Determination of concentrations of ENMs in biological samples using spICP-MS

Rachel Smith, Adam Laycock
Public Health England

Richard Handy, Nathaniel Clark, Robert Clough
University of Plymouth

NanoHarmony

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

Data Requirements in Test Guideline and Guidance Document development for nanomaterials, Webinar 16th December 2020

Objectives and Content

NanoHarmony

OBJECTIVES

Workshop Outcomes and Next Steps

- Summarise the outcomes of the two sessions on the **Determination of concentrations of ENMs in biological samples using spICP-MS** held as part of the NanoHarmony Workshop on Gap Analysis and Data Requirements to support TG and GD Development, 3-5 November
- Describe the next stages of the work

Provide a further Opportunity for Engagement with Stakeholders

- Answer questions from participants

CONTENT

- Background to the OECD project and NanoHarmony activities in support of it
- Main points from the Workshop Sessions
- The way forward
- Questions

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

Background

NanoHarmony

OECD Project -

- Information on the presence and concentration of nanoparticles within biological samples can be important in a range of circumstances, especially in the interpretation of the potential toxicological or ecotoxicological significance of exposures. But currently limited guidance on appropriate approaches is available.
- **Development of a new Guidance Document on the determination of concentrations of nanoparticles in biological samples for (eco)toxicity studies**
- Data Collection, Analysis and Interpretation → Generation of new data → Guidance Document
- **Primary focus spICP-MS** but other techniques will also be addressed

NanoHarmony Project –

- Work alongside OECD projects to support and accelerate the development of Test Guidelines and Guidance Documents related to nanomaterial test methods. Covers data collection, experimental studies, workshops
- **NH Task 1.4 supports this project.**

For more information on NanoHarmony including webinars on - *The pathway to Test Guidelines: from science to standards for nanomaterials* and *Introduction to NanoHarmony*, go to nanoharmony.eu

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

Workshop Session Objectives

NanoHarmony

Objective

Identify additional data, and consider priority areas to focus on to develop spICP-MS as a robust measurement approach for a range of ENMs and biological sample matrices to support eco(tox) studies

Involvement

Over 25 experts joined the sessions from 9 EU 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.

Session A - Extraction Protocols

Existing Data

- Overlooked/unpublished/unsuccessful approaches/experiences?
- Relevant lessons from other sample types /or other analytical areas

Data Gaps

- What are key data gaps and what exp studies are required to fill them?
- Would more extraction comparison studies be fruitful?

Preferred Approach

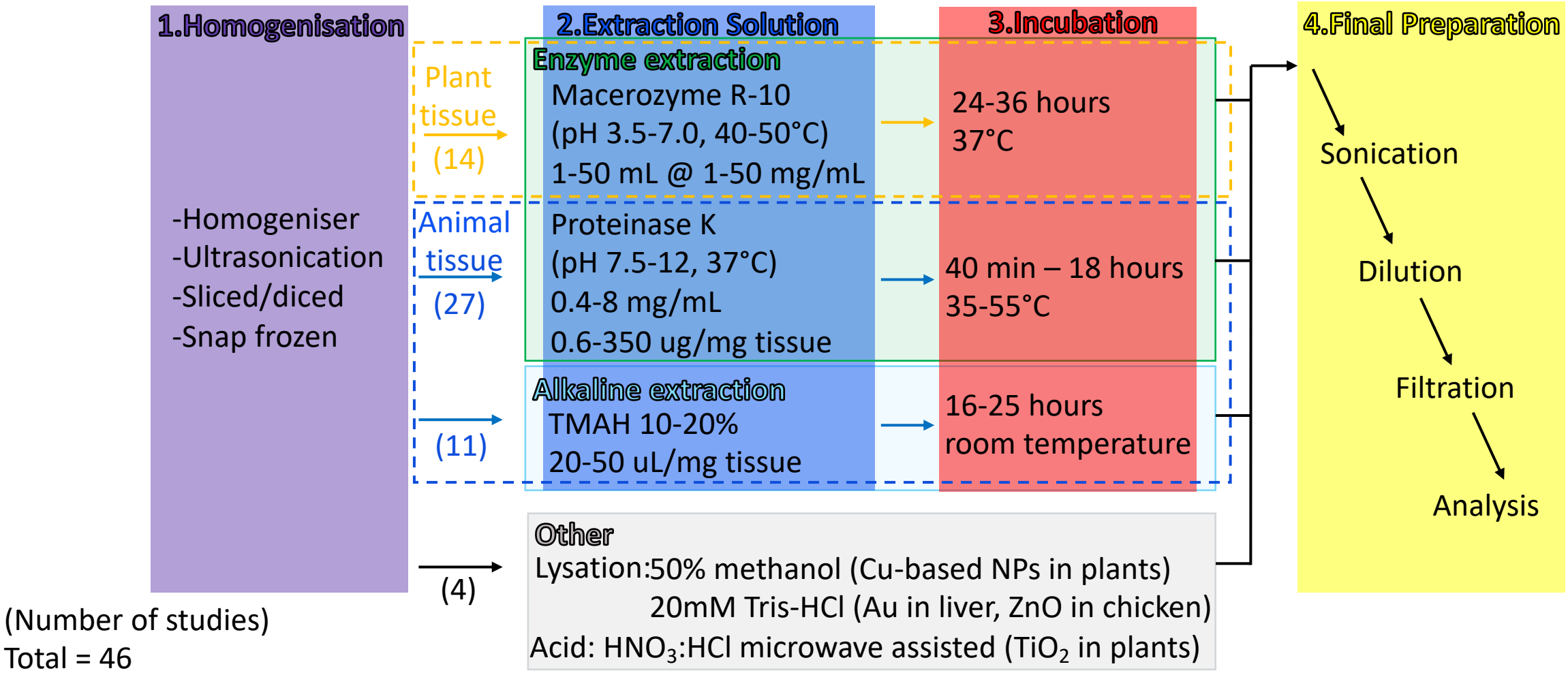
- Is it possible to make recommendations re most appropriate protocol?
- Considerations based on cost, simplicity, and H&S in recommendations?

Session B - Measurement and Validation

- In the absence of a Certified Reference Material (CRM), is the spike recovery 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?

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

Session A – Extraction protocol data gaps

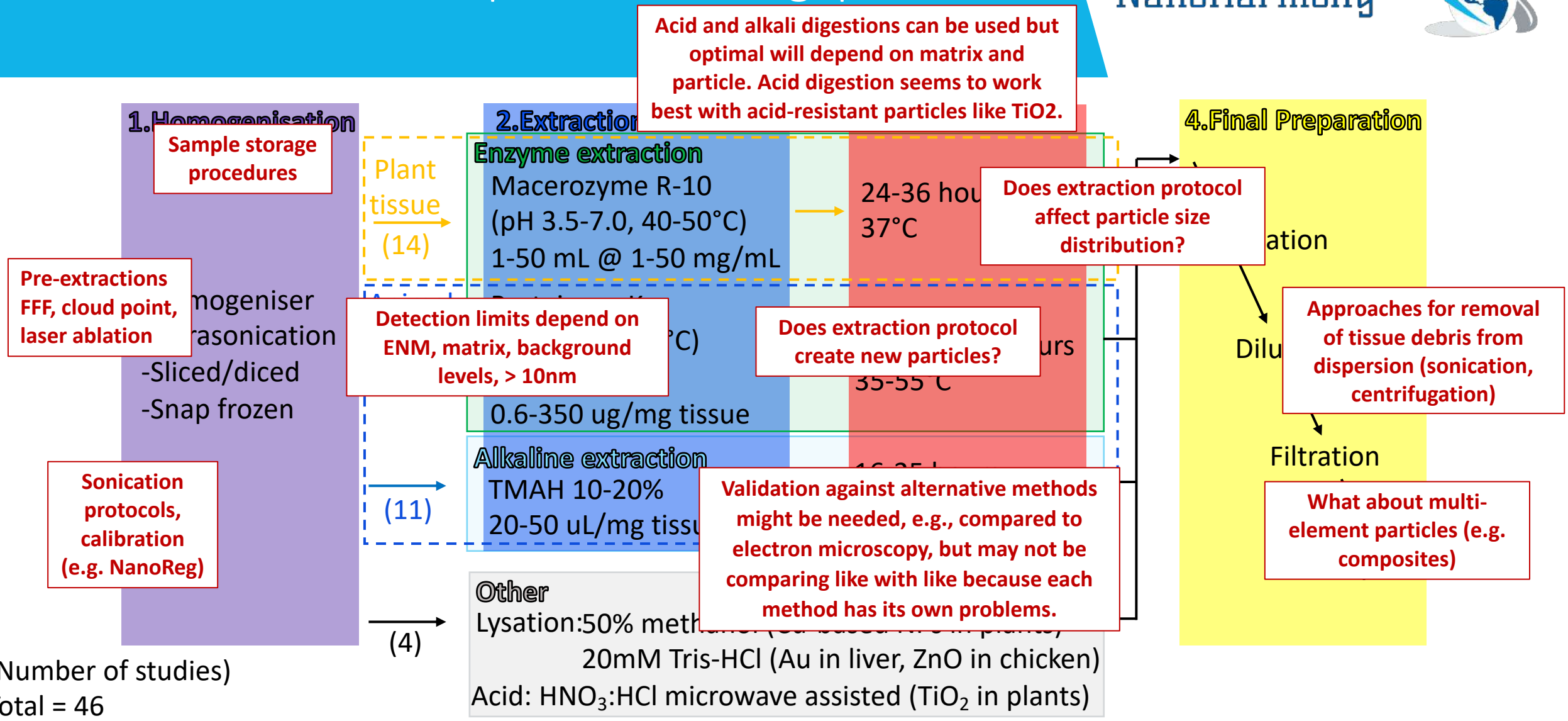


(Number of studies)
Total = 46

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

Session A – Extraction protocol data gaps

NanoHarmony



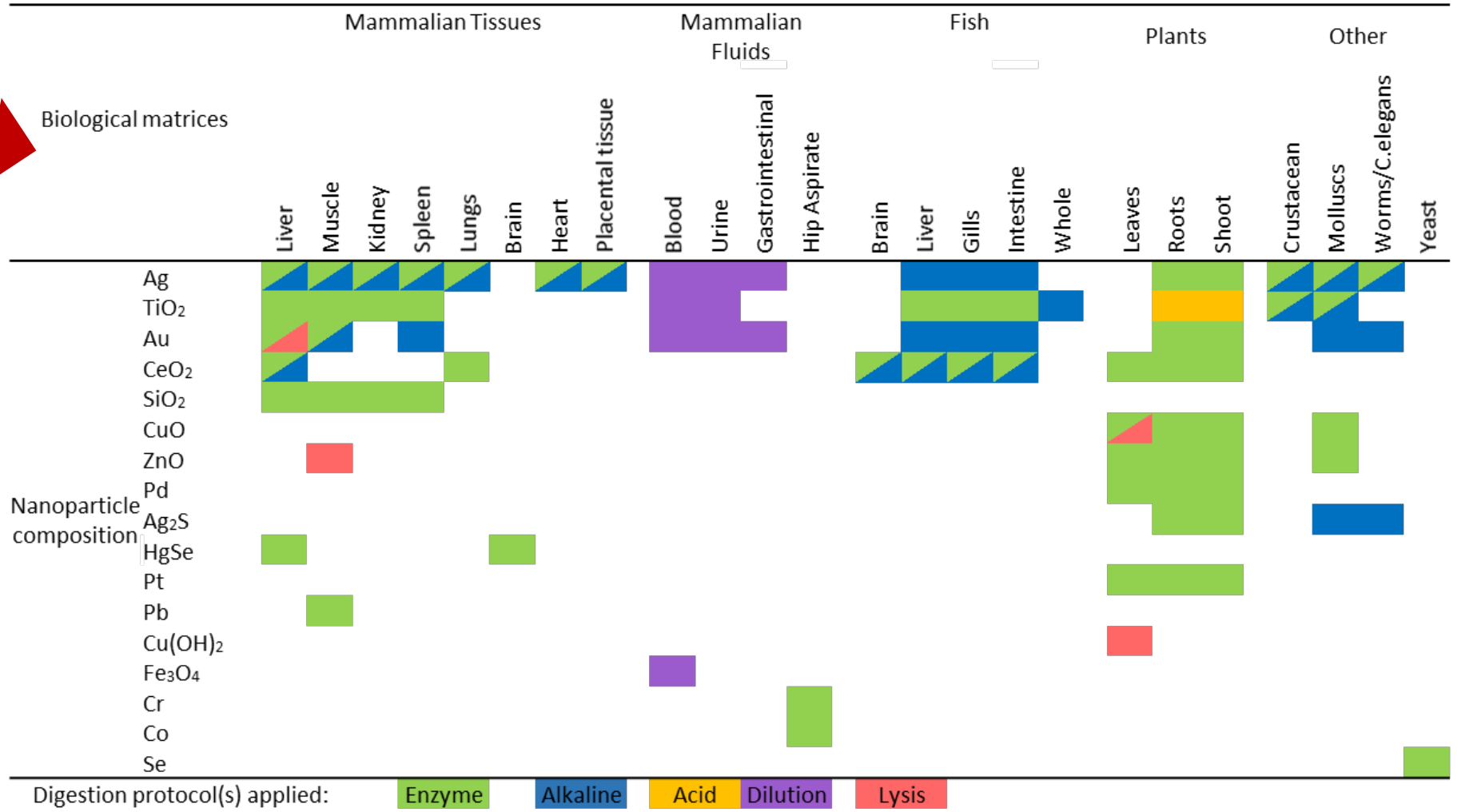
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 885931

Session A Data Gaps – ENMS/Matrices

NanoHarmony

Bone, muscle, skin, biofluids (ecotox), hip aspirate

AlO3, BaSO4, SnO, Ni, Mn, Fe



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

Session B – Validation

- In the absence of a Certified Reference Material (CRM), is the spike recovery 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?

YES to spike recovery, but …

Also consider other approaches/checks …

- Mass balance, i.e. total metal (complete digestion, ICP-MS) = nanoparticle + dissolved
- Test particle stability using extract protocol before undertaking (eco)tox study
- Round Robins to build consensus
- Comparison with other techniques (e.g. NAA)
- Compare size distribution of original particles vs extracted

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

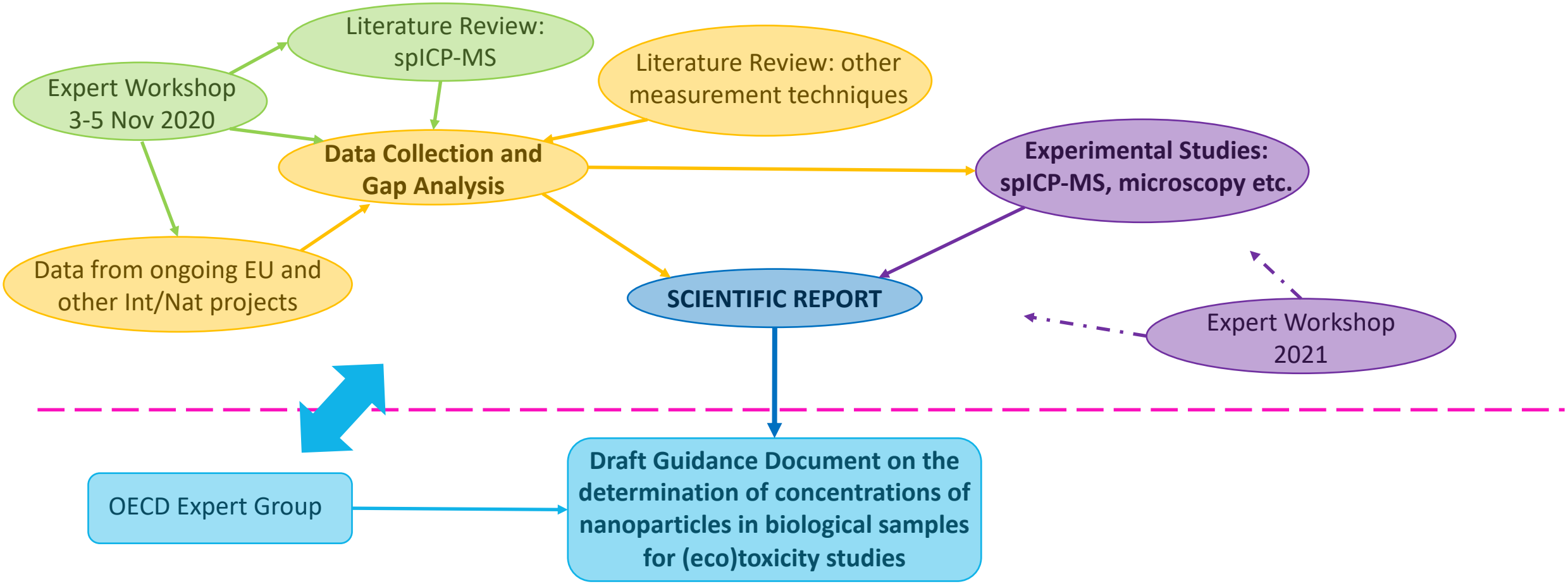
Multi-particle CRMs may be popular and efficient

Engage with CRM producer accredited to ISO17034

This project has received fun
research and innovation pro

Next Steps

NanoHarmony

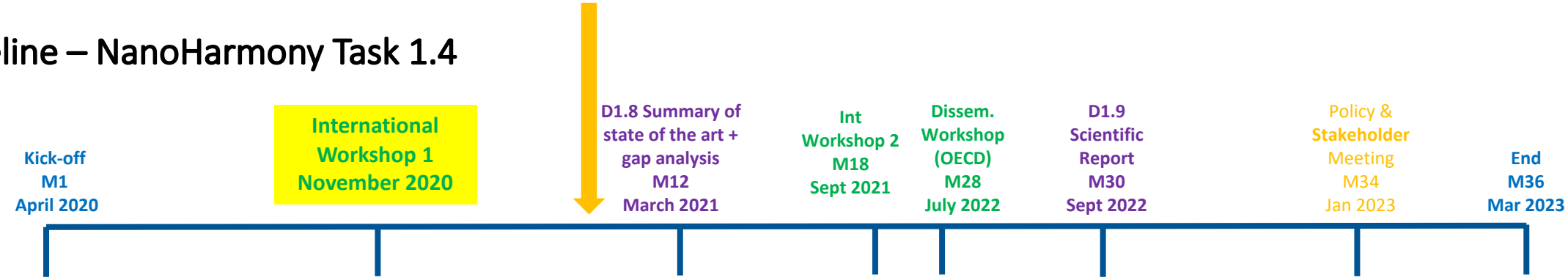


This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 885931

Timelines: NH Task 1.4 and Associated OECD project

NanoHarmony

Timeline – NanoHarmony Task 1.4



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

Feb 2019	7 May 2020	Sept 2020	Sept 2020 – mid-Nov 2020	mid-Nov 2020	mid-Nov 2020 – April 2021	April 2021	April 2021 – May 2022	June – Sept 2022	Oct – Nov 2022	Dec 2022 – mid-Feb 2023	mid-Feb 2023	April 2023
Project Proposal APPROVED WPMN	Ad Hoc Expert Group TC (SPSF discussion etc.)	Draft SPSF at WPMN	SPSF Commenting Round at WPMN	Submission of SPSF at WNT	SPSF Commenting Round at WNT	Formal approval at WNT	Project at WNT with 1 or more WNT Expert Group Meetings	Draft GD 1st Commenting Round at WNT	Potential WNT Expert Group Meeting	Draft GD 2nd Commenting Round at WNT	Final submission of GD to WNT	Approval by WNT

General timeline for the development of a nanomaterial focussed OECD Test Guideline or Guidance Document

June (previously Feb)	1 or more years	June (Sept 2020)	June – mid-November	mid-November	mid-November – April	April	2months + 1 or more years	June – September	October – November	December – mid-February	mid-February	April
Project Proposal at WPMN	Project at WPMN	Draft SPSF at WPMN	SPSF Commenting Round at WPMN	Submission of SPSF at WNT	SPSF Commenting Round at WNT	Formal approval at WNT	Project at WNT with 1 or more WNT Expert Group Meetings	1st Commenting Round at WNT	Potential WNT Expert Group Meeting	2nd Commenting Round at WNT	Final submission to WNT	Approval by WNT

OECD WPMN

OECD WNT

Start

End

NanoHarmony

THANK YOU FOR LISTENING

ANY QUESTIONS?

If you have any additional comments and suggestions please e-mail them to:
Rachel.smith@phe.gov.uk and Richard.handy@plymouth.ac.uk

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