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# Development of an Integrated Approach to Testing and Assessment for grouping High Aspect Ratio Nanomaterials within The EU Project GRACIOUS

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## Introduction

The **GRACIOUS** project aims to develop a highly innovative science-based framework to facilitate the adoption of grouping and read-across and support the assessment of risk posed by an increasing array of nanomaterials (NMs) and their nanoforms (NFs) on the market and under development.

The GRACIOUS framework will be underpinned by **scientific hypotheses** which aim to identify descriptors relevant to grouping of different NM. Hypotheses for grouping will be substantiated by **Integrated Approaches to Testing and Assessment (IATA)** which rely on an integrated analysis of existing information coupled with the generation of new information to support a grouping decision. A tiered testing strategy has been developed to guide the gathering of the most relevant evidence (from literature and experimentation) required to assess similarity and support grouping based on the descriptors identified in the grouping hypothesis.

**Aim: to group high aspect ratio nanomaterials (HARN) based on their structural similarities to pathogenic asbestos fibres and potential to cause mesothelioma; a tumour linked with exposure to pathogenic fibres.**

A template is used to generate the HARN hypothesis including information relevant to:

- Lifecycle, release and exposure
- What they are: Physicochemical identity
- Where they go: Environmental fate, uptake and toxicokinetics
- What they do: human and environmental toxicity.

| | |
|---|---|
| **Purpose:** Precautionary, Regulatory, Safety by Design, Targeted testing<br>**Exposure Context:** Occupational | |
| **Input from life cycle**<br>Generated as a respirable aerosol during production or use<br>Workplace atmosphere<br>Inhalation exposure | **What they are**<br>High aspect ratio, rigid NF with slow dissolution rate and aerodynamic diameter to allow deposition in the distal lung |
| | **Where they go**<br>Deposit in the distal lung and translocate to the pleural cavity. Retained in the pleural cavity due to size-restricted clearance through stomata in the chest wall and diaphragm |
| | **What they do**<br>Cause frustrated phagocytosis as pleural macrophages attempt to remove them and result in chronic inflammation, mesothelial cell proliferation, fibrosis and, overtime, mesothelioma |

**Hypothesis:** Respirable, biopersistent, rigid HARN: Following inhalation exposure and translocation of HARN to the pleura, mesothelioma development can occur.

The **purpose** for grouping is linked to the potential **implications** of being placed in a group

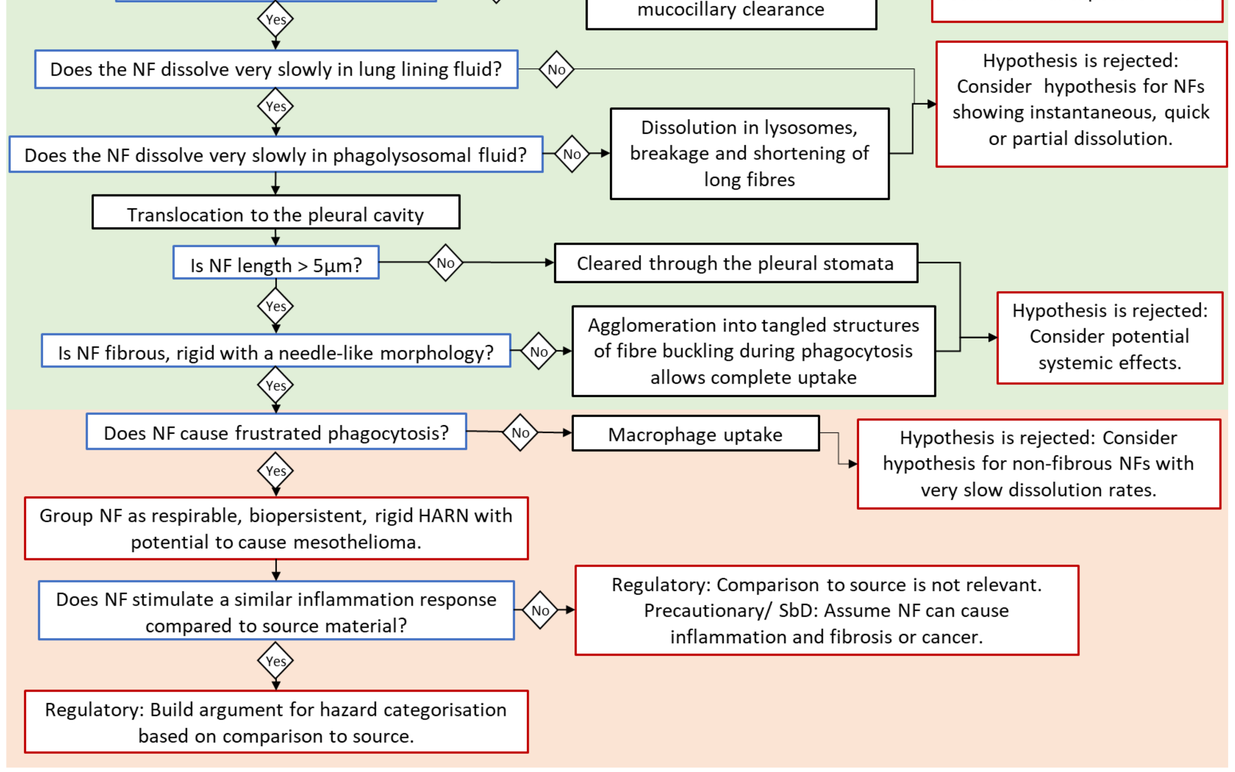
- Facilitating streamlined **risk assessment**.
- Hazard categorisation based on grouping with pathogenic source materials e.g. asbestos
- Waiving *in vivo* studies for mesothelioma

- Grouping to promote the adoption of **precautionary measures** for materials on which limited hazard data is available
- Adoption of occupational exposure levels or hygiene controls based on information on other members of the group

- Grouping to guide and support the innovation of **Safe(r)-by-Design** materials.
- Alter the PC characteristics that contribute to hazard potential for members of group

- Fill data gaps and increase **scientific understanding** of key mechanisms of fibre pathogenicity
- Contribute to refinement of the group

## Integrated Approach to Testing and Assessment




An **IATA** will guide information gathering and the testing strategy required to make an evidence-based decision on whether the grouping hypothesis should be accepted or rejected.

The representation of the pathway from exposure to the target site in a **decision tree** format allows the key PC characteristics directing fate and hazard to be identified along with potential points of departure where the user may deviate from, and therefore reject, the grouping hypothesis.

Based on the fibre pathogenicity paradigm, the HARN IATA prompts users to address relevant questions regarding the **morphology, biopersistence and inflammatory potential of the HARN** under investigation.

The IATA testing strategy is structured in a **tiered** manner, allowing each decision to be made on simple *in vitro* or *in silico* methods in the lowest tier or on complex *in vivo* approaches in the highest tier, depending on the purpose of the grouping.

-Lower tiers may be sufficient for Safe(r)-by-Design decision making
-Higher tiers may be required to support grouping for regulatory purposes

## Tiered Testing Strategy

| Can NF deposit in the distal lung? | Does the NF dissolve very slowly in lung lining fluid? | Does the NF dissolve very slowly in lysosomal fluid? | Is NF length >5µm? | Is the NF fibrous, rigid with needle-like morphology? | Does the NF cause frustrated phagocytosis? | Does NF stimulate a similar inflammation response to source material? |
|---|---|---|---|---|---|---|
| **Tier 1** | | | | | | |
| Review existing data sets | | | | | | |
| Estimation of $D_{ae}$ from NF size measurements by TEM/SEM and density measurement | Batch dissolution test in lung lining fluid (pH 7.4) or Dissolution in continuous flow system in lung lining fluid (pH 7.4) | Batch dissolution test in lysosomal fluid (pH 4.5) or Dissolution in continuous flow system in lysosomal fluid (pH4.5) | NF size measurements by TEM/SEM | Measure diameter of NF by TEM | Inflammasome activation:<br>• IL-1β release<br>• CathepsinB activity and /or release<br>• Lysosomal Disruption | Inflammation potency: *in vitro* testing using cell lines<br>Acute Endpoints:<br>• Cytotoxicty<br>• Cytokine release |
| **Tier 2** | | | | | | |
| Review existing data sets | | | | | | |
| Measurement of MMAD by cascade impactor from an airborne dispersion of the material | Durability in cellular systems | | NF size measurements by TEM/SEM from an airborne dispersion of the material | Measurement of flexural rigidity by dynamic scanning electron microscopy | *In vitro* incubation with co-culture models of macrophages and mesothelial cells or 3D microtissue models<br>Acute Endpoints:<br>· Cytokine release<br>Chronic:<br>· Granuloma formation | |
| **Tier 3** | | | | | | |
| Review existing data sets | | | | | | |
| Quantification of lung burden after in vivo inhalation studies (OECD TG 412/413).<br>Initial timepoint to measure deposition in distal lung<br>Longer timepoint to measure biopersistence | ‘Biologically stiff’ NF determined experimentally by morphological assessment and size measurements after *in vitro* incubation with macrophages. | | | | Intraperitoneal/ Intrapleural instillation:<br>Acute Endpoint:<br>• inflammation,<br>Chronic:<br>• Fibrotic lesion<br>• Mesothelioma | |

### Case study to test IATA performance

Aim: to test the capability of the IATA to differentiate between ‘mesothelioma-positive’ and ‘mesothelioma-negative’ MWCNT based on studies from the literature

| MWCNT<br>Meso-Hazard | Deposition in the distal lung | Dissolution/ Biopersistence | Fibre length >5µm | Rigid, needle-like morphology | Frustrated Phagocytosis | IATA outcome |
|---|---|---|---|---|---|---|
| **Mitsui-7**<br>Meso + | **YES (Tier 3)** | **YES (Tier1)** | **YES (Tier 2)** | **YES (Tier1)** | **YES (Tier2)** | **Accept** |
| **SD1**<br>Meso + | **Not reported** | **YES (Tier1)** | **YES (Tier 1)** | **YES (Tier1)** | **YES (Tier 1)** | **Provisionally Accept** |
| **N-CNT**<br>Meso + | **YES (Tier 3)** | **YES (Tier 3)** | **YES (Tier 2)** | **YES (Tier1)** | **Not reported** | **Provisionally Accept** |
| **NTlong**<br>Meso + | **Not reported** | **YES (Tier1)** | **YES (Tier1)** | **YES (Tier1)** | **YES (Tier 1)** | **Provisionally Accept** |
| **MWCNT+**<br>Meso - | **YES (Tier3)** | **NO (Tier3)** | **NO (Tier 2)** | **NO (Tier 1)** | **NO (Tier 1)** | **Reject** |
| **NTtng1**<br>Meso - | **Not reported** | **YES (Tier1)** | **NO (Tier 1)** | **NO (Tier 1)** | **NO (Tier 1)** | **Reject** |

Does the IATA correctly identify ‘mesothelioma-positive’ MWCNT?
**-YES**

Is the IATA sufficiently stringent to exclude the ‘mesothelioma-negative’ MWCNT?
**-YES**

## Conclusions

Following the GRACIOUS HARN IATA will support the evidence-based grouping of HARN with similar potential to pose a mesothelioma hazard.

By promoting the use of alternative, non-rodent approaches such as *in silico* modelling, *in vitro* and cell-free tests in the initial tiers, the IATA testing strategy will enable practical and more efficient risk analysis while reducing the ethical and economical burden of testing.

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## GRACIOUS Partners