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Nanoplastics: reference and test materials production and characterisation, environmental fate and toxicity testing methods, report of an OECD state-of-the-art workshop.

OECD Series on Safety of Manufactured Nanomaterials and other Advanced Materials,

This is the report of the OECD workshop on the state-of-the-art on nanoplastics: reference and test materials production and characterisation, environmental fate and toxicity testing methods. This event was held at the OECD Headquarters on 12-14 November 2025.

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About the OECD

The Organisation for Economic Co-operation and Development (OECD) is an intergovernmental organisation in which representatives of 38 countries in North and South America, Europe and the Asia and Pacific region, as well as the European Union, meet to co-ordinate and harmonise policies, discuss issues of mutual concern, and work together to respond to international problems. Most of the OECD's work is carried out by more than 200 specialised committees and working groups composed of member country delegates. Observers from several Partner countries and from interested international organisations attend many of the OECD's workshops and other meetings. Committees and working groups are served by the OECD Secretariat, located in Paris, France, which is organised into directorates and divisions.

The Environment, Health and Safety Division publishes free-of-charge documents in twelve different series: **Testing and Assessment**; **Good Laboratory Practice and Compliance Monitoring**; **Pesticides**; **Biocides**; **Risk Management**; **Harmonisation of Regulatory Oversight in Biotechnology**; **Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents; Safety of Manufactured Nanomaterials;** and **Adverse Outcome Pathways.** More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (https://www.oecd.org/en/topics/chemical-safety-and-biosafety.html).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank, Basel, Rotterdam and Stockholm Conventions and OECD. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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Abbreviations and Acronyms

AF4-MALS	Asymmetric Flow Field-Flow Fractionation-MultiAngle Light Scattering
AFM	Atomic Force Microscopy
AFM-IR	Atomic Force Microscopy–Infrared Spectroscopy
AOP	Adverse Outcome Pathway
CBC	OECD Chemicals and Biotechnology Committee
CEAP	Circular Economy Action Plan
CRM	Certified Reference Material
CUSP	The European Research Cluster to Understand the Health Impacts of Micro- and Nanoplastics
DLS	Dynamic Light Scattering
FFF	Field-Flow Fractionation
FTIR	Fourier Transform Infrared Spectroscopy
IATA	Integrated Approaches to Testing and Assessment
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ILC	Interlaboratory Comparison
ISO	International Organization for Standardization
LDIR	Laser direct infrared imaging
LOD	Limit of Detection
LOQ	Limit of Quantification
MALS	Multi-Angle Light Scattering
MDL	Method Detection Limit
MNPs	Micro- and Nanoplastics
MPs	Microplastics
NMR	Nuclear Magnetic Resonance
NTA	Nanoparticle Tracking Analysis

NPs	Nanoplastics
PE	Polyethylene
PET	Polyethylene terephthalate
PLA	Polylactic acid
PLC	Polymer of Low Concern
PP	Polypropylene
PS	Polystyrene
PSLT	Particle Size-Limited Testing
PTA	Particle Tracking Analysis
PVA	Poly vinyl Alcohol
PVC	Polyvinyl Chloride
Py-GC/MS	Pyrolysis–Gas Chromatography/Mass Spectrometry
QA/QC	Quality Assurance / Quality Control
RMs	Reference Materials
SDS	Sodium Dodecyl Sulphate
SEM	Scanning Electron Microscopy
SRS	Simulated Raman Scattering
TED-GC/MS	Thermal Extraction Desorption–Gas Chromatography/Mass Spectrometry
TEM	Transmission Electron Microscopy
TFF	Tangential Flow Filtration
TGs	Test Guidelines
TWA	Technical Working Areas
VAMAS	Versailles Project on Advanced Materials and Standards
XPS	X-ray Photoelectron Spectroscopy

Nanoplastics: reference and test materials production and characterisation, environmental fate and toxicity testing methods, report of an OECD state-of-the-art workshop

Background

Since its establishment by an OECD Council Decision in 1971, the OECD Chemicals Programme, under the remit of the OECD Chemicals and Biotechnology Committee (hereafter CBC), has played a central role in advancing global chemical safety and biosafety. The core work of OECD Chemicals Programme includes developing standardized testing guidelines for chemical safety, fostering mutual acceptance of data, harmonizing risk assessment methodologies to protect both human health and the environment, and promoting efficient, cost-effective, and environmentally protective chemical management across member countries.

In November 2024, the CBC discussed the issue of nanoplastics present in the environment. It was agreed to organise a workshop to better understand the state-of-the-art research, any existing risk assessment activities and identify any potential regulatory needs. To support the organisation, an ad hoc steering group was launched, comprising experts in the field from industry, research and government agencies. The workshop was held on 12- 14 November 2025 at the OECD and was attended by 121 participants from academic research, national environmental research institutes, metrology organisations, national standardisation bodies, industry and animal welfare organisations.

A total of 28 presentations were delivered to participants, with opportunities for questions and answers, and panel discussions addressing the following topics:

- current landscape on international, regional and national activities on safety testing of nanoplastics,
- ongoing research on test methods for production characterisation of reference and test materials,
- analytical methodologies and measurement challenges, and
- weathering and physical/chemical transformation techniques for environmentally relevant material

Discussion on a definition of nanoplastics was not included in the workshop agenda, as the properties of reference and test materials—as well as the choice of testing methods—are highly dependent on the specific context and objectives of a given study. In this context, establishing a single global definition for nanoplastics, including precise size ranges and shapes, was considered outside the scope of the workshop.

Workshop Overview

The workshop was opened by Bob Diderich, Head of Environment, Health and Safety Division at OECD. He explained that this workshop was organised following a recommendation from the CBC to scope the issue of toxicity and stability of fragmented nanoplastics and to better understand how the OECD can contribute to further harmonisation of tools and methods for their safety testing. This was followed by opening remarks from Jung-Kwan Seo, Director of Environmental Risk Division at National Institute of Environmental Research (NIER), Korea. Mr Seo highlighted that Korea has several research institutes addressing the potential hazards of nanoplastics to human health and environment. As such, they saw an

opportunity to support the workshop as a platform for sharing knowledge and experience, laying the foundation for strong international cooperation to mitigate issues related to nanoplastics.

The workshop agenda was divided in six sessions held over three days (see Annex B for the Workshop Agenda). The topics addressed were as follows:

Setting the Scene. This session featured three keynote presentations that provided a high-level overview of nanoplastics testing. Topics ranged from the processes and challenges associated with generating reference materials to the applicability of currently available OECD Test Guidelines to micro- and nanoplastics.

Standardization and international coordination for safety testing of nanoplastics. This was a panel discussion on efforts towards standardisation and international coordination for the safety testing of nanoplastics. The panel included three representatives from validation bodies and research consortia working on nanoplastics, as well as one representative from industry, who shared their perspectives on current activities and future needs.

Production and characterization of Reference Materials and Test Materials included presentations covering a range of particle generation methods representing both top-down and bottom-up approaches. The presentations and follow-up questions discussed the respective advantages of these production methods, their limitations and technical challenges.

Analytical Methodologies and Measurement Challenges which highlighted issues associated with detecting and characterizing nanoplastics, arising from their small size, low concentrations, polymeric nature, and the complexity of surrounding matrices such as soil and biological tissues.

Environmental Relevance & Fate: Weathering and Physical/Chemical Transformation Techniques addressed different weathering techniques used to prepare environmentally relevant nanoplastics samples, with a focus on exposure routes (e.g., airborne) and release mechanisms (e.g., mechanical wear).

Challenges in steps towards risk assessment framework for safety testing of nanoplastics was a panel session dedicated to discussions on existing risk assessment frameworks and on how tools and resources from nanomaterials risk assessment could be applied to nanoplastics.

1 Session 1: Setting the Scene

This Session intended to provide workshop participants with an overview of current issues with the testing of nanomaterials associated with the use of test and reference materials and the applicability of currently available methods for both human health and environmental toxicity testing.

Reference Material Production at JRC and Reference Materials for Microplastics / Nanoplastics with quantitative and qualitative properties - Håkan Emteborg[1]

The presenter provided an overview on how the JRC prepares and characterizes reference materials (RMs) for micro- and nanoplastics (MNPs) to improve interlaboratory comparability and traceability. MNPs differ widely in size, polymer type, and surface properties, and environmental aging alters the properties (e.g., increased surface hydrophilicity). As a result, studies apply different criteria and struggle to compare or consolidate findings. Drawing on long experience with matrix certificate reference materials (CRMs), the JRC provides common reference points so laboratories measure under comparable conditions and record the same core items. Although new measurement fields face limited supply and lengthy verification of homogeneity, stability, and characterization, the JRC continues to supply RMs that directly support comparability and traceability, helping laboratories build stable foundational datasets for subsequent risk assessment.

To keep RMs aligned with real samples, environmentally induced surface changes need to be reflected. The JRC applies surface treatment to polyethylene terephthalate (PET) and polyethylene (PE) to adjust hydrophobicity and verifies the resulting changes with dark-field microscopy-based methods. In this context, the hydrophobicity index (Hy-index) derived under OECD Test No. 126 (DF microscopy) was used as a common indicator to record hydrophilicity shifts associated with artificial aging, enabling batches and laboratories to compare different degrees of aging on the same basis.

For large-scale production, continuously stirred NaCl suspensions are aliquoted to ensure uniform distribution; ultra-microbalances, and quantitative 1H-NMR are used to check mass trueness, and homogeneity over time. In practice, mass and particle number remain the basic definitions, and surface characterization results (e.g., Hy-index) are recorded alongside these metrics to address surface heterogeneity and improve interpretation.

Certification requires verifying homogeneity, transport and storage stability, and comprehensive characterization for each production lot, with testing conducted in accordance with ISO 17034 and the referenced ISO/IEC 17025 requirements. New measurement fields face a circular dilemma: reliable methods are needed to produce reliable RMs, yet reliable RMs are needed to validate those methods. The JRC feed results back into the next batch and confirm performance with the same protocols. In parallel, results are reported in a consistent format and compared across laboratories, to increase consistency and traceability of the measurement results.

[1] European Commission, Joint Research Centre

The presenter indicated that additional studies are needed to produce RMs based on artificially aged PET MNPs, including homogeneity and stability testing. The JRC is also considering controlling the degree of artificial aging to adjust particle hydrophilicity and offering materials across a range of Hy-indices.

OECD Test Guidelines for human health toxicity: Applicability to currently available micro- and nanoplastics - Wendel Wohlleben[2]

The presenter evaluated how current OECD Test Guidelines (TG) apply to MNPs and outlined the test designs, key observations, and current limitations. He reported results from inhalation, oral, and in vitro studies and discussed what constrains reproducibility and comparability. Because MNPs are insoluble, highly heterogeneous, and transform under environmental conditions, some of the guidelines created for soluble chemicals need adaptation to produce meaningful toxicity assessments.

In inhalation testing (OECD TG 412) using polystyrene (PS-Nile Red) and polyamide-6 (PA-6) nanoplastics were administered for 28 days, followed by recovery assessments as presented. Lung inflammation markers consistent with particle exposure were reported, including neutrophil and macrophage infiltration, with signs of gradual recovery. A central difficulty was quantifying particle deposition and biokinetics in organs, as several analytical approaches showed background interference in certain tissues. Fluorescence labelling was usable only for some organs in the examples presented, and stability under biological conditions was not assessed. Pyrolysis-GC/MS was applicable for selected tissues in these studies.

In oral testing (OECD TG 407), cryomilled microplastics (MPs) with a mean size of (~7 µm) were administered up to 1,000 ppm, and no treatment-related effect were identified in the example presented. Dosing strategies including food matrix and higher sensitivity analytics are needed for future oral studies. The study design was applicable for assessing effects in the material tested, but for biokinetics the lack of standardized quantification methods limited reproducibility and cross-study comparison. Other guidelines showed differing applicability: skin irritation (TG 439) was straightforward, whereas the micronucleus test (TG 487) was incompatible due to particle precipitation in the example presented. In vitro assays also faced dosimetry challenges because well-dispersed nanoplastics did not readily deposit on cells, making exposure estimation uncertain.

A realistic assessment framework is required. The presenter proposed a practical path: secure diverse, well characterized test materials (polymer types, sizes, surfaces, aging states); standardize quantification workflows (possibly Py- GC/MS, fluorescence labelling) with robust quality assurance / quality control (QA/QC) and defined reporting elements; and run international ring trials under harmonized conditions to compare results and refine protocols.

The presenter's proposal on possible future directions include:

- Build an inventory of 'representative' test materials covering polymer type, size distribution, surface chemistry, and aging or transformation states.
- Establish standardized quantification workflows with interlaboratory QA/QC and minimum reporting elements for insoluble particles.
- Organise ring trials under harmonized conditions to evaluate performance and adapt protocols.
- Develop dosing strategies and dosimetry models that ensure controlled exposure and verifiable tissue detection.

Following the first two talks of Session 1, the Q&A session addressed the following issues:

[2] BASF, BIAC

- Additives and contamination control: Additives characterization is underway, with contamination control and long-term storage measures integrated into production. Reports of low-dose gamma irradiation sterilizing complex RMs without damaging plastics suggest a candidate approach for evaluation.
- Methods documentation and reporting level: the JRC does not issue SOPs for purchased analytical methods; measurement parameters are clarified at a general level. Because mass and particle number alone do not capture surface heterogeneity, further surface characterization and method development are being pursued.
- Defining items by mass and particle number if helpful for practical use, but it does not capture surface heterogeneity within-vial and between-vial; additional method development is needed to characterize these properties.
- Verification and comparability: Combining mass-based trueness checks with interlaboratory comparison (ILC) reduces uncertainty and yields more consistent interpretations, improving comparability across studies.

Key learnings on environmental sub-micro- and nanoplastic's analysis and ecotoxicity tests from LABPLAS project - Begoña Espiña[3]

The presenter reported on the experimentally verified impacts of MNPs on the environment and living organisms. Smaller particles (for example, nanoplastics <1 µm) posed greater physical risks through ingestion, and leachates—chemicals released from plastics under environmental conditions—introduced additional toxicity. These observations suggest that current testing guidelines may miss combined particle and chemical effects, reinforcing the need for precise quantification and robust control group design. At the same time, interpretation remains limited by sample heterogeneity and differences in exposure conditions.

Most of the environmental samples exhibited low toxicity, but a subset showed strong toxicity across all test species. For example, sample collected from the Elbe River in Germany demonstrated high toxicity across aquatic species; chemical analysis identified copper likely originating from an electric cable (without the wire), as main contributor. In other natural samples showing intermediate toxicity and containing cigarette butts, high concentrations of polycyclic aromatic hydrocarbons (PAHs) were detected. Another sample showing moderate toxicity presented high concentrations of phthalates. These findings suggest that additives or surface treatments, rather than the plastic polymer itself, may be primary drivers of toxicity.

Toxicity scores were always higher for aquatic species when exposed to leachates than to particles. However, earthworms showed higher sensitivity for particles than leachates on samples displaying toxicity.

In risk assessment, the risk quotient (RQ) values were generally below 1—indicating low immediate concern at first glance—but results varied by up to five folds depending on whether plastic concentration was calculated based on particle surface area, volume, or chemical tracing. This highlights an urgent need to standardize quantification methods.

The release of nanoparticles from 250 µm particles was confirmed using Nanoparticle Tracking Analysis (NTA). In applying OECD TG 201 on algal growth test, growth rates showed little change, but toxin production increased significantly meaning that relying solely on growth rate can overlook indirect or sublethal effects. In OECD TG 236 on fish embryo test using commercial polystyrene nanoparticles (commercial calibration standards), early growth delays and adhesion to neuromasts (sensory organs) were observed in the example presented.

[3] International Iberian Nanotechnology Laboratory, Portugal

The presenter recommended to standardize quantification protocols so that inconsistencies between mass, area, and volume-based measurements are resolved; to strengthen control group design by including multiple controls such as natural particles with similar size, density, and surface properties; and to integrate sublethal and indirect endpoints (for example, toxin production and adhesion to sensory organs) into test designs. Without these considerations, testing may continue to overlook “invisible dangers” present in complex environmental samples.

Following the presentation, the Q&A session provided additional information as follows:

- Leachates were generated without chemical extraction by mixing plastics in the same medium used for the toxicity tests under identical conditions for 24 hours, allowing chemicals to leach naturally. This approach aimed to replicate environmental release and to distinguish chemical effects from particle effects; a separate test assessed the direct effects of the plastics themselves.
- Although some samples were collected from natural environments, the analysis focused on additives originating from the plastics. External contaminants adsorbed from surrounding water or soil were not included in this study. Future work should address interactions between adsorbed pollutants and plastic additives for more comprehensive risk assessment.

2 Session 2: Standardization and International Coordination for safety testing of nanoplastics

This session was a panel discussion on efforts underway towards standardisation and international coordination for the safety testing of nanoplastics. The session was opened by Anne Gourmelon, Principal administrator of the OECD Test Guideline Programme, who presented an overview on the remit of OECD work on Test Guidelines and taking informed decisions on standards development. She explained that OECD Test Guidelines are harmonised and standardised tools used for the testing of industrial chemicals, including manufactured nanomaterials, pesticides, biocides, personal care products that are placed on the market in various countries. She explained the aim, scope, and the process for establishing OECD Test Guidelines and how they differ from other standards from other organisations as they guarantee mutual acceptance of data, when combined with good laboratory practices.

Panellists were asked to prepare an intervention addressing the following questions:

- Are current projects and organisation sufficiently coordinated to avoid overlap but enable synergies?
- Do current projects and organisations reach sufficiently to regulators and other stakeholders?
- What can be the impact of more/better standardised methods on nanoplastics?
    - Improve reproducibility of research findings and facilitate international research collaboration?
    - Better regulate (limit) the incidental release of nanoplastics?
    - Limit the placing on the market of problematic nanoplastics?
    - Other?
- Is there a regional/international forum/organisation addressing the policy issue regarding nanoplastics (beyond the technical standards)
- What are the short-term, medium-term, and long-term priorities for standardisation?
    - Method development for detection considering low limit exposure in the environment?
    - Method development/adaptations for toxicity testing? Validation of methods?
    - Environmentally relevant reference materials development?
- What are the challenges in achieving standardisation for testing, quantifying, measuring, characterising nanoplastics? Is standardisation the best way forward, or are there other avenues?
- What kinds of harmonisation efforts are there?

Harmonization Initiatives for Micro- and Nanoplastics Analysis: Insights from PlasticTrace, ISO, and VAMAS4 - Andrea Mario Giovannozzi[5]

PlasticTrace aimed to build a full harmonization and standardization chain for testing MNPs—from reference material development and method validation to interlaboratory comparisons and formal standardization. The project's core approach was to strengthen the reliability of MNPs analysis by pairing new technologies with international collaboration across drinking water, food, and environmental applications.

Current MNP research explores a wide range of materials and testing methodologies. To make the transition from research to regulation, reliable reference materials and validated test methods are essential. Aligned with the EU Circular Economy Action Plan (CEAP), PlasticTrace focused on laying the metrological foundations needed for consistent quantification and characterization of MNPs.

The project produced water soluble tablet forms of homogeneous PET, Low density polyethylene (LD-PE) and polypropylene (PP) microplastic particles (10–100 µm) and developed PE and PP nanoplastic suspensions for use in ISO and VAMAS standardization activities. These materials underwent homogeneity and stability testing in accordance with ISO Guide 35 on reference materials. For method validation, infant formula and surface water samples including PET tablet spiking were used for evaluation of thermo-analytical techniques (TED-GC/MS, Py-GC/MS) and spectroscopic methods (µFTIR, µRaman, LDIR). An interlaboratory comparison (ILC) with 12 laboratories on Raman/IR based vibrational spectroscopy validation was performed according to ISO 160942 (a drinking water microplastics analysis standard). ILCs on nanoplastics measurement techniques are ongoing within VAMAS Technical Working Areas (TWA) 45.

For the path forward, three priorities were highlighted: improving accuracy for particles below 20 µm in complex food and environmental matrices; strengthening chemical identification through DEP (dieletrophoresis)-Raman fingerprinting of oxidation and surface properties; and expanding international collaboration with ISO, CEN, and VAMAS, including more ILCs and standardization activities to underpin future regulatory frameworks.

Following the presentation, the Q&A session provided additional information as follows:

- All laboratories used the same operating procedure, even if their instruments differed. If a lab could not follow the procedure, it documented the reason; instrument differences are treated as normal method variation and will be analyzed.
- Sample handling depends on the technique: PTA/NTA requires dilution, while other methods measured the stabilized suspension as is. Choices in dispersion and dilution can change the reported particle size distribution.
- Each method defines "size" differently. For instance, DLS reports the hydrodynamic diameter, while Field-Flow Fraction (FFF) combined with Multi-Angle Light Scattering (MALS) reports the radius of gyration. For nonspherical particles these are not equivalent, so the methods are not interchangeable. The goal is to standardize procedures and assess method performance, not to assign a single "true" size.

[4] http://www.vamas.org/VAMAS: Versailles Project on Advanced Materials and Standards (https://www.vamas.org/)

[5] INRIM: Istituto Nazionale di Ricerca Metrologica (National Institute of Metrological Research)

Advancing Standardisation in Micro- and Nanoplastics Analysis via Interlaboratory Comparisons - Enrica Alasonati[6]

ILC was presented as a practical tool to verify test method reliability under real conditions and to accelerate international standardization. Complex challenges that a single institution cannot resolve must be tackled through global collaboration, centred on the development of reference materials, high sensitivity analytical techniques, and improvements in data quality.

ILC goes beyond simple performance checks—it is a key step for systematically verifying repeatability and reproducibility and for supporting the development of international standards. Standardization is about building a trustworthy data ecosystem, and ILCs designed and analysed under ISO 13528 and ISO 5725 show how method performance can be assessed and contribute to regulatory frameworks.

An ILC with 12 laboratories on Raman and μFTIR vibrational spectroscopy validation according to ISO 160942 (a drinking water microplastics analysis standard) ran in 2023–2024. Three reference materials (ISOA, ISOB, ISOC) were designed to reflect different polymer compositions, particle sizes (20–500 μm), and concentrations. Repeatability was 20–45%, and reproducibility reached up to 70% depending on particle size and material type.

The Nanoplastics Analysis ILC (VAMAS TWA 45) uses the Nano-polypropylene reference material to compare multiple techniques, including DLS, MADLS, FFFMALS, PTA, and PyGC/MS. Size measurement methods (DLS, FFF) showed repeatability of 2–5% and reproducibility of 8–23%, whereas concentration and mass based methods (PTA, thermal analysis) showed repeatability around 30% and reproducibility of 41–60%.

For future work, measurement accuracy for ultrasmall particles (below 20 μm) in environmental samples needs to be improved, where current technologies struggle. Developing measurement techniques with higher precision is essential. Chemical identification strategies should be strengthened to assess oxidation and surface properties. Finally, expanded international collaboration—stronger engagement with ISO, CEN, and VAMAS, and more ILCs and standardization activities—will help secure global data reliability.

Following the presentation, the Q&A session provided additional information as follows:

- Most materials were conventional plastics such as polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), and polystyrene (PS). Polylactic acid (PLA), a biobased material, was included in some projects, but only to a very limited extent.
- The five projects aimed to test similar materials to make results easier to compare. Some harmonization was achieved, but differences in project objectives led to variations. In the end, all results were compiled into a single dataset covering multiple plastic types.

CUSP research roadmap 2026 - 2032 State of the art, gaps, and future needs in micro- and nanoplastic and health research – Alberto Katsumiti[7]

The EU funded CUSP [8] cluster linked five collaborative projects—Aurora, ImpTox, PlasticsFate, PlasticHeal, and Polyrisk[9]—to comprehensively examine the impacts of micro and nanoplastics on human

[6] Laboratoire national de métrologie et d'essais (LNE), France

[7] Gaiker, Spain

[8] https://cusp-research.eu/

[9] **AURORA** – Actionable European Roadmap for early-life health Risk Assessment of micro- and nanoplastics

health. Each project addressed a distinct axis (early life exposure, inflammatory responses, carcinogenicity and mutagenicity, broad health effects, and inhalation related allergenicity) and combined *in silico*, *in vitro*, and *in vivo* approaches to conduct hazard assessments and build individual risk assessment frameworks. The newly released research roadmap integrates these outcomes and sets out a structured path for future standardization and policy development.

The roadmap's central message is that the foundations for standardization are still insufficient. Current research lacks harmonized criteria for analytical methods, exposure assessment, and hazard evaluation, and laboratories use different materials and protocols, making it difficult to compare results or set regulatory benchmarks. For example, while Aurora analysed early life exposure, other projects focused on inflammation or carcinogenicity, leading to divergent methodologies. This diversity advances scientific progress, yet it also underscores the urgent need for "usable standards"—common reference materials and unified reporting elements—to enable industrial application and regulatory compliance.

Translating research into practice faces several real-world barriers. Advanced analytical techniques discussed in academia are difficult to deploy in commercial laboratories due to high costs and limited access to specialized personnel, and an even greater challenge is contamination control. Preventing bacterial, endotoxin, and chemical contamination throughout sampling, storage, and analysis is extremely demanding. CUSP worked to lower these barriers by developing interlaboratory protocols and producing standardized reference materials but faithfully reproducing real world particles under laboratory conditions remains a fundamental limitation.

Investigations of realistic exposure scenarios—urban traffic, indoor artificial turf fields, and occupational environments—detected micro and nanoplastics across air, water, food, and personal care products. Some particles carried bacteria, producing a "Trojan horse" effect where chemical additives and microbial contaminants cooccur. Both acute and chronic effects were observed, along with microbiome alterations and long-term biological responses; however, quantitative data on real world exposure levels and associated health risks are still scarce, leaving considerable uncertainty.

Consequently, short term priorities include standardized reference materials, stronger quality assurance, robust *in vitro* and *in silico* testing systems, and reporting standards for exposure studies; midterm goals involve harmonizing analytical methods, advancing high throughput testing, improving measurement of polymer types in human tissues, and generating hazard data for key plastic types; and long term objectives focus on characterizing hazards of real world particles, scaling up biomonitoring, building predictive exposure models, and integrating social determinants into risk frameworks. In parallel, designing safer, more sustainable plastics and assessing the economic impacts of mitigation policies will underpin future regulatory strategies.

Contextualization of standardization needs and applications - Denise Mitrano[10]

Standardization is about striking a balance between scientific rigor and industrial practicality. Methods must ensure accuracy and reproducibility while remaining feasible for commercial laboratories. Reliable results require robust analytical methods agreed upon by all stakeholders. Such standards form the foundation not only for academic publications but also for regulatory compliance and industry reporting. Without them,

ImpTox – An Innovative Analytical Platform to Investigate the Effect and Toxicity of Micro and Nano Plastics Combined with Environmental Contaminants

PlasticsFate – Plastics Fate and Effects

PlasticHeal – Innovative tools to study the impact and mode of action of micro- and nanoplastics on human health

POLYRISK – Understanding human exposure and health hazard of micro- and nanoplastic contaminants

[10] Nestle, Switzerland

there is no benchmark to judge research outcomes, making it difficult to compare studies or set regulatory thresholds. Therefore, the goal should be to establish standardized test methods that define baseline exposure across various matrices—food, water, and environmental samples.

While advanced analytical techniques are widely discussed in academia, their adoption in commercial labs faces significant hurdles.

- Most industrial settings prioritize cost and operational efficiency due to limited access to cutting-edge instruments and specialized personnel. If methods are overly complex or resource-intensive, they risk being abandoned. Thus, solutions must maintain accuracy while ensuring practicality.
- This challenge is particularly evident in the food sector. Reports of microplastics detected in bottled water or common food items often alarm consumers, yet these findings frequently raise questions about quality. Without standardized criteria, distinguishing flawed research from credible results becomes nearly impossible.

Despite current limitations, there is room for optimism. Just as automobiles evolved from the Model T to today's advanced vehicles, methods that seem impractical now may become tomorrow's standards. The key is to define sufficiently robust criteria for the near future while continuing to advance technology.

3 Session 3A: Production and characterization of Reference Materials and Test Materials: Top-Down Production

The first part of the Session 3 was dedicated to presentations on top-down production of reference materials and test materials. Top-down approaches involve physical or mechanical fragmentation of larger plastic materials into nanoscale particles, aiming to generate materials that more closely resemble nanoplastics formed through environmental degradation. The presentations in this session showed that there is active research work in employing top-down approaches to produce nanoplastics reference materials, although the limited particle stability and production yield continues to be a big hurdle in producing reference materials that meet the existing standards (e.g., ISO Guide 35). There was an agreement that the most urgent need for nanoplastics reference materials is in standardizing detection methods to reduce measurement uncertainty from size. A stepwise approach to making test materials to test hypothesis and to decouple combined toxicological effects from different sources (e.g., polymer effect vs. size effect vs. additive effect) was suggested.

Micro- and nano-plastic test/reference material production via hybrid top-down degradation methods - Jaewoong Lee[11]

A project led by NIER, Korea targets the safety assessment of MNPs by developing standardized test methods and reference materials, with a long-term goal to build an international standardization framework. The current work involves reviewing exposure data in environmental and human contexts and producing test and/or reference materials via a top-down approach using polyethylene terephthalate (PET) and polypropylene (PP).

Reliable reference materials and validated test methods are essential to move from research to regulation. According to ISO standards, reference materials must demonstrate homogeneity and stability, and test materials must maintain batch level consistency. To meet these requirements, the project combines international collaboration with targeted technical development.

A 2025 literature review of PubMed and ScienceDirect reported PET, PP, and PS as dominant polymers in environmental samples; PET was most frequently detected in air following by PP and PS, while PP and PS were prevalent in sediments. In human related toxicity studies, PS was most used, followed by PET, PE, and PP. Exposure from food and drinking water was primarily associated with PP, with PE, PS, and

[11] NIER: National Institute of Environmental Research, Korea

PET also reported. Recent studies have further indicated that microplastics (PE, PVC, PET, PP and PS) have been found in human organs such as liver, kidney, brain, lungs and blood.

Test and/or reference materials were generated through a hybrid top-down workflow that combines cutting, grinding, filtration, and sonication. After filtration, the average particle sizes were approximately 380 nm for PET and 268 nm for PP. DLS showed distinct peaks, and particle morphology was observed by Transmission Electron Microscopy/Scanning Transmission Electron Microscopy (TEM/STEM) to verify the presence of nanoscale fragments. The next step is homogeneity and stability testing in accordance with ISO Guide 35, which will assess whether the produced PET and PP materials may be suitable as candidate reference materials. Future work will examine nanoplastic fragmentation under environmental conditions, characterize chemical properties, and conduct toxicity screening tests using test and/or reference materials.

Top-down cryo-ground nanoplastics: the pros and the cons of their use as reference materials for (eco)toxicological studies vs. bottom-up synthesized polymer nanoparticles - Olivier Sandre[12]

Two approaches for producing nanoplastics—top-down (cryo-grinding) and bottom-up (nanoprecipitation) methods— were compared to analyse effects of nanoplastics on marine ecosystem pollution and (eco)toxicity assessments. Contamination experiments and toxicity evaluations using freshwaters or marine algae, clams, and oysters were conducted to provide insight on the impact of environmentally relevant test materials.

The top-down route physically reduces existing plastics to the nanoscale through cryogenic grinding, sieving, and filtration. The yield is very low, around 1%, with some studies reporting improvements to 3–5%. For example, when beach litter was reduced from 2 mm to 200 µm, the yield exceeded 50%, but most particles were lost at the nanoscale stage. Polyolefin fibers (PE, PP) from fishing nets are particularly difficult to sieve. FTIR identified PE as the most common polymer, and laser scattering granulometry indicated that particles below 10 µm accounted for only 0.4%. Top-down approach produces materials that mimic the physical characteristics of particles found in the environment, but its low yield, high energy consumption, and challenges in size control remain clear limitations.

The bottom-up route forms particles by dissolving polymers in a solvent and dispersing them into water, enabling high yield (up to 0.3% solid content suspensions) and precise control over size and morphology. PLA and PS particles ranging from 80 nm to 80 µm were produced and mixed with iron and gold nanoparticles to enhance characterization. Conditions such as salt addition, ultrasound, and freeze thaw cycles were optimized to improve stability and size distribution. However, these particles may differ in physical and chemical properties from those formed naturally by erosion in the environment, which should be considered when interpreting (eco)toxicity results, particularly when mixture or labelled particles are used, as they may not be directly appropriate for toxicity testing.

In toxicity testing, a microfluidic chip was used to control salt gradients and to prevent particle precipitation. Multi-angle dynamic light scattering (MADLS/DLS) showed particles averaging about 553 nm, and dense aggregates accumulated in oyster tissues, triggering inflammation. Gene expression analysis (qPCR) indicated that PLA induced relatively lower stress responses, whereas polystyrene caused pronounced activation of stress markers. Particle size, morphology, and surface properties were identified as key factors influencing toxicity.

In biodegradability tests based on OECD TG 301F, nanoplastics exhibited minimal degradation compared with macro-sized particles. Future work include assessing how size, crystallinity, and density affect

[12] University of Bordeaux, France

biodegradability and developing AI-based predictive models to forecast nanoplastics behaviour in the environment.

Following the talk, the Q&A session provided additional information on the following:

- On whether non-solvents or safe surfactants help stabilize products in simplified processes, several studies have used ethanol as a suspending medium. Future plans include applying poly vinyl alcohol (PVA) as a safe surfactant. Ideally, intermediary solvents could eliminate the need for surfactants altogether, streamlining production.
- On using thermodynamic models and AI to predict particle properties and to improve system design, trends in particle size and production efficiency indicate these tools can optimize formulations and forecast biodegradability. For example, preliminary data show that nanoprecipitation process lowers crystallinity but increases density *via* formation of compact globules in poor solvents, which affects biodegradability; multi-parametric studies coupled with AI could be used to clarify these relationships.

Laboratory-scale generation of micro/nanoplastics for toxicological testing - Sabina Halappanavar[13]

The presence of diverse microplastics was confirmed in archived atmospheric samples from Montreal (Canada), but extracting sufficient quantities for toxicity testing was not feasible. The air contains abundant carbon-based materials such as dust and soot, which dilute plastic signals during analysis, and the few isolated particles were insufficient in quantity and quality to serve as test materials. The team therefore chose to produce laboratory test materials that could simulate environmental exposure.

Using PET water bottles as the feedstock, the researchers combined top-down (blending- cryomilling–filtration) and bottom-up (solvent precipitation) approaches to generate four types of test materials: PET Small (sharp fragments under 3 µm), PET Large (smooth-surfaced particles under 10 µm), PET Fiber (fibre-like structures, did not retain fibre shape post-cryomilling), and PET Nano (spherical particles 4–40 nm). Chemical identity was verified by Raman spectroscopy, and surface characteristics and size were analyzed with STEM and AFM, ensuring consistent information on particle morphology, size, and surface features. These production and characterization results were then used to design toxicity tests, providing a basis for comparing how each particle type behaves in model systems.

Overall, toxicity testing showed that PET Small with sharp surface topography exhibited the highest toxicity, PET Nano with smooth surface topography showed low toxicity despite its nano size, compared to PET small. PET Large with smooth surface elicited nearly no response. This pattern is consistent with the interpretation that sharp fragments with rough surfaces can induce mechanical stress and increase reactivity, whereas smooth spherical nanoparticles tend to be less reactive.

Differences in toxicity were observed to depend on physical properties and intracellular localization. Particles with sharp edges and rough surfaces were more likely to cause physical damage to cellular structures, and TEM revealed that significant number of PET Small particles penetrated critical organelles such as the mitochondria and endoplasmic reticulum. In contrast, smooth spherical nanoparticles were predominantly dispersed in the cytoplasm, a location associated with milder toxic effects.

Following the talk, the Q&A session provided additional information as follows:

- On sodium dodecyl sulphate (SDS) used in bottom-up approach, the amount of SDS found in the final microplastics preparations were not high enough to induce toxicity. Other process

[13] Health Canada, Canada

induced impurities were detected, however, their levels were insignificant in the exposure medium to cause any notable toxicity.

- Whether the Small PET is more toxic due to their reactivity arising from the rough surfaces can easily be determined using reactivity assays, which are planned.
- On the status of exposure studies and the role of particle characteristics, more than 10 different microplastics types have been prepared in house and their toxicity tested along the side of several commercially purchased microplastics types. In vitro testing has been completed, and in vivo studies are planned, keeping in mind the types that have shown response in vitro; fiber shape was expected to influence toxicity, but milling removed the fiber structure and complicated AFM analysis, while sharp edges are likely to increase physical damage and reactivity.
- On particle uptake, dose relevance, and waste management, TEM showed PET Small particles in mitochondrial matrix and ER, PET nano particles inside the cells, whereas larger (PET Large) particles exhibited minimal uptake; doses were derived from nanomaterial literature and their relevance to human exposure remains uncertain; efforts were made to minimize waste. The left over plastic sediment and slurry are reused to minimize waste. All of this work was completed using as little as 12 grams of PET pieces from disposable water bottles. Multiple batches are prepared from individual bottles to assess reproducibility and comparability.

Production of reference materials by Quality of Design - Korinna Altmann[14]

Current micro- and nanoplastics (MNP) research relies on diverse analytical methods that often yield variable results. For example, Raman spectroscopy has shown an uncertainty of ±9%, while FTIR and GC-MS exhibit higher variability. Transitioning from exploratory studies to regulatory frameworks requires validated detection techniques and standardized reference materials that meet strict criteria for homogeneity, stability, and reproducibility to support accurate calibration and reliable toxicological testing. The Quality by Design (QbD) framework, whose effectiveness has been proven by the industry could contribute to this process.

Development of reference materials begins by defining the target product profile—size (e.g., 10 100 µm), mass (e.g., 19 µg ±9%), production method, and desired aging state—which determines the manufacturing approach and aligns the material with experimental objectives. For instance, cryomilling PET pellets yields smoother fragments due to heat, whereas using PET films produces distinctly different shapes; these choices directly affect surface properties and toxicological relevance.

Microplastic particles can be produced via top-down routes such as cryomilling and sieving to achieve 10 100 µm size ranges with sufficient yield. Nanoplastics are more challenging because they must remain stable in suspension, be free of additives and contaminants, and be sterile. A combination of ultrasonic fragmentation, laser ablation, and chemical precipitation produced particles of 4–40 nm, with surface characterisation using XPS. These nanoplastics particles remained stable for 12 months without agglomeration or changes in physical properties, supporting long-term experimental use. Five consecutive production batches yielded identical results, demonstrating reproducibility. Endotoxin levels were reduced below detection limits, although achieving completely endotoxin-free materials remains difficult and calls for interdisciplinary collaboration.

Near-term priorities include scaling up production (from 50 mg to kilogram quantities), incorporating aging effects, and improving dispersion in biological media. Harmonized SOPs and validated methods such as Raman spectroscopy will be central to reducing uncertainty and enabling global standardization.

[14] BAM, Germany

Following the talk, the Q&A session provided additional information as follows:

- On contamination control and batch reliability, complete removal of endotoxins remained extremely difficult despite sterile water, ethanol cleaning, and heat treatment; real-world microplastics often carry microbes, complicating interpretation, and proper controls with large, standardized batches are essential because small lab-made quantities are insufficient for consistent testing.
- On material design for toxicology, a stepwise approach was outlined: begin with additive-free polymers to isolate polymer effects, then add known additives to reflect realistic conditions. Reference materials are used for standardizing detection methods, while test materials are for testing hypothesis.
- Near-term priorities include scaling up production, incorporating aging effects, improving dispersion in biological media, and relying on harmonized SOPs with validated methods such as Raman to reduce uncertainty and enable global standardization.

4 Session 3B: Production and characterization of Reference Materials and Test Materials: Bottom-Up Production

The second part of the Session 3 was dedicated to presentations on bottom-up production of reference materials and test materials, which involves generating nanoscale plastic particles through controlled synthesis rather than by fragmenting larger plastics. Particles generated from bottom-up production do not mimic the environmentally found nanoplastics in their physical and chemical properties, but their size, shape, and surface chemistry can be controlled with high precision. The presentations in this session showed how the advantages of bottom-up production approach can be used to build a material library and to make sample with characteristics that meet the specific needs of the study.

Bottom-up nanoplastics: tunable parameters, particle diversity, and suitability for method development and risk assessment, Stephanie Reynaud[15]

Commercial nanoplastics were originally developed as calibration standards and often do not fit environmental research needs. Their chemical composition is frequently unclear, “pristine” products may still be surface-functionalized, the concentration and location of functional groups and labels are seldom reported, and some labels can leach, distorting results. The team therefore opted for custom production to fully control size, surface properties, composition and covalently attached labels whenever required.

Two bottom-up strategies were used. Emulsion polymerization with or without surfactants generates monodisperse particles with precise control over size and quantified surface functionality (to mimic aged particles) and chemical composition (additive may be included) and achieves high concentrations but is mostly limited to polystyrene. Nanoprecipitation, which relies on dissolving a formulated plastic followed by its controlled precipitation, allows the production of nanoplastics from a wider variety of polymers with internal additives, offering flexibility, but size dispersity is less narrow, surface functionality cannot be quantified, and yields are low. Particles produced from these two approaches were used to examine the impact of particle shape and chemical composition.

To lower detection limits, particles may be labelled to enable sensitive detection and quantification of the introduced nanoplastics in complex matrices. Palladium-labelled nanoplastics have become important in this field, enabling ecotoxicological assessments of biodistribution and bioavailability. Metallic, fluorescent, and isotopic labelling with stable attachment was implemented. Embedding one gold (Au) nanoparticle per nanoplastics enabled accurate nanoplastics quantification both by particle number and by mass in complex

[15] University of Pau

media; 2H and 13C isotopic labelling allowed quantitative analysis in biological matrices such as mussels, Artemia, and Daphnia. Covalent bonding of fluorescent and isotopic probes was used to prevent leaching and preserve particle properties.

Fit-for-purpose nanoplastics can be produced using both approaches. Bottom-up methods provide tight control over size and surface properties and allow high-concentration production but yield shapes less representative of environmental particles. Top-down methods better reflect environmental realism but lack control over size, surface characteristics and have limited scalability. The approaches are complementary and should be selected according to research objectives and analysis requirements. Particle characteristics, concentration, and surface functionality should be clearly reported, with both mass concentration and particle number concentration provided to support interpretation of surface interactions and mechanism-driven effects.

A bottom-up particle fabrication approach for the development of a library of micro- and nanoplastics - Kosuke Tanaka[16]

The presented library of MNPs include 110 types of particles, that cover a range of shapes (e.g., spherical, fragment, and fibrous) and physical states (e.g., pristine, degraded, and labelled).

An additive free precipitation method was used to generate LDPE, HDPE, and PP particles with average sizes of about 400 nm, and PVC and PS particles around 200 nm. The molecular weight distribution matched the raw materials, and properties such as crystallinity, melting point, and glass transition temperature fell within the same range as commercial products. However, achieving uniform sizes below 100 nm was difficult, and the size distribution remained broad.

To overcome these limitations, a micro reactor based continuous flow process was used. By mixing polymer solutions and antisolvent phases in extremely narrow microfluidic channels, uniform blending, precise control of temperature, concentration, and reaction time were ensured. This enabled reliable production of sub100nm particles—previously proven to be challenging in batch processes. Production efficiency also improved to about 1 gram per week.

For accurate quantification, the team incorporated carbon13 stable isotopes into the particles, allowing them to serve as internal standards in mass spectrometry and to compensate for analytical losses. Quantum dots were embedded inside the particles for fluorescent detection. These labels are stable, do not leach into solvents, and facilitate tracking during exposure experiments.

The particles produced were spherical, which differs from the irregular shapes found in real environmental samples. Nevertheless, their well-defined size and shape allows for high reproducibility, a strong advantage for standardization. Grams cale production is currently feasible, making these particles suitable as reference materials for repeated measurements and comparisons. Future priorities include scaling up production.

Following the talk, the Q&A session provided additional information as follows:

- Stable isotope libelling levels were clarified: ^{13}C enrichment is near complete (>99%).
- Scalability for continuous flow production: current output is about 1 gram per week, and improving scalability remains a key development priority.

[16] NIES, Japan

Reprecipitation method for synthesis of polyethylene and polypropylene nanoplastics and small microplastics - Jinyoung Jeong[17]

A bottom up reprecipitation approach was used to meet the targeted particle size, polymer type, and production yield for toxicity assessment, while enabling precise control of size, shape, and chemical composition.

Toxicity research for MNPs has long been biased toward using polystyrene (PS) because standardized PS materials are readily available, yet polyethylene (PE) and polypropylene (PP) dominate real world production and waste. Therefore, PE and PP-based nanoplastics were generated as target test materials.

Focusing on (i) the relevant size range for human health toxicity (about 100 nm to 10 μm), (ii) environmentally representative polymers (PE and PP), and (iii) sufficient yield for toxicology research, a reprecipitation method to produce PE and PP nanoplastics was optimized to reach gram scale production. example, the reported method could be used recently to supply 5 g of PE nanoplastics for a plant toxicity study.

Furthermore, solvent ratios were tuned to produce multiple PP size classes. The reported method was also extended to fabricating nano-sized PE particles (100–500 nm) and microparticles (1–10 μm). The final product included spherical and elongated particles as well as irregular morphologies. FTIR and DLS confirmed polymer identity and stable, nanometer-scale aqueous dispersions.

Fluorescent libelling was applied to track biodistribution *in vivo*, and zebrafish assays enabled real-time imaging of excretion and semi-quantitative analysis of excreted particles. Ongoing studies are addressing effects on the gut, brain, and immune system as well as mixture toxicity, working with organoids, plants, and other models. Future work planned includes clarifying interactions with additives, adsorbents, and contaminants.

Following the talk, the Q&A session addressed the following issues:

- Additive and contaminant strategy: no additives were used during synthesis at this stage; only plain particles were prepared. Introducing additives is in the plan for future work.
- Beyond PS and the role of additives: shifting from PS to other polymers is complicated due to the lack of test materials of nanoplastics (e.g., PE, PP). The toxicological effects of plastic additives (including target molecules, fraction in plastics, and their biological effects) should be carefully considered.

[17] KRIBB: Korea Research Institute of Bioscience and Biotechnology

5 Session 4: Analytical Methodologies and Measurement Challenges

Session 4 was composed of five presentations that address the current status, strength and limitation of available analytical methodologies. All presentations had a consensus that a single-method approach cannot provide an access to quantification, size, or chemical composition of samples all together, hence multi-method workflow was recommended. However, multi-method workflow would be resource intensive and require special expertise; whether such an approach is feasible outside of research laboratories should be reflected. There was also a general agreement that currently available methodologies have limited applicability to nanoplastics, and especially for nanoplastics found in the complex environmental matrices (e.g., soil), where even sampling step was proven to be challenging. Developing standardized protocols and harmonized minimum reporting criteria was emphasized in all presentations.

Challenges in spectroscopy-based nanoplastic detection and the role of OPTIR in advancing analysis - Shima Ziajahromi[18]

Due to their polymeric nature, nanoplastics are difficult to detect and analyse due to interference from organic matter, dyes, and contaminants. Conventional pretreatment to remove organic matter is essential but may alter particle characteristics and therefore requires further verification. Their tendency to aggregate through surface charges complicates single particle analysis.

FTIR and LDIR typically detect particles down to about 10–20 micrometers (μm), while conventional Raman spectroscopy reaches ~1 μm—still above the nanoscale. Detection limit for Advanced Raman methods can reach ~20 nm but could face fluorescence interference and suffer from long scanning time and reproducibility issues; for example, a recent soil study could detect only ~10% of extracted particles because fluorescence masked signals. Environmental samples often contain pigments and additives that emit natural fluorescence, further complicating the analysis.

Atomic force microscopy based infrared spectroscopy (AFMIR) provides nanoscale spatial resolution for size and functional group identification but is limited by organic matter interference and insufficient selectivity of plastic markers. In contrast, Optical Photothermal Infrared Spectroscopy (OPTIR) combines infrared and photothermal imaging to analyse individual nanoplastics without fluorescence interference, with key advantages:

- High resolution: Hundreds of nanometers, superior to Confocal Raman Microscopy (CRM) and Surface Enhanced Raman Spectroscopy (SERS)
- Reduced interference: Less affected by organic matter in complex matrices such as soil, sediments, and biosolids
- Speed: Faster scanning over larger areas compared to AFM-IR

[18] Griffith University, Australia

- Comprehensive data: Provides chemical functional group identification along with particle size, morphology, and surface area

Sample preparation workflows for complex matrices were optimized using hydrogen peroxide, Fenton's reagent, and enzymatic treatments to remove organic matter, followed by density separation and filtration. OPTIR achieves approximately 500 nm resolution and has recently detected 200 nm PS beads. In drinking water, the instrument identified 400 nm polymethyl methacrylate (PMMA); in soil, even with incomplete filter cleaning, automated analysis could distinguish plastics from organic matter and provide details on particle shape (fibers, fragments, beads) and surface area. Current applications of OPTIR include detecting polymers such as PE, PP, and PMMA in water, wastewater, soil, sediments, and biosolids.

While OPTIR offers faster and more reliable analysis than FTIR and Raman, further validation is needed for pretreatment efficiency and detection limits. Flotation for ~400 nm particles can take up to 798 hours, indicating a need for improved extraction methods. to the plan for future work includes spiking reference materials with known sizes and shapes to evaluate recovery and accuracy, applying quality assurance/quality control (QA/QC) protocols similar to those used for FTIR and Raman to ensure robust performance.

Physicochemical Characterization and Quantification of Nanoplastics: Challenges, Advanced Methods and Perspectives - Natalia P. Ivleva[19]

Nanoplastics vary widely in size, shape, and chemistry, and occur at low environmental concentrations. Methods developed for engineered nanoparticles—optimized for inorganic materials—often do not directly transfer to plastics due to different optical, thermal, and surface properties. Even simple pretreatment can reduce recoveries, underscoring the need for effective pre-concentration and enrichment strategies. No single method is sufficient; a multi-method approach is essential.

DLS yields intensity weighted hydrodynamic diameters from total signals and the results can be biased toward larger particles. Nanoparticle Tracking Analysis (NTA) tracks individual Brownian motion to provide number-based size and concentration, subject to thresholding and aggregation effects. Tunable Resistive Pulse Sensing (TRPS) measures resistive pulses through a nanopore, requiring electrolyte control and calibration standards. Centrifugal Liquid Sedimentation (CLS) relies on accurate density assumptions and is sensitive to matrix properties. Field-Flow Fraction (FFF), coupled with detectors such as Multiangle Light Scattering (MALS), Ultraviolet-Visible spectroscopy (UV–Vis), or Fluorescence spectroscopy, can deliver high resolution size separation for irregular or polydisperse populations when appropriately calibrated.-

Size analysis alone is insufficient. FFF can be analysed by Raman micro spectroscopy or pyrolysis GC/MS. Raman enables single particle polymer identification but is prone to fluorescence interference. Pyrolysis GC/MS is destructive to samples and mass based, providing polymer composition without morphology. Online FFF–Raman enables size resolved identification of particles from approximately 100 nm to 5 µm under favourable signal conditions, for both monodisperse and polydisperse samples. Ongoing work aims to extend size-resolved identification of nanoplastics below 100 nm using more powerful lasers and optimized set-up, while managing potential heating and background signals.

Simulated Raman Scattering (SRS) suppresses fluorescence interference and enhances vibrational signals, supporting rapid analysis of nanoplastics in flow. Under controlled conditions, a linear relationship between particle number concentration and detected events has been demonstrated, enabling number-based quantification. In the future the application of broad band SRS should enable simultaneous analysis of nanoplastic particles of different polymer types.

[19] Technical University of Munich, Germany

Comprehensive nanoplastic analysis requires integrating fractionation with chemical identification, with consideration for sensitivity and preconcentration improvements. Recommended QA/QC protocols includes field blanks, spike and recovery analysis with polymer standards of known size and shape, polymer specific LOD/LOQ, replicate analyses, and interlaboratory comparison. Reports should include, at minimum, the size metric used, concentration metric (number vs mass), polymer identity, morphology, and associated uncertainty.

Analytical Insight into Nanoplastic Analysis: Strengths and Challenges of Mass Spectrometry Approaches - Javier Jiménez[20]

Pyrolysis-Gas Chromatography/Mass Spectrometry (PyGC/MS) thermally decomposes plastics to volatile products that are separated by GC and detected by MS, providing polymer identity and mass-based concentration. PS nanoplastics produces a characteristic fragment at m/z 104, but this fragment is not specific and other compounds (for example, phenylalanine) can interfere. Isotopic labelling with deuterium or ^{13}C shifts the most abundant fragments (e.g., m/z 109), enabling detection of nanoplastics in *Daphnia magna* with limits around 18 ng. The method is destructive to samples, uses small injection volumes, is susceptible to interference, and does not report particle size or morphology; protocol standardization is still limited.

Elemental Analyzer – Isotope Ratio Mass Spectrometry (EA-IRMS) oxidizes organic carbon to CO_2 and measures $^{13}C/^{12}C$ ratios for quantification. The approach can be used for ^{13}C-labeled nanoplastics and is not suitable for unlabelled environmental samples. In controlled biological matrices, uptake of ^{13}C-labeled PS nanoplastics were quantified with a detection limit near 42 ng.

Single particle Inductively Coupled Plasma Mass Spectrometry (spICPMS) introduces particles individually into plasma and detects each particle as a single transient peak, yielding number based concentrations. Metals ionize efficiently, but carbon-based plastics exhibit low ionization efficiency and strong ^{12}C background. Metal tagging by surface coating or core doping enables indirect measurement via metal signals at ng/L levels in labelled environmental and biological samples.

The three MS approaches address complementary objectives: PyGC/MS supports polymer identification and mass quantification, EAIRMS enables isotope-based quantification for labelled samples, and spICPMS provides sensitive single particle counts via metal tags. Further works are needed in resolving the issues with interference and labelling, especially for environmental samples, improving sensitivity of the method and establishing harmonized protocols

Size versus abundance: Where are the major limitations? - Ralf Kägi (EAWAG, Switzerland)

Optical methods are constrained by the diffraction limit and struggle to detect particles below roughly 5–10 µm in infrared systems; instrument speed has increased significantly over two decades, but the fundamental limit remains. To extend analysis into the nanoscale, two routes are pursued: using shorter wavelengths (electrons) and adopting non-optical approaches, with electron microscopy and atomic force microscopy–infrared spectroscopy (AFM-IR) emerging as key options.

Scanning electron microscopy (SEM) offers high spatial resolution and automated sizing for sub-micrometre particles; in practice, it is reliable around 1 µm, and while measurement down to 500nm is possible, the accuracy degrades below ~100 nm due to substrate signal interference. Thin conductive

[20] University of Pau, France

coatings (~1–2 nm) can enhance imaging but will alter surface properties. SEM measures size but does not provide polymer identity; mixtures of PE, PVC, silica, and polytetrafluoroethylene (PTFE) particles could not be distinguished via chemical composition. Elemental ratio differentiation could help at ~1 µm but not for smaller particles.-

Transmission electron microscopy (TEM) complements SEM with structural and chemical contrasts when combined with Xray analysis and electron energy loss spectroscopy (EELS). EELS is effective for light elements such as carbon, enabling distinction between silica and PS. These capabilities require specialized expertise and infrastructure, and throughput declines significantly when chemical information is acquired, typically limiting analyses to hundreds or thousands of particles.

AFMIR circumvents the diffraction limit by mapping surfaces with AFM while probing polymer absorption using an IR laser; when the wavelength matches an absorption band, local thermal expansion is detected by the cantilever to generate chemical maps. PS nanoplastics have been identified down to ~18 nm, with spectra comparable to that of FTIR. Mixed aerosol samples containing soot, PE, and PS particles were distinguished, with PS exhibiting a notable band near 1492 cm^{-1}. Processing speed remains a constraint; newer instrument generations improve automation but do not yet deliver High throughput screening (HTS).

Method selection depends on the analytical objective: size only workflows can cover very large particle counts, whereas adding chemical identification reduces throughput. Approaches suited to homogeneous reference materials are difficult to use for environmental samples such as a mixture of soot and nanoplastics found in the atmosphere. Further work is needed for improving the method sensitivity and applicability (e.g., to environmental samples) and establishing standardized protocols; automation in AFMIR and TEM is advancing, but there is still a long way to go for large-scale and high-speed processing.

Quantifying plastics with Py-GC-MS in complex matrices – analytical challenges and limitations - Cassandra Rauert[21]

Nanoplastics are difficult to quantify in complex matrices due to their small size and low concentrations. PyGC/MS is a mass-based approach that reports total polymer mass without particle size, count, or morphology; it is not limited by particle size and therefore applies to the nanoscale, while requiring careful control of interferences.

Py-GC/MS thermally decomposes polymers into smaller molecules that are separated by GC and detected by MS, enabling polymer identification and mass calculation. Time-series analyses of archived biosolids and sediment cores with Py-GC/MS have shown increasing plastic burdens consistent with rising global production; these trend inferences depend on robust calibration, blanks, and consistent workflows.

The method is indirect, measuring degradation products rather than intact polymers. In complex samples, non-plastic organics can generate overlapping signatures: PE produces patterns of alkanes, alkenes, and alkadienes, but the same breakdown products from pyrolyzed triglycerides and other compounds with long chain alkane components. Monitoring the ratios of a range of breakdown products will flag irregular patterns and identify the presence of potential interference.

Biological matrices (e.g., blood) present significant interference signals; enzyme coupled with hydrogen peroxide digestion decreases interferences but does not eliminate them, constraining accurate quantification of PE and PVC particles. Spike and recovery experiments indicate >50% recoveries for PE, PP, PET, and nylon6 particles, with low recoveries for PS and PMMA particles. Surface modified PS (carboxylated) shows higher recovery (up to ~70%), underscoring the need for environmentally relevant reference materials and matrix matched validations.

[21] University of Queensland, Australia

PyGC/MS is a useful mass-based tool within a multimethod framework; but it should be complemented by size/morphology techniques such as SEM, NTA, and AF4 to reduce uncertainty in nanoplastic detection. Future work is needed for improved pretreatment process to minimize interferences, reference materials that reflect environmental aging and additives, and standardized methods and harmonized data reporting (calibration, blanks, polymer specific LOD/LOQ, replicate analyses, and reporting of uncertainty) to ensure reliable results.

An often-overlooked part of the analytical chain: nano- and microplastics sampling and extraction - Denise Mitrano[22]

As with all contaminant quantification, in nanoplastic analysis, reliable results begin with representative sampling and extraction. To separate upstream sampling issues from downstream analytics, model plastics were synthesized by incorporating trace amounts of environmentally rare metals (~0.2%) into polymers. Metal proxy was measured using ICPMS. This approach enabled rapid, precise testing under controlled conditions and accelerated protocol development by decoupling sampling/extraction performance from instrument specific constraints.

Soil spiking experiments showed that sampling strategy strongly affects representativeness. Grab sampling was least representative, while the riffle splitter performed best. During homogenization, a household mixer (KitchenAid) outperformed an industrial tumbler, yet complete uniformity was not achieved. Monte Carlo simulations indicated that approximately 20% of the total sample mass was needed to reach acceptable representativeness. Fiber shaped plastics yielded lower recoveries than fragments, likely due to entanglement with soil aggregates through wetting and drying cycles, indicating morphology should be considered in sampling design and extraction. Consequently, consideration needs to be given to homogenization and sample size to ensure a representative sample. Test materials, such as metal doped plastics, would allow for faster assessment of the approach.

Extraction of plastics from soil demonstrated high recoveries for microplastics (~80%) but very low recoveries for nanoplastics (~1.4%). Challenges arose across the entire analytical chain—sampling, pretreatment, and extraction—with nonuniform sampling, strong binding of soil particles to nanoplastics, and the extremely small size limiting separation and filtration effectiveness. Isolating nanoplastics from soil is therefore a multi-layered problem requiring tailored workflows.

Sampling and extraction remain amongst the most understudied and uncertain steps in the nano- and microplastics analytical chain, and downstream characterization lacks value without representative inputs. A typical microplastic extraction protocol exceeded two weeks, limiting routine use.

[22] Nestle, Switzerland

6 Session 5: Environmental Relevance & Fate: Weathering and Physical/Chemical Transformation Techniques

Session 5 was composed of five presentations that discussed fate of MNPs in marine environment, aging process and risk assessment with aged samples considering real-life exposure scenarios, investigating abrasion process, release quantification and material characteristics at nanoscale and developing aging scoring framework to produce environmentally relevant reference materials for nanoplastics. The study outcomes demonstrated their relevance to support development risk assessment framework, for example, by providing insights to variables involved in the fate of nanoplastics (e.g., long-term aging), the impact of additives and aging on the hazard assessment as well as on particle release rate from mechanical abrasion.

Environment Fate as the Compass: Micro- and Nanoplastics in Marine Ecotoxicology - Sander Scheffers[23]

Offshore pipeline coatings made of PE, PP, and epoxy deteriorate as steel corrodes and sacrificial anodes deplete, leading to coating failure and fragmentation into particles and fibres. Degradation spans centuries (roughly 300–1,000 years), generating microplastics and non-polymeric fibres that can be positively or negatively buoyant. The initially positively buoyant particles (e.g., PP particles) sink as biofilm increases density. Concurrent oxidation and surface roughening enhance adsorption of metals and organic pollutants, shifting initially inert particle surfaces to chemically active ones.

Real-world exposure involves mixtures of polymers with additives and fillers undergoing simultaneous oxidation, biofilm colonization, and mechanical abrasion, continuously altering particle properties. Combined risks include pollutant adsorption, biological interactions, and mechanical effects such as gastrointestinal blockage, while typical laboratory tests use clean, spherical particles unlike those found in the environment. A modelling work with pipelines suggests background concentrations near 0.02 particles/mL and per-pipeline contributions around 10^{-8} particles/mL; these are below toxicity thresholds for a single pipeline but cumulative impacts across thousands of kilometres require mixture- and region-based assessments.

Testing should reflect mixtures and cumulative exposure according to a harmonized framework. Accelerated aging protocols that reproduce ultraviolet (UV) exposure, abrasion, and biofilm colonization are needed, with FTIR and Raman spectroscopy paired with hydrodynamic modelling to identify polymers,

[23] Hydrobiology, Australia

characterize oxidation states, and size distributions. Site-specific guideline values can be derived with Species Sensitivity Distributions (SSD) and regional standards (e.g., Australia and New Zealand), with cumulative risk assessment providing scientifically defensible regulatory guidance.

Introduction to Environment Micro and Nanoplastics Ageing Relevant for Human Exposure Scenarios - Martin Leonard[24]

Particle aging is a critical variable in hazard assessment of nanoplastics. Most real-world exposures involve aged particles that are smaller with altered surface chemistry, eliciting different biological responses. Phagocytic activity depends on size, shape, and rigidity, and studies report aged particles can reduce mucosal barrier resistance due to increased surface oxidation and changes in chemical characteristics.

Photo-oxidation is one of the most studied aging drivers; UVA exposure increases carbonyl index, and experiments using UVB and UVC have been performed, though UVC does not reach the Earth's surface, raising methodological questions about environmental relevance. Aging is typically characterized with SEM for surface morphology and FTIR spectroscopy for oxidation state, while temperature—both long-term environmental variation and short-term high-temperature events linked to human activities—significantly modulates aging metrics.

Studies report applying machine learning to SEM images and FTIR data to classify aging types, with dimensionality reduction revealing distinct clusters for UV exposure, heat, and mechanical abrasion. Natural aging datasets partially overlap with experimental aging, indicating certain laboratory conditions replicate aspects of environmental aging but not its full complexity due to multiple interacting factors. This approach helps identify key aging factors relevant to human exposure scenarios and informs the design of aging protocols for developing reference materials.

Further research should address the bioavailability and tissue penetration of volatile organic compounds (VOCs) and dissolved organic matter (DOM) retained in plastics, and improve characterization of exposure levels, particle forms, and polymer types across scenarios. Producing nanoplastic reference materials for clinical analysis and creating test materials that reflect polymer diversity and degrees of aging are essential. The aging process should be reported with clear criteria, including the intended study, clear guidelines on protocols, and the targeted property change.

Synthesis of airborne nanoplastics RM (or TM) and development of exposure platform - Ki-Joon Jeon[25]

Airborne nanoplastics are likely undercounted due to spectroscopic detection limits, while reports of particles in human tissues (kidneys, lungs, intestines, brain, eyes, sperm) suggest possible transgenerational toxicity without defined thresholds. Real-world exposure involves sub2.5 μm particles capable of deep lung penetration originating from tire wear, plastic waste incineration (small particles up to 100fold increase), plumbing repairs, emissions from 3D printers (e.g., VOCs and particulates), coastal plastic weathering, and long-range transport, indicating diverse sources and elevated exposure potential.

The study synthesized test/reference materials via thermal evaporation–condensation of acrylonitrile butadiene styrene (ABS), polylactic acid (PLA), and nylon at 225 °C, yielding ~220 nm particles with an effective density of 0.4 g/cm³ that can remain airborne. Raman mapping confirmed ABS spectral signatures, and particles remained stable for four weeks in liquid matrices. Biological evaluations employed

[24] UK Health Security Agency, United Kingdom

[25] Inha University, Korea

an air liquid interface (ALI) exposure platform for human relevant lung delivery and nasal inhalation studies in mice, supporting mechanistic insight into formation and persistence.

Acute toxicity was observed on A549 human lung cells exposed for four hours that includes cell death, tight junction disruption, mitochondrial damage, and elevated interleukin-6 (IL-6) and interleukin-8 (IL-8). Bronchoalveolar lavage fluid indicated increased inflammatory cytokines, with liver damage and elevated blood glucose. Comparative analysis found particulate matter ≤2.5 µm (PM2.5) required ~17× higher concentrations to induce ~30% cell death, whereas nanoplastics produced similar effects at only 5 µg/m³, with signals consistent with apoptosis and TNFRP36 expression.

Thermal synthesis achieved controlled size, distribution, and concentration. Simulating photooxidative aging on nanoplastics over 6–30 days to assess their potential toxicological hazards is ongoing. The findings point to significant biological effects after minimal exposure and underscore the need to investigate chronic risks and to incorporate aging into the production of test/reference materials to produce meaningful data for regulatory risk assessment of nanoplastics.

Novel insights into nanoplastic release via mechanical wear - Boya Xiong[26]

Plastics generate and release nanoplastics through everyday mechanical abrasion; this process is pervasive in real-world settings yet remains under-studied. Quantitative methods were developed to link applied sliding forces to wear outcomes and to the properties of the released particles. Tests were done in the typical scenarios — laundry, sand friction, and sediment movement —to investigate how plastics generate nanoplastics through abrasion and how release mechanisms depend on wear mode, applied force, and material state.

Two complementary, multiscale approaches were established to quantify mechanical wear under controlled and environmentally relevant conditions. AFM-based nano scratch test applied normal loads 2–6 µN to plastic surfaces to examine how repeated abrasion promotes pile-up formation and subsequent detachment into nanoplastics. Sand abrasion experiments reproduce realistic sediment-plastic interactions; input energy was quantified by measuring sediment velocity and sliding friction coefficients. Cross-validation of force ranges across the two platforms provided a coherent framework for analyzing nanoplastic release from environmentally relevant mechanical abrasion.

Repeated abrasion of LDPE predominantly produced nanoplastics via a plowing wear mechanism: material first piles up along the edges of the wear track and later detaches as particles during sliding. Pile-up heights were approximately 200–500 nm, comparable to AFM tip size. NTA of sand-abrasion samples confirmed particle size in the hundreds of nanometers, and PE release was quantified at approximately 100–420 mg/m². UV aging accelerated surface oxidation and chain scission, shifted wear from plowing to cutting, and increased release rates by up to tenfold. In addition, 5–20% of nanoplastics were released via transfer and adhesion to the counter face, observed across both platforms, potentially serving as new sources of unbound nanoplastics.

Mechanically generated nanoplastics were about threefold softer than the bulk polymer and exhibited approximately threefold greater viscoelastic dissipation. These features are consistent with increased free volume and chain scission during abrasion, indicating that real-world nanoplastics are not merely size-reduced fragments of the bulk but possess distinct properties beyond differences in surface chemistry. Consequently, risk assessment and exposure modeling should consider these property changes in addition to particle size.

[26] University of Minnesota, USA

UV aging doubled crystallinity and produced highly oxidized particles with an approximately tenfold reduction in molecular weight. Sand abrasion transferred mineral grains onto plastic surfaces, forming heterogeneous layers within as little as 15 days, from which hybrid sand-polymer nanoplastics were detached and released. Such particles are likely to display altered degradation pathways and pollutant interactions. Moving forward, harmonized test protocols that quantify input power and define applied forces, and samples aged under environmentally relevant UV and microbial conditions, are needed to build reliable studies and datasets.

Linking Simulated Weathering to Laser-Ablated Nanoscale Plastic Reference Materials - Shan Zou[27]

A data-driven multivariate aging framework was developed that integrates structural, thermal, chemical, and spectroscopic descriptors to capture changes on PE, PP, and PET microplastics under controlled UVC irradiation. Principal Component Analysis (PCA) was used to identify the dominant modes of variation in aging features, and SHapley Additive exPlanations (SHAP) method from machine learning was applied to quantify each property's contribution to the aging score. This approach moves beyond traditional single-parameter indicators and links early lamellar destabilization, surface oxidation, and loss of crystallinity to the fragmentation potential of semi-crystalline and amorphous polymers. It provides a reproducible, transferable way to harmonize aging assessments across polymer types and laboratories. Importantly, the study demonstrates that polymer additives—such as pigment and stabilizer content—modulates the depth of surface oxidation and crack propagation, influencing aging dynamics. For example, transparent PP particles exhibited more extensive surface cracking than pigmented microplastics. Capturing such effects from additives is critical for assessing the environmental fate of microplastics and designing representative reference materials.

Building on these mechanistic insights, the study established an environmentally relevant aged nanoscale PP reference material (NPPP-1) to improve measurement reliability. Femtosecond laser ablation produced a discoidal morphology with nanometer-scale thickness (< 5 nm), heterogeneous lateral dimensions spanning tens to approximately 200 nm, and oxidative carbonyl functionalities consistent with environmental weathering. Biological assays in 3D intestinal models showed that NPPP-1 induces ROS accumulation, ferroptotic stress, oxidative injury, and secondary apoptotic and pyroptotic responses across intestinal models. Notably, activation of the Wnt/β-catenin axis has been identified, revealing a compensatory regenerative epithelial program that cells deploy under oxidative burden.

Developing reference materials with other polymers (i.e., NPPE-1 and NPPET-1) supported validation of detection methods. The particles produced resembled those found in the environment, exhibiting heterogeneous sizes and shapes, as well as modified chemical fingerprints associated with environmental weathering, while remaining stable in different media, allowing their use in biological and ecotoxicology testing.

The data-driven aging framework and the laser-fabricated nanoplastic reference materials could support development of a scalable, reproducible route to produce aged nanoplastic reference materials for risk assessment based on real exposure scenarios, although whether particles aged in the laboratory truly mimic those found in the environment should be investigated. Future work includes building a capacity to produce reference materials across all relevant polymer types, standardising characterization protocols, and investigating impact of long-term aging.

[27] National Research Council Canada, Canada

7 Session 6: Challenges in steps towards risk assessment framework for safety testing of nanoplastics

Session 6 was dedicated to a panel discussion on challenges in steps towards a risk assessment framework for the safety testing of nanoplastics. Two panellists were invited to share their experience in leading development of risk assessment framework for MNPs and their insights on how the work on building risk assessment framework for nanomaterials could contribute to the ongoing discussion on MNPs. The panel members were also asked to address following key questions:

- Is there a need to define nanoplastics in the context of risk assessment for secondary particles with large heterogeneity in their size, shape, polymer types, and other parameters?
- Are current risk assessment frameworks based on hazard and exposure assessment applicable to secondary nanoplastics management? If not, what are the adaptations needed?
- Given the uncertainty of hazard data, limitation of analytical and detection methods which also complicates the exposure assessment, how can risk assessment of nanoplastics produce meaningful recommendation for policy makers?
- Given the OECD's strengths in developing international standards, harmonised test methods, and guidance on assessment strategies, where do you see the opportunity for OECD to add values in risk assessment for nanoplastics?

The POLYRISK Risk Assessment Framework for Micro- and Nanoplastic Particles (MNPs) and its Application in Selected Case Studies - Andrea Haase[28]

The POLYRISK project addressed core obstacles in MNPs risk assessment—uncertain particle identity, threshold questions, analytical limits, and sparse data—by developing a dedicated framework and verifying it with real world case studies rather than remaining purely theoretical.

Building on nanomaterial assessment experience, the framework is modular and scenario flexible (single particles, mixtures, pristine, aged) with route specific decision nodes for oral and inhalation exposures. It draws on concepts such as OECD "polymer of low concern (PLC)" and the fibre toxicity paradigm, adapts EFSA nanomaterial guidance for MNP needs, combines several existing Integrated Approaches to Testing

[28] BfR: The German Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung)

and Assessments (IATAs) and introduces a new IATA for assessing immunotoxicity, enabling categorization of MNP across exposure routes.

Verification used two types of case studies (lab-based in vitro, real-world human). Different in vitro systems (primary cells and established cell lines) were applied, focusing on important particle-related key events such as inflammation and oxidative stress. To the extent possible, existing OECD TGs were used and if necessary, adapted to MNP characteristics. The IATA to assess immune responses included different test particles covering different polymers, sizes and partially morphologies, including weathered materials to demonstrate that the framework operates effectively with real data. In addition, POLYRISK addressed several real-world scenarios (MNPs in bottled drinking water, MNPs in urban and rural outdoor air, rubber-granulate MNPs for indoor soccer players, and MNPs in textile fibre workplace).

The framework groups particles by shared properties to streamline data use and integrated evaluations, contributing to efficient prioritization of MNPs that need further assessments. Further work is needed on developing validated test methods and readiness criteria that reflect particle specific traits, certified (reference) materials (including aged forms), and interdisciplinary collaboration. Continuing alignment with OECD initiatives (e.g., AOPs, IATAs) and regular module updates will keep the framework adaptable.

Following the talk, the Q&A session addressed the following issues:

- Hazard characterization should cover the full concentration range—from very low to accident level high—to establish dose response; for bottled water, measured concentrations were too low to justify a study in humans. Risk assessment typically relies primarily on in vitro and in vivo toxicity data, with human studies used as complementary information.
- International and European guidance documents (e.g., OECD, ISO, EFSA) were referenced in a general methodological sense. Case studies were selected to reflect real-life scenarios, which included measuring particle levels in bottled water and assessment of collected particles in textile fibre workplaces under test conditions. The results from these studies did not indicate the presence of critical polymer fibers but many particles that originated from outdoors (e.g., tire abrasion). Future work will refine methods and the framework.

Friends or Foes? Manufactured Nanomaterials for and against Nanoplastic - Steffi Friedrichs[29]

Nanoplastics and engineered nanomaterials share size ranges and some exposure characteristics, but they differ fundamentally in origin and composition. Nanoplastics are mostly unintended by-products of production and end-of-life processes, whereas nanomaterials are deliberately engineered. Nanoplastics are polymer-based and may carry additives or adsorbed species, introducing transport and interaction variables. Early nanomaterial studies showed that certain particles could migrate along the olfactory nerve into the brain—an observation that suggested pharmaceutical potential but also risk—yet not all particles behave this way, underscoring the need for precise characterization. The work should prioritize scientific consistency over premature definitions or regulations.

To resolve inconsistent toxicity and exposure results in the literature, academia, regulators, and industry collaborated under a shared responsibility, standardization first approach. Laboratories were required to demonstrate reproducibility on standardized reference materials before testing their own samples. Industrially relevant materials were sourced in large quantities (about 20–30 kg) from single batches, processed under GLP conditions, and distributed globally in sealed vials. Strict control of solvents, concentrations, and matrices addressed reproducibility issues seen in early toxicity studies of carbon

[29] AcumenIST, Belgium

nanotube, where results ranged from null effects to effects at unrealistic concentrations and were sometimes skewed by solvent artifacts (e.g., toluene).

For the Sponsorship programme of OECD Working Party on Manufactured Nanomaterials (WPMN), methodologies and endpoints were agreed to cover physicochemical properties, environmental fate, and toxicity. OECD TGs were reviewed—most were deemed applicable with minor adaptations, while some (e.g., boiling point, viscosity) were irrelevant for nanoscale solids. Exposure scenario design leveraged lessons from nanomaterials to nanoplastics but confirmed that not all nanomaterials or nanoplastics translate into meaningful exposure. Attempts to identify a single type of nanomaterial or nanoplastics were not successful. Moreover, because many nanoplastics arise from microplastic degradation, effects are often inseparable across size fractions; programme design therefore adopted an integrated approach that considers both microplastic and nanoplastic fractions.

Based on the WPMN's sponsorship program, global efforts avoided rushing into nanospecific regulations, focusing instead on harmonized scientific evidence. International standardization activities (e.g., ISO TC229) were referenced at a general level to support agreed characterization sets. The EU funded MACRAMÉ initiative delivered three systems: a control material library, standardized sampling protocols, and a data sharing platform. These measures reduced interlaboratory variability, enforced strict control of fractionation, matrices, and solvents, and required reproducibility on reference materials— improving comparability and transparency in global testing.

By prioritizing standardized materials and methods, validating reproducibility, and integrating micro–nano continuity into program design, this work provided a practical pathway to reconcile conflicting data from the literature. Future priorities include expanding the spectrum of environmentally relevant reference materials, automating sample preparation and fractionation to minimize matrix interference, defining realistic exposure scenarios emphasizing long-term, low dose evaluations, and strengthening interoperability of data platforms—enabling reliable, science-based decisions while recognizing that nanoplastics and nanomaterials are "same but different."

Following the talk, the Q&A session provided additional clarifications as follows:

- Focus on risk related properties, not the origin: Because most nanoplastics result from degradation of larger plastics, separating primary versus secondary classes has limited practical value for risk assessment. Properties that influence risk—such as density, translocation potential, and surface chemistry—should be prioritized, and testing programs should include both micro and nanofractions to capture degradation pathways and combined effects.
- Definitions can wait: Definitions may support future risk management and exposure limits but prioritizing them now could delay essential empirical work. International standard definitions (e.g., ISO) serve technical and terminological purposes; enforceable regulations should follow clear evidence of harm. The first step is identifying which particles pose risks before setting thresholds.

8 Workshop Conclusion

The workshop provided an opportunity for regulators, researchers, and industry representatives to discuss the current landscape of available technologies for producing, analysing and characterising nanoplastic reference and test materials and the associated strengths and limitations.

Session 1 keynote presentation from the European Commission Joint Research Centre showed the rigorous process that goes into developing and producing reference materials. The presentation also highlighted the common dilemma between standardised reference materials vs. standardised analytical methods, especially for a field with large uncertainty such as nanoplastics, where one is needed to develop the other. It was emphasised that developing reference materials is a collaborative effort, and the publicly funded national and regional bodies for reference material production collaborate together30.The keynote presentation from BASF shared practical insights learned from applying OECD Test Guidelines for several human health endpoints and recommended that for hazard assessment, the priority is to develop 'representative' test materials tailored for the study need.

Session 2 highlighted that the field has many active international collaborations. There are several research projects and consortia (e.g., EU CUSP, POLYRISKs), supported by international collaboration and standardisation bodies such as VAMAS, where many OECD countries are members, including Australia, Canada, France, Germany, Italy, Japan, Korea, Mexico, the United Kingdom, the United States and the European Commission. The panels provided an overview on completed, ongoing and planned activities, spanning from standardisation of reference materials and test methods, performing hazard assessment on representative test materials and steps towards developing risk assessment framework for nanoplastics.

Session 3 showcased ongoing research work on producing reference and test materials for nanoplastics either via a top-down or a bottom-up approach. All presentations agreed on the associated strengths and limitations for different methods. For example, top-down approaches allow for generation of materials that mimic 'real-world' samples (e.g., secondary MNPs formed via environmental degradation), but suffer from low yield; bottom-up approaches allow for precise control of size, shape, and morphology, but may not be relevant for risk assessment of secondary MNPs. The field is evolving with improvement and refinement of methods, but the work is still at a research level. Reference materials are mostly generated via 'in-house' processes without standardised protocols and well-defined characterisation criteria.

Session 4 emphasized the fundamental challenge in the context of risk assessment of nanoplastics – extracting, detecting, and measuring nanoplastics from complex matrices. Applying current technologies for reliably detecting and quantifying nanoplastics from environmental and biological matrices is essentially impossible, and there was a general agreement that a combination of different methods should be applied to compensate the limitation from individual methods. However, it was also questioned whether the resource and expertise needs for a multimodal approach would be feasible outside of a research laboratory. Concerns on practical issues including long scanning time and low throughput were also raised.

Session 5 provided insights gained from studies on impact of aging and additives on nanoplastic toxicology and physical characterisation. There was a consensus that impacts of aging on material property and toxicity potential could be significant and developing a standardized protocol and harmonized criteria for aging process could lead to the validation of the aging process methodology and increase the reproducibility of the studies.

Session 6 provided an overview of steps towards a risk assessment framework for nanoplastics developed by POLYRISK[30] and discussion on how experience from developing risk assessment framework for nanomaterials could translate to the ongoing discussion on nanoplastics. It was emphasized that defining and applying realistic exposure scenarios with long-term and low-dose evaluations is more important than defining what nanoplastics are, as existing international standard definitions (e.g., ISO) serve the technical purposes. Applying existing risk assessment framework such as the one from POLYRISK to realistic exposure scenarios, as has been demonstrated via POLYRISK case studies on bottled drinking water and on fiber production work condition, will support evidence-based decision. Ultimately, the priority is to develop standardized and harmonized criteria for essential steps in the risk assessment framework and for the test methods and reference/test materials to be validated for an increased reproducibility of study results; this echoes key messages from all presentations given at this workshop.

[30] https://polyrisk.science/ (Consortium with membership representing seven countries, including Netherlands, Germany, Norway, Italy, Belgium, Romania, and Bulgaria)

Annex A. Participant list

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Annex B. Agenda of the workshop

Time	Topic	Presenter/ Chair
12th November 2025 **Auditorium, 13:00-18:00**		
13:00	Welcome	Bob Diderich (OECD Secretariat)
13:15	Opening Remarks	Jung-Kwan Seo (NIER, Korea)
13:30-15:30	**Session 1 -** Keynote Speeches	Chair: Bob Diderich (OECD Secretariat)
13:30-14:15 (45 min)	Reference Material Production at JRC and RMs for MP/NP with quantitative and qualitative properties	Håkan Emteborg, European (Commission, Joint Research Centre)
14:15-14:50 (35 min)	OECD Test Guidelines for human health toxicity: Applicability to currently available micro- and nanoplastics	Wendel Wohlleben (BASF, BIAC)
14:50-15:20 (30 min)	Key learnings on environmental sub-micro- and nanoplastic's analysis and ecotoxicity tests from LABPLAS project	Begoña Espiña (International Iberian Nanotechnology Laboratory, Portugal)
15:25-15:30 (10 min)	Q&A	Chair: Bob Diderich (OECD Secretariat)
15:30-16:00	*Coffee Break*	
16:00-	**Session 2** - Panel Discussion on Ongoing Work towards Standardization and International Coordination for safety testing of nanoplastics	Chair: Anne Gourmelon (OECD Secretariat)
16:00-16:15	Presentation by the secretariat on scope of different 'standard-setting' organizations	Anne Gourmelon (OECD Secretariat)
16:15-16:35	Harmonization Initiatives for Micro- and Nanoplastics Analysis: Insights from PlasticTrace, ISO, and VAMAS	Andrea Mario Giovannozzi (IRIM, Italy)
16:35-16:55	Advancing Standardisation in Micro- and Nanoplastics Analysis via Interlaboratory Comparisons	Enrica Alasonati (LNE, France)
16:55-17:15	CUSP research roadmap 2026 - 2032	Alberto Katsumiti (Gaiker, Spain)

	State of the art, gaps, and future needs in micro- and nanoplastic and health research	
17:15-17:35	Contextualization of standardization needs and applications	Denise Mitrano (Nestle, Switzerland)
17:35-18:00	Wrap up & End of the Day 1	Chair: Anne Gourmelon (OECD Secretariat)
13th November 2024 **Conference Centre Room 7**		
Session 3 A– Production and characterization of Reference Materials and Test Materials		
09:32-09:47	Micro- and nano-plastic test/reference material production via hybrid top-down degradation methods	Jaewoong Lee (NIER, Korea)
09:49-10:04	Top-down cryo-ground nanoplastics: the pros and the cons of their use as reference materials for (eco)toxicological studies vs. bottom-up synthesized polymer nanoplarticles	Olivier Sandre (University of Bordeaux, France)
10:06-10:21	Laboratory-scale generation of micro/nanoplastics for toxicological testing	Sabina Halappanavar (University of Ottawa, Canada)
10:23-10:38	Production of reference materials by Quality-of-Design	Korinna Altmann (BAM, Germany)
10:38-11:00	Q&A Discussion	
11:00-11:10	*Coffee Break*	
Session 3 B - Production and characterization of Reference Materials and Test Materials (Bottom-Up Production)		
11:22-11:37	Bottom-up nanoplastics: tunable parameters, particle diversity, and suitability for method development and risk assessment	Stephanie Reynaud (University of Pau, France)
11:39-11:54	A bottom-up particle fabrication approach for the development of a library of micro- and nanoplastics	Kosuke Tanaka (NIES, Japan)
11:56-12:11	Reprecipitation method for synthesis of polyethylene and polypropylene nanoplastics and small microplastics	Jinyoung Jeong (KRIBB, Korea)
12:11-12:30	Q&A Discussion	
12:30-14:00	*Lunch Break*	
Session 4 - Analytical Methodologies and Measurement Challenges		
14:02-14:17	Challenges in spectroscopy-based nanoplastic detection and the role of OPTIR in advancing analysis	Shima Ziajahromi (Griffith University, Australia) (Online)
14:19-14:34	Physicochemical Characterization and Quantification of Nanoplastics: Challenges, Advanced Methods and Perspectives	Natalia P. Ivleva (Technical University of Munich, Germany)

14:36-14:51	Analytical Insight into Nanoplastic Analysis: Strengths and Challenges of Mass Spectrometry Approaches	Javier Jiménez (University of Pau, France)
14:53-15:08	Size versus abundance: Where are the major limitations?	Ralf Kägi (EAWAG, Switzerland)
15:10-15:25	Quantifying plastics with Py-GC-MS in complex matrices – analytical challenges and limitations	Cassandra Rauert (University of Queensland, Australia) (Online)
15:27-15:42	An often-overlooked part of the analytical chain: nano- and microplastics sampling and extraction	Denise Mitrano (Nestle, Switzerland)
15:42-16:05	Q&A Discussion	
16:05-16:20	*Coffee Break*	
Session 5 - Environmental Relevance & Fate: Weathering and Physical/Chemical Transformation Techniques		
16:22-16:37	Environment Fate as the Compass: Micro- and Nanoplastics in Marine Ecotoxicology	Sander Scheffers (Hydrobiology, Australia)
16:39-16:54	Introduction to Environment Micro and Nanoplastics Ageing Relevant for Human Exposure Scenarios	Martin Leonard (UK Health Security Agency) (Online)
16:56-17:11	Synthesis of airborne nanoplastics RM (or TM) and development of exposure platform	Ki-Joon Jeon (Inha University, Korea) (Online)
17:13-17:28	Novel insights into nanoplastic release via mechanical wear	Boya Xiong (University of Minnesota, USA) (Online)
17:30-17:45	Linking Simulated Weathering to Laser-Ablated Nanoscale Plastic Reference Materials	Shan Zou (National Research Council) Canada (Online)
18:05-18:20	Q&A Discussion	
14th November 2024 **Auditorium**		
Session 6 - Challenges in steps towards risk assessment framework for safety testing of nanoplastics		
09:30-09:50	The POLYRISK Risk Assessment Framework for Micro- and Nanoplastic Particles (MNPs) and its Application in Selected Case Studies	Andrea Haase (BfR, Germany)
09:50-10:10	Risk assessment and regulation of nanoplastics	Kristian Syberg (Roskilde University, Denmark) (Online)
10:10-10:30	Friends or Foes? Manufactured Nanomaterials for and against Nanoplastic	Steffi Friedrichs (AcumenIST, Belgium)
10:30-11:20	Panel Discussion	
11:20-12:00	Closing Remarks	