NanoHarmony 2nd Project Workshop November 2021

Determination of concentrations of ENMs in biological samples (Task 1.4)

25th November 2021

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 885931

Task 1.4 Scientific basis for a GD on the determination of concentrations of ENMs in biological samples

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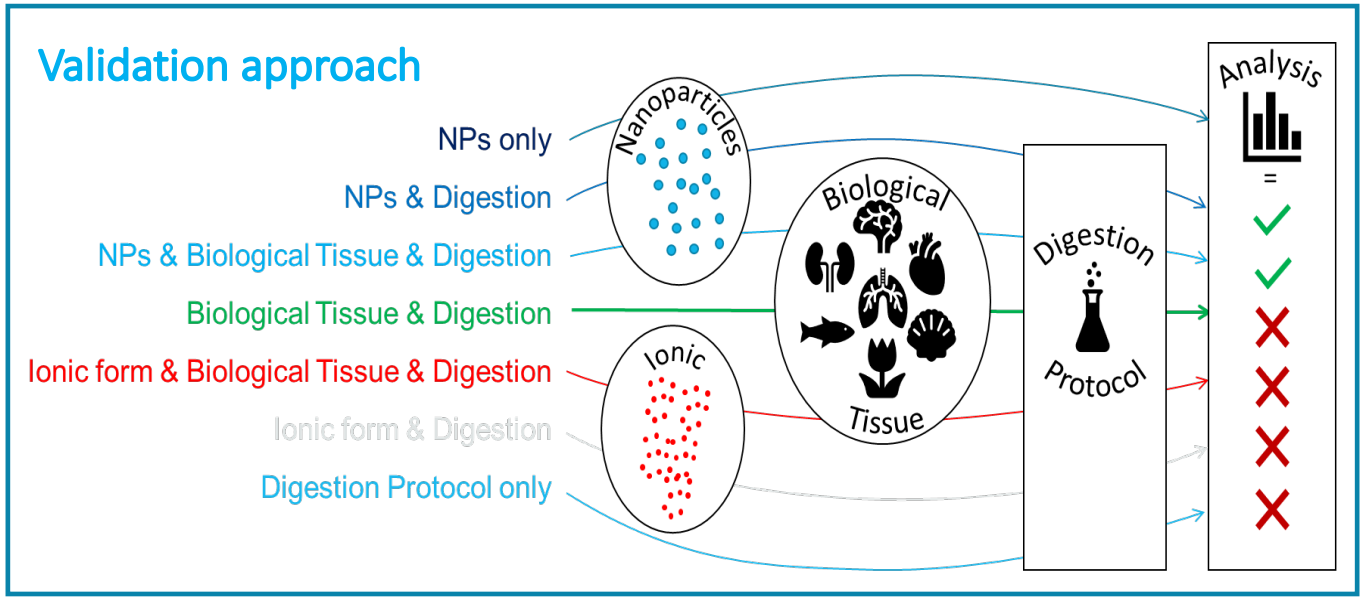
OECD Project: Development of a new Guidance Document on the determination of concentrations of nanoparticles in biological samples for (eco)toxicity studies. UK lead

Reason for inclusion in NanoHarmony - a number of existing and proposed Test Guidelines (e.g. ecotoxicity and toxicokinetics) identify a requirement to determine concentrations of nanoparticles in biological samples, but do not provide detailed guidance on how this should be undertaken. This is a gap that needs to be addressed by reviewing available information and undertaking targeted experimental studies.

Activities

- Identification of the state of the art and data gaps
- Experimental studies on spICP-MS and enhanced dark-field microscopy
- Workshops to identify key data and obtain expert input

Outcome Scientific document with a broad consensus to support OECD Guidance Document development, addressing current state-of-the-art techniques and protocols. Reflecting new data generated and focussing on sample treatment (e.g. digestion protocols), detection limits, validation approaches.



Partners UK Health Security Agency, University of Plymouth, National Research Centre for the Working Environment (NFA), UK Centre for Ecology & Hydrology

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Methodology and Deliverables

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Data Collection, Analysis and Interpretation

- Ongoing throughout project
- Initial data capture phase to (a) define state of the art and (b) identify gaps
- Literature, Projects (national and international)
- Expert input (workshop sessions)

Generation of new data

- single particle ICP-MS, enhanced darkfield microscopy

Deliverables

D1.8 Summary of current state of the art and report on the data gaps analysis on methods for determination of concentrations of ENMs in biological materials (M12). Completed on time, March 2021

D1.9 Scientific document to support OECD activities on the development of GD on the determination of concentrations of ENMs in biological materials (M30). Due September 2022

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Scope of OECD project

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- Nanomaterials
- Primary focus on simple (non-complex) manufactured nanoparticles (e.g. metallic nanoparticles) as they are those most commonly in use and for which the most extensive development of techniques has been undertaken.
- Application to more complex and advanced materials will be addressed to the extent possible:
    - Multi-component (e.g. alloys, coated)
    - Fibres/platelets
    - Carbon-based (e.g. MWCNT, graphene)
    - Other advanced nanomaterials (e.g. polymers)

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Single Particle ICP-MS

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- Systematic literature review
- Expert Workshops

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NanoHarmony International Workshop on Gap Analysis and Data Requirements to support Test Guideline and Guidance Document Development, 3-5 November, 2020

Determination of concentrations in biological samples using spICP-MS

Extraction Protocols and Data Gaps (Session A) 13:00-14:30 November 3rd 2020
Measurement and Validation (Session B) 15:00-16:30 November 4th 2020

https://nanoharmony.eu/2020/11/09/workshop-on-gap-analysis-and-data-requirements-to-support-tg-and-gd-development/

Determination of metallic nanoparticles in biological samples by single particle ICP-MS: A systematic review from sample collection to analysis
Adam Laycock, Nathaniel J. Clark, Robert Clough, Rachel Smith and Richard D. Handy (submitted)

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WORKSHOPS November 2020

> 25 experts joined the Task 1.4 sessions from 9 European countries plus South Africa, Australia, Korea and USA. The experts were from academia, Government Institutes, EU bodies (including JRC and ECHA) and large and small commercial companies.

approach the most useful to determine procedural recovery during the extraction and analysis of a tissue sample by spICP-MS? Are there other approaches?

- What are the appropriate criteria for acceptance of an analysis?
- What can we learn from other areas of science that are exploring the detection of ENMs in other types of samples (e.g., in soil, water, medicines)?
- What is an appropriate roadmap for the development of a CRM?

Potential problems/issues ...

- Extract protocol and consumables may affect recovery (e.g. purity of TMAH led to differences in recovery of AuNPs in woodlice samples)
- Spike recovery may depend on type and mass of spike added
- How to demonstrate complete digestion
- Spike may promote formation of particles
- Biologically incorporated NPs typically harder to extract than spike NPs

Less strict than the 10-20% for ICP-MS metal extraction and will vary depending on purpose

Options for making reference materials

- Soft gelatine capsules
- Growing cells with NPs (e.g. skin keratinocytes)
- Using cancer tissue models, e.g. spheroids
- Some CRMs may already contain NPs (diatoms – silica?) – investigate using CRM database (also BAM and NIST)

Funding perhaps from EUROMET?
Demonstrate potential demand to convince manufacturers
Multi-particle CRMs may be popular and efficient
Engage with CRM producer accredited to ISO17034

This project has received fun
research and innovation pro

spICP-MS – next steps

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RESEARCH		
IDENTIFY APPROPRIATE PROTOCOLS & CONDITIONS FOR:		
Storage	ENM & Matrix Combination	Extraction Protocol
Effect of duration and conditions on ENMs in biological matrices	Expand the range of ENM and matrix combinations addressed	Comparison of sample preparation techniques for different ENM & matrix combinations

DEVELOPMENT OF GUIDANCE		
PRODUCE STANDARD METHODS & NOMENCLATURE FOR:		
Approach & Reporting	Terminology & Controls	Sample Preparation
How to determine and report particle parameters (e.g. LOD_{size}) and metrics	Logical and consistent use of terminology and control samples	A systematic approach to the selection of a protocol

PRODUCTION OF REFERENCE MATERIALS
Development of CRMs representing a select few types of ENM & matrix compositions

→ Project - Effects on samples of repeated freezing and thawing, storage time, freeze drying, collection tube types UoP 2022

Expand range of materials – e.g. steel nanomaterials under consideration

Limited ILC under consideration, 2022

→ Input to final Task 1.4 deliverable
Further discussion with experts

→ Project - To assess the viability of using existing fish tissue CRMs as representative test materials for engineered nanomaterials (ENMs) in fish, UoP 2022

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Microscopy Techniques

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Literature Review

- Electron microscopy techniques are widely used for the characterisation of pristine nanomaterials and are generally considered the gold standard in this respect
- Various microscopy techniques (e.g. electron, dark-field, raman scattering, laser confocal) have also been used in many toxicity and ecotoxicity studies to explore (qualitatively) the localisation of ENMs within subcellular structures, cells, tissues, organs and organisms. However, there are few examples of their use in a quantitative manner.
- Two general quantitative approaches:
  - counting such objects in situ, i.e. in biological sample sections. Appropriate sample selection and morphometric/stereological approaches for counting particles in biological sample sections are available, but to date there are limited examples in the literature of the use of such for ENMs
  - digestion of the biological matrix and extraction of the particles, which are then mounted on a substrate for counting by microscopy (limited examples mainly for fibres).
- A complicating factor for most microscopy approaches is the need for additional analysis to confirm that the particles are of the expected type and chemical composition (e.g. EDX analysis).

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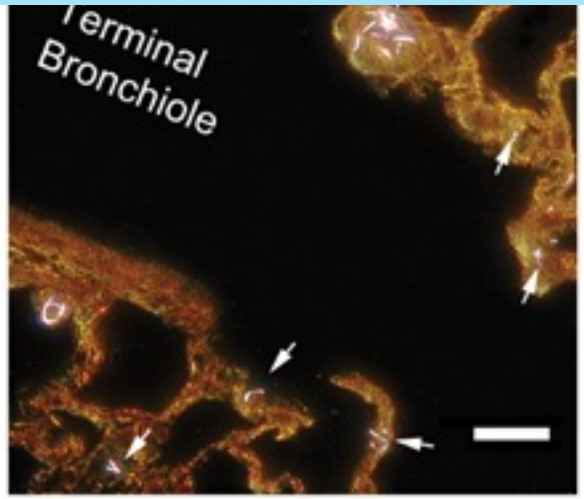
Microscopy T

….dose producing 12,000 fibres in an ED would only produced 6 in TEM ….
Mercer at al (2018) The Fate of Inhaled Detection and Measurement by Enhanc Microscopy Toxicol Pathol. 2018 January 46. doi:10.1177/0192623317732321.

…..it would take over four hundred million hours to analyze a total single mouse liver using STEM …
Kempen et al (2013) A scanning transmission electron microscopy approach to analyzing large volumes of tissue to detect nanoparticles. Microsc Microanal. 2013;19(5):1290-1297.

Literature Review (c

- Clearly it is necessary for the microscopy technique used to be capable of resolving th
- Electron microscopy approaches, in particular transmission electron microscopy, can b types and sizes, however, this is a very time consuming technique for quantification w areas imaged. This has implications for the levels of doses that may be required in any detection and quantification via this route.
- Advanced automated image analysis systems would clearly help in this area but there adoption (e.g. FibreDetect).



Conclusions

1. Lack of practical guidance on the use of quantitative EM approaches in study design, in particular on appropriate dose levels, to enable judgements to be made on its usefulness in particular situations.

2. Other microscopy techniques may offer a better solution (e.g. enhanced dark-field microscopy and raman microscopy) and have been used in studies involving both metallic and carbon based materials. However, the equipment is not yet widely available and more detailed consideration needs to be given to the practical use of these and other microscopy approaches before they could be more widely adopted for quantitative (semi-quantitative) application in toxicity or ecotoxicity tests.

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Carbon-based ENMs

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Analysing carbon based ENMs (e.g. carbon black, carbon nanotubes) within biological matrices for toxicity and ecotoxicity studies is a challenge but is an area of increasing importance given the growing production of such materials.

Literature Review

- Building from a number of key review papers on analytical approaches to detect, characterise and quantify carbon nanomaterials (Petersen et al., 2016 and Goodwin et al., 2018)
- Range of established methods capable of characterising pristine carbon nanomaterials, and this is reflected in the number of ISO standards documents available in this area.
- The detection, characterisation and quantification of carbon-based nanomaterials within biological samples (i.e. carbon-on-carbon) presents additional analytical challenges. There are examples in the literature but there are currently no international standard approaches in this area (note: ISO project in this area).
- Wide range of techniques with wide range of reported detection limits

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Carbon-based ENMs

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The main conclusions of the literature review are that:

- Currently no established routine techniques or standard methods for the detection, characterisation and quantification of carbon-based nanomaterials in biological matrices.
- Lack of practical information for many of the techniques required for their routine application in toxicity or ecotoxicity tests, including detection limits and validation approaches.
- The establishment of standard, internationally agreed, methods suitable within the regulatory context for the quantification (detection and characterisation) of carbon-based nanomaterials in biological matrices is some years away.

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Workshop

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The determination of carbon-based nanomaterials in biological samples – the way forward for regulatory purposes - Friday 26th November 2021 12:00-14:00 (CET)

12:00 Introduction

Part A – Scene Setting

12:10 “Developing robust analytical methods to measure the bioaccumulation of different carbon nanomaterials” (Elijah Petersen, NIST)

12:30 – 12:45 "Quantification of carbon nanotubes in lung tissue for toxicology purposes" (Frédéric Cosnier, INRS)

12:45 – 13:00 “Detection of carbon-based nanomaterials in tissues by enhanced darkfield microscopy” (Trine Berthing, NFA)

Part B - Discussion

13:00 – 13:50 Focussed Discussion

13:50 – 14:00 Summary of Discussion

14:00 Close

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Techniques and Limits

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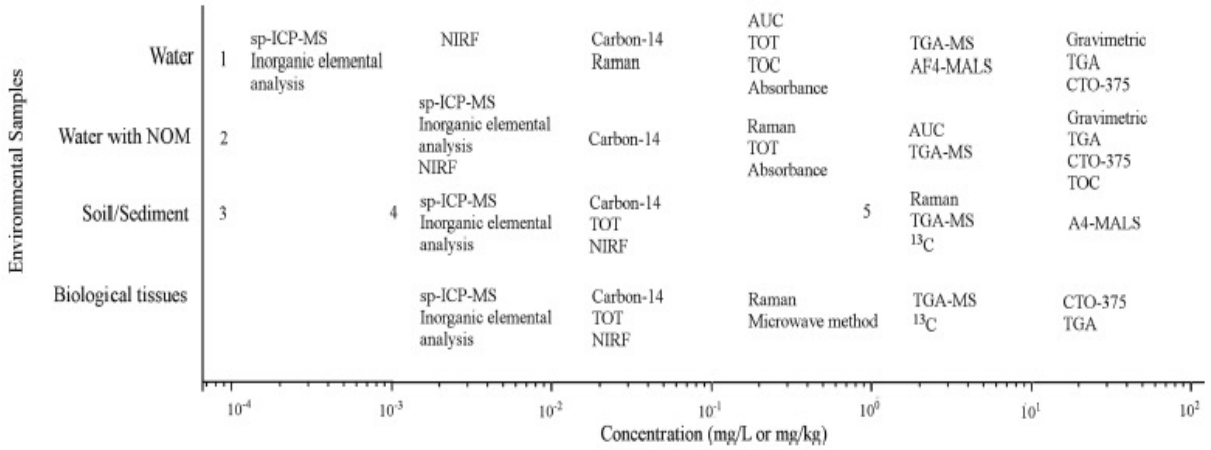
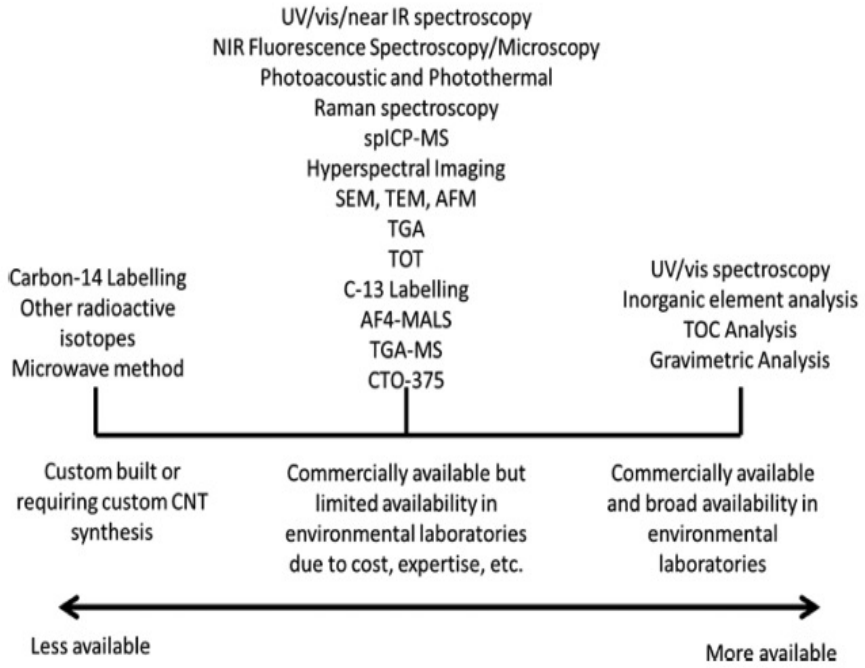


Expected detection limits for analytical techniques in various media (from Petersen et al., 2016).



Availability of CNT quantification techniques (from Petersen et al., 2016).

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Timelines: Task 1.4 and Associated OECD project

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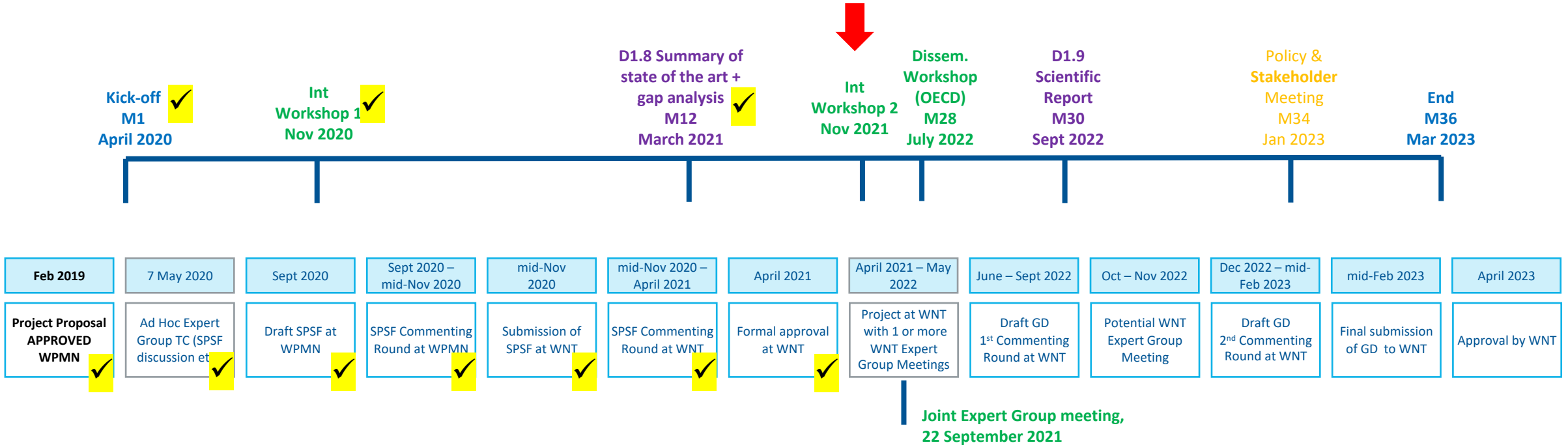

Proposed Timeline - OECD project to develop GD on ENM concentration determination in Biological Samples

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Outlook

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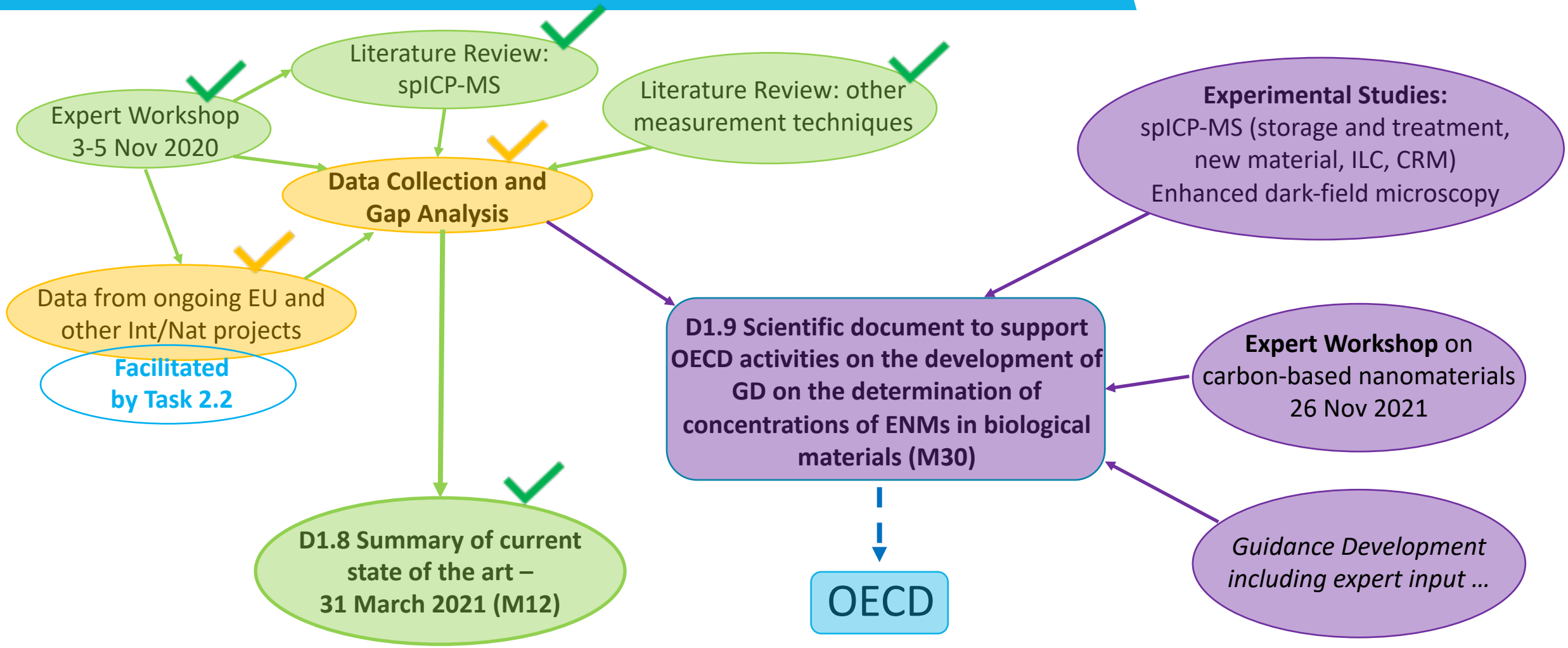

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Thank you for listening

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