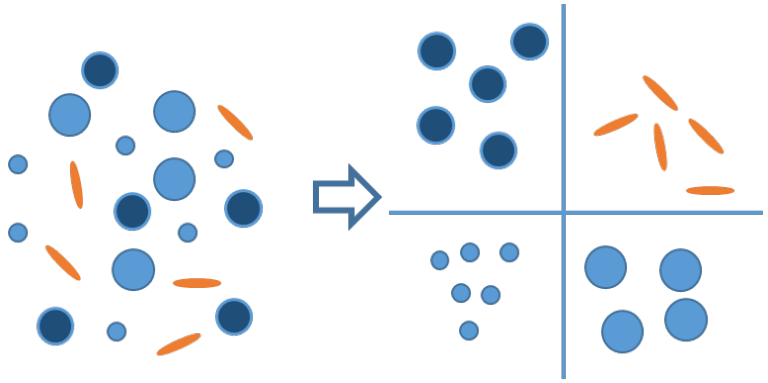



- Started January 2018
- Finish end Sept 2021
- 23 partners

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

Existing Knowledge

DRAFT FRAMEWORK

Stakeholder engagement

GRACIOUS FRAMEWORK


- 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

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

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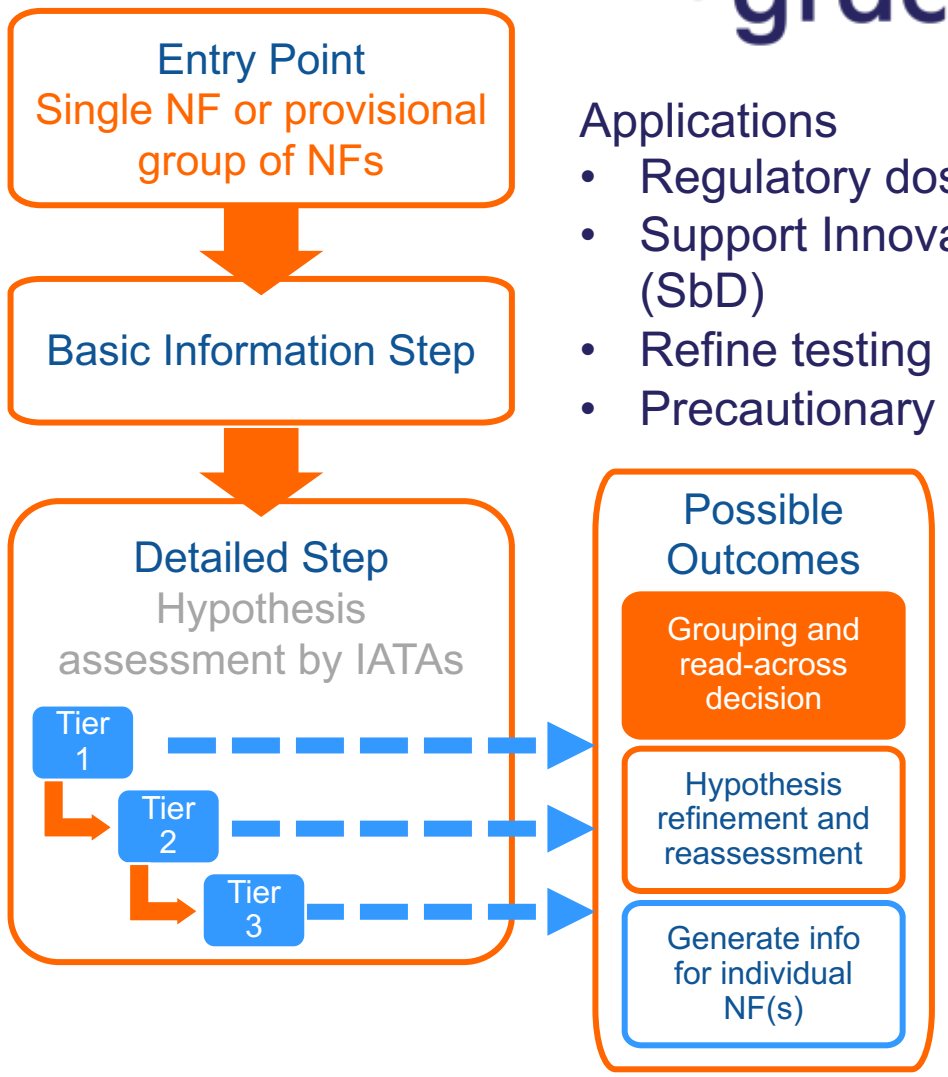


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Applications

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

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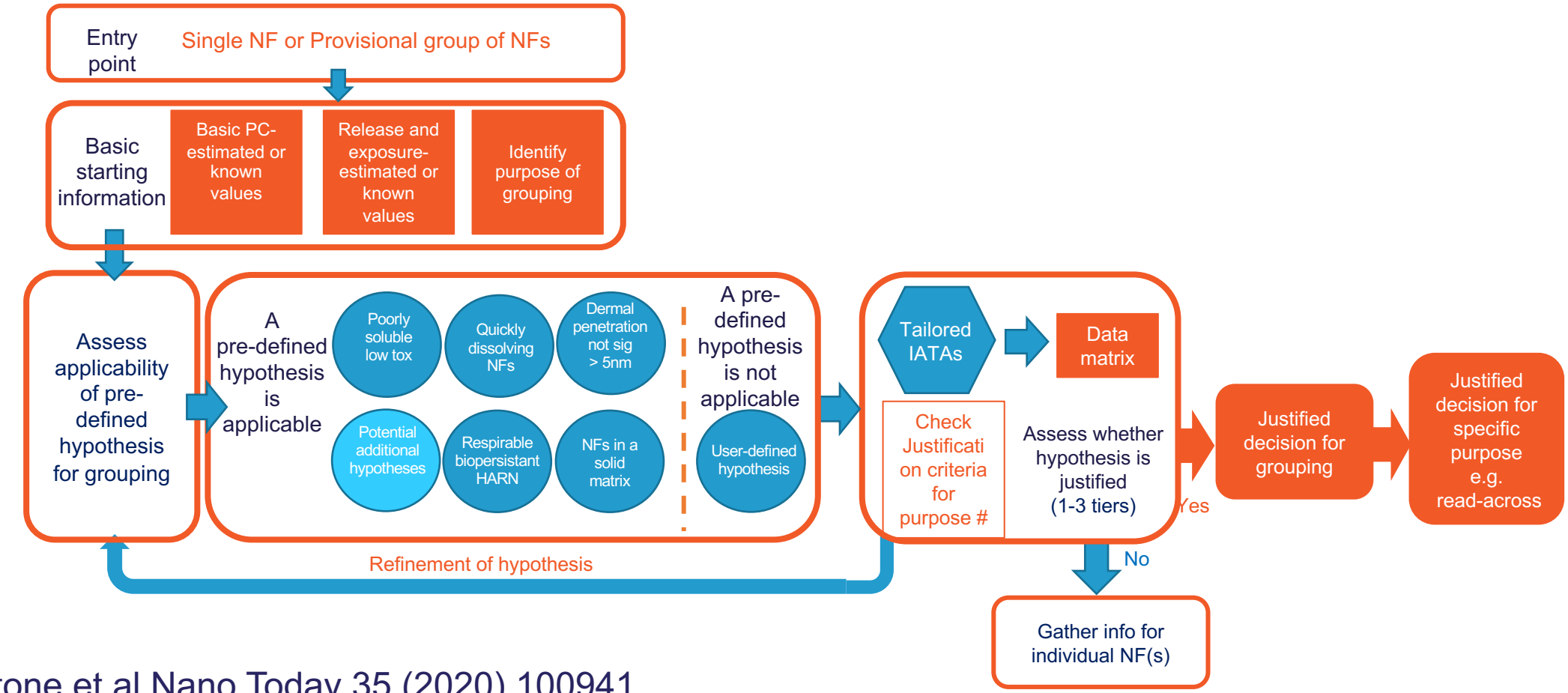
Detailed form

# GRACIOUS Framework

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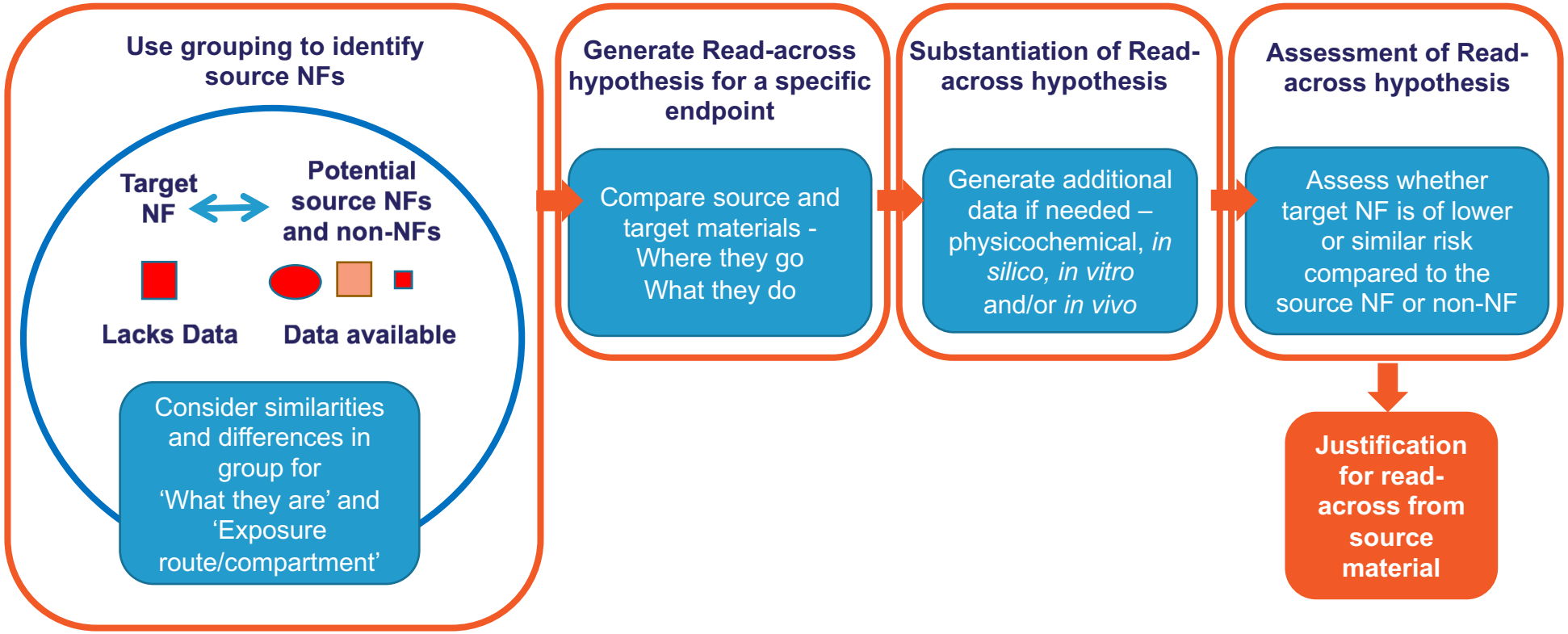
# Using the Framework for Read-Across

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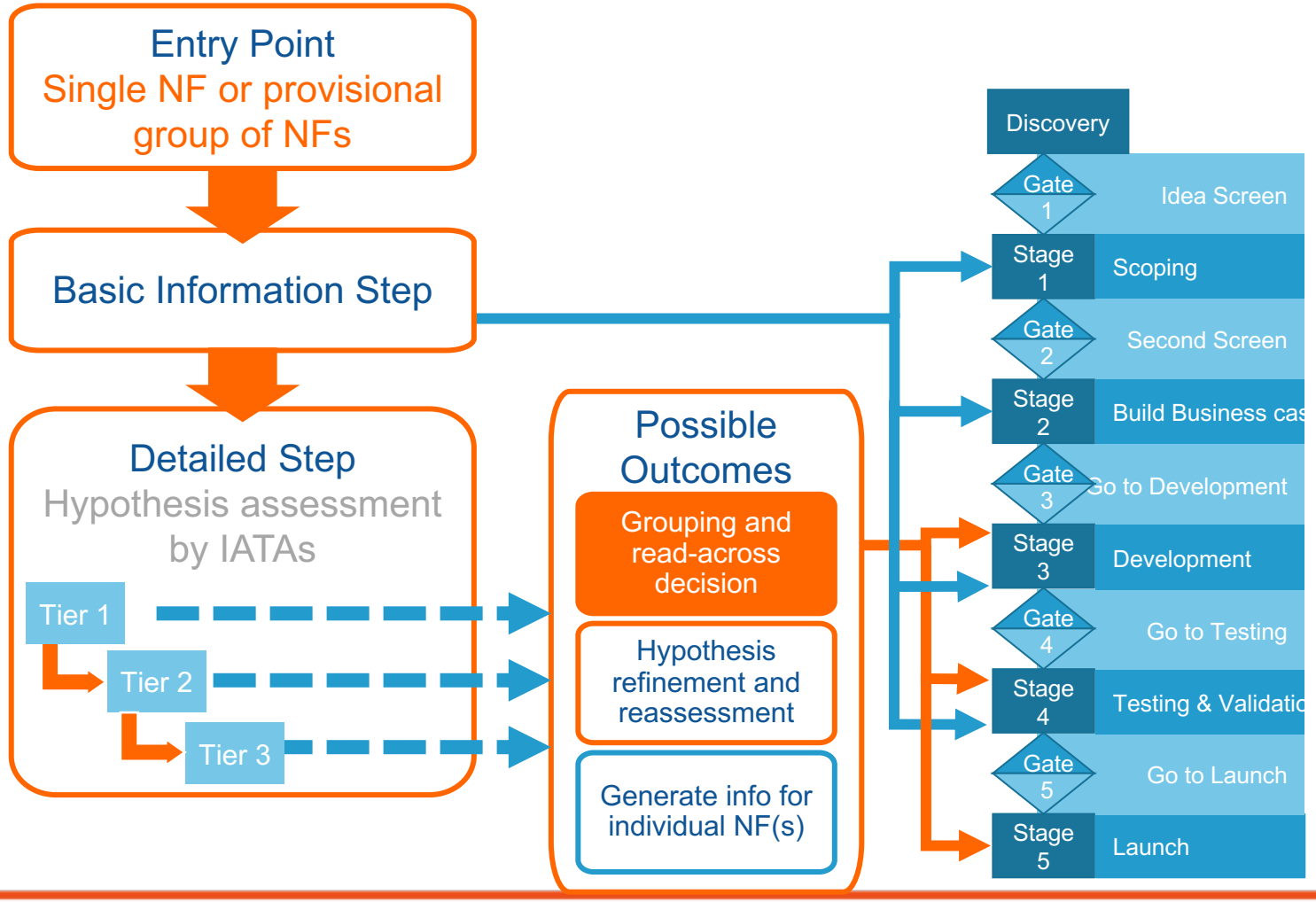
# Using the Framework for Safe(r) by Design




- 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

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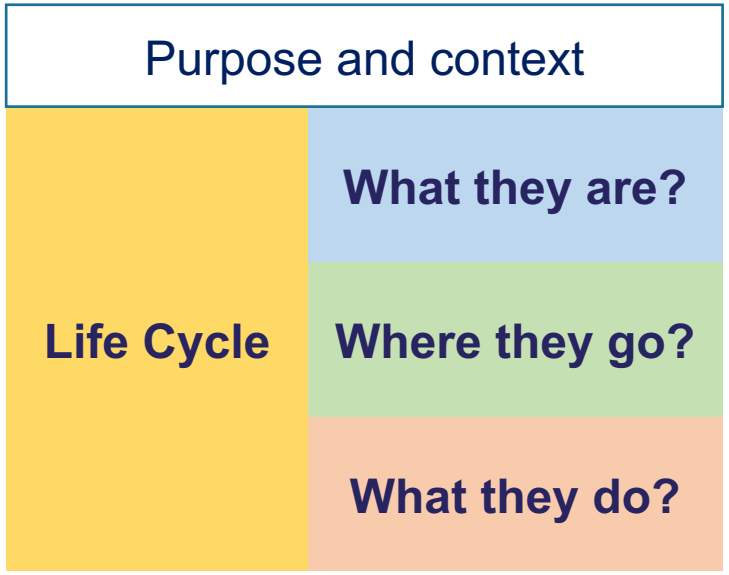
Grouping is Hypothesis Driven

# Hypothesis Template

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



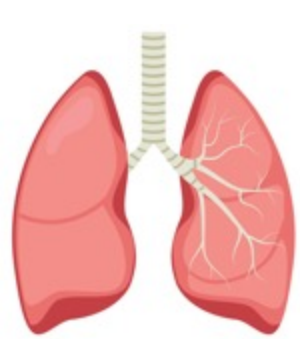

The Framework includes approx. 40 pre-defined hypotheses

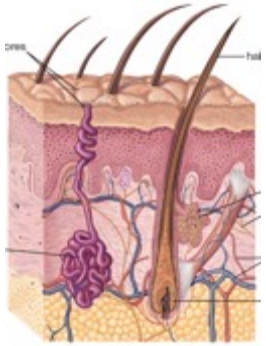
- Based upon the literature and available data
  - 17 for human hazard
  - 23 for environmental hazard

Grouping

# Hypothesis examples

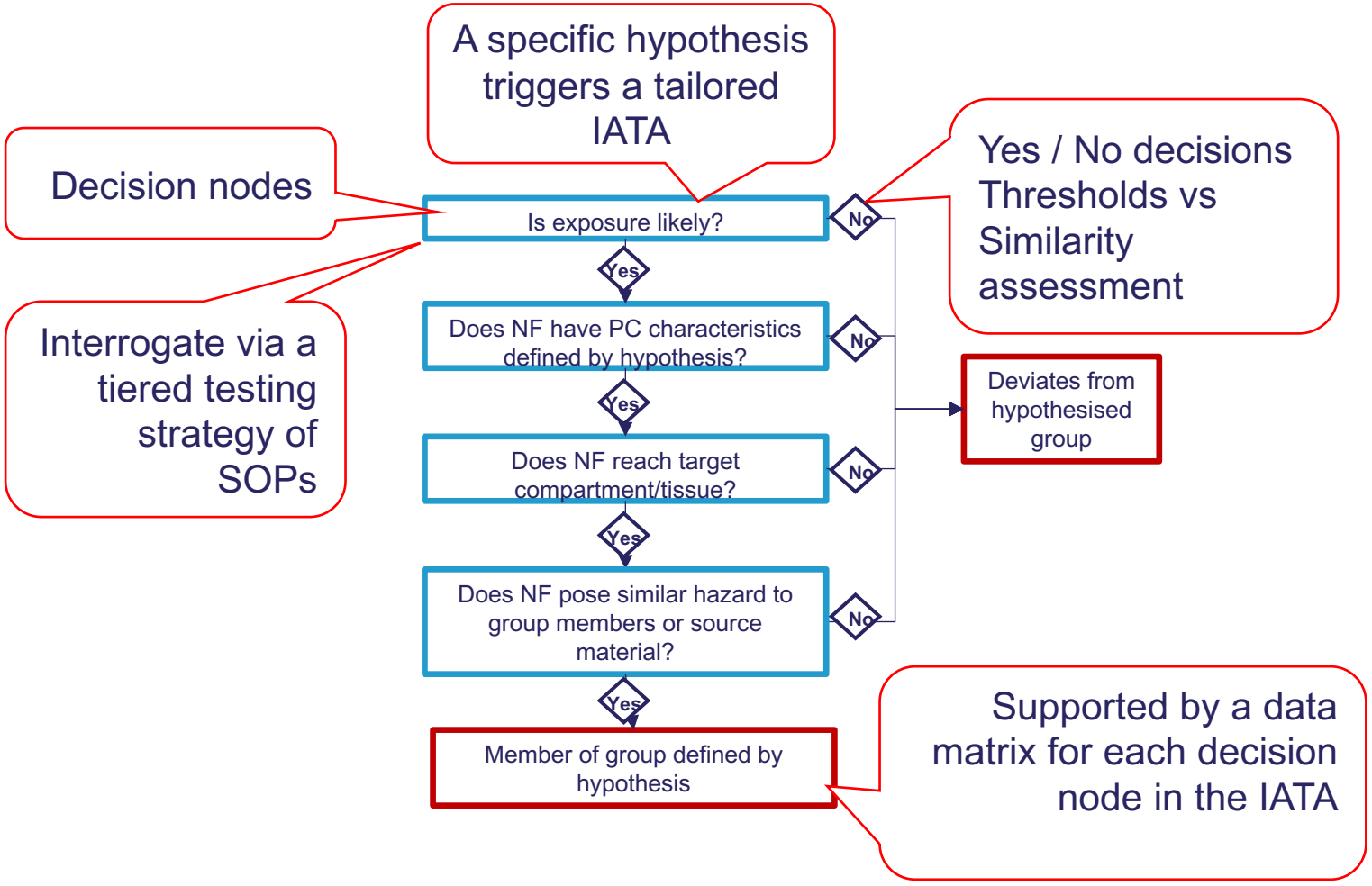
- Respirable NFs showing quick dissolution: Following inhalation both NFs and constituent ions or molecules may contribute to toxicity, but there is no concern for accumulation. Toxicity (also) depends on the location of the ionic or molecular release.
- NFs with a chemical coating that is lost from 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





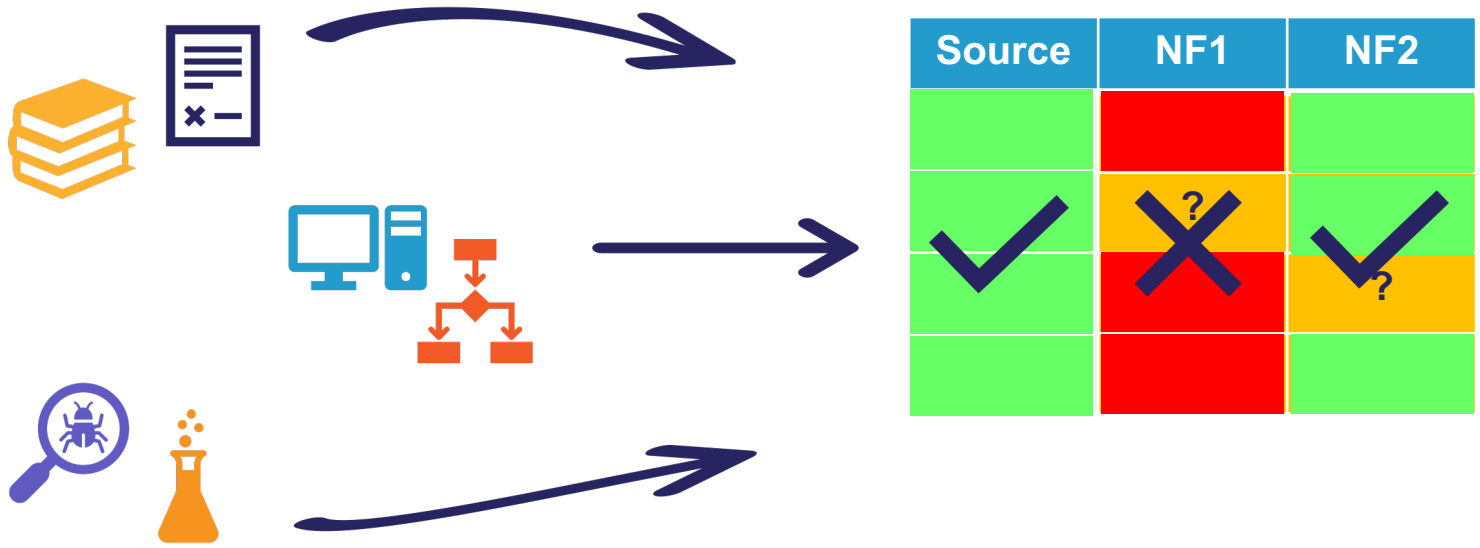

# Integrated Approaches to Testing and Assessment

- 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



## 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

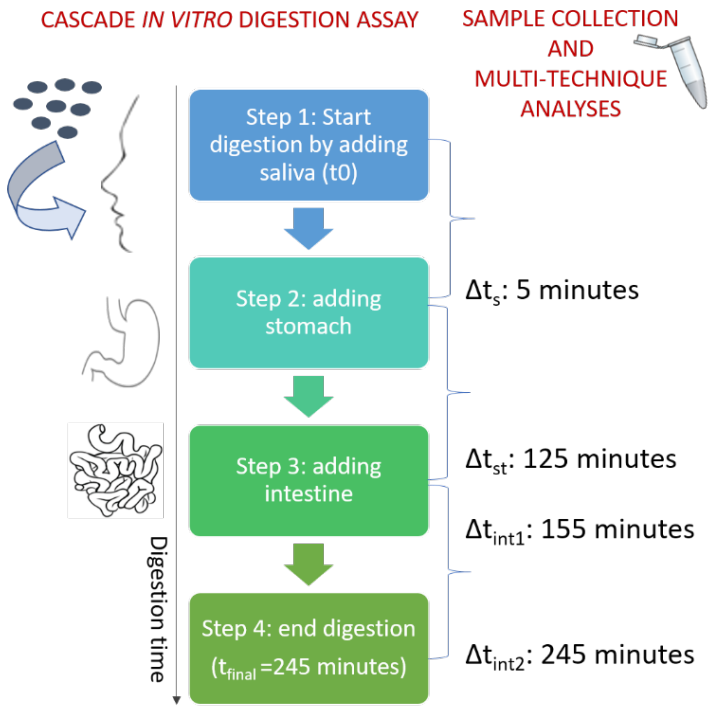
# Hypotheses relevant to grouping

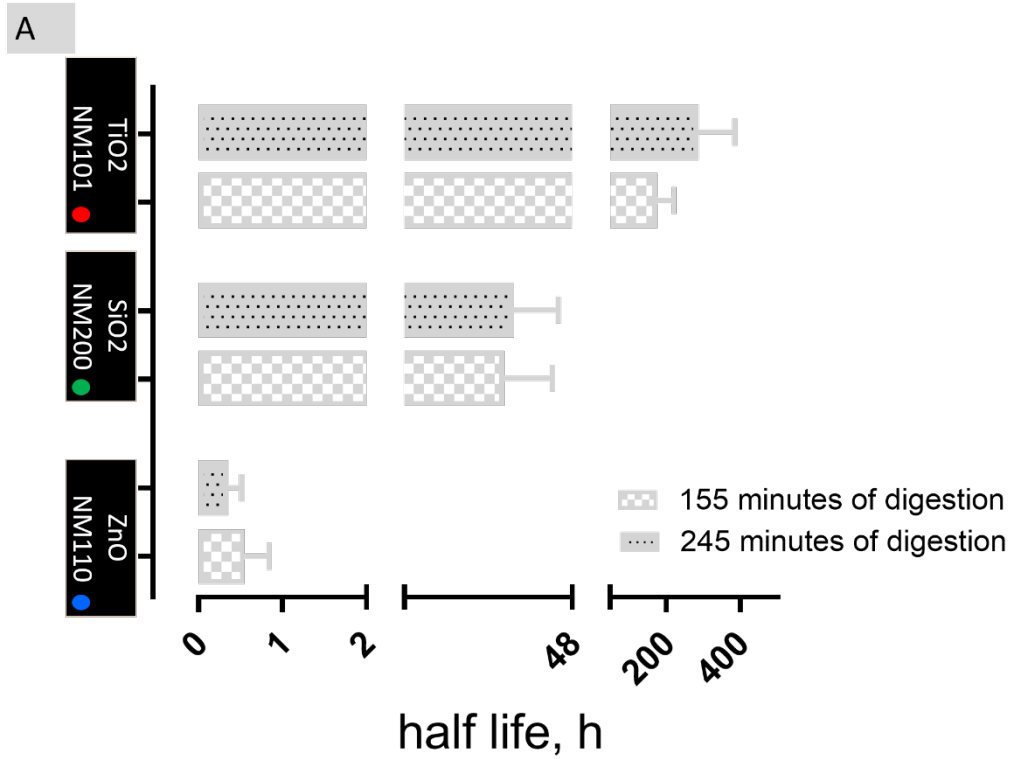
Dissolution in GIT fluids:

| | Human oral hypotheses | |
|---|---|---|
| Instantaneous | H-O-I | 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. |
| Quick | H-O-Q1 | 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. |
| | H-O-Q3 | 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. |
| Partial | H-O-P1 | NFs showing partial dissolution: Following oral exposure both NFs and constituent ions or molecules may lead to local inflammation in the OGI tract. |
| | H-O-P2 | 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. |
| | H-O-P3 | 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 |
| Very slow | H-O-S1 | 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. |
| | H-O-S2 | 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. |
| | H-O-S3 | 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

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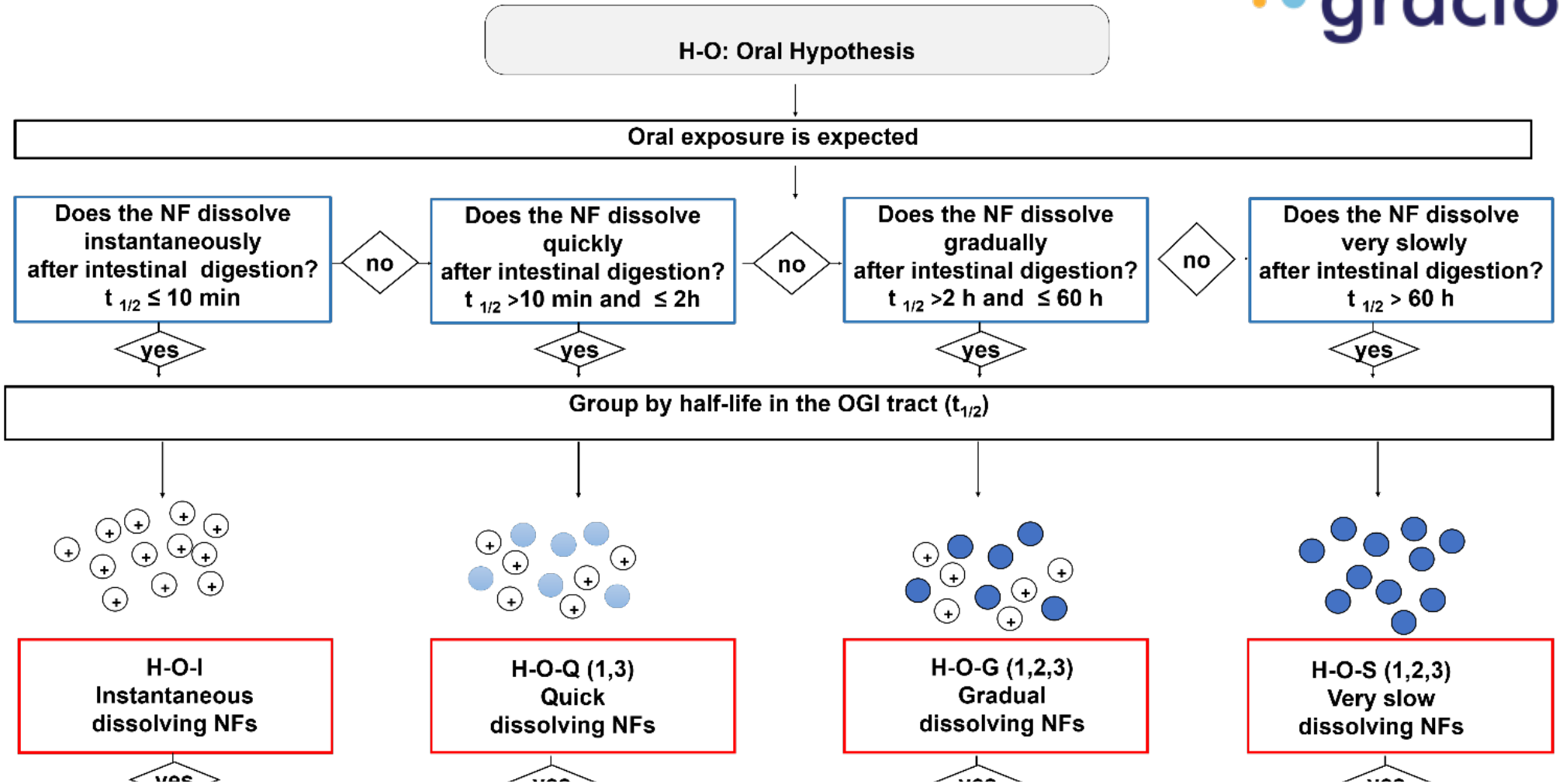
*Di Cristo et al. in preparation*

Oral IATA

# Dissolution in GIT fluids

Where they go

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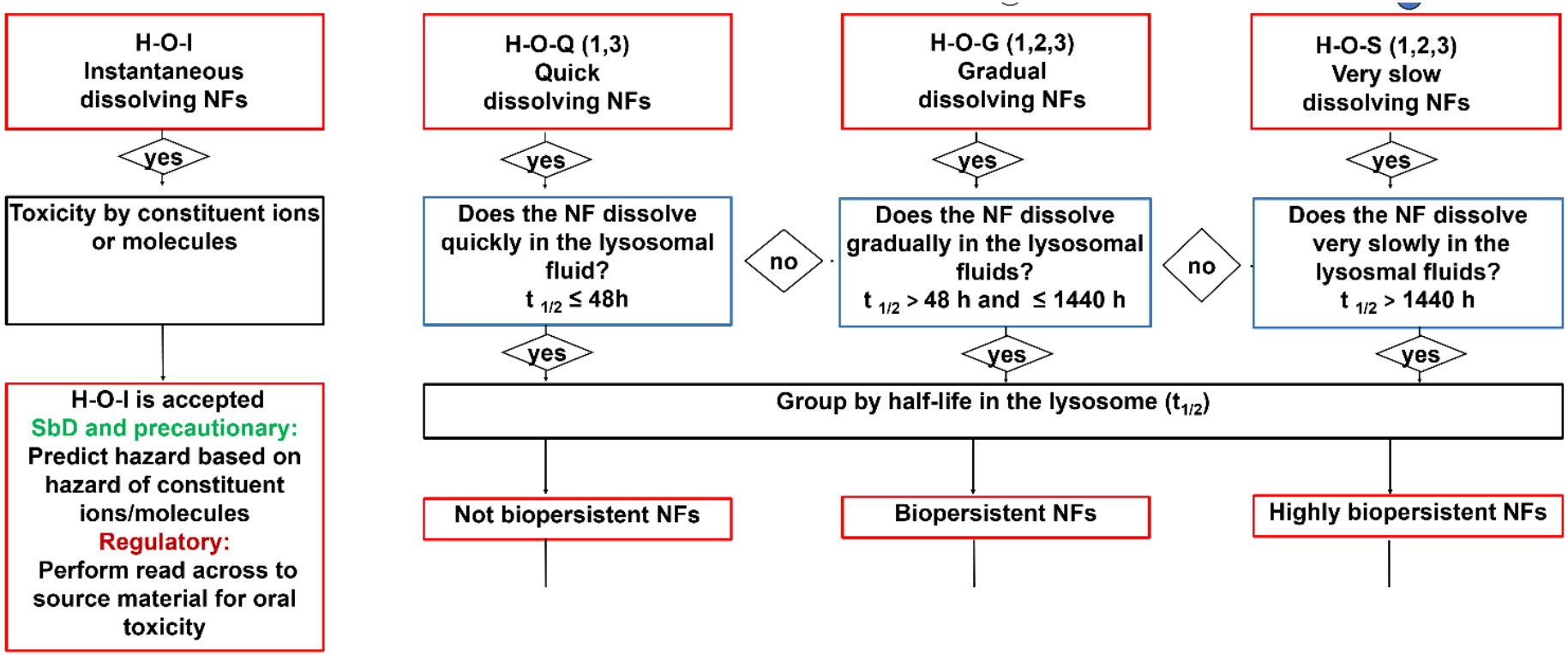
DRUG DISCOVERY AND DEVELOPMENT

*Di Cristo et al. in preparation*

Oral IATA

# Dissolution in cells



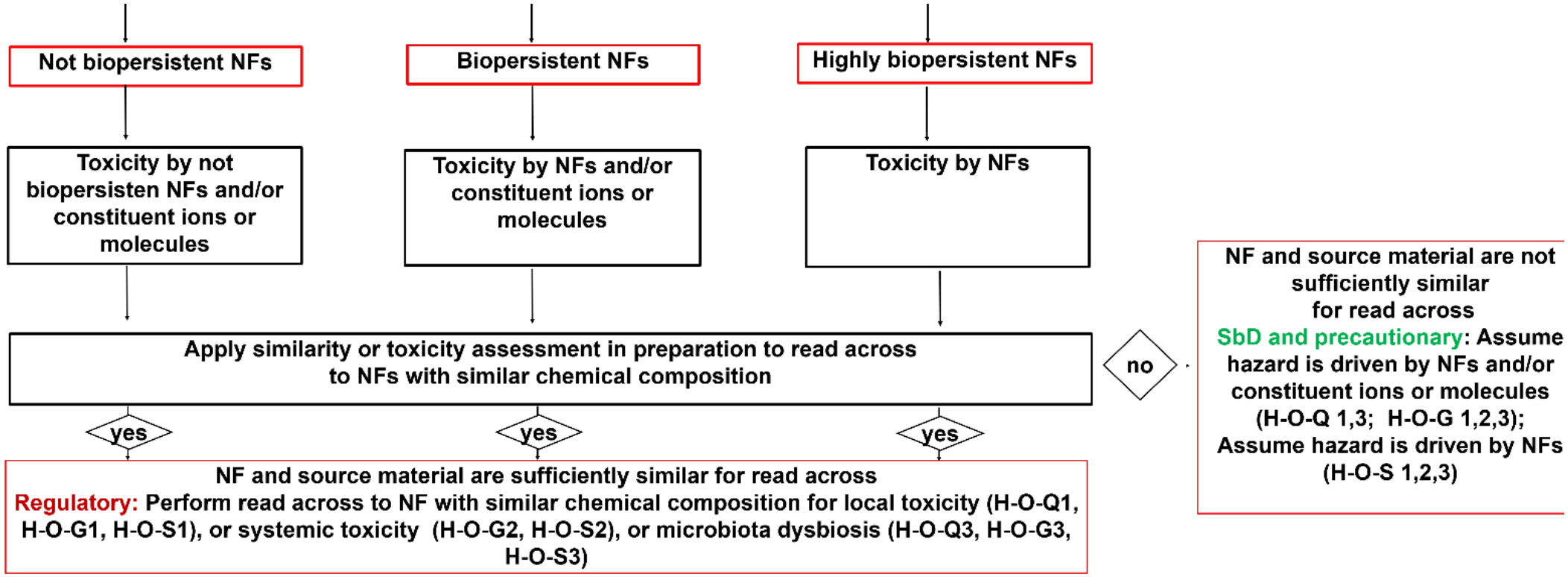

What they do



*Di Cristo et al. in preparation*

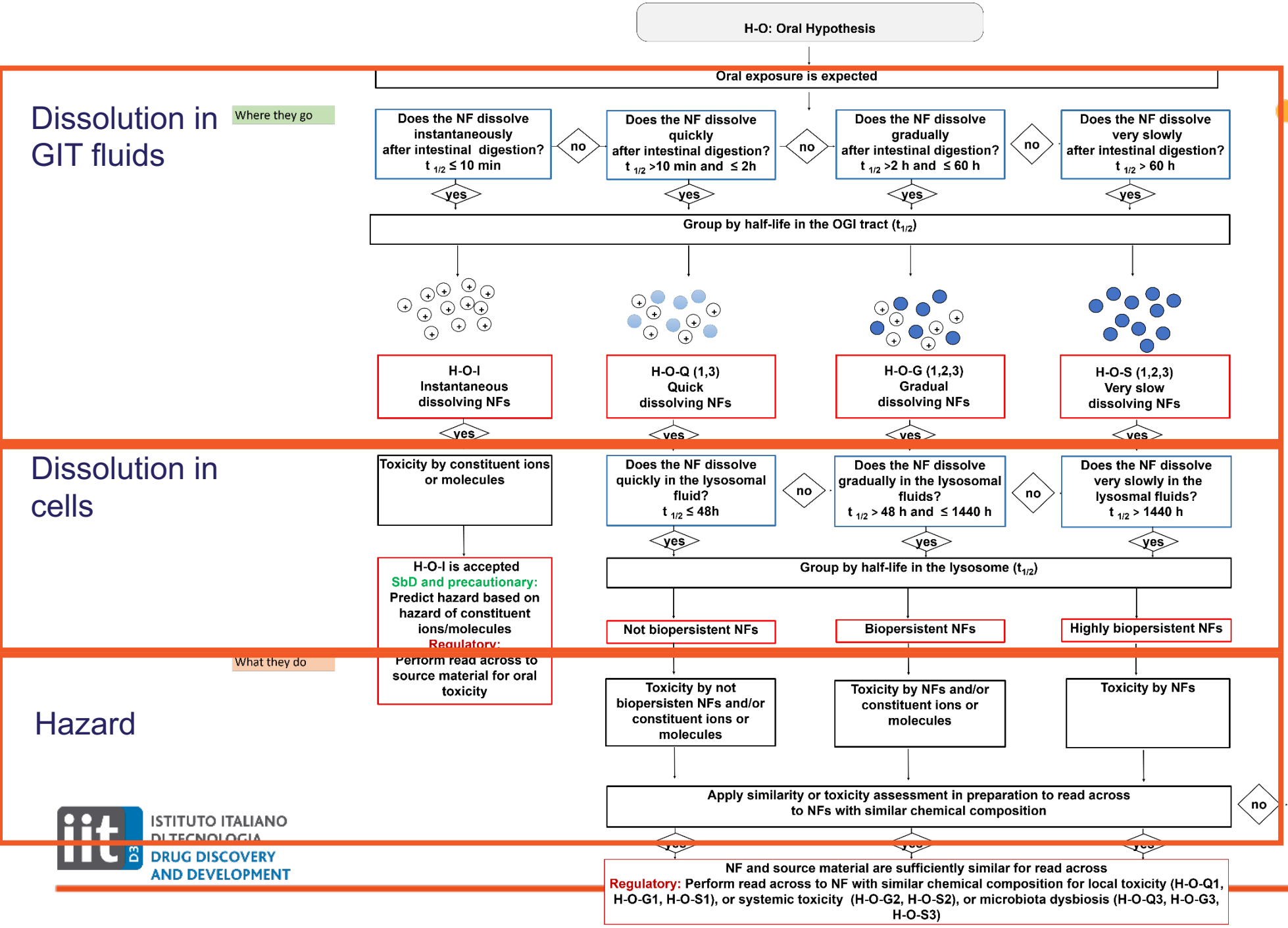
Oral IATA

# Hazard






*Di Cristo et al. in preparation*

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Oral IATA

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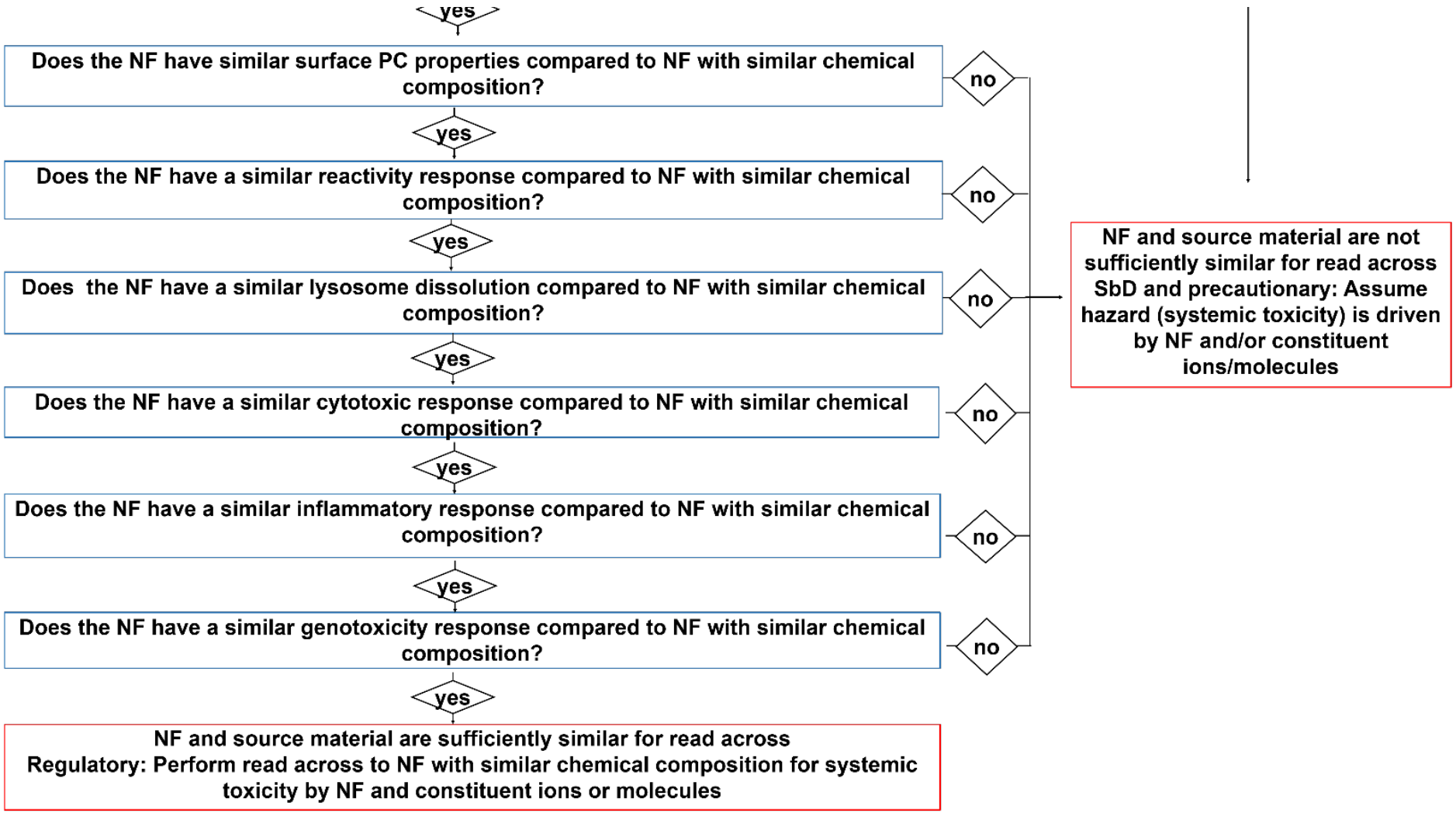
*Di Cristo et al. in preparation*

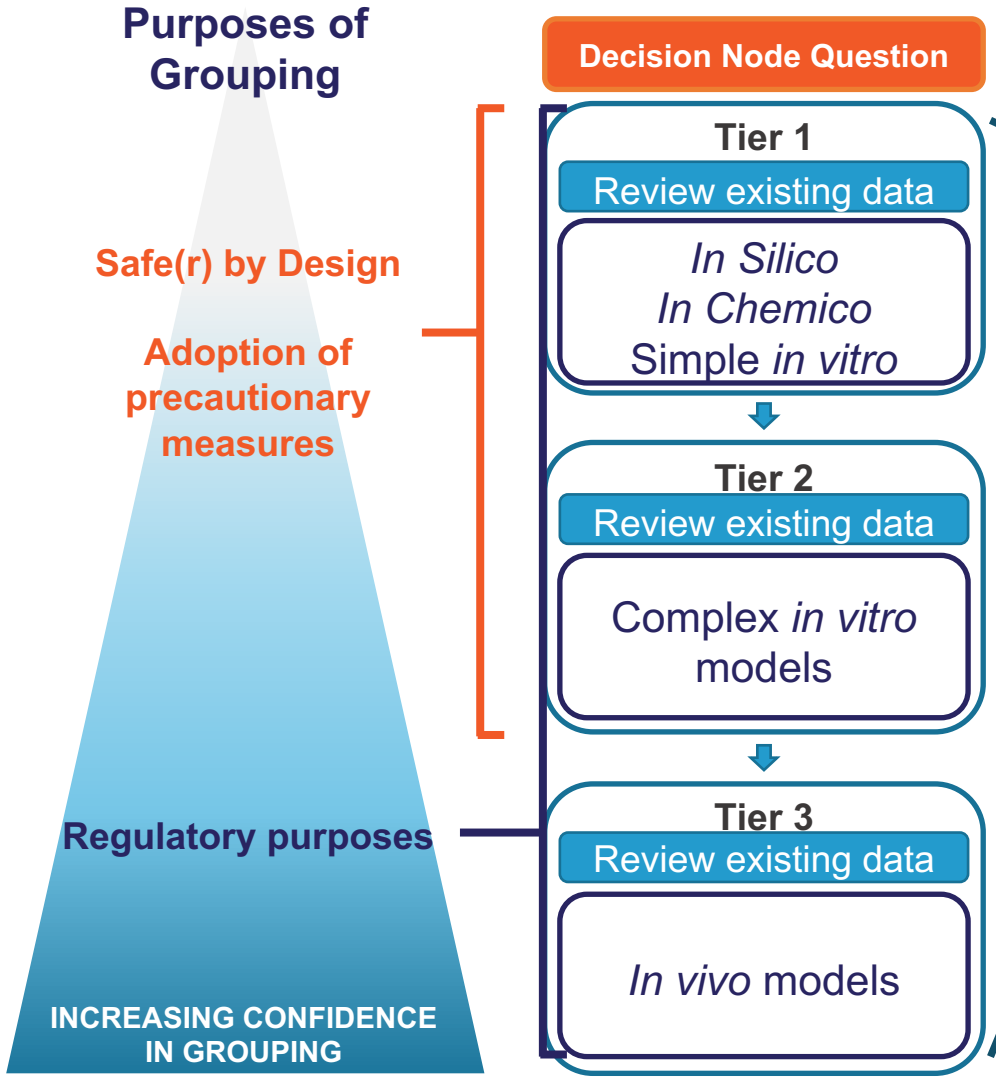
Oral IATA

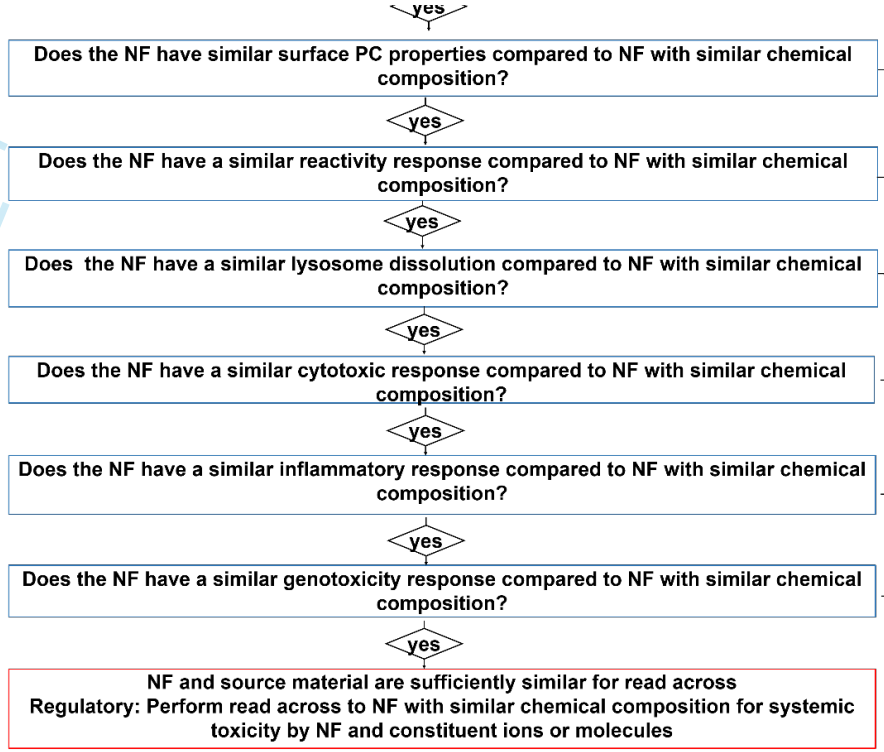
# Hazard decision nodes



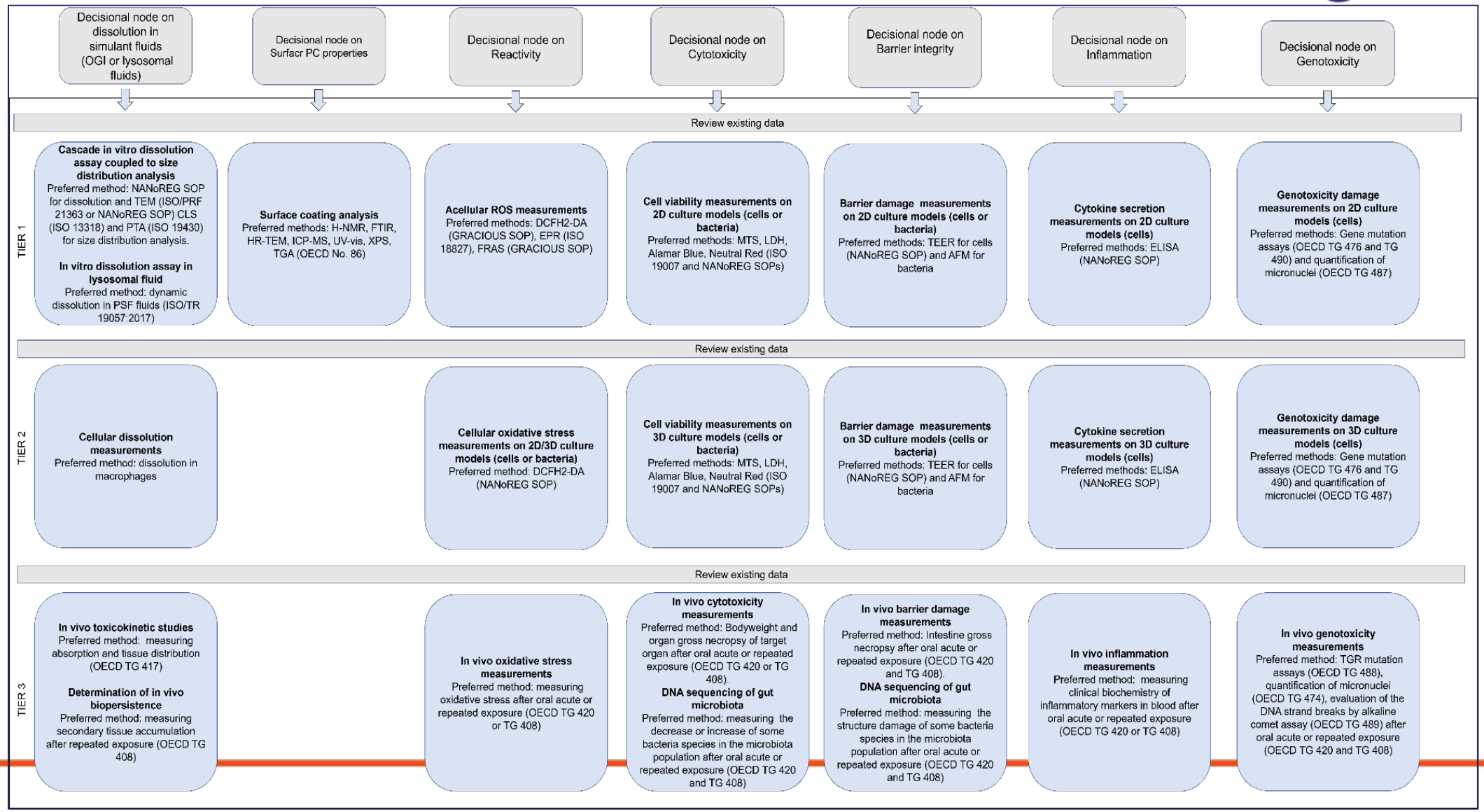
Tiered testing strategy

# 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)

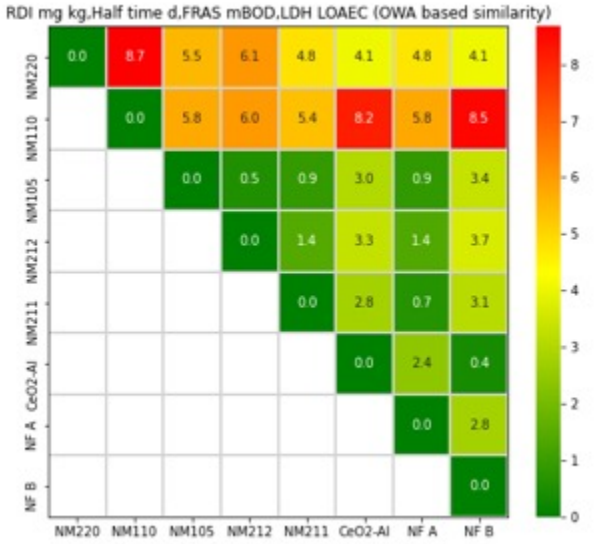
**How can we justify hazard assessment by grouping instead of animal testing? Concepts and usable tools to quantify the similarity of nanoforms**

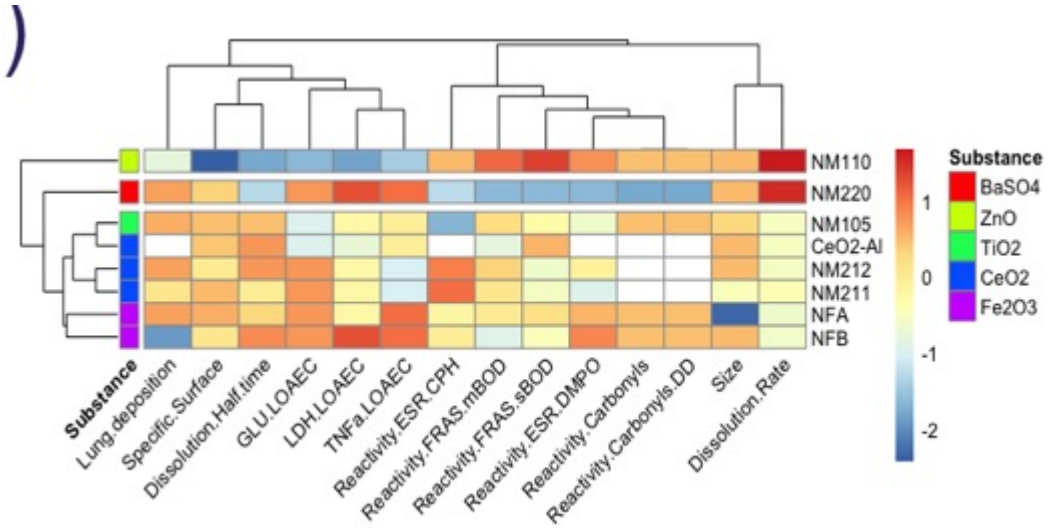
Nina Jeliazkova[a], Eric Bleeker[b], Richard Cross[c], Andrea Haase[d], Gemma Janer[e], Willie Peijnenburg[b], Mario Pink[d], Hubert Rauscher[f], Claus Svendsen[c], Georgia Tsiliki[g], Alex Zabeo[h], Danail Hristozov[h], Vicki Stone[i], Wendel Wohlleben[j]*

NanoImpact special issue
To be submitted June 2021

# 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)



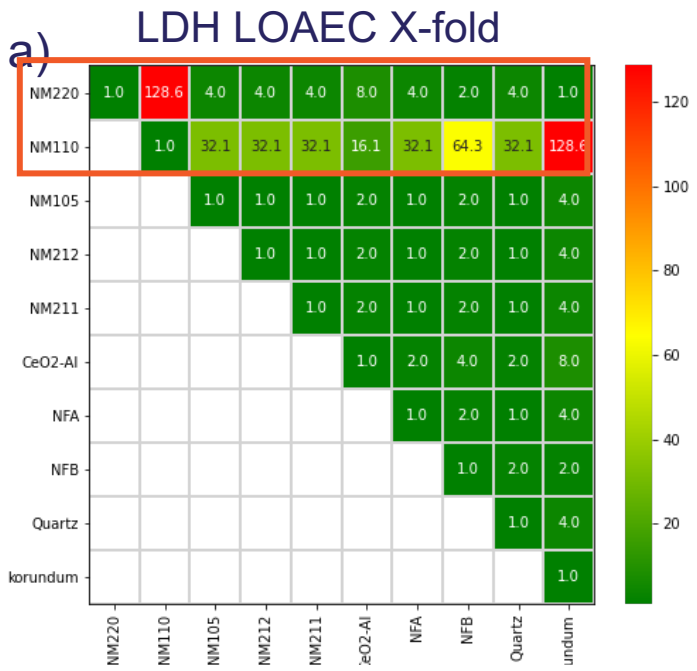

Results: Not robust between different algorithms.

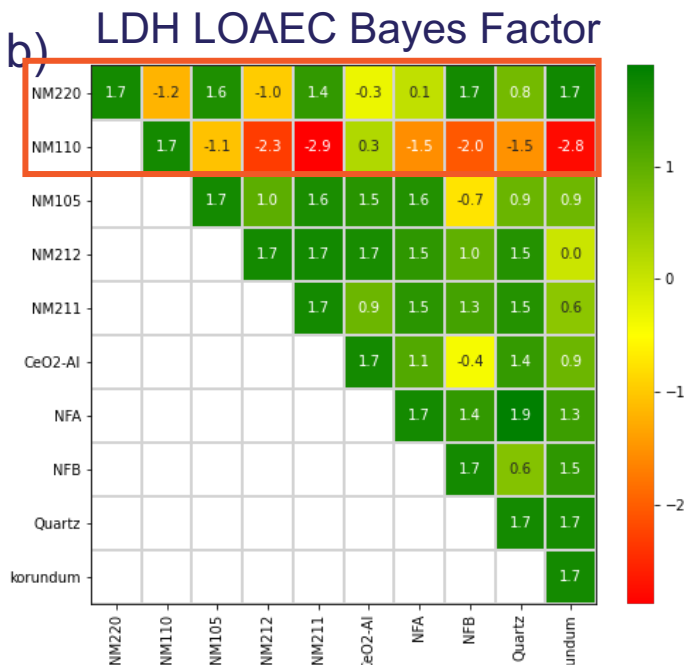
Conclusion: Multidimensional approaches are considered to be tools of discovery rather than regulatory methods.

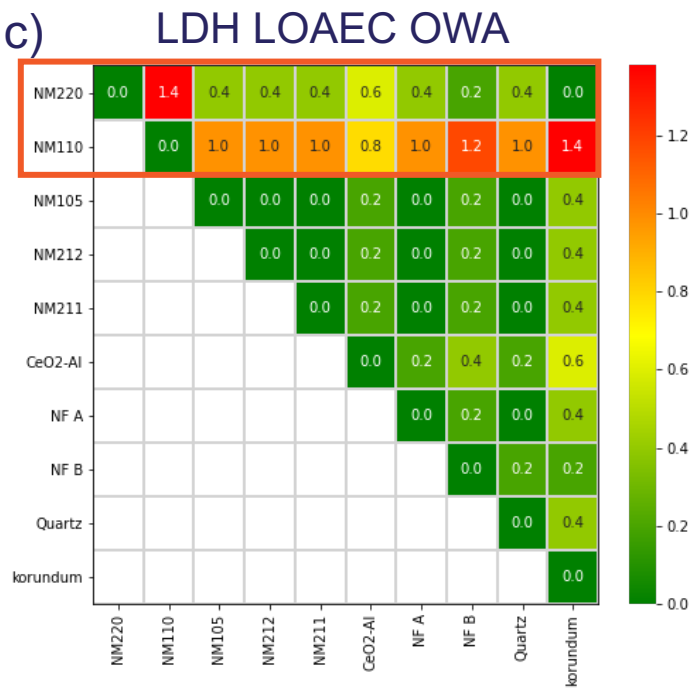
Pairwise property-by-property

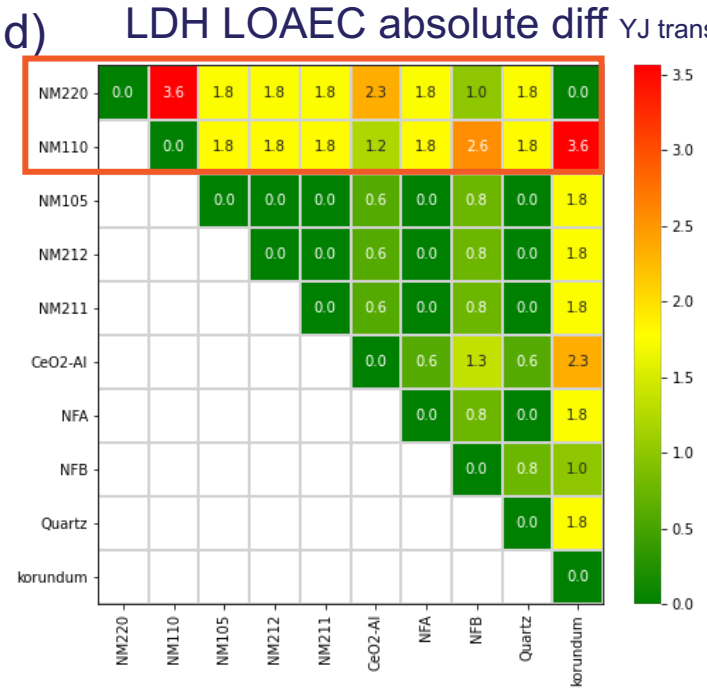
# In vitro toxicology

- Four algorythms compared
- The $CeO_2$ NFs and $Fe_2O_3$ NFs
  - similar within each substance,
  - clearly different from benchmarks.
- Similarity results for x-fold, OWA-based, Bayes are mostly consistent
- Numerical values used to assess similarity are different per algorithm.
- Absolute difference (1D Euclidean) required YJ transformation of data to give comparible results



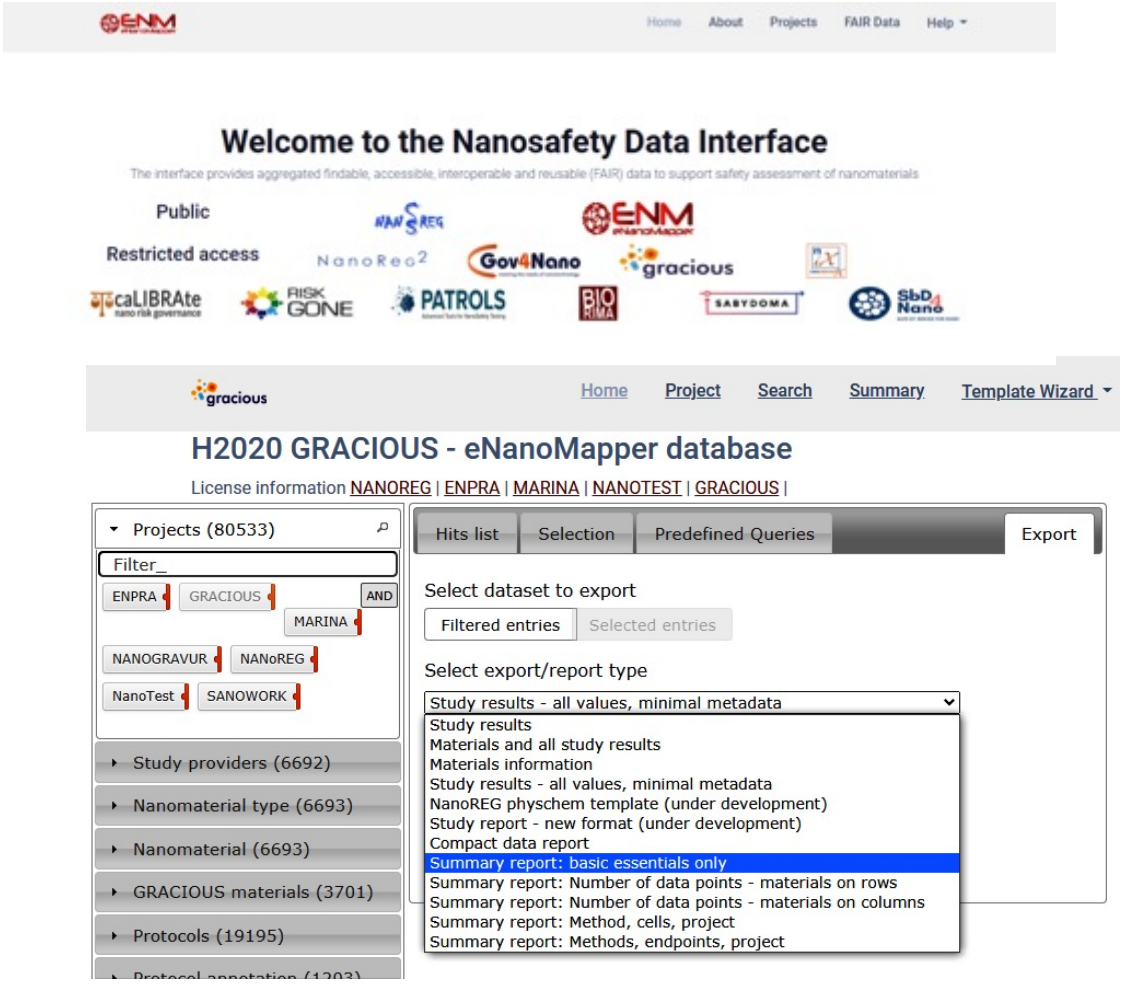



GRACIOUS links to eNanoMapper

# Using existing and new data




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

https://search.data.enanomapper.net/projects/gracious

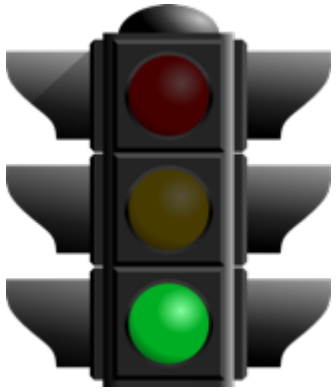
New and existing data

# 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**.

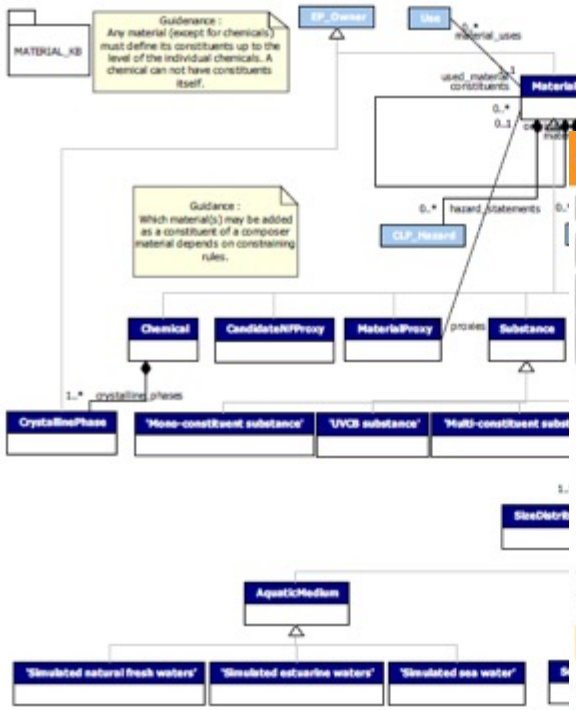


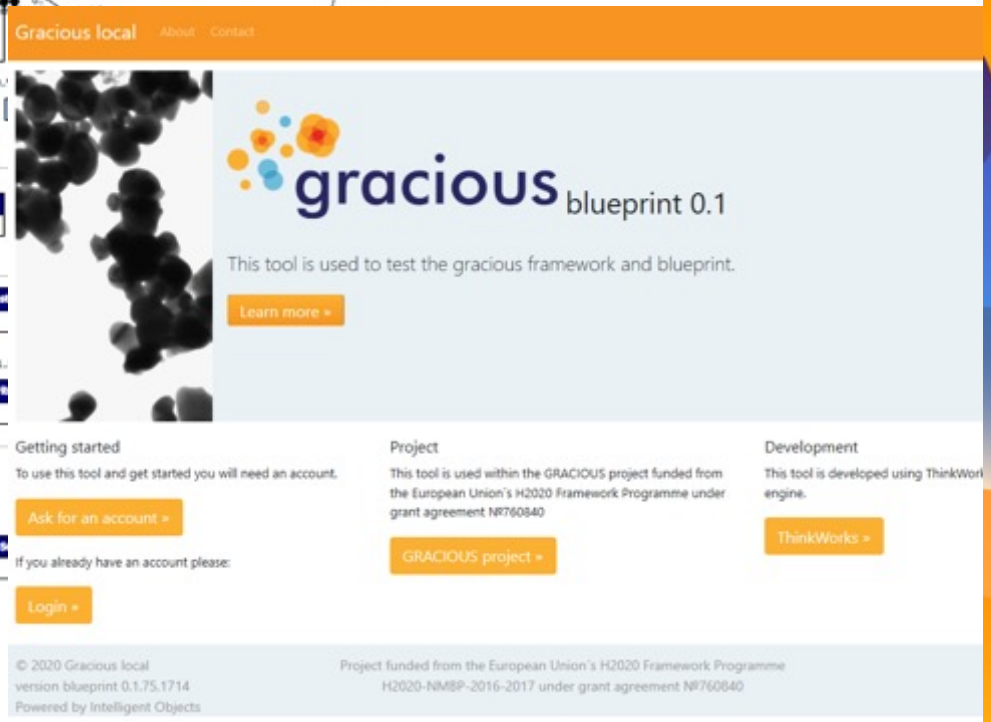
Red – data is of insufficient quality.

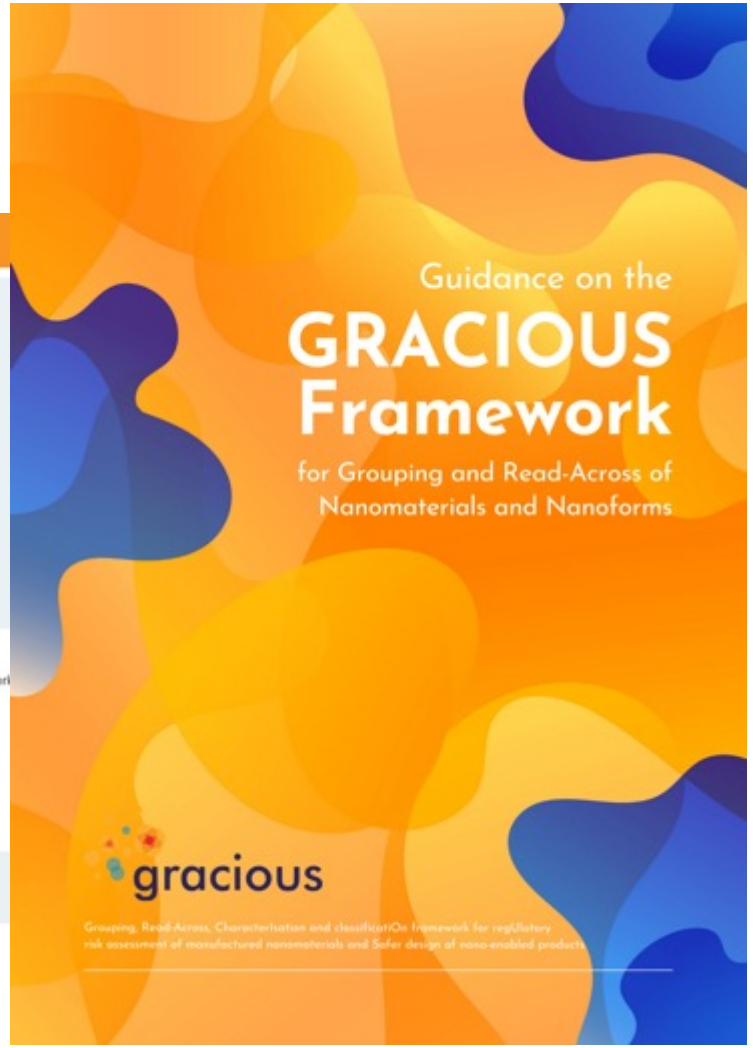
Yellow – data is sufficient quality, but needs further consideration to be used for a specific task.

Green – data high quality.

# Blueprint and Guidance Document

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Testing the Framework

# Case studies

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Research

Consultants
industry

Regulators &
Policy makers

REPÚBLICA PORTUGUESA
SAÚDE

Instituto_Nacional de Saúde
Doutor Ricardo Jorge

ARCHE
CONSULTING

CDC

BfR
Bundesinstitut für Risikobewertung

NANORIGO

BLUE FROG
SCIENTIFIC

JRC
EUROPEAN COMMISSION

ECHA
EUROPEAN CHEMICALS AGENCY

GRACIOUS Grouping Framework Design

# Outputs

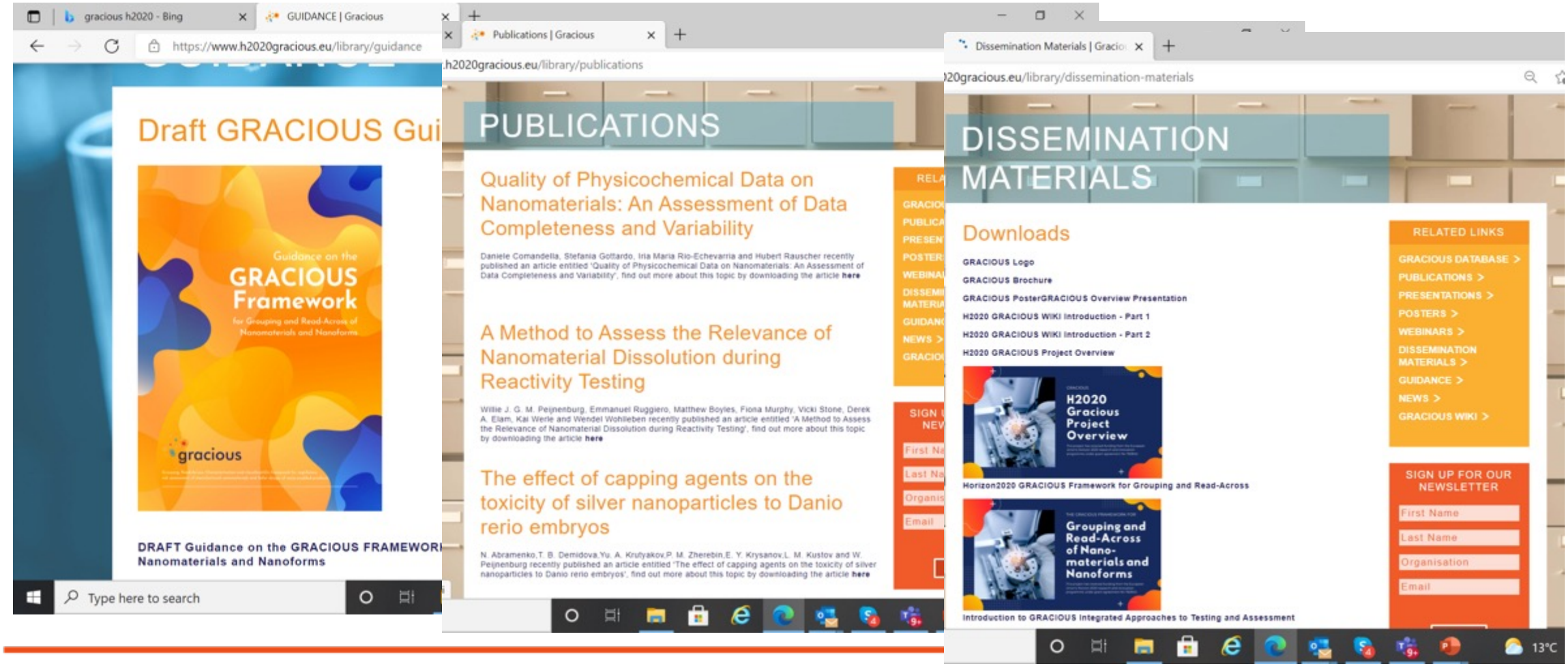
- Hypotheses
  - Pre-defined list complete
  - User-defined template complete
- IATAs
  - Human complete
  - Environment complete

- 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



# Thank you!

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