MALTA INITIATIVE

Priority List for making OECD Test Guidelines and Guidance Documents applicable for Nanomaterials and Advanced Materials

Version 1

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The preparation of the Malta Initiative Priority List was a collective effort with multiple steps.

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Content

Introduction

Aim of the Malta Initiative Priority List

The Malta Initiative Priority List aims to define priorities for making OECD Test Guidelines (TGs) and Guidance Documents (GDs) applicable for nanomaterials and (other) advanced materials. Priorities are set with the aim to:

- Help making legislation implementable and supporting industry with their regulatory compliance.
- Highlight the importance of these activities by listing important topics and rating them.
- Provide guidance for decision makers towards funding.
- Guide and encourage scientists to support work towards OECD documents.
- Support the work performed by OECD.
- Collect resources to support the activities.

To serve this aim, the Malta Initiative Priority List will be actively shared with scientists, decision makers, industry, regulators and the OECD to help guide their work.

What is the Malta Initiative Priority list?

The list prioritises actions to support the development and amendment of OECD TGs for nanomaterials and (other) advanced materials, i.e. materials that come with specific properties and behaviour due to their size, shape and structure. The list is intended as a living document reflecting the current situation. Actions that are currently ongoing in the OECD Test Guidelines Programme are not included in the Priority List; as its purpose is to highlight needs that are not already covered by an ongoing OECD project.[1] It is foreseen that the Malta Priority List will need regular updating (e.g. every 3 years) to include progress made, either by work performed towards the actions themselves or by outside factors (e.g. changes in regulation may prompt new needs/prioritisations, new materials may question applicability of test methods for them).

What information is provided in the Malta Initiative Priority List

For each topic in the list, it indicates the specific products aimed for (e.g. amended / new TG, GD or scoping review) and a tentative estimated timeframe. This is complemented by a brief overview of the state of the art, resources to be considered and a description of the action required. The topics were rated as highly relevant according to the following questions:

(a) Is the action relevant for multiple TGs or endpoints?
(b) Is it broadly relevant for regulation?
(c) Is it industrially relevant?
(d) Can an output that supports regulatory testing be created quickly?

An overall score for relevance was calculated according to the following equation: *(a+b+c)*d* (Table 1 provides an overview of the different topics in the list with their scoring). The list is sorted within each section according to relevance, starting with the action that achieved the highest overall score.

[1] For an overview of ongoing and finished (OECD) projects consider the Status Report by Heunisch et al.

Steps towards the Malta Initiative Priority List

The description and the rating of topics was a collective effort that was coordinated by the authors of this list and involved the following steps. Initial discussions on preliminary topics to be included in the list were based on the expert knowledge of the authors. These authors are experts in the field of physical chemical properties, human and environmental toxicity and are industry representatives, scientists and regulators.

In the next steps, discussions in the OECD WPMN and Malta Initiative, as well as different publications were considered. Key publications include a review by Bleeker et al. (2023), a roadmap by Rasmussen et al. (2023), and a report from ECHA on "Key Areas of Regulatory Challenge".

A further step involved a broad stakeholder consultation using an online survey that was broadly distributed. Feedback was also collected during different meetings e.g. from ECHA's Nanomaterials Expert Group, the NanoMesureFrance Association and the NanoHarmony consortium. Valuable feedback on both the relevance and the content of the topics were received. In general, a broad support for the Malta Initiative List was expressed.

Feedback from online survey

A total of 31 experts participated in the online survey. Participants were well distributed over the different stakeholder groups and the different countries in and outside of Europe (see Figure 1). Comments were also well distributed over the different topics. 26 participants commented on the physical chemical section, whereas 13 answered on health effects, 12 on the environmental endpoints and 11 on the other topics.

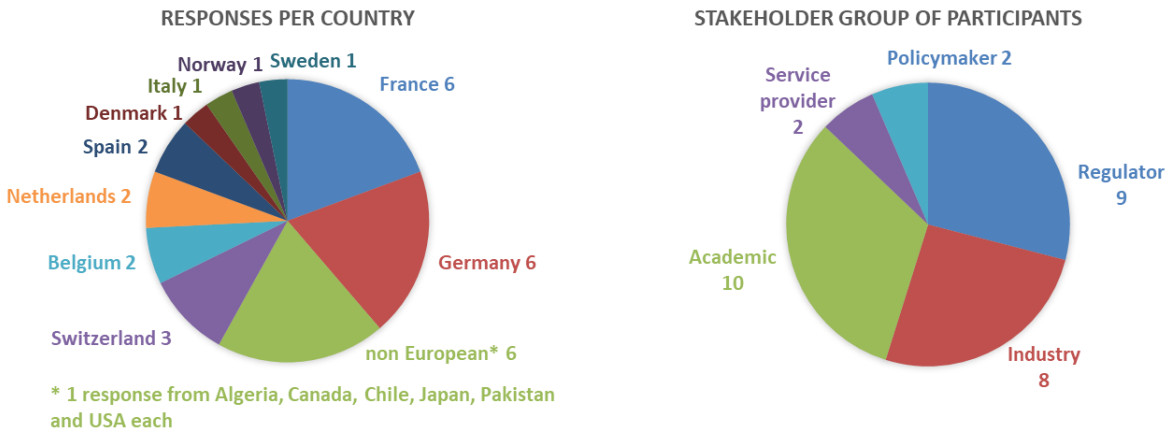

Figure 1: Overview of survey participants.

Call for action

With this Priority List, the Malta Initiative calls for action to ensure that the TGs needed to ensure regulatory compliance for nanomaterials and (other) advanced materials are available. For citizens to benefit from the advances being made in materials research and development whilst ensuring the safety of humans and the environment, there needs to be equal focus given to the innovations required in the regulatory space. New materials will not reach the market without the availability of harmonised test methods that can be used to ensure quick and effective regulatory compliance.

The European Commission, OECD Member Countries and all concerned stakeholders are encouraged to pick up one or more actions needed for topics on the list. As developments in materials and testing methodologies emerge, new topics and actions may also be identified to be added to the list. In this way, the Malta Initiative aims to push the development/adaptation of OECD TGs/GDs and ensure that these remain up to date. In the end this will ensure legislation remains implementable and industry is supported in their regulatory compliance.

The following recommendations are therefore suggested:

1) That relevant funding agencies in Europe and other OECD Member Countries take this list into account when setting funding opportunities to support the development of harmonised test methods.
2) That project consortia applying for funding opportunities prioritise their objectives to focus upon the test methods with the highest priority.
3) That policymakers understand the need to focus their efforts to help ensure the availability of test methods when considering regulation of nanomaterials and (other) advanced materials.
4) That industry, including Contract Research Organisations, testing laboratories and other industry service providers, continues to aid the development of test methods and focus upon the most needed and most important methods and encourage others to do so to help ensure these methods are made available.
5) That researchers focus their attention on the priorities identified in the Malta Initiative Priority List when considering how they advance their research for use in validation, standardisation and harmonisation.

Table 1: Overview and scoring of topics

Topic	Scoring for relevance: 1 (low), 2 (medium), 3 (high)			d) Scoring for time needed*	Total score (a+b+c)*d
	a) for TGs/ endpoints	b) for regulation	c) for industry		
Section 1: Physical Chemical Properties					
1.1 Preparation and measurement of stable dispersions in liquid test media	3	3	3	3	27
1.2 Aerosol generation for toxicity testing for in vivo and in vitro	3	3	3	2	18
1.3 Determination of concentration of carbon-based materials in biological media / tissues	3	2	3	2	16
1.4 Determination of concentration of carbon-based materials in environmental test compartments	3	2	3	1	8
1.5 Determination of critical fibre rigidity	1	2	2	1	5
Section 2: Effects on Biotic Systems					
2.1 Effects on terrestrial organisms	3	2	3	2	16
Section 3: Environmental Fate and Behaviour					
3.1 Bioaccumulation potential	2	3	1	2	12
3.2 Biotic degradation	3	2	3	1	8
Section 4: Health Effects					
4.1 Testing nanomaterials in in vitro assays	3	3	2	3	24
4.2 Acute toxicity inhalation (in vivo)	2	2	3	3	21
4.3 Genotoxicity / mutagenicity (in vitro)	3	3	3	2	18
4.4 Developmental neurotoxicity (in vitro)	2	3	3	2	16
4.5 Acute toxicity inhalation / respiratory sensitisation (in vitro)	3	2	2	2	14
4.6 Skin sensitisation (in vitro)	3	2	2	2	14
4.7 Fibre toxicity	2	2	3	2	14
4.8 Testing the reactivity of nanomaterials	2	2	2	2	12
4.9 Reproductive toxicity	3	3	3	1	9
4.10 Inflammation induction (in vitro)	1	2	1	2	8
Other connected issues					
5.1 Exposure assessment	2	3	2	3	21
5.2 Predictivity and sensitivity of in vitro assays and other NAMs for nanomaterials	3	2	2	2	14

**3 (≤ 3 years); 2 (4 – 6 years); 1 (> 6 years) for the development of a product that supports regulatory testing (specific Guidance Document or Test Guideline)*

1 Actions to support the applicability of OECD TGs and GDs in Section 1: Physical Chemical Properties

1.1 Preparation and measurement of stable dispersions in liquid test media

Section 1: Physical Chemical Properties

Estimated duration: **3 years**	Foreseen product(s): » **OECD Guidance Document** on preparation and measurement of dispersions of nano- and advanced materials in relevant media

State of the art and resources to be considered

Test guidelines have been developed for chemicals which are soluble, hence using those with dispersed samples represents a challenge, since nanomaterials may precipitate over time, changing sample concentrations and leading to differences in behaviour. Sample dispersion also represents the first step in any testing approach, and non-optimal dispersions are one main cause of lack of reproducible results. Sample preparation is also dependent on the final test to be used, and will be different among physical-chemical tests, human toxicity or ecotoxicological testing, while route of exposure in the body is also relevant. Besides, different nanomaterials may also show differences in sample preparation due to e.g. changes in hydrophobicity and particle stability. This needs to be considered in both, surfactants used and energy applied. The effect of surfactants and their compatibility with tests (e.g. *in vitro* tests) needs to be considered. A number of nanomaterial dispersion protocols are currently available (NIST, PROSPEcT, Nanogenotox, ENPRA, NanoDefine, OECD GD 317, enhanced NANoREG ECOTOX, OECD TG 318, DeLoid et al.) however the high variety of nanomaterials available, suggests that a general dispersion protocol applicable to all is unlikely to be developed. For the measurement of dispersion several methods are available (ISO 19337:2023, ISO13097:2013).

Action required and output / product(s) foreseen

Currently under the WPMN Steering Group on Testing and Assessment, a new guidance on Sample Preparation and Dosimetry is being developed which covers sample preparation requirements for physical-chemical, ecotoxicological and mammalian end points. This guidance represents a large and ambitious effort from several OECD delegations, and it is slowly progressing due to lack of resources. An updated overarching guidance is required that includes the following parts:

1. A clarification of the stability of the dispersion required for its targeted endpoint and test method, versus realistic exposure scenarios and how to deal with and report on unstable dispersions, including (minimum) quality criteria (e.g. stability, homogeneity, agglomerates versus single particles, additives, etc.) of dispersions.
2. Protocols on characterisation of the dispersion in terms of stability, homogeneity, concentration, effective density, size distribution etc. and protocols on how to determine the dissolved fraction.
3. A decision tree for the preparation of stable dispersions, that guides the user step by step depending on material classes and targeted test method/endpoint to an appropriate dispersion protocol and links to protocols for dispersion e.g. ISO and CEN as well as scientifically developed.

Next to the overarching guidance additional protocols for the dispersion of e.g. hydrophobic or 2D- and 1D-materials or nanoplastics need to be developed and standardised (see also Connolly et al. 2023).

1.2 Aerosol generation for toxicity testing for *in vivo* and *in vitro*

Section 1: Physical Chemical Properties

Estimated duration:	Foreseen product(s):
5 years	» **Guidance Document** on aerosol generation for toxicity testing

State of the art and resources to be considered

Controlled generation of aerosols and measurements of relevant doses are pivotal for *in vivo* lung toxicity as well as for *in vitro* testing at the air-liquid interface (ALI). As stated in the Guidance on Sample Preparation and Dosimetry dry powder generation methods are to be preferred for those materials that are in dry status. The generation of aerosols from dry powders has the advantage of using the powder without prior sample treatment (e.g. dispersion in liquid) that will alter the properties of the material. However, more and more materials are generated in dispersion (e.g. by polymerization and co-precipitation in the case of several nano-carriers) and dedicated aerosolization systems have been developed to dispense liquid dispersions. Choosing the right parameters and techniques for aerosol generation will allow testing of realistic aerosols that represent exposure scenarios (Kuhlbusch et al. 2011). The Guidance on Sample Preparation and Dosimetry lists different techniques for generation of an exposure atmosphere. Possible techniques are for example the venturi (Cheng et al. 2008) and a jet-mill (Cheng et al. 2010) for dry materials or vibrating mesh nebulizer or "chemical printing" for liquid dispersions. These techniques are however to a large extent not standardised. Challenges differ for testing *in vitro* and *in vivo*. For *in vivo* tests stable exposure conditions are needed to be maintained and monitored over a long period. For *in vitro* tests, deposition on cells and (quantitative and qualitative) determination of the dose are challenging.

The question of how to create stable aerosols from nanomaterials in a reproducible manner that mirrors possible exposure scenarios remains open. Standardised SOPs on aerosol generation, monitoring of aerosols, application on cells and dose characterisation are missing. Definition of quality standards for both *in vivo* and *in vitro* testing is needed and guidance on how to reach those standards.

Action required and output / product(s) foreseen

Existing SOPs on aerosol generation, monitoring of aerosols, application on cells and dose characterisation are to be collected, (further) developed and standardised. The action can also build on SOPs developed for dustiness testing of nanomaterials (e.g. Broßell et al, EN 17199-1:2019, ISO/TS 12025:2021, NANoREG framework for the safety assessment of nanomaterials) and develop them further for generation of stable aerosols with sufficient quantity.

Quality standards and approaches towards generation, characterisation, application and dose characterisation (considering different metrices) as well as dispersion and homogeneous distribution of the aerosol are to be described in a Guidance Document including and linking (standardised) SOPs.

1.3 Determination of concentration of carbon-based materials in biological media / tissues

Section 1: Physical Chemical Properties

Estimated duration:	Foreseen product(s):
3 years	» **Overarching Guidance Document** on issues to be considered for determination of concentration of carbon-based materials in biological media/tissues
6 years	» Research and further **Guidance Document**

State of the art and resources to be considered

Much of the research on nanomaterials in the last decades has focused on development, use and impact of metallic nanomaterials. This has pushed the technological boundaries for certain analytical techniques, such as single particle-Inductively Coupled Plasma Mass Spectrometry (sp-ICP-MS,) which however, is of no or very limited use when applied to the carbon-based materials that are an emerging trend in nanotechnology. Therefore, new techniques and/or adaptations of techniques already in use should be explored in order to allow the detection and, possibly, quantification of carbon-based nanomaterials once embedded in biological matrices. This links directly with the OECD WNT Project 1.10 on new Guidance Document on the determination of concentrations of nanomaterials in biological samples. A draft Guidance Document already exists that represents an excellent basis for metallic materials. However, further research is needed in order to improve, understand limitations and applicability of less conventional techniques. Techniques that can support the detection and quantification of carbon-based nanomaterials in biological tissues are for example as dark field microscopy coupled to Hyperspectral Imaging (DFM-HIS), Raman, confocal fluorescence microscopy, different mass spectrometry techniques, new machine learning tools for image recognition and Secondary Ions Mass Spectrometry (SIMS) coupled to different detectors such as ionization sources.

Action required and output / product(s) foreseen

Using as a starting point the overarching Guidance Document that is under preparation and that covers metallic and carbon-based materials, further research and technical developments should be achieved in order to understand potential and limitations of current techniques. Further understanding is required on how to use them for the detection and quantification of carbon-based material classes in biological matrices. Examples of carbon-based material classes are graphene-related materials, fullerenes, carbon nanotubes, carbon black, polymer particles, etc.. These activities should be summarised in an overarching guidance and can be partially supported by running international activities, such as NAMs4NANO (EFSA Tender), European projects like MACRAMÉ, POTENTIAL, and an ongoing ECHA EUON study on C-based nanomaterial detection and quantification in environmental and biological matrices, etc.

1.4 Determination of concentration of carbon-based materials in environmental test compartments

Section 1: Physical Chemical Properties

Estimated duration:	Foreseen product(s):
2 years **6 years**	» First a **scoping review** to establish state of the art of protocols available » Standardisation of protocols to determine the concentration of carbon-based nanomaterials and advanced materials in the environment (soils/sediment) for **OECD Guidance Document** or input in Guidance Document

State of the art and resources to be considered

So far, the development of the OECD documents for nanomaterials is based on the experiences gained from metal and metal oxides. Main challenges come with the identification of carbon-based nanomaterials against a rich organic background in complex organic matrices. Only very limited methods are available (e.g. radio-labelled nanomaterials like carbon-14). There is a strong need for specific and multiple analytical and spectroscopic methods for the detection and quantification of graphene related materials and other carbon-based nanomaterials in environmental compartments (soils / sediments). This was e.g. highlighted in a recent report by ECHA on graphene and other 2D materials and in an overview of regulatory needs (Bleeker et al. 2023). The nanospecific OECD Guidance Documents 317, 318 and 342 also flag the issue.

There is one ongoing OECD-project (TGP-project 1.10) aiming to provide guidance on detecting and measuring nanomaterials in biological tissues, but the topic needs to be widened to also cover different environmental compartments such as sediments or soils. The urgency comes also from the increasing number of carbon-based materials entering the European market, e.g. nanoplastics, nanotubes, graphene, modified cellulose. Furthermore, these different categories may require different approaches. This is putting pressure on the development of acceptable standards to detect nanomaterials in complex organic matrices to fulfil the regulatory information requirements. Insights from agricultural chemistry and the pesticides area may provide starting points.

Action required and output / product(s) foreseen

A scoping review on the current state of art on carbon-based nanomaterial detection and quantification in environmental test compartments could provide a systematic overview on detection and quantification of different carbon-based material classes (e.g. graphene-related materials, fullerenes, carbon nanotubes, carbon black, polymer particles, etc). An ongoing ECHA EUON study on C-based nanomaterial detection and quantification in environmental and biological matrices could serve as a basis for the scoping review. This will provide a clear indication of what is currently possible for which category of materials and where limitations are (e.g., issues with background levels), while in parallel identifying next steps for development efforts. Such a systematic overview will help to prioritise the work while clearly indicating the steps to be taken by relevant key players to progress these developments. This would then be followed by the development of standardised methods and protocols leading to OECD Guidance Document in the long run.

1.5 Determination of critical fibre rigidity

Section 1: Physical Chemical Properties

Estimated duration:	Foreseen product(s):
2 years	» **OECD scoping review** and subsequently further OECD Guidance Document or Test Guideline

State of the art and resources to be considered

Rigidity is an important material property of fibres. It is hypothesised that rigid fibres have a higher probability to cause adverse health effects than flexible fibres. These effects are in line with the fibre-pathological paradigm. The rigidity of fibres depends on the bending modulus, and the thickness of the fibres. The specific synthesis method used to produce the fibres also partly determines the rigidity. However, the determination whether (nano)fibres fall into the category of rigid or flexible fibres is difficult as the actual threshold value is still under discussion. Furthermore, (standardised) test-methods for determination of rigidity are still missing. Various promising approaches to measure fibre rigidity and to determine the critical rigidity are currently discussed and under development. The methods range from measurement of electromechanical resonance (Fortini et al. 2020) to *in vitro* approaches and modelling based on material parameters and morphology of fibres.

Action required and output / product(s) foreseen

An OECD scoping review is foreseen as a first action with the following two objectives:

a) To create a common understanding of the relevance of fibre rigidity for hazard assessment and a definition of the measurand.
b) To get an overview of test-methods for the determination of fibre rigidity including their associated uncertainties, to determine the correlation between different factors influencing the rigidity and to identify the potentials and limitations of these methods, as well as to explore additional/alternative (indirect) methods.

Based on this an OECD Guidance Document and/or Test Guideline should be developed to provide detailed information on how to determine the rigidity of fibres.

2 Actions to support the applicability of OECD TGs and GDs in Section 2: Effects on Biotic Systems

2.1 Effects on terrestrial organisms

Section 2: Effects on Biotic Systems

Estimated duration:	Foreseen product(s):
3 years	» **Overarching Guidance Document** on issues to be considered for determination of terrestrial toxicity
6 years	» Research and further **Guidance Document**

State of the art and resources to be considered

Much of the focus so far has been towards the aquatic environment (including sediment systems). However, soils are a major sink for nanomaterials. Furthermore, the particulate nature of these materials prohibits the use of equilibrium partitioning approaches to predict terrestrial toxicity from aquatic toxicity data. This will increase the need for terrestrial toxicity testing. Therefore, potential adaptations for soils testing of nanomaterials needs to be further examined. Most issues with soil testing are likely related to dosing and determining actual exposures. OECD GD 317 provides guidance on spiking sediments which may be applicable to soils as well. Research results from European projects such as PROSPEcT, NanoFATE, NanoFASE, GUIDEnano, or from the UK-US Initiative TINE may provide further insides, such as SOPs for methods used, the need for more long-term exposures (Diez-Ortiz et al. 2015) or potential influence of different spiking procedures on test outcomes (Waalewijn-Kool et al. 2012).

Action required and output / product(s) foreseen

Based on current knowledge an overarching Guidance Document on issues to be considered for determination of terrestrial toxicity appears needed, similar to OECD Guidance Document 317 for aquatic and sediment toxicological testing of nanomaterials. In addition, and (partly) in parallel a gap analysis may be needed to identify (further) gaps in toxicity testing of terrestrial organisms for regulatory purposes.

3 Actions to support the applicability of OECD TGs and GDs in Section 3: Environmental Fate and Behaviour

3.1 Bioaccumulation potential

Section 3: Environmental Fate and Behaviour

Estimated duration:	Foreseen product(s):
3 year	» **Overarching Guidance Document** that outlines the steps in a tiered approach to determine bioaccumulation potential of particulate materials.
6 years	» Further specific **Test Guidelines** for some of the tiers in this approach.

State of the art and resources to be considered

For particulate materials that form colloidal dispersions the octanol-water partitioning coefficient is inappropriate for many of such materials. As a result, there is currently no trigger for bioaccumulation testing strategies for these particulate materials, and to proceed directly to *in vivo* bioaccumulation tests with fish is then the only option. With support from the NanoHarmony project an OECD WPMN scoping review was written to explore the possible options for a tiered approach to bioaccumulation testing. It outlines an example scheme, based on available tools or test methods. In addition, it provides data to show potential linkages between the possible tiers in the testing strategy, together with an evidence-base for seeking alternatives to using live fish (e.g. the use of the freshwater arthropod *Hyalella azteca*). While this tiered approach is promising, it needs further development and research. Currently, the scientific basis stems mainly from metal or metal oxide nanomaterials. To explore whether the tiered approach is suitable for carbon-based materials further research is needed. For some of the tiers (additional) Test Guidelines need to be developed as well.

Action required and output / product(s) foreseen

To explore whether the tiered approach is suitable for carbon-based materials further research is needed (including the setting of triggers to move to a next tier). This also requires suitable methods to determine such materials in biological media, which is closely linked to ongoing activities on the topic. For some of the tiers (additional) Test Guidelines need to be developed as well, most notably on the gut-sac approach to determine potential uptake from fish gut into the organism. Also, for other TGs the applicability or nanomaterials and other advanced materials may need further research.

3.2 Biotic degradation

Section 3: Environmental Fate and Behaviour

Estimated duration: **6 -8 years**	Foreseen product(s): » **Overarching OECD Guidance Document** on the biodegradability of nanomaterials including physical changes of particles

State of the art and resources to be considered

The principles and strategies for testing degradation of organic chemicals described in the revised introduction to the OECD guidelines for testing of chemicals, Section 3 are generally applicable to nanomaterials that are organic or have an organic component that could be biologically degraded. Extension is required to provide guidance on when it is necessary to test for biodegradability of a nanomaterial / component(s) of a nanomaterial and how to interpret this information (e.g. where degradation results in physical changes of the nanomaterial). The Physical-Chemical Decision Framework to Inform Decisions for Risk Assessment of Manufactured Nanomaterials does not provide clear guidance on when to test for biodegradability of nanomaterials or how to do so.

Screening tests can in principle be applied to organic nanomaterials or to test degradation of organic components of more complex nanomaterials. There are, however, physical limitations to the applicability of these tests on such materials:

- Screening tests require a high concentration of organic substrate (10-1000 mg/L), as biodegradation is measured indirectly.
- The degradable component of nanomaterials may make up a relatively small fraction of the material, but can have a significant impact on the exposure/hazard profile of the material (e.g. the encapsulations of encapsulated pesticides may degrade and toxic pesticides released in locally high concentrations).
- At such high concentrations, nanomaterials and the inoculum may be unstable due to aggregation/agglomeration or may be toxic to the inoculum, potentially precluding the use of screening tests for many nanomaterials.

Simulation tests therefore may be necessary for the testing of nanomaterials where only a component of the material is under assessment for biodegradability. This provides the benefit, however, that simulation tests allow for testing under conditions representative of specific environments. Soils in particular have been highlighted as a knowledge gap.

Action required and output / product(s) foreseen

To what extent degradation of nanomaterials and/or their organic constituent parts alters the exposure and hazard profile of the material is an important question in risk assessment. This includes questions on the role of physical changes of the material. The existing biodegradation tests are not designed to address this question. Knowledge on the predictivity of such tests on how biodegradability drives directional changes in exposure and hazard profiles is needed. Likewise, predictivity of existing information on biodegradability of surface treatment chemicals is unknown for when such chemicals are associated with the nanomaterial as a surface treatment. In context of sustainability issues, also difference in life cycle stages may have an influence on when and how to test the biodegradation. Insights on these topics may first be captured in a review before providing guidance in a Guidance Document.

4 Actions to support the applicability of OECD TGs and GDs in Section 4: Health Effects

4.1 Testing nanomaterials in *in vitro* assays

Section 4: Health effects

Estimated duration:	Foreseen product(s):
3 years	» **OECD WPMN Guidance** on important aspects to consider when testing nanomaterials in *in vitro* assays (dosimetry and detection)

State of the art and resources to be considered

Several *in vitro* testing assays are available to test toxicity of chemicals in a cost-efficient way. Such assays may be used to provide mechanistic information regarding toxicological mode of action and supporting further implementation of New Approach Methodologies (NAMs) when such assays are combined into testing strategies (IATAs). Such *in vitro* assays include cell viability, cell proliferation, generation of reactive oxygen species or inflammation, and represent common end points found recurrently in different Adverse Outcome Pathways (AOPs). Such assays are also widely used with nanomaterials, though in those cases may be used with caution, since nano specific issues may need to be addressed. It is also important to note that in order for such methods to be relevant and implementable, they should be mechanistically anchored to the mechanism of action. Such issues have been highlighted in different publications or OECD documents from the OECD Sponsorship Programme on nanomaterials dating back to 2009. Issues include nanomaterial precipitation affecting cell detection, interference of nanomaterials with fluorescence or colour detection, or deposition rate leading to adaptation of exposure times. An up-to-date document stating the state of the art for the most used assays will represent a very useful tool for *in vitro* testing of chemicals and nanomaterials, facilitating subsequent IATA implementation and identifying existing gaps and modifications needed to enable testing and assessment of nanomaterials.

Action required and output / product(s) foreseen

There is a general interest in the implementation of NAMs due to costs, time, and more humane testing. *In vitro* approaches, as NAMs, are being widely encouraged yet there are still adaptations to be made regarding nanomaterials testing in otherwise well-established assays. A thorough evaluation of the output from H2020 projects and other relevant initiatives is therefore needed, since these results are currently useful but scattered. A Guidance Document collecting the state of the art of such initiatives is proposed herein, which will facilitate and harmonise the use of *in vitro* testing approaches across different laboratories. Such Guidance Document should also consider guidance on how properly select realistic dose-ranges, exposure times and relevant biological endpoints.

4.2 Acute toxicity inhalation (*in vivo*)

Section 4: Health effects

Estimated duration: **1-2 years**	Foreseen product(s): » **Harmonisation** of texts in **different Test Guidelines**. » Potential (further) update of Guidance Document

State of the art and resources to be considered

Test Guidelines for inhalation toxicity testing include description on the size range of a test aerosol in order to guarantee that the test substances can be inhaled and also are respirable for the animals used for toxicity assessment. Some Test Guidelines (TG 412, TG 413) have been adapted to accommodate testing of materials that have dimensions in the nanometre size range. This recommended range, however, is not harmonised among all inhalation toxicity Test Guidelines (see Table). In addition, paragraphs on sample or test article preparations, including the vehicle used, are not harmonised over the different test guidelines. This is critically important, as changes in the preparation and characterization of the aerosol can induce relevant changes in the biological effects.

OECD Test Guideline number	Title	Size range (mass median aerodynamic diameter)	Geometric standard deviation (σ_g)
403	Acute inhalation toxicity	1 to 4 µm	1.5 to 3.0
433	Acute inhalation toxicity: Fixed concentration procedure	≤ 4 µm	1.0 to 3.0
436	Acute inhalation toxicity: Acute toxic class method	1 to 4 µm	1.5 to 3.0
412	28-day (subacute) inhalation toxicity study	≤ 2 µm	1-3
413	Subchronic inhalation toxicity: 90-day study	≤ 2 µm	1-3
452	Chronic toxicity studies	Not specified	Not specified

Albeit that OECD GD 39 is currently being revised, the specific (recommended) size ranges should be harmonised. It is a highly relevant research need to investigate and adapt the protocols, with regard to dosing, administration, toxicity criteria, and 3R compliance where GD 39 is not adequate.

Action required and output / product(s) foreseen

Since required size ranges have been extensively discussed during the revision of OECD TG 412/413, it is strongly recommended to use exactly the same information in all other test guidelines that can be used for inhalation toxicity testing. As such, the OECD secretariat can adopt the text and the WNT can approve these changes accordingly.

All text on sample preparation including the use, pros and cons on vehicles used to generate aerosols should be taken out and reference should be made to OECD GD 39 and the WPMN "Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials" once its ongoing update has been finalised.

4.3 Genotoxicity / mutagenicity (*in vitro*)

Section 4: Health effects

Estimated duration:	Foreseen product(s):
5 years	» **Adaptation** of available **Test Guidelines** to nanomaterials (TG 487 and TG 489 in a first step)

State of the art and resources to be considered

Mutagenicity/genotoxicity testing is required by REACH for novel chemicals including nanomaterials. Genotoxicity endpoints are critical for risk assessment, since they give yes/no information, which is relevant because direct-acting genotoxic carcinogens are considered to have a no effect threshold. The testing approach currently in use for standard chemicals starts with *in vitro* assays followed by *in vivo* (except for cosmetic testing for which animal testing is not allowed); however, owing to the unique features of nanomaterials, guidelines require modification with respect to length of exposure, maximum concentration (avoiding agglomeration), presence/absence of S9 metabolising system, etc. Also, additional information is needed on physicochemical characterisation, behaviour in test medium, and uptake by cells. Past and present research projects including national and EU initiatives have made good progress on adaptation of different assays such as the micronucleus assay, mammalian gene mutation test, and comet assay, and on the development of NAMs based on advanced *in vitro* models of lung, liver, skin etc. with simultaneous assessment by both micronucleus and comet assays.

Action required and output / product(s) foreseen

The UK and Norway are working on an imminent SPSF submission regarding modification of the micronucleus test for nanomaterials (OECD TG 487), hence adaptation of this guideline is achievable in the near future providing funding is available. Regarding the *in vivo* COMET assay (OECD TG 489) minor adaptations were implemented under the Graphene Flagship which proved to be successful and thus the Test Guideline is now applicable to nanomaterials. Further work would require adaptation of TG 489 to *in vitro* testing of nanomaterials and including critical reviewing of TG 474, 475 and 488 towards their applicability for nanomaterials.

4.4 Developmental neurotoxicity (*in vitro*)

Section 4: Health effects

Estimated duration: **5 years**	Foreseen product(s): » **Guidance Document or Test Guideline** on testing of (developmental) neurotoxicity of nanomaterials

State of the art and resources to be considered

Animal experiments with rats are currently the gold standard in developmental neurotoxicity (DNT) testing. These are specified in the OECD Test Guideline 426 (OECD TG 426) or in the DNT cohort (cohort 2) of the Extended One-Generation Reproductive Toxicity Study (EOGRTS, OECD TG 443). Such animal tests are currently required as so-called Tier II studies in Europe for pesticides, biocides and chemicals, and in USA for pesticides, upon triggers like e.g. an endocrine or neurotoxic mode-of-action (MoA). However, they are not fit-for-purpose for assessing a large number of compounds because i) they are time- and cost-intensive (1 year/compound may cost more than 1.000.000 EUR), ii) it is ethically questionable (testing one substance may require up to 140 dams and 1000 juveniles), iii) there are uncertainties in its methodologies, evaluation, and regulation; iv) their predictivity for protection of the human brain is questionable due to the differences in brain function/complexity, exposure, neurodevelopmental timing, toxicokinetics and toxicodynamics between rodents and humans (Dorman et al. 2001; Kaufmann 2003; Tsuji and Crofton 2012; Paparella et al. 2020). For closing the huge existing data gap of compounds with no DNT information available, a DNT *in vitro* battery has been set up that allows investigation of compounds much faster, cheaper, with higher human relevance and without using animals. This *in vitro* battery was recently acknowledged by the European Food Safety Authority (EFSA; Crofton and Mundy 2021) and recommended by the OECD (2023). So far, the chemical applicability domain of the *in vitro* battery covers chemicals dissolved in water or solvents like DMSO, e.g. plant protection products. There is so far no experience how the DNT *in vitro* battery deals with nanomaterials. Experiences with model materials like carbon black will give insight into the applicability domain of the DNT *in vitro* battery concerning nanomaterials. Moreover, the addition of microglia to the cell systems will strengthen a possible secondary MoA via inflammatory mediators.

Action required and output / product(s) foreseen

Different nanomaterials need to be tested in the DNT *in vitro* battery. These data will be a case study for the suitability of the DNT *in vitro* battery for nanomaterials. Microglia will either be added to the DNT *in vitro* battery assays or the battery will be challenged with supernatants of nanomaterial-exposed microglia. The output will be a gain of knowledge on the applicability domain of the DNT *in vitro* battery for nanomaterials leading to an adoption of the *in vitro* battery to nanomaterials. This will support the work on consolidating the initial OECD recommendations (for chemical) into a Guidance Document or Test Guideline on testing of (developmental) neurotoxicity of nanomaterials.

4.5 Acute toxicity inhalation / respiratory sensitisation (*in vitro*)

Section 4: Health effects

Estimated duration:	Foreseen product(s):
1 year	» **Detailed Review Paper** on respiratory sensitization (SPSF already accepted at WNT)
1-2 years	» **Test Guideline** on development of methods for respiratory sensitization (expected for 2025)
5 years	» **Guidance Document** on *in vitro* inhalation toxicology (submission of SFSF expected in 2025-2026)

State of the art and resources to be considered

Inhalation is considered to be one of most relevant exposure routes for nano-materials, especially when considering occupational exposure scenarios. Exposure to airborne chemicals and nanomaterials can lead to various consequences such as local acute and long-term toxicity, including inflammation, genotoxic effects and/or immune-related effects (e.g. respiratory sensitization). Alternatively, inhaled nanoparticles can be translocated from the alveolar lumen into the bloodstream and finally to secondary target organs, where they can induce systemic effects and secondary toxicity. Traditionally, inhalation toxicity is performed using *in vivo* methods (OECD TG 403 - Acute Inhalation Toxicity; OECD TG 412 Subacute Inhalation Toxicity: 28-Day Study; OECD TG 413 - Subchronic Inhalation Toxicity: 90-day Study; OECD TG 436: Acute Inhalation Toxicity – Acute Toxic Class Method). However, especially when applied to nanomaterials and specific biological endpoints (e.g. respiratory sensitization) such methods have been shown to be problematic and requiring modification and adaptations. For these reasons, in recent time, there has been tremendous development of Air-Liquid-Interface methods and of methodologies for the prediction of long-term human biological effects using *in vitro* inhalation methods. The EU-funded project MACRAMÉ and POTENTIAL, together with other projects such as CHIASMA, are developing and testing *in vitro* methods for the assessment of nano-materials inhalation risk, with particular focus on acute toxicity and respiratory sensitization. It is of critical importance that the samples preparation, characterization, exposure and dosimetry methods are properly developed, since small differences in the protocol could lead to great differences in the induced biological effects.

Action required and output / product(s) foreseen

The OECD WNT Project 4.166 - Detailed Review Paper (DRP) to facilitate the Development of Test Methods to Predict the Respiratory Sensitisation Potential of Substances, is supported by several member countries and supported by the EU project MACRAMÉ. The first draft of the DRP is expected for early 2024. Following the DRP, there is a plan to develop a Test Guideline on *in vitro* prediction of respiratory sensitization potential. In a later moment, based on the results generated by the EU projects MACRAMÉ, POTENTIAL and other future projects, the plan is to support the development of a Guidance Document on *in vitro* inhalation toxicity.

4.6 Skin sensitisation (*in vitro*)

Section 4: Health effects

Estimated duration:	Foreseen product(s):
5 years	» **Guidance** on how to apply OECD TG 442C-E to nanomaterials

State of the art and resources to be considered

Molecular and cellular biology have shown that nanomaterials can exhibit sensitisation capacity even in the absence of damaged skin (Yoshioka et al. 2017), via non-covalently binding to skin proteins (Dwivedi et al. 2011; Dykman et al. 2017), or through the release of free chemicals which might have skin sensitising properties (Kim et al. 2021). Scientific evidence also supports that nanomaterial can exhibit effects on the immune system even if they are not able to get into viable skin layers. (Yoshioka et al. 2017). Skin sensitisation is a required end point in several chemical regulations worldwide, including nanomaterials. The ban on animal experimentation imposed in cosmetics in Europe, led to timely research on the molecular events behind skin sensitisation, which allowed the development of a series of *in vitro* tests that, when applied together, are able to predict the *in vivo* situation. These series of tests start with the implementation of an *in chemico* test predicting the molecular initiating event which mimics the covalent interaction of chemicals with cellular proteins (OECD TG 442C). This test may not be applicable to nanomaterials since their interaction with cells may be different to bulk chemicals. Secondly, OECD TG 442D addresses the mechanism described under the second Key Event of the AOP for skin sensitisation, namely keratinocyte activation or more specifically induction of cytoprotective genes. This Test Guideline, in particular the Keratinosens approach, was studied under the Gov4Nano EU project regarding its applicability to nanomaterials, and our results indicated that, following a few adaptations, it may be applicable. Finally, OECD TG 442E addresses the activation of dendritic cells, which represents the subsequent Key Event on the skin sensitisation AOP. Different test methods included in the test guideline may be used and are based on the activation of different cell lines and expression of specific surface markers link to dendritic cells (h-CLAT, U-SENSTM, IL-8 Luc Assay). It should be noticed that testing of inorganic materials often leads to interferences with the readout. Thus, the availability of additional assay would be a great advantage.

Action required and output / product(s) foreseen

Testing protocols to address skin sensitisation are well described for chemicals but still need adaptations to nanomaterials, due to their potential alternative routes to cell internalisation, potential interferences in the readout (e.g. the nano-materials can interfere with cytofluorimetry analysis) or their leaching capabilities in case of metallic particles. Initial studies under Gov4Nano indicated that some protocols may be directly applicable whereas others may need adaptations, and therefore further studies are required to fulfil REACH requirements to nanomaterials. Actions required:

- OECD TG 442C: Develop a new *in chemico* test which better mimics nanomaterial interactions with cell receptors
- OECD TG 442E: Adapt current testing strategies so there is no interference with detection methodologies

4.7 Fibre toxicity

Section 4: Health effects

Estimated duration:	Foreseen product(s):
3 years	» **Scoping review** on mechanisms behind fibre toxicity
6 years	» **Guidance Document on testing of fibre toxicity**

State of the art and resources to be considered

It is well known that inhalation of naturally occurring mineral fibres such as asbestos can lead to the development of lung diseases and even incurable mesothelioma. With the increasing use of new fibre materials such as carbon nanotubes, there is growing concern that negative effects similar to those observed with asbestos may occur.

Extensive studies on the toxicity of fibres after inhalation have led to the development of the fibre pathogenicity paradigm. Important determinants of potentially hazardous fibres are dose, dimensions (diameter less than 3 µm, length greater than 5 µm, and aspect ratio 3:1 (WHO counting rule for criteria fibre)), biopersistence, and possibly the rigidity. Current research indicates that fibres meeting the above-described criteria, also known as high aspect-ratio materials (HARMs), exhibit toxic properties (Donaldson et al. 2013). Materials having sufficient length and biopersistence tend to accumulate in the lung and can be retained in the pleural cavity. There, oxidative stress and persistent inflammation are induced, resulting in diseases like lung and pleural fibrosis, cardiovascular disease and cancer.

Adverse Outcome Pathways (AOP) have been used as frameworks built on available mechanistic information concerning a toxicological response. AOPs establish a mechanistic relationship between a molecular initiating event (MIE) and a sequence of intermediate key events (KE) that ultimately lead to an adverse outcome (AO). Utilising alternative testing methods to investigate MIE and KE facilitates the development of tiered testing strategies that offer data relevant to the proposed AO endpoint.

This thorough understanding can aid in formulating hypotheses as the foundation for Integrated Approaches for Testing and Assessment (IATAs) to facilitate nanofibre grouping. IATAs involve gathering information, addressing data descriptors, and following a structured approach to ultimately accept or reject a (grouping) hypothesis (Murphy et al. 2021). Current AOPs aimed at inhalation toxicity from nanomaterials are being adapted, which could determine *in vitro* toxicological outcomes. (Halappanavar et al. 2020). Further research is needed to elucidate the mechanisms of fibre toxicity and to develop accurate test methods to distinguish toxic particles from non-toxic ones.

Action required and output / product(s) foreseen

The large wide-spread use of fibre-like materials, including plastic fibres, and their several industrial and medical applications call for an assessment of fibre toxicity that includes a detailed mechanistic understanding. Currently testing strategies and approaches are scattered due to the different chemical nature, shape, or size of such materials. We propose to produce a scoping review document gathering all current information regarding fibre-driven toxicity mechanism and testing approaches. This could lead to a discussion with experts (workshop) to address those, which could guide further development of harmonised testing strategies (including NAMs) in connection with the already existing ones or the currently ongoing ones. In addition, this will foster the development of advanced innovative materials by safe-by design approaches.

4.8 Testing the reactivity of nanomaterials

Section 4: Health effects

Estimated duration:	Foreseen product(s):
2 years	» **OECD scoping review** and subsequently an OECD Test Guideline

State of the art and resources to be considered

Many nanomaterials (NMs) show a higher surface reactivity compared to the respective bulk material and can generate reactive oxygen species (ROS) via different mechanisms. Fenton-like reactions leading to the generation of hydroxyl radicals are among the most common ones. Other relevant mechanisms are catalytic processes at the nanomaterial surface or radical production via dissolved (metal) ions. The imbalance between ROS generation and ROS detoxification results in elevated ROS levels within cells, which is referred to as oxidative stress. Oxidative stress has been frequently connected to various adverse outcomes such as cytotoxicity or genotoxicity.

It should be noted that “inflammation”, “oxidative stress”, and “cytotoxicity” are the most reported “biological events” for NMs in the NanoAOP database. Moreover, almost every grouping framework for NMs considers “particle surface reactivity” as one of the most crucial parameters.

Thus, surface reactivity and the ability to induce cellular oxidative stress are important parameters to assess for nanotoxicology. Many nanosafety projects have done substantial work already. For instance, a comprehensive, tiered methodology to assess reactivity and oxidative stress was proposed by the EU project GRACIOUS, which also has been included in several of the GRACIOUS IATAs. In the first tier of the GRACIOUS testing strategy different assays that can assess ROS formation are suggested, e.g. electron paramagnetic resonance (EPR), ferric reduction ability of serum (FRAS), dichlorodihydrofluorescin diacetate assay (DCFDA). Here, SOPs from different nanosafety projects are available, some of which already have been verified in interlaboratory comparisons (ILCs). The second tier of the GRACIOUS testing strategy suggests applying different cell-based oxidative stress assays (Braakhuis et al. 2021). Suggested assays include measuring Nrf2 activation, protein carbonylation or the induction of oxidative stress markers.

Action required and output / product(s) foreseen

An OECD scoping review is foreseen as a first action to get a comprehensive overview of existing test methods for the determination of reactivity and oxidative stress, to identify the potentials and limitations of these methods as well as to explore additional/alternative methods.

This could be utilised as a basis for a new OECD Test Guideline.

4.9 Reproductive toxicity

Section 4: Health effects

Estimated duration:	Foreseen product(s):
3 years	» **Scoping review**

State of the art and resources to be considered

Reproduction is one of the most fundamental characteristics of living organisms. Harmful exposures affecting sensitive processes such as fertilization, oocytes maturation, sperm generation and maturation, and embryo-foetal development, can jeopardize successful pregnancy and increase the risk of disease later in life (Developmental Origin of Health and Disease concept) (Heindel et al. 2017; Schmitz-Felten et al. 2016). Nanomaterials are increasingly associated with adverse pregnancy outcomes and the development of postnatal (chronic) diseases, (Larsen et al. 2020; Hougaard et al. 2015), hence, reproductive and developmental toxicity testing should be a priority. However, current OECD Test Guidelines (e.g. TG 414, 415, 416, 421, 422, 426 and 443) largely rely on animal studies, which are highly cost- and time-intensive, of ethical concerns, and associated with considerable species-specific uncertainties. Moreover, for nanomaterials, the currently assessed gestational and litter parameters appear to be less sensitive to maternal particle exposure than offspring organ function in postnatal life (Larsen et al. 2020; Hougaard et al. 2015), which are, however, only covered to a limited extent in the existing TG. In addition, nanoparticles can accumulate and persist in the placental tissue and induce indirect developmental toxicity even in the absence of fetal transfer (Dugershaw et al. 2020). Although alternative non-animal test systems have been developed (e.g ReProGlo assay for body axis patterning and cell fate specification, embryonic stem cell test, zebrafish embryotoxicity assay), the placenta is lacking in most models to cover placenta-mediated indirect fetotoxicity.

Action required and output / product(s) foreseen

Overall, there is a need for rethinking reproductive and developmental toxicity testing (Hougaard et al. 2021) and adapting it to nanomaterials. Besides refinement of existing protocols, there is great potential to apply novel hypothesis driven testing strategies involving new experimental (non-animal) models (e.g. microphysiological, organoid and multi-organ models) as well as AI-based chemocentric and biocentric *in silico* models (e.g. QSAR, read-across, MFA, MoA, AOP and PBK models) in the development of alternative test methods. Therefore, in a first step a scoping review on alternative test methods and their applicability towards testing reproductive toxicity of nanomaterials is sought, followed by the development of a TG/GD on reproductive toxicity testing of nanomaterials.

4.10 Inflammation induction (*in vitro*)

Section 4: Health effects

Estimated duration:	Foreseen product(s):
2-3 years	» **Scoping review**, which might pave the way to new or updated Test Guidelines

State of the art and resources to be considered

In recent years, development in toxicological sciences and in the perception towards animal welfare have pushed for a paradigm shift from *in vivo* testing towards *in vitro* testing. The concepts of NAMs (New Approach Methodologies), 3Rs (Reduction, Refinement and Replacement), AOPs (Adverse Outcome Pathways) and IATA (Integrated Testing to Testing and Assessment) are nowadays very common and relevant in modern toxicology. *In vitro* systems can be of different complexity and are very useful in predicting *in vivo* biological outcomes. However, it should be kept in mind that all *in vitro* models, from the simpler 2D models to the more advanced NAMs, are an approximation of the *in vivo* tissues and, in most cases, they need to be anchored to AOP and to the mechanistic understanding of the biological process involved. One of the most valuable biological readouts that can be used to predict toxicity using *in vitro* systems is the induction of inflammation, which is a very dynamic process that evolves in time and include the cross-talk and feedback mechanisms. Often, inflammation, e.g. via release of pro-inflammatory cytokines (e.g. IL-8, IL-6, IL-1β, etc.) or activation of pro-inflammatory signalling pathways (e.g. activation of the Nf-Kb pathway (Koganti et al. 2023)) is used as a key event for the prediction of adverse outcomes. I.e. OECD TG 442E - *in vitro* skin sensitization, describes the measurement of IL-8 for distinguishing skin sensitizers from non-sensitizers in accordance with the UN GHS. However, in order to widely use the induction of inflammation as a key event for the prediction of adverse outcomes, it is necessary to further develop the AOPs related to specific biological effects and anchor them to existing or new NAMs.

Action required and output / product(s) foreseen

An OECD scoping review is foreseen as a first action to get a comprehensive overview of existing test methods for the determination of *in vitro* inflammation and understand how this relates to *in vivo* outcomes regarding different adverse outcomes/apical endpoints. Additionally, the review should identify potentials and limitations, including the possibility of interferences in the assay induced by nanomaterials, and include critical aspect of inflammation, such as the kinetic of inflammatory response and differentiation between adaptative and biological response versus first steps toward chronic inflammation.

This could be utilised as a basis for a new OECD Test Guidelines and/or refinement of existing ones and it could leverage on the approval of other NAMs.

5 Other Activities

5.1 Exposure assessment

Estimated duration:	Foreseen product(s):
3 years	» **OECD WPMN Guidance**

State of the art and resources to be considered

Exposure is the possible contact of an organism or the environment with an agent and its derivatives. Profound exposure assessment, according to REACH (EC 2006), includes an understanding of the properties and life cycle of substances in order to determine the scope of the exposure assessment, describing relevant uses and key determinants, the generation of exposure scenarios, as well as the estimation of exposure. Exposure estimates are derived from either direct measurement of exposure, data on similar materials (read-across) or the use of exposure modelling tools.

A framework to measure and assess the potential exposure to airborne emissions of engineered nano-objects and their agglomerates and aggregates at workplaces is provided in the harmonised tiered approach developed by OECD (2015). A formalised exposure assessment is demanded in order to capture exposure comprehensively and to disseminate regulatory decisions (Kuhlbusch et al. 2018) and an overview of strategies to assess occupational exposure to airborne nanoparticles was published (Galey et al. 2023). A measurement strategy to assess inhalation exposure is standardised (CEN. pr EN 17058; 2019). The standard includes a very comprehensive approach involving the use of multiple equipment to measure different metrics of exposure. However, detailed concepts on how to implement the measurement strategy also for routine monitoring using the measurement devices described is yet to be standardised or harmonised.

Besides exposure measurements, exposure models are another approach to estimate the exposure. An evaluation of such tools and models applicable for occupational, consumer and environmental exposure settings was performed by OECD (2021). Subsequently, OECD WPMN Steering Group 8 is currently working on two projects: “The identification of factors that can be measured to evaluate exposure to Nano-Objects and their Aggregates and Agglomerates (NOAA) in the workplace” and a “Guidance on Exposure Models/tools for Manufactured Nanomaterials and Advanced Materials for Consumer Exposure Scenarios”. In addition, an OECD Guidance Document for the use of dustiness data for exposure modelling is under development.

Action required and output / product(s) foreseen

In addition to the framework towards exposure assessment at the workplace, a detailed OECD WPMN guidance on how to perform routine exposure measurements at workplaces is needed. Harmonised strategies taking into account the type of nanomaterial and the presence of nanoscale background materials will facilitate occupational hygienists the monitoring of exposure, will enhance comparability and significance of the obtained measurement results and will contribute to protect workers’ health.

Furthermore, besides the accomplished and ongoing developments of guidance on exposure models and tools for manufactured nanomaterials and advanced materials for occupational and environmental scenarios is missing, similar to the OECD WPMN guidance for consumer scenarios in development. Most of the tools available to date are not taking into account the exposure to fibrous materials and advanced materials (e.g. multi-component materials). Here further development of new and advancement of existing tools is needed.

5.2 Predictivity and sensitivity of NAMs for nanomaterials

Estimated duration:	Foreseen product(s):
2 years	» **Scoping review** on how to assess predictivity and sensitivity of NAMs (in the absence of reference nanomaterials)
4 years	» **Guidance Document** on assessing the predictivity and sensitivity of NAMs for nanomaterials

State of the art and resources to be considered

During the last years, great efforts have been made to unravel the mechanistic basis behind the adverse effects induced by nanomaterials. These efforts have been supported by the development of adverse outcome pathways (AOPs), for which the OECD launched a program for chemicals in 2012. The development of AOPs is aligned with the concept of Toxicity Testing in the 21st Century (Hartung et al. 2009) which was born out of the need to improve the safety assessment of chemicals. The drivers are twofold 1) there is a recognised need to improve reproducibility in testing, e.g. for complex endpoints the correlation with animal experiments is only about 60% (Basketter et al. 2012) and 2) there is a need to develop cost-efficient and fast testing strategies to deal with the increased number of chemicals (including nanomaterials) reaching the market. Development of animal-free alternative strategies, represented by New Approach Methodologies (NAMs), which can mimic human biology and provide mechanistic information about how a chemical may cause toxicity in humans, is a strategy currently being explored by different stakeholders including regulatory agencies (van der Zalm et al. 2022). A recent report by ECHA concluded that adapting existing NAMs accepted for chemicals to nanomaterials was the most promising path ahead and the next steps should consider (i) adapting exposure-driven scenarios to account for diverse routes of nanomaterial exposure; (ii) adjusting test systems to emulate human biology; (iii) developing appropriate *in vitro* exposure protocols that consider nanomaterial behaviour; (iv) developing effective methods for characterizing nanomaterials in their pure forms and within culture media; and (v) utilizing existing data and accessible databases to endorse the creation and validation of *in silico* methods. As in the case with *in vitro* and *ex vivo* testing the use of chemical controls for toxicity has also allowed to demonstrate the effectiveness of NAMs. They also allow identification of a band-width for effects of a certain NAM and help in comparisons for similar tests for the same endpoint. However, the use of positive chemical controls, in the absence of nanomaterial controls, particularly when the NAMs are developed with specific consideration of nano-related effects, is tricky and does not allow to remove the uncertainties around the fit-for-purpose of the newly developed assays for nanomaterials.

Action required and output / product(s) foreseen

In a first step information is gathered on approaches for determining the predictivity and sensitivity of NAMs for nanomaterial testing to identify pitfalls and gaps. The information will be summarised in a scoping review. In a second step a strategy should be proposed for assessing the validity of the newly proposed NAMs for nanomaterials. This could include, for instance, considering mixture toxicity by employing co-exposure of known chemicals and materials, which would allow at least to determine if the assay is resistant to interferences. The establishment of a materials library is advisable, together with a Guidance Document on how to assess the sensitivity/robustness of NAMs for nanotoxicological applications.