MACRAMÉ – Advanced Characterisation Methodologies to assess and predict the Health and Environmental Risks of Advanced Materials

List of participants

Participant		Country
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3	LNE - Laboratoire National de Métrologie et d'Essais	France
4	LIST – Luxembourg Institute of Technology	Luxembourg
5	7P9-SI – Seven Past Nine d.o.o.	Slovenia
5.1	7P9-DE – Seven Past Nine GmbH (Entity Affiliated with 7P9-SI)	Germany
6	BAuA – Bundesanstalt für Arbeitsschutz und Arbeitsmedizin	Germany
7	RIVM – Rijksinstituut voor Volksgezondheid en Milieu	Netherlands
8	IBE – IBE R&D Institute for Lung Health gGmbH	Germany
9	TASCON – Tascon Gesellschaft für Oberflächen- und Materialcharakterisierung mbH	Germany
10	GAIKER – Fundacion GAIKER	Spain
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12	BASF – BASF SE	Germany
13	MEDICA – Medica S.P.A.	Italy
14	CARBON WATERS – Carbon Waters SAS	France
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Table of Contents

1. MACRAMÉ Excellence

The MACRAMÉ Project is fully aligned with the EU ambitions to secure the safety and sustainability of new chemicals, materials, products and processes in order to strive for zero pollution and toxic-free environments, as addressed in the EU's Chemical Strategy for Sustainability (2020), and in the European Green Deal (2019 & 2021); in doing so, the Project concentrates on methodologies that are applicable to nanomaterials, and widens them to '**Advanced Materials**' (AdMas) – a material category that includes but surpasses that of 'nanomaterials' (EU, 'Definition of a Nanomaterial') - in commercialised products and that are aligned with the future-oriented innovation, safety and sustainability considerations of the OECD (OECD (2020)), the EU (EU (2022)), and several of its Member States (e.g. Germany (2021)). This will be achieved through development and demonstration of novel methodologies, and by advancing their harmonisation & standardisation on **three MACRAMÉ Material Families** of **inhalable carbon-based AdMas** of various morphologies and dimensions (Tiwari *et al.* (2012)), beyond spherical particles: **(a) graphene-related material** (GRM), **(b) carbon nanofibres** (CNFs), e.g., carbon nanotubes (CNTs), and **(c) Poly Lactic-co-Glycolic Acid (nano)particles** (PLGA). The focus on carbon-based AdMas addresses unsolved detection and characterisation issues, especially in complex media. In doing so, MACRAMÉ builds on >15 years of research and innovation (R&I) and knowledge pooling in nanosafety, formed through numerous European and international collaborations. MACRAMÉ will add value to the results of collaborations, such as the Malta-Initiative, and the Graphene Flagship Validation Service and Standardisation Committee, to proactively support EU industries in becoming world-leaders in clean technologies and products and achieving the Green Deal's ambitious timeline.

The **MACRAMÉ R&I Approach** (Figure 1) aims to widen the development of harmonised test guidelines (TGs) and guidance documents (GDs) (OECD) and standards (CEN, ISO) to **market-relevant AdMas in their complex product matrices**. This will be achieved by defining the R&I Strategy through life-cycle assessment for **five market-relevant industrial MACRAMÉ Use-Cases.** These **define the selection** of the **MACRAMÉ R&I Activities** and development of **MACRAMÉ Methods**, and the benchmarks chosen for monitoring the progress R&I. MACRAMÉ R&I Activities include a range of novel sample preparation techniques and ambitious quantitative detection and imaging methodologies that support reliable and reproducible determination of AdMas in different complex matrices (**AdMa@CMs**) and using **inhalation as their main exposure route**. By applying, combining and evaluating both established and novel inhalation toxicity tests a tiered approach to toxicity testing will be developed that will provide data on state-of-the-art characterised control materials for the **MACRAMÉ Control Material Library**. The library will serve future AdMa toxicological research. The ultimate **MACRAMÉ Outcomes** are proposals for harmonisation and (pre-)standardisation projects to be provided to and further elaborated with the relevant bodies, (i.e. OECD, VAMAS/CEN/ISO). The proposals will be founded on **robust summary datasets**, **scientific documents** and **recommendations for hazard- and risk-assessment methodologies for AdMas in complex product matrices** (AdMa@CMs). All data and information, obtained from external sources and generated during the Project, will be handled and stored in the **MACRAMÉ Information Hub** - the Project's central information processor, whose interoperability is based on a **Data Stewardship** concept, designed according to IndustryCommons principles.

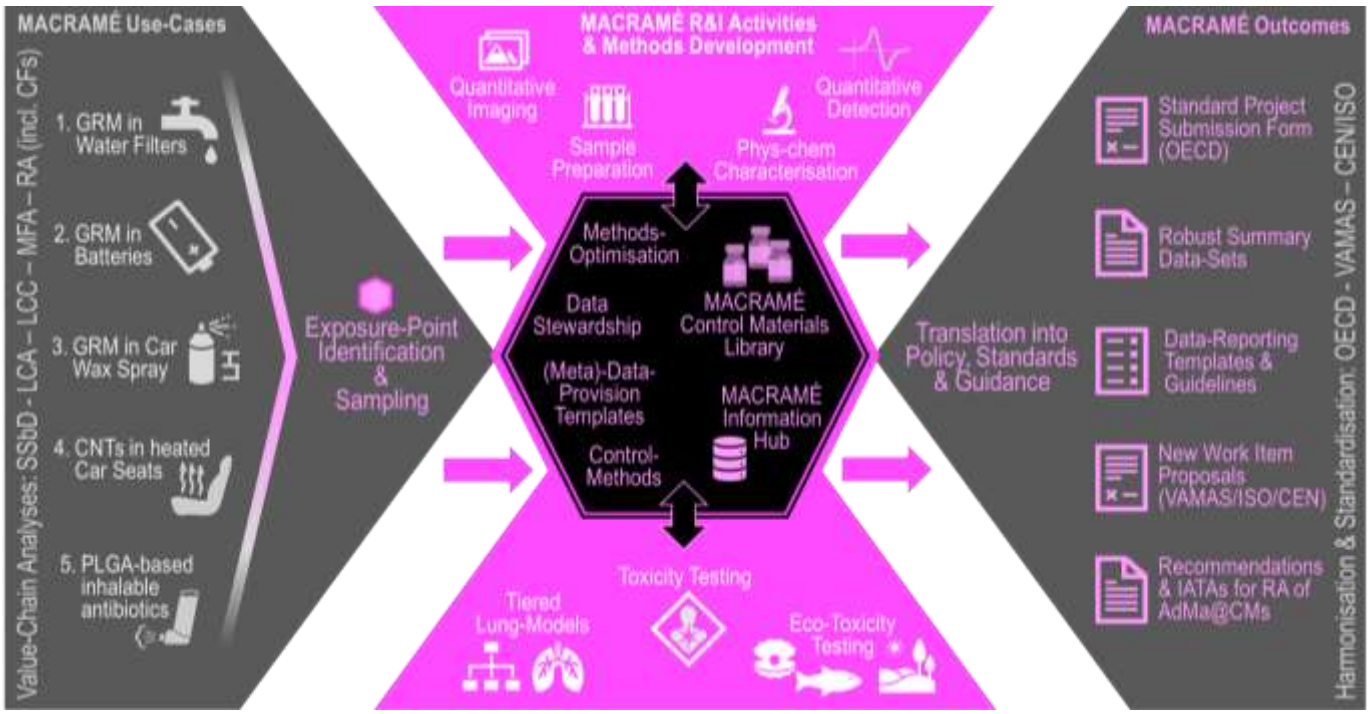

Figure 1: Illustration of the MACRAMÉ R&I Approach (AdMa@CMs: Advanced Materials in complex matrices; CF: Characterisation Factor; GRM: graphene-related material; IATA: integrated approaches to testing and assessment; LCA: Life-Cycle Assessment; LCC: Life-Cycle-Costing; MFA: Material-Flow Analysis; RA: Risk-Assessment; SSbD: Safe-&-Sustainable-by-Design).

The resulting efficiency and effectiveness of MACRAMÉ Methods will be demonstrated through their application in Use-Case evaluations, using LCA-, LCC- and 'Safe & Sustainable by Design' (SSbD)-based (EWARN (2022)), highlighting benefits like reduced costs of regulatory compliance, by following a **MACRAMÉ Safety & Sustainability Matrix**. This matrix will be a modular building block for MACRAMÉ's information-transferring interfaces for different scientific and regulatory communities, and thus provide a stepping-stone for Europe's route towards a ***'one substance – one assessment'*** approach (European Green Deal (2019)) and promote an open strategic autonomy (ETUI (2021)) through key enabling and emerging technologies, including digital ones.

1.1. MACRAMÉ Objectives & Ambitions

The dynamic nature and rapid uptake of advanced materials in industrial, consumer and medical products requires the development of reliable and practical tools to ensure the safe and sustainable use of the products. Accordingly, **MACRAMÉ's Central Objective** is to:

- **detect, characterise and quantify AdMas during handling and processing** along the **product life-cycle**,
- **assess** potential **impacts on (human) health and the environment in** intended or unintended **exposure situations** (i.e. 'Exposure Points') in the product value-chain,
- **advance** the wide-spread applicability of the developed **test and characterisation methods**, by **demonstrating their effectiveness and efficiency in the context of existing, market-relevant industrial AdMas containing products**, and
- **prepare and initiate standardisation, harmonisation and technological & regulatory validation** of test- and characterisation-methods.

This will be achieved in a stepwise approach, by pursuing the following **MACRAMÉ High-Level Objectives** (HLOs); their accomplishments represent **Project Milestones (**M1.1 to M5.2**)** on the path towards the Expected Impacts (see Section 2.1). Each HLO is characterised by Key Performance **Indicator(s)** (KPIs), enhancing the HLO's clarity and pertinence, and enabling the monitoring of the Project's progress towards its Central Objectives.

HLO1: Interfacing Communities and Streamlining Methodological Approaches to assess the Risk of AdMa@CMs

MACRAMÉ's first aim is to provide a state-of-the-art scientific, technical, regulatory, and political background by establishing interfaces among regulatory, industry, and technology communities. This requires developing communication and translation strategies and tools for relevant ongoing EU projects and policy makers. The **detailed MACRAMÉ R&I** Strategy will define necessary benchmarks, time-critical work processes and responsibilities within and between MACRAMÉ WPs. To ensure the maximum impact and engagement with industrial actors, policy-informing, -making and standardising bodies, a strategic **MACRAMÉ Engagement Roadmap** will be defined to enhance the acceptance and advancement of the methods with regard to standardisation and harmonisation; this is supported by the **needs assessment of policy frameworks**, in connection with two **Risk Assessors Summits**.

Indicator	
	KPIs: Refined MACRAMÉ R&I Strategy (D1.1; M02); Needs Assessment Report of Regulation & Policy Frameworks (D1.2; M24); Summary Report: 1st Risk Assessors' Summit (D1.3; M12) Final Recommendations: Needs for TG & Standards Developments (D5.4; M36)
	M1.1: Confirmed & specified MACRAMÉ R&I Strategy agreed (M02).
	M1.2: Presentation of results at the 2nd Risk Assessors Summit (M36).

HLO2: Assessment of market-relevant Exposure Points and representative Sampling along the Life-Cycle of five MACRAMÉ Use-Cases

Five market-relevant industrial use-cases of AdMas have been selected, introducing real-life materials and products for subsequent development of methods, to assess the mutable nature of the AdMas in products along their life-cycle and potential impacts on humans and the environment. The AdMas were chosen in line with the EU Green Deal's regard of their pivotal role in green technologies and to represent **different morphologies of respirable carbon- or polymer-based particles (0D), fibres (1D) and platelets/graphene (2D).** In **aerosol or matrix-bound applications,** they harbour different release potential along their life-cycle. End-of-life (EoL) phases such as incineration, pyrolysis, shredding and landfilling are of particular interest with respect to release and will be investigated in detail. For a recycling-based future EoL is the most relevant but least studied life-cycle phase. Here, effective safety concepts are key for designing a sustainable economy. For all **MACRAMÉ Use-Case**, specific **MACRAMÉ Exposure** scenarios, ranging from occupational to the end-of-life, and corresponding exposure **Points** have been defined; sample collection and preparation procedures will be developed for subsequent characterisation and (eco) toxicological analyses. MACRAMÉ will study the ecotoxicological effects for risk- and sustainability-assessments of the most relevant stages of the life-cycle of the Use-Cases using OECD guidelines and extending some of the standard protocols (TG 429), to determine not only acute, but also subchronic and chronic effects.

Indicator	
	KPIs: Development of at least 5 sample collection and preparation protocols (D4.1; M12). Report on Life-Cycle-Inventory Data-Set and Characterisation Factors of the AdMas in market-relevant Use-Cases (D4.3; M24); Assessment Report of environmental & economic Sustainability of Use-Cases (D4.5; M36); **M4.1**: Sampling & sample-provision procedures set (M06). **M4.2**: LCI dataset & development of CFs completed (M22) **M4.3**: Establishment of *in-vitro* ecotox model for subchronic and chronic effect (M18)

HLO3: Characterisation - Development & Harmonisation of Methodological Approaches for the Identification, Physical-Chemical Characterisation and Quantification of inhalable AdMa@CMs

One of the most important exposure routes for AdMas is inhalation; MACRAMÉ will develop and harmonise methodological approaches for **controlled aerosol generation**, aerosol characterisation and *in-vitro* exposure. All approaches will be assessed and optimised for their capacity to characterise physical-chemical properties of aerosol, dust and airborne material generated during the different phases of production, use and disposal of AdMas. State-of-the-art complementary **qualitative** and **quantitative imaging methods** and **mass spectrometry** approaches will be combined, **correlated** and fully extended to characterise and image AdMas in their pristine or early state, or upon integration in complex matrices (incl. complex (biological) matrices and different physical/chemical states of the AdMas as a whole, or their individual components). The methods are usable with a multitude of different materials and will be **benchmarked and validated** against the **MACRAMÉ Control Material Library** and Use-Cases.

Indicator	
	KPIs: Report on the SOPs developed by WP2 (D2.2; M18); Report on the Performances & regulatory Readiness of SOPs for Aerosol Generation (D2.3; M18). **M2.1**: Criteria for the MACRAMÉ Control Material Library established (M03). **M2.3**: Draft version of SOPs for exposure and characterisation measurements available (M10).

HLO4: Toxicity Tests - Development of new Advanced Methodologies & Harmonisation of existing Methodological Approaches for human and environmental Hazard Characterisation of inhalable AdMa@CMs

MACRAMÉ will optimise and develop biological assays/systems with increasing complexity for inhalation *in-vitro* toxicology, including single cells, bi-culture systems, advanced *in-vitro* and *ex-vivo* models, representative of upper (bronchi) and lower (alveoli) airways, for regulatory hazard assessment and/or exploratory studies, thus enabling the formulation of new data-driven hypotheses. The methods will be organised in a **hazard assessment framework** that will allow toxicological assessment of AdMas life-cycles, by harmonising different throughputs and biological readouts (e.g. early signs of toxicity or detection of long-term effects such as genotoxicity). **Air-liquid-interface (ALI) exposure**, a critical aspect of *in-vitro* inhalation studies, will be harmonised to ensure that the **different methods for exposure of biological systems** provide similar and comparable exposure conditions. The ***in-vitro*/*ex-vivo* methods will be benchmarked** against the **MACRAMÉ Control Material Library**, comprising materials with different physical-chemical properties and known biological effects.

Indic.	
	KPIs: At least eight methods evaluated for their applicability in R&D and in regulatory applications or exploratory studies (D2.2: M18; D2.4-6: all M24) **M2.2**: *In-vitro* & *ex-vivo* models for toxicity assessment established and qualified (M12)

HLO5: Hazard Assessment - Development of the scientific Background to foster regulatory Acceptability of Methods and Methodologies for the Physical-Chemical and Hazard Characterisation of AdMa@CMs

MACRAMÉ will work in close liaison with regulatory bodies (OECD, VAMAS and ISO/CEN) to ensure that all development and progress achieved by the Project will be "regulatory relevant" and performed in a way that they can be picked up by regulators and by all other stakeholders. In order to produce **robust methodologies**, all experiments will be benchmarked first against the **MACRAMÉ Control Material Library**, which will ensure sufficient coverage of all possible physical-chemical properties of AdMas and nanomaterials (e.g. size, shapes, aspect ratio, solubility, aggregability, dispersibility, toxicity endpoints) ensuring appropriate experimental controls. In a second stage, the **benchmarked assays and methods** will be applied for the physical-chemical and hazard characterisation of **MACRAMÉ Use-Cases**, which are representative of realistic uses of AdMas in **industrial and consumers' applications**.

Indicator	
	KPIs: Scientific document to support the development a Guidance Document on inhalation toxicology of AdMas to regulatory bodies (e.g. OECD WNT) (D2.8; M34); Final informed Recommendations on future Needs for Test Guideline- & Standards Development (D5.4; M36). **M5.1:** At least five MACRAMÉ Methods for TGs, GDs, standards mapped into roadmap (M12).

HLO6: Establish improved Data-Reporting Guidelines and deliver harmonised, FAIR Data for the Characterisation of AdMas at different Life-Cycle Stages

Full documentation of the methods, their development and reliability of the data produced are pre-requirements for standardisation and regulatory validation. MACRAMÉ will develop **improved reporting guidelines** combining community standards for (nano)materials characterisation and safety data with detailed descriptions of the complex

product, biological and environmental matrices and apply them to document method development and Use-Cases. The **MACRAMÉ Information Hub** will be the one-stop sharing platform with all stakeholders at the earliest time, directly integrated into **advanced analysis**, **grouping and read-across** applications, with the aim to strengthen European competitiveness. MACRAMÉ Result will be processed and integrated into computational models for automatic data-gap filling and simulations developed within previous EU projects (ECETOC NanoAPP, the various IATAs developed and validated *via* OECD IATA Case-Studies, NanoInformaTIX, NanoSolveIT, NanoCommons) allowing the extension of models and approaches towards **semi-automated digital workflows** and guidance for future developments of decision support systems based on AI and machine learning.

Indicator	
	KPIs: MACRAMÉ SOPs and data available in public repositories and data warehouses (M36) **M3.1**: MACRAMÉ Information Hub fully operational (M12) **M3.2**: Data Stewardship established within the MACRAMÉ Consortium (M04) and embedded (M15) **M3.3**: Human- & machine-readable data-exchange formats defined & integrated (M18)

HLO7: Translation of Detection, Characterisation and Test Methods and combined novel Methodological Approaches into Standards and OECD Test Guidelines & Guidance Documents

The translation of detection, characterisation and test methods is addressed through several actions in the Project: **MACRAMÉ Methods** are advanced for harmonisation & standardisation by the preparation of the strategic **MACRAMÉ Summary Roadmap** showing possible implementation of Project results for regulatory purposes and with the aim to draft recommendations on further development needs to steer the research towards standards and TGs required for enforceable regulation. MACRAMÉ will engage as early as possible with stakeholders in standardisation & harmonisation and will implement the roadmaps and propose topics to the relevant standardisation/harmonisation bodies (VAMAS/ISO/CEN, OECD). MACRAMÉ will organise **international workshops with stakeholders relevant to harmonisation and standardisation**, and training webinars for scientists and industries, thus extending the exchange and dialogue started in the H2020 projects NanoHarmony and REFINE.

Indicator	
	KPIs: MACRAMÉ Harmonisation & Standardisation Roadmap Summary Report for MACRAMÉ Methods and Models (D5.1; M12); Four reports on 'Performance & regulatory Readiness of [methods]' (D2.3-6; M18 and M24); Scientific Doc. to support the Development of a GD for *in-vitro* Hazard Assessment of inhalable Materials (D2.8; M34); Science-based Recommendations to develop new TGs & Standards (D4.6; M30). **M2.3**: Draft version of SOPs for exposure and characterisation measurements available (M10). **M5.1**: At least five MACRAMÉ Methods for TGs, GDs, standards mapped in roadmap (M12). **M5.2**: Draft Recommendations on future Needs for TG & Standards Developments (M24).

1.1.1. Beyond the State-of-the-Art: the MACRAMÉ Ambition

The current state-of-the-art is to characterise and assess AdMas in their pristine form; MACRAMÉ will surpass this by looking at **AdMas and AdMa-containing products in their market-relevant** form in complex matrices (**CMs**). Based on this, one of MACRAMÉ's central challenges is posed by its focus on **three carbon-based MACRAMÉ Material Families**, which are hard to detect against a background of biological or environmental complex matrices. The following **scientific & technical ambitions** will be pursued in MACRAMÉ to tackle this formidable challenge:

- To support development, harmonisation, and benchmarking of testing methods applied within the Project, a MACRAMÉ **Control Material Library** (CML) will be established. The CML will contain established control/reference materials (of known properties) and new samples from the MACRAMÉ Material Families, prepared using standardised methods. The CML will be made available as **a new service to collaborating and future projects,** extending repositories (e.g. the JRC's repositories of certified reference materials or representative nanomaterials), by adding well characterised AdMas and AdMa@CMs (in complex matrices).
- To measure the physical stability and aggregation propensity in biological and environmental liquid media, MACRAMÉ will go beyond current methods that are limited in their applicability to dark/opaque samples, such as GRM suspensions: **novel, robust methods**, including static multiple light scattering (SMLS) will be validated by comparison to complementary approaches, e.g. cryo-TEM (transmission electron microscopy).
- To detect, spatially co-localise and characterise GRMs and CNTs in composites and biological matrices, **innovative object localisation and correlative microscopic techniques** will be further developed to overlay SEM, SEM/EDS, optical microscopy (OM) and confocal Raman chemical images with high accuracy by using image object recognition and pattern constellation matching algorithms (based on ISO-G-SCoPe results).
- To **determine the surface oxygen content state of GRM and CNTs**, high-sensitivity EDS (<100 nm lateral resolution) will be used and complemented by XPS (x-ray photoelectron spectroscopy) and Raman analyses.
- To **quantitatively measure the uptake of PLGA doped with metal and/or metal oxide particles** in cells and tissues from 3D cultures, state-of the-art laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) will be used at subcellular resolution (< 5µm). Combining histological images and spatially resolved results by LA-ICP-MS will allow for the evaluation of cellular uptake/tissue distribution of AdMas.
- To **assess the biological impact of AdMas upon inhalation**, MACRAMÉ will further develop and optimise biological systems with increasing complexity representative of upper and lower airways, benchmarked against

reference materials and AdMas from the MACRAMÉ CML, demonstrate that the models are fit for purpose for regulatory applications and foster the development of a GD for hazard assessment of inhalable AdMas.

- MACRAMÉ will develop harmonised protocols for the generation and characterisation of aerosols for the *in-vitro* exposure of biological systems for inhalation toxicology. This will benefit the entire inhalation toxicology field, by providing **harmonised approaches for the generation of comparable experimental results**.
- To assess the impact in the environment of the ADMas in relevant stages of the life-cycle of the use cases, MACRAMÉ will **further develop existing OECD ecotoxicity assays with increased complexity to allow assessment of acute, subchronic and chronic effects**, and to assess internalised doses *via in-vitro* gill barriers.
- MACRAMÉ will use *in-vitro/in-vivo* data extrapolation approaches and LCA inventories to produce data in a cost-efficient way to support the evaluation of the environmental footprint of products and processes.
- **A laboratory test bench will be developed** by using a controlled-Atmosphere Cone Calorimeter (CACC) and a tube furnace, to harmonise the simulation of the incineration processes and associated release at different oxygen concentrations and advance the sampling and online/offline characterisation of gas and soot particles emitted during the thermal degradation process.

1.1.2. MACRAMÉ TRL-Advancement

Figure 2 provides an overview of the anticipated TRL advances in each R&I Activities, positioning the MACRAMÉ Project firmly in the **'lab to market'** field. R&I activities with starting TRLs lower than the required level 3 are of exploratory nature; critical risks pertaining to their advancement within the Project are discussed in Table 3.1.e.

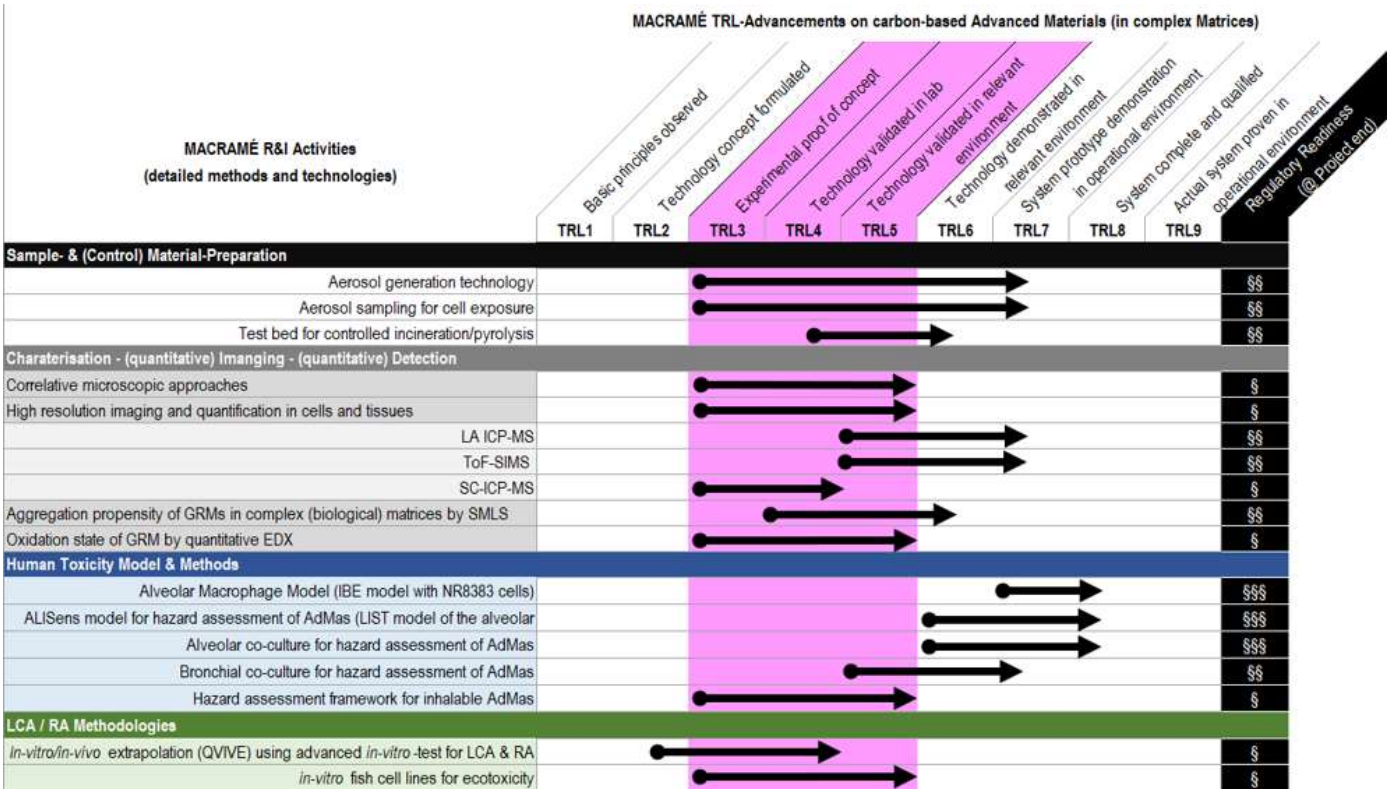

Figure 2: Schematic overview of the main MACRAMÉ R&I Activities, their detailed methods and technologies applied, and their TRL start- and end-levels, as well as their 'Regulatory Readiness'-levels at Project end (§=low to §§§=high).

MACRAMÉ will **push current regulatory boundaries and the availability of standards, TGs and GDs** pertaining to them through the integration of different technologies into intelligent, tiered testing approaches that address market-relevant AdMas both at possible Exposure Points and in their relevant complex matrices:

- Novel conceptual and technical approaches to aerosol generation, with controlled dosimetry & particle morphology, developed and harmonised, leading to standardised experimental frameworks with well-defined modules adapted to the desired study design (sample nature, cell model requirements, etc.) as described in task 2.2. MACRAMÉ's work will develop an OECD GD for risk assessment of inhalable AdMas.
- Life-cycle-relevant **MACRAMÉ Exposure Points,** defined in collaboration with industrial partners providing MACRAMÉ with AdMa@CMs in the **five MACRAMÉ Use-Cases.** Samples at **Exposure Points** will be obtained and characterised using advanced quantitative imaging and detection techniques (in WP2) to overcome the current limitation of release assessment being largely based on literature-derived assumptions.
- MACRAMÉ will **stress-test, validate and implement several biological models representative of the human respiratory system**, including models of upper (bronchial region) and lower (alveolar region) airways. MACRAMÉ's biological systems use existing technology at different TRLs (see Figures 2 and 4), some commercially available (i.e., Mucilair™, Alveolair™), or almost commercial (e.g., the ALIsens (LIST) and the

Alveolar Macrophage Model (IBE)). So far, validation of such models has been performed on chemicals, respirable particles, and nanomaterials rather than AdMa@CMs. MACRAMÉ will demonstrate the usefulness of the proposed biological systems for hazard assessment of AdMas and regulatory practice.
- MACRAMÉ will **extend** the existing OECD TG249 Rainbow Trout Fish gill cell toxicity tests by implementing it as **an *in-vitro* barrier model, enabling acute and sub-chronic evaluation** of AdMas.

Life-cycle costing (LCC) assessments and the creation of life-cycle-inventories (LCIs) will be conducted on the MACRAMÉ Use-Cases with regard to the economics of incumbent materials and test methods, allowing to demonstrate that **the novel methodologies/assays developed** within the Project are also able to **foster industrial innovation by a reduction and rationalisation of cost for characterisation, assessment and testing.**

MACRAMÉ will develop and implement a novel concept of **data-stewardship** that will digitally enable state-of-the-art Safe and Sustainable by Design (SSbD) approaches towards the foundations of a newly conceptualised **'MACRAMÉ Safety & Sustainability Matrix'** to sustain the **MACRAMÉ Approach** efforts towards a **circular, climate-neutral and sustainable economy** and to contribute to the overarching EU's plan to move towards a policy of *'one substance – one assessment'* as part of the European Green Deal (2019).

1.2. MACRAMÉ Methodology

I. The MACRAMÉ Drawing Board - collaborative Strategy Setting & Adoption with all Partners

The MACRAMÉ workflow is highly complex, because it aims to analyse the physical-chemical characteristics and impact on humans and the environment of four different advanced materials during the life-cycle of five products. The Project will start from a collaborative planning exercise in a **WP1**, in order to establish collaborations and to re-confirm and further detail the underlying **MACRAMÉ R&I Strategy**, to set time-critical benchmarks to oversee the performance and achievements and to guarantee the maximisation of MACRAMÉ's highly interdisciplinary approach and its impacts. This approach will guarantee resilience of the Project delivery and enables the detailed consideration of the re-use of and value-add to previous project results, arrangements of collaborative approaches with sister projects (i.e. DIGITAL-EMERGING-01-35), as well as the engagement of the wider relevant stakeholder community (elaborated in the **MACRAMÉ Stakeholder Roadmap**). MACRAMÉ will consider ongoing and future projects in OECD, ISO and CEN on nanomaterials and AdMas for input from MACRAMÉ and vice versa; a close collaboration is thus established with the Malta-Initiative, the NanoHarmony and NMBP13 projects (Gov4Nano, NANORIGO, and RiskGONE); relevant OECD projects already identified for input from MACRAMÉ are: (a) WNT project 1.10 GD on the determination of concentration of nanoparticles in biological samples for (eco)toxicity studies, (b) WPMN project on Guidance on Release Tests for Manufactured Nanomaterials, (c) WPMN project on Recommendations for guidance on adaptations needed when using OECD TG 201, 202 and 203 for the determination of the Ecotoxicity of MNs, (d) WNT project 3.10 TG on dissolution of nanomaterials in aquatic environment, (e) WNT project 3.16 GD on environmental abiotic transformation of nanomaterials, (f) MACRAMÉ will also exchange with a future German project envisioned on acute inhalation toxicity. During the first collaborative Project months, and following the IndustryCommons initiative, MACRAMÉ **bridge between communities** by emphasising the interdisciplinary knowledge exchange, acquisition of skills, innovation methodologies and new knowledge creation, demonstrated through the efficient and effective application of the MACRAMÉ results and outputs in various industry sectors (i.e. chemicals, medicine, electronics, and consumer goods).

II. The MACRAMÉ Foundations - Use-Case Definitions & Sample Provision

WP1 will reconfirm the **MACRAMÉ Exposure Points**, at which exposure of humans and/or the environment to advanced materials may occur, based on the product-relevant value-chain considerations of **five market-relevant MACRAMÉ Use-Cases**, chosen to be representative of the **three carbon-based MACRAMÉ Material Families** (i.e. (a) GRM (incl. GO (graphene oxide) and FLG (few-layer graphene)), (b) carbon fibres (i.e. CNTs), and PLGA). The resulting detailed MACRAMÉ R&I Strategy (M02) refines the product-relevant exposure points, control materials, sampling strategy, operating procedures for sampling, sample-size (i.e. quantity of a sample) and -provision arrangements, specific R&I activities to be conducted for each sample (i.e. exact characterisation and (quantitative) imaging and detection methods) and instrument-exchange. Part of this strategy is the *'Sampling and Sample-Provision (to WP2 Partners) Procedures of representative inhalable Materials from the five Use-Cases established'* (Milestone M4.1, to implemented and completed in WP4). The **five market-relevant MACRAMÉ Use-Cases** are:

1. **MACRAMÉ Use-Case 1 (UC1) - Graphene oxide (GO) flakes in drinking-water filters** [Material Family: GRM; providing partner: MEDICA]
   UC1 deals with water filters made from polysulfone and graphene oxide (5% w/w). Previous studies under the Safegraph project (Graphene Flagship) have shown that upon usage, no graphene is released or detected at 50 ppb (limit of detection). Risk assessment is currently being performed in the Safegraph project by feeding the Precautionary Matrix for nanomaterials and the LICARA-Nanoscan with data from the producing company (MEDICA). MACRAMÉ will focus on the end-of-life of the filters, which includes through incineration, land

filling and recycling. WP2A advanced imaging techniques will be used in the context of the end-of-life of the filters to detect the possible presence of graphene and its physical-chemical status.

2. **MACRAMÉ Use-Case 2 (UC2) - Few-layer graphene (FLG) in battery management systems (BMS)** [Material Family: GRM; providing partner: Carbon Waters]
   UC2 studies few-layers graphene dispersed in epoxy resins used in a battery management system (BMS). BMS are designed to improve security and shelf life of electric batteries. Carbon Waters will provide non-reticulated (liquid samples) and reticulated (solid samples) of epoxy resins with FLG. During production or use, i.e. during normal use, fire or extreme heat, the adhesives can generate gases from different solvent and in particular epoxy, enhancing the formation of vapour containing the FLG with potential toxic effects by inhalation. During use or end-of-life thermal adhesives can enter in contact with the water leading to degradation and to release of the adhesives in water or in the soil with potential toxic effects for the environment.
3. **MACRAMÉ Use-Case 3 (UC3) – GRM-bearing sprays** [Material Family: GRM; (no industrial partner; consumer product sprays are purchased); UC-Lead: GAIKER]
   GRMs are incorporated into applications such as paints, coatings, lubricants, bikes and car polish to add superior performances (durability, better protection, reduction of friction, heat and wear, long lasting parts, and even self-cleaning properties). Table 1 shows a selection of the products that have been identified; the composition is not available, and questions sent to the manufacturers were left unanswered. UC3 will start with a characterisation of each spray, in order to verify the producers' claim and quantify any GRM content.

   Table 1: Selection of different GRM-bearing consumer products already identified; descriptions are quotes from the product websites.

Product Type	Product Name - **Functionality** - Intended Use - Description
Spray Paint	ECP 573/JSF180/4124 Permanently Conductive Transparent: **Static elimination:** Application in lighting, windows, clear covers and lids, displays. Single component permanently conductive graphene hybrid clear acrylic coating. Contains CNTs and graphene derivatives.
Car-wax Spray	Hybrid solutions pro graphene flex wax: **Surface protection:** Simple spray-on solution can be used as a dry wax, rinse wax or even both! Gloss, slickness, water repellence and heat dissipation for increased UV protection.
Tire-shine spray	Hybrid solutions graphene acrylic tire shine spray coating: **Gloss and protection:** preserves and protects the rubber in one of the most important safety features of your car – your tires! Tiny graphene particles penetrate and bond with the tire surface for greater durability.
Lubricant oil	100% graphene lubricant spray: **Synthetic lubricant:** To apply on mobile parts of bikes. Lubricating oil with graphene nanoparticles and antioxidants for chain lubrication on a bike. Longer life of parts, super silent gears and chains and minimum friction between parts.

   UC3 will focus on two life stages: (a) professional or private use and (b) end-of-life. UC3 will provide an assessment of the suitability of the MACRAMÉ Methods to characterise the commercial aerosols. Aspects to investigate are aerosol characteristics (i.e. graphene concentration, droplet dispersion, droplet size, stability), as well as toxicity, in particular inhalation acute, repeated dose, and chronic. After use, residues are usually washed off, so the waste-water will be investigated for GRM residues, and assessed for potential ecotoxicity.
4. **MACRAMÉ Use-Case 4 (UC4) - Carbon nanotubes (CNTs) in Polymer Foils for thermal Applications (e.g. heated Car Seats)** [Material Family: carbon fibres (i.e. CNTs); providing partner: FILK]
   Electrically heated polymer foils based on multi-walled CNT-PU (polyurethane) nanocomposites improve energy efficiency by body contact heating (e.g. in car seats). UC4 will evaluate the suitability of MACRAMÉ methodologies to detect, identify and characterise CNTs in polymer foils and in aerosols, which can be released at different moments such as (a) during manufacturing, (b) during use by abrasion or malfunctioning (e.g. fire or overheating or, (c) end-of-life, i.e. by incineration, pyrolysis or shredding. Samples will be provided to answer questions on abrasion during use and failure due to overheating. Aerosol particles released in these scenarios will be characterised and tested for toxicity. Results will be compared to those for pristine CNTs.
5. **MACRAMÉ Use-Case 5 (UC5) – PLGA-based inhalable Antibiotics** [Material Family: PLGA (nano)particles; providing partner: MyB]
   Lung infections are predominantly treated by oral or systemic antibiotic administration which are associated with side effects on other organs. An inhalation product can overcome the treatment bottlenecks by locally delivering the antibiotic directly to the sites of infection. UC5 will focus on an assessment of inhalable drug where ciprofloxacin loaded PLGA nanoparticles will be used for proof-of-concept of treatment for antibiotic resistant lung bacterial infections. In addition to ciprofloxacin-loaded PLGA nanoparticles (NPs), controls will be prepared for imaging purposes, loaded with Fe_xO_y- or gold-NPs or labelled with Lumogen-red®, to verify the suitability of the MACRAMÉ approach to quantify and characterise the aerosols upon exposure of the *in-vitro* lung models. Figure 3 provides an overview of the identified **product- and life-cycle-relevant MACRAMÉ Exposure Points** and the sampling, characterisation/imaging/detection and testing to be conducted at these points.

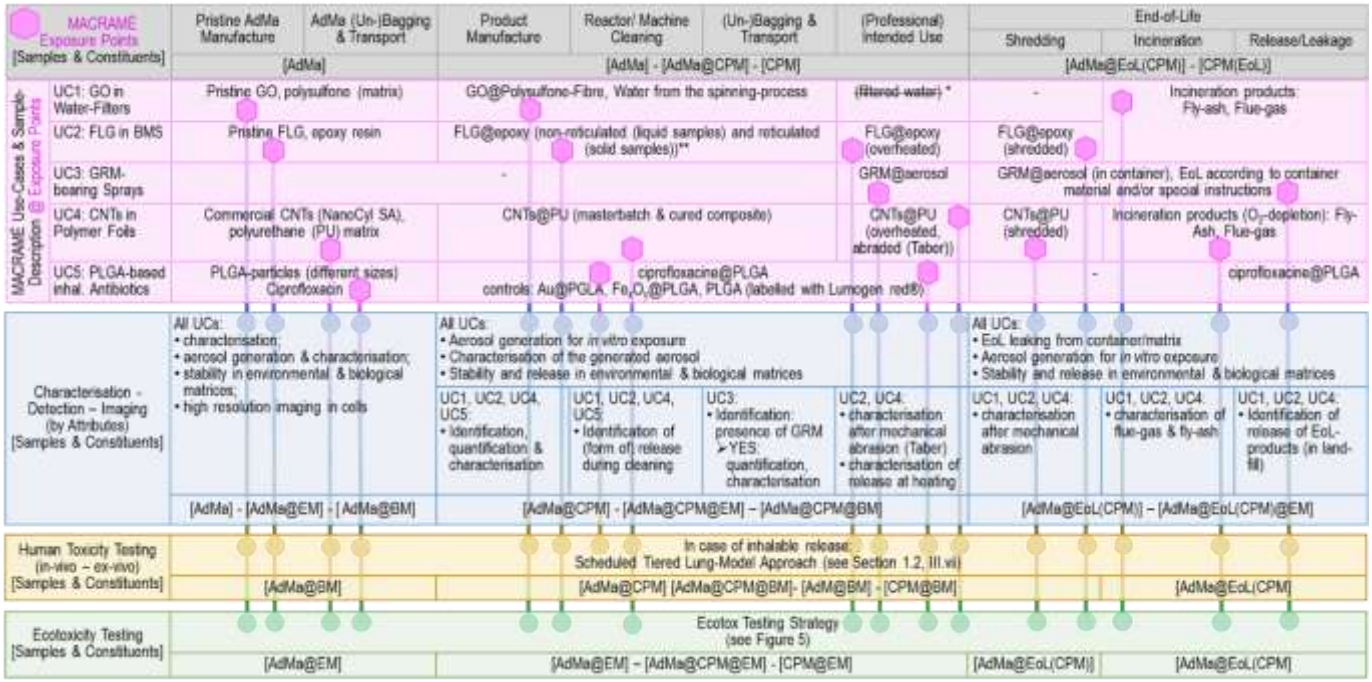

*Figure 3: Schematic overview of the identified product-relevant MACRAMÉ Exposure-Points and the sampling, characterisation/imaging/detection and testing to be conducted at these points; *3 GRMs will be processed to a GRM reinforced epoxy composite according to Netkueakul et al. (2020).*

The MACRAMÉ Exposure- Points have been used to define the samples that need to be taken from the industrial Use-Case processes by the relevant providers (in WP4) and sent to the characterisation- and testing partners (in WP2), adhering to the *'Sampling & Sample-Provision Protocols for AdMas in complex Matrices'* (D4.1, Task 4.1), agreed by all participating partners. The combinations of **advanced materials (AdMas)** in **complex matrices (CM)** to be studied in MACRAMÉ include all life-cycle-relevant occurrences of both (a) complex product matrices (**AdMa@CPM**), (b) degraded complex product matrices at the product's end-of-life (EoL)(**AdMa@EoL(CPM)**), (c) regulatory relevant biological matrices for human toxicity testing (**AdMa@BM**), (d) environmental matrices for ecotox testing (**AdMa@EM**), and relevant forms of the different complex matrices, such as soot and char, and aerosols generated from compounding, machining, use, weathering, degradation or incineration of products.

III. The MACRAMÉ Laboratory - Characterisation & Testing of AdMas in complex Matrices

The ambitious MACRAMÉ objectives will be achieved through the development of **(a)** a **MACRAMÉ Control Material Library (CML)** and **(b) MACRAMÉ Validated Protocols**, established in collaboration between WP2 and WP4. Based on exposure points defined by the five MACRAMÉ Use-Cases, the CML will provide reference materials and AdMas, matrices and their composites to partners to enable R&I measurement- and test-transferability studies of the MACRAMÉ Project. The CML will contain samples of all combinations of **AdMas** in **complex matrices (AdMa@CMs)** described above (cf. Figure 3). Once material quality, stability and traceability are established, the CML will be offered to other projects and standardisation and harmonisation communities. Library AdMas will be catalogued with respect to their life-cycle state and matrix; the AdMas will include platelet-shaped (2D) (i.e. GRM, namely GO, FLG (4-5 layers), MLG (multi-layer graphene) (10-20 layers)), fibre-shaped (1D) (i.e. CNTs) and spherical (0D) (i.e. GMP-compliant polymer) (nano)particles. The CML will also compile characterisation data of all relevant samples, based on the measurements described in Task 2.1: known critical attributes of AdMas, affecting their toxicological profile, will be characterised at an early Project stage to ensure appropriate reference measurements to follow their modifications, when subjected to compounding, machining, degradation or cell exposure studies. The selection of applied methods will be optimised and validated for the different material classes. **MACRAMÉ Validated Protocols** will be defined and validated with respect to robustness, repeatability and reproducibility. This way, information on strengths and weaknesses of approaches and details of their constraints, including limits of detection (both material- and particle size-specific) and their robustness/reliability is obtained. The developed and validated methods will be focusing on:

i. Measurement of **critical attributes of GRM**, incl. (i) their **stability, biotransformation and release** in liquid media by SMLS for simultaneous screening of size, agglomeration state, stability and dosimetry of not spherical particles like FLG in turbid solution (Sentis *et al.* (2020)), in relevant liquid mixture (water-solvent), environmental matrices and biological fluids; (ii) measurement of **the oxidation state of GRM** by high-sensitivity EDS detector complemented by XPS and Raman analyses.
ii. **Detection, spatial co-localisation, characterisation and quantification** of GRMs and CNTs **in polymer composites and cells by correlative microscopic techniques**. This will be achieved by spatially-accurate overlaying SEM, SEM/EDS, atomic force microscopy (AFM), optical microscopy (OM) and/or **confocal Raman chemical images** using image object recognition and constellation matching algorithms. The

quantitative approach developed by BAuA for correlative imaging of CNTs lying (1) on track-etched filter membranes or (2) on top or inside of macrophages using SEM, AFM, OM and Raman spectroscopy will be further developed for matching patterns of arbitrary, optically resolvable structures, to detect and quantify GRMs and CNTs in thin cell layers and polymer composite thin films or surface layers. The achievable detection reliability will depend on the intensities of spectral characteristics of the particle, matrix and background spectra. The integration duration required for reliable identification governs the limit of detection (LoD) by restricting the achievable area or volume scan speed. Different matrix materials of the MACRAMÉ Control Material Library will be spiked with known particle numbers to study resulting LoDs.

iii. Approaches for **controlled aerosol generation** include wet techniques already established in the framework of inhalation studies, and dry techniques that allow re-designing aerosols generation from dust and powder materials representative of real-life exposure scenarios. For materials in liquid or suspension brought to cells as droplets, established aerosol generation protocols using atomisation & nebulisation (LIST, RIVM, EPITHELIX) will be adapted to yield aerosols that are similar in droplet size or emission rate, even if apparatuses differ. An interlaboratory comparison (ILC) will be conducted, initialising a harmonisation process. Aerosol generation from dry powders is conducted with the vortex shaker established at LNE and the fluidiser developed by BAuA, both originally applied as dustiness tests and subject to harmonisation in the project NanoHarmony. To be applicable in the framework of inhalation studies, the SOPs will be adapted, guiding testing powders under standardised conditions and optimisation of conditions to produce aerosols with desired properties. The fluidiser method will be extended to all AdMas to provide a comprehensive picture of the dust morphology released under different gas flow and powder agitation energy regimes. For dose generation and material testing, BAuA's artificial neural network-based object recognition and measurement image analysis toolbox will be extended.

iv. A **modular approach** for ***in-vitro* cell exposure to aerosols** of controlled morphology and dosimetry will be developed to ensure that the cell exposure dose is well-defined and -characterised. The modular framework will include: *(i) sample preparation* prior to aerosol generation, performed using established protocols to create stable particle suspensions for wet aerosolisation or homogeneous powders for dust generation; (ii) *aerosol generation* including wet and dry techniques, as described above; (iii) *aerosol manipulation* required to expose *in-vitro* models to selected particle morphologies for controlled experiments, e.g. to select fibres within a specific length range; (iv) *aerosol characterisation* comprises common aerosol monitors like the SMPS, CPC, APS, ELPI and NSAM, and morphological characterisation by Raman and EM as experimental controls; and, finally, (v) *exposure* to aerosolised particles, of cells, support or dishes depending on the used cell model. In this regard, MACRAMÉ will explore multiple exposure options: (a) Cyto-TP for fibres and other dry aerosols and (b) the Cloud system for droplets. The latter is an established commercial exposure device for tissues hosted at the air liquid interface (ALI). The Cyto-TP is a minor modification of a commercial exposure system, common within the MACRAMÉ laboratories, that allows a strongly augmented deposition of dry nanoparticles and fibres onto living cells hosted at the ALI. Aerosolised particles will be deposited on different ALI cell configurations of the partners by direct particle exposure (e.g. epithelial monocultures, co-cultures with epithelial and immune cells as well as 3D-MucilAir model by Epithelix), or by pre-loading µ-dishes with fibres, a novel approach to investigate alveolar macrophage responses to fibres (prototype developed in InnoMat.Life). Advantages of this approach include: (i) dispersion of single fibres in the aerosol is largely maintained, making them accessible to cells under submerged conditions; (ii) fibre-loaded µ-dishes can be distributed to Partners, enabling a comparison of effects among different labs and eases standardisation; (iii) the uptake of fibres can be monitored with time-lapse imaging of living cells and other imaging methods (darkfield microscopy, Raman, ToF-SIMS, LA-ICP-MS); (iv) the method allows investigation of the effects of coatings of the µ-dishes (with proteins or lung surfactant) on fibre uptake and cell responses, thus mimicking the conditions of the inner lung surface.

v. **Approaches for detection and semi-quantitative measurement of nanomaterials in biological fluids, cells and tissue** will be developed using a combination of advanced imaging techniques relying on light microscopy (i.e. enhanced darkfield microscopy (DFM), hyperspectral microscopy (HSI), confocal Raman microscopy (CRM)), and mass spectrometry (i.e. laser ablation induced-coupled plasma mass spectroscopy (LA-ICP-MS) or Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS)). Importantly, DFM, HSI and CRM reach a lateral resolution <1 µm and can be sequentially applied to screen and identify AdMas in cells and materials. For the quantitative determination of particle uptake into cells, LA-ICP-MS will be used and further standardised. The technique shall mainly be used to quantify gold-doped PLGA and metal oxide particles in cells, to measure the cellular particle uptake in *in-vitro* experiments. To unravel the cell-type specific uptake of materials in 3D cultures, a mass labelling of cell types (by lanthanide-coupled antibodies against surface markers) along with LA-ICP-MS will be used. Overall, quantitative analyses of AdMas in submerged and ALI cultures will rely on LA-ICP-MS technology. Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) will be used to detect PLGA in single cells. ToF-SIMS is a marker-free technique which allows detection of different types of ions (Na^+, K^+, Ca^{2+}) and biomolecules characteristic for cellular compartments (nucleus, cytoplasm) in a semi-quantitative fashion. High-resolution detection of PLGA in cells by ToF-SIMS and

Orbitrap-SIMS is the main goal. For this, an optimisation of measurement parameters and data evaluation routines is necessary: energy and pulsing scheme of primary and sputter ions will be adjusted, and analyser parameters will be optimised and MS/MS routines have to be adapted, with the help of automation.

vi. Controlled **protocols for the simulation of end-of-life scenario** and further toxicological testing will be developed according to the product-relevant MACRAMÉ Exposure-Points reconfirmed and detailed in WP1 and during the LCA (WP4) (e.g. incineration/pyrolysis/ weathering, for the detection of released of AdMas from finished products and for the characterisation of their degradation (i.e. applying different oxygen concentration in a controlled-Atmosphere Cone Calorimeter (CACC) or in a tube furnace flushed to simulate incineration and/or pyrolysis)). The produced materials will be included as part of the CML. Moreover, approaches for direct exposure of *in-vitro* lung cultures or sampling and collection of materials released prior to toxicological testing will be developed and validated with a focus on control dosimetry and material recovery in the different steps.

vii. Biological systems of increasing complexity (i.e. from single cell culture to advanced multi cellular cultures and *ex-vivo* human tissue), will be used to generate a **MACRAMÉ Tiered Lung-Model Approach** for hazard assessment of inhalable advanced materials (see Figure 4). At first the biological systems will be challenged with reference materials from the CML to allow the assessment of their performances and to understand the field of applicability and if modifications and adaptations are necessary prior of the assessment of AdMas from the Use-Cases from WP4. This will allow to generate a hazard assessment framework for inhalable materials covering different level of biological complexity i.e. from single cell assays and simple co-culture models, to *ex-vivo* human biological systems (precision cut slices from the bronchial and alveolar region) passing through advanced *in-vitro* multi-cellular system. Some of the models are more suitable for fast screening and early assessment, while other models are less suitable for regulatory applications and more adapted for exploratory studies and data driven hypothesis generation. Part of the work performed in MACRAMÉ is devoted to extract information that would allow prediction of long-term effects; regulatory bodies (e.g. ECVAM, OECD) will received proposals of our methods and approaches for formal validation and inclusion in official TGs/GDs (WP5).

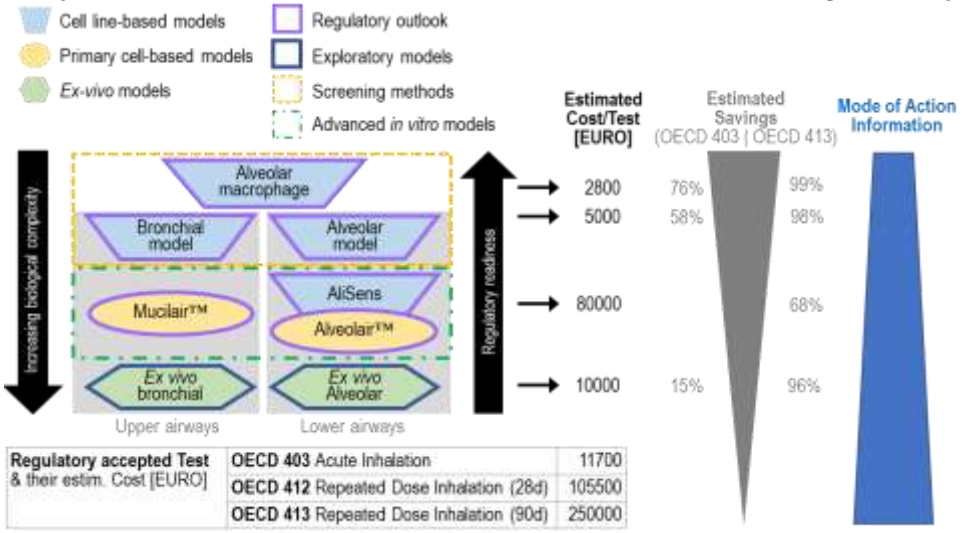

Figure 4: In-vitro and ex-vivo Models that will be assessed within the Project, indicating the estimated cost that can be saved and MoA- Information that can be obtained at the different tiers.

viii. **Innovation in ecotoxicological models of increasing complexity**, allowing assessment of acute, sub-chronic and chronic impacts leading to an improved and extended version of the Threshold Approach for Acute Fish Toxicity Testing that accounts for the specific features of AdMas. The MACRAMÉ ecotox approach, shown in Figure 5, includes an innovative extension to the existing OECD TG249 (Rainbow Trout Fish gill cell tests) over acute and sub-chronic timescales. (24 h - 2 weeks) with the existing acute OECD TG201 and TG202 tests (microalgae and daphnids, respectively), and the subchronic and chronic TG 211 (Daphnia reproduction test) utilising a conditioned medium approach to disperse the materials. The approach allows the cells / algae / daphnids to interact with the medium and secrete proteins for a period of 24 hours, which are quantified using a BCA assay for the total protein content, for foxed times, and then this conditioned medium is used to disperse the materials, allowing more realistic and environmentally relevant environment exposure conditions.

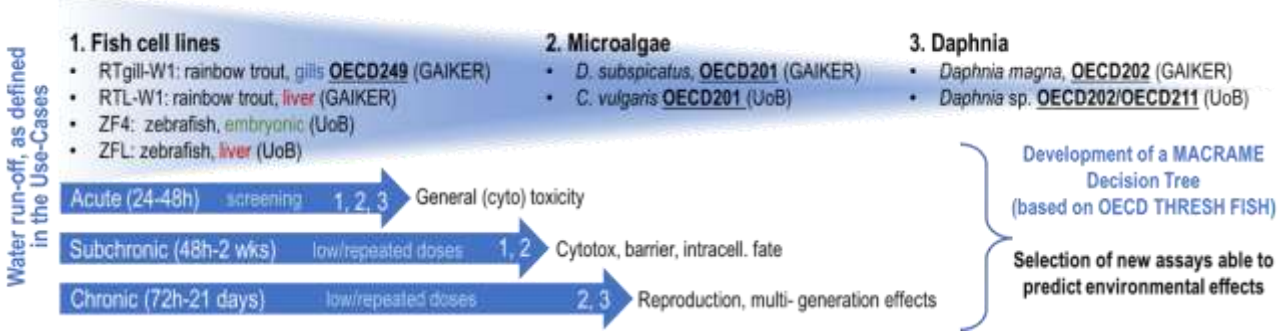

Figure 5: Schematic illustration of the MACRAMÉ ecotoxicity testing approach and outcomes.

The OECD 249 assay will be extended to enable assessment of accumulation in, and transport across the gill, using transwell systems, and impacts on fish embryonic cells and/or fish liver cells will be incorporated by co-culturing on or other of these cells on the bottom surface of the basolateral chamber to assess impacts from materials in or crossing through the gill barrier. The OECD 201 and 202 assays will be extended to include sub-chronic and chronic multi-generational exposures also, providing further parameterisation of the decision tool for when to perform Acute Fish Toxicity testing, and to provide mechanistic grouping for advanced materials. By the end of the Project, we will approach validation and regulatory bodies (e.g. ECVAM, OECD) to propose our methods and approaches for formal validation and inclusion in official TGs/GDs. The ecotoxicity data generated will feed into the LCA analysis and support grouping and read-across.

IV. The MACRAMÉ Processor – Centralised Knowledge-Generation

Data from the MACRAMÉ data characterisation techniques will be enriched with information from a multitude of other sources and aggregated for knowledge discovery (Task 3.3). This will be done on different levels:

i. provide information on materials study design and data analysis stage for specific MACRAMÉ methods;
ii. test for transferability of analysis approaches from one method to the other; and
iii. evaluate data aggregation approaches applied in WP4 Use-Cases for automatisation and generalisation.

Collected data will ultimately be applied in existing approaches for grouping and read-across (ECETOC NanoApp, GRACIOUS, NanoSolveIT, NanoInformaTIX and RiskGONE approaches and tools) (Task 3.4). This will assess the relevance of the data produced in MACRAMÉ for setting the boundaries between nanoforms and grouping. To develop these modified approaches into standard alternative methods, each MACRAMÉ method will define applicability domains and constraints based on and constantly updated with the data produced in the Project including calibration measurements and negative/positive result samples. Full provenance trails to underlying data will be generated over time; to provide a seal of quality for each method, to guide its selection and assay parameters, giving the most reliable result for the material and grouping/read-across application at hand, and ultimately guiding the development of SOPs for standardisation/validation of the method. Grouping and read-across approaches, developed to assist industry with REACH registrations regarding nanomaterials, have been recognised as very useful by ECHA and presented at the OECD respectively. Replacing established, validated characterisation methods with the advanced MACRAMÉ methods as input for these methods has the potential to increase the predictability and reliability of these methods but will highly increase the information requirement to be provided with each data point. While following standardised test guidelines is often enough to trust the quality of the results, the new, advanced methods will show the quality by providing information on the robustness, reliability and validity of the method. This data generation process is depicted in Figure 6: it requires a very intense two-way exchange (of information and knowledge) between the WPs and is based on the development of new characterisation methods and their validation of them *via* industry Use-Cases. This exchange will occur in the form of materials and sampling plans, study designs translating needs into actions, method specifications describing the measurement principles, protocols and SOPs, and finally the data (raw, transformed and robust summary), all which we will call **MACRAMÉ (Meta)Data** in the following. For each Use-Case, information is initially generated by WP4 (incl. materials flow analyse (MFA), exposure points, sampling points and protocols, and sample preparation SOPs. Then, the data generation is started with the sampling protocols on WP4, and then continued on characterisation and human toxicity testing (WP2) and on ecotoxicity and life-cycle-based assessments, mass-flow-analyses (MFA) and risk assessment (RA) (WP4); this will be documented with detailed descriptions of the methods, their development and development stages, and the corresponding protocols and SOPs detailing the specific variations needed and the effect of changes to the protocol on the resulting data (e.g. absolute values, uncertainties, repeatability). This is further complemented by gap filling using existing data from public resources identified by WP1, mined, curated and integrated by WP3, and *via* the modelling approaches of WP3 generating computational data. All of this information will be

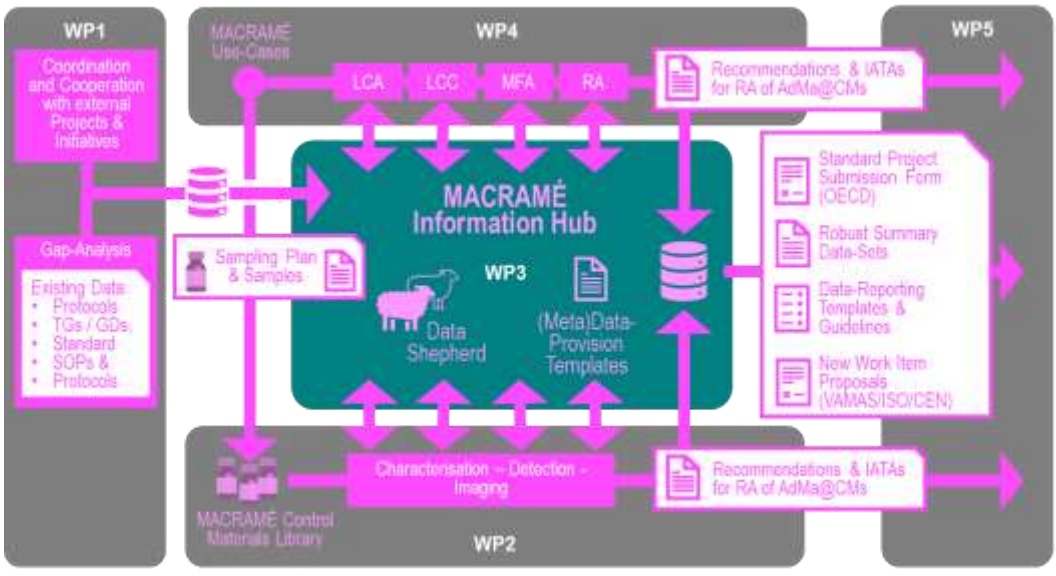

Figure 6: Schematic illustration of the flow- of and value-add to data within the MACRAMÉ Project.

integrated, harmonised and handed back to the Use-Cases (WP4) for analysis against LCC criteria, and in a condensed (summary) form to WP5 and WP6 as basis for the development of updated guidelines and standards, and for dissemination of the Project results to target stakeholders. All information will be managed and exchanged *via* WP3, as depicted in Figure 6, guaranteeing safeguarding of all MACRAMÉ (Meta)Data with full provenance trails, and creating an first implementation of example of the concept of **'Materials as a Service'-concept (MaaS) for the safety & sustainability of AdMa@CMs**. Data to be shared include not only data generated by the fully developed method as used in the Use-Cases of WP4 but every produced data point with their specific protocols from early development, calibration and control measurements, thus collecting knowledge about the applicability domain and reliability of the methods, and forming the basis for the (pre-)evaluation of the methods and for evaluation of their applicability in risk assessment, grouping and read-across approaches. All partners will work together to define the specific MACRAMÉ (Meta)Data reporting and sharing standards and their customisation to the specific methods (Task 3.2). These will be translated into metadata-rich, highly structured and semantically annotated curation templates. To support this creation and usage of the method-specific templates covering all aspects (method specifications, SOPs, data), a **MACRAMÉ data shepherd** located within 7P9-DE and supported by 7P9-SI will be established. A data shepherd is an enhanced version of a data steward, who not only oversees the data management, handling and quality control processes, but can mediate between parties in need of data management solutions and parties providing them and resolve any misunderstandings (Papadiamantis *et al*., 2020). In this way, implementation of the FAIR principles at every stage and harmonisation with major European Initiatives (EU NanoSafety Cluster, European Materials Characterisation Council and IndustryCommons) by reusing and extending community interoperability standards (computer-readable and annotated CHADA, CEN CWA 17815 Materials, VAMAS-CODATA UDS, NanoFASE-like instance maps for describing complex environments and experimental workflows) can be guaranteed. Curation, storage and sharing tools will be selected optimally for the Project and implemented and customised to generate the **MACRAMÉ Information Hub** (Task 3.1) as a one-stop interface to access all information, with each data point clearly associated with the life-cycle state of the material, the environment in which it was studied, as well as the method and protocol used to create it. The MACRAMÉ Knowledge Hub will serve three main purposes:

i. fostering the timely documentation and sharing of all Project information among all consortium partners and specifically building the knowledge exchange interface between the 'characterisation and testing'-WP (WP2) and the 'Use-Case & demonstration'-WP (WP4);
ii. providing the data to the MACRAMÉ Interpreter (WP5), which will build the basis for pre-validation (i.e. first 'technical validation' done in some laboratories indicating that this method may be ready to be transformed into a Test Guideline (TG) or Standard) and validation activities and stakeholder uptake of the MACRAMÉ Project outputs; and
iii. becoming, in the long run, the interface to provide MACRAMÉ Information (method specifications, SOPs and data) as a public resource used by the community to share research outputs.

V. The MACRAMÉ Demonstrator – Analyses, Assessments & Validations in industrial Value-Chains

Based on the identified exposure points and the characterisation of AdMas in complex media performed in WP2, WP4 will conduct a detailed Material flow analysis (MFA), in order to obtain an overall assessment of the releases of materials at each stage of their lifecycle for all possible applications and the possible exposure to humans. The evaluation of AdMas degradation or transformation in the matrix of the final product, or during use and at the end-of-life or ageing under environmental conditions is, although extremely challenging, highly relevant, as currently a worst-case scenario is assumed, where AdMas stay in their pristine form. The MFA will be based on previous developments performed within NanoFASE, caLIBRAte and NanoInformaTIX to include the form of materials and their released form into MFA models. MACRAMÉ will apply these models for the first time to specific Use-Cases; the resulting data will prove the effectiveness and efficiency of the materials and products following the developed MACRAMÉ methods, which are regulatory-based and aim at cost- and time-efficient testing and hence drastically reduce time to market while addressing regulatory-based endpoints. Life-Cycle Assessment (LCA) will be applied to compare different strategies to the MACRAMÉ Use-Cases, in order to achieve safer and environmentally compliant products. To achieve this, the input from other tasks (i.e. the exposure assessment along each life-cycle stage of the product and the hazard assessment) will be applied to implement the evaluation of the potential human and environmental impact due to the (possible) release of AdMas, along each life-cycle stage of the AdMas. The application of LCA to novel materials such as AdMas is hampered by the absence of characterisation factors (CF) which allow comparison of environmental impacts of various substances. Traditionally, CF are developed based on *in-vivo* toxicological data. In NANORIGO, EMPA has proposed an integrated pathway on how *in-vitro* data can be used for the human hazard assessment of AdMas. A combined *in-vitro/in-vivo* dosimetry approach was developed which enables the extrapolation of *in-vitro* doses to human exposure levels for inhaled AdMas. A step-by-step procedure was then developed to calculate *in-vitro/in-vivo* extrapolation factors that can be used to estimate *in-vitro*-based effect factors for inhaled AdMas needed for LCA. The limited available data pool from the literature led to the conclusion that dedicated experiments are needed to improve the comparability of the results. The combined *in-*

vitro/in-vivo dosimetry approach presents a large step towards more realistic exposure conditions compared to the results from single cell lines used in the work performed in NANORIGO. The use of advanced *in-vitro* models in **MACRAMÉ** is expected to result in a much better predictiveness of *in-vitro* data for hazard assessment of AdMas. Information collected in MACRAMÉ will support the 3Rs strategy on reduction on animal experimentation and will feed risk assessment and LCA, which traditionally rely on *in-vivo* data. WP4 thus defines, demonstrates and documents the validity of the advanced **MACRAMÉ Methods** (i.e. characterisation and (quantitative) imaging and detection techniques): methodologies developed in WP4 will both embed existing SSbD approaches (EWARN (2022)) and support the SSbD strategies currently developed in EU projects (e.g. HARMLESS, SUNSHINE and IRISS), extending them to real-life industrial settings, and enabling the conceptualisation of a novel '**MACRAMÉ Safety & Sustainability Matrix**' – a mathematical matrix combining several parameters from RA and LCA with sustainability criteria to derive a value, which can be visualised by consumers in a simple colour bar scale.

VI. The MACRAMÉ Interpreter – Supporting Science towards Harmonisation & Standardisation

WP5 will elaborate the results of the MACRAMÉ Project in a fashion that is specifically useful and relevant to the standardisation community (i.e. standardisation (TR/TS) project-item proposals), and the policy-making and policy-informing communities (such as the OECD WPMN and WNT). The characterisation and test methods developed in WP2 are at a relatively low TRL level (3-5), as requested by the call. Some of the methods cannot yet be validated by an ILC or brought forward to the standardisation bodies or OECD. Therefore, **MACRAMÉ** will prepare **strategic Roadmaps for the harmonisation and standardisation of MACRAMÉ Methods**, building on the MACRAMÉ (Meta)Data, and advance them within the Project as far as possible. WP5 will coordinate the input from MACRAMÉ into new and ongoing TG-, GD- and standard-developments. MACRAMÉ´s method development will only cover some aspects of AdMas characterisation and testing. Thus, informed recommendations will be developed on future R&I needs with a focus on the MACRAMÉ Material Families and the needs for testing in (nano)medicine of market-relevant AdMas in their complex matrices and value-chains. The informed recommendations will build on the background analysis from WP1. Risk-Assessors Summits and (online) workshops will make MACRAMÉ results attractive and accessible to regulatory risk assessors.

VII. The MACRAMÉ Facilitator – Streamlined Project Coordination & Impact Maximisation

In order to efficiently support this short, but highly ambitious R&I Project through both optimum management and impact-enhancing measures, WP6 has been streamlined to provide combined scientific coordination, project management and dissemination, communication, exploitation measures for the Project, thus guaranteeing a stream-lined project-support process that maximises the performance and resources in the technical WPs (WP1-WP5).

1.2.1. Value-Addition from and to past and ongoing national and international Projects

The **established elements of the MACRAMÉ R&I processes** (i.e. sample- & control-material preparation, advanced characterisation methods, human *in-vitro*-toxicology models, and LCA/RA methodologies) are brought into the Project through the individual expertise and strengths of the MACRAMÉ Partners. Table 2 provides a list of the specific expertise that the Partners bring from past and ongoing projects, as well as the latter's central objective, indicating how this expertise will be used and further developed in the MACRAMÉ Project.

Table 2: National and international R&I activities that will be linked to the MACRAMÉ Project through utilisation of their results, adding value to their outputs and results.

Project name (*Project call*) [Coordinator, partner]. Value-Add to and by MACRAMÉ
NanoHarmony (H2020 NMBP-34) [**BAuA**, LIST, RIVM]: The liaison between research and regulators established in NanoHarmony will be continued, with a specific view to provide (a) guidance on how to translate results into regulatory relevant information, (b) a continuation of harmonisation and standardisation workshops and use and advancement of the training material, and (c) further development of BAuA´s fluidiser, standardised in NanoHarmony, to become a standardised method for creating aerosols for inhalation and *in-vitro* testing.
Gov4Nano, RiskGONE, NANORIGO (H2020, NMBP-13) [LIST, UoB, BAuA, **RIVM**, AIST, EMPA, GAIKER] The NMBP-13 project results will be used through (a) provision of a framework for the risk/hazard assessment of AdMas for the exploitation of the *in-vitro/in-vivo* MACRAMÉ results, (b) estimation of the exposure levels corresponding to the concentrations of particles used in *in-vitro* submerged systems (incl. a dose equivalence library), and (c) application of the *in-vitro*/*in-vivo* extrapolation model for toxicity data to AdMas.
PHOENIX (H2020-DT-NMBP-06) [**LIST, MyB**]: Strong collaboration with PHOENIX and utilisation of its results develops the market for the commercial exploitation of MACRAMÉ's results for inhalation toxicology of AdMas.
SCENARIOS (H2020-LG-GD) [LIST, UoB]: Building on the knowledge gained in the SECNARIOS project offers a link for the exploitation of toxicological methods to sectors other than nanomaterials and AdMas.
NanoBioDetect (German BMBF) [**IBE**, Tascon]: MACRAMÉ will build on the experience and results gained development of methods for the detection of nanomaterials lung and remote tissues; MACRAMÉ will advance the quantitative detection of gold and silver NMs tissue sections with LA-ICP-MS for the ambitious quantitative detection of AdMas in complex matrices.

Project name (*Project call*) [Coordinator, partner]. Value-Add to and by MACRAMÉ
NanoBioQuant (German BMBF) [**IBE**, Tascon, BASF]: MACRAMÉ will advance the quantitative imaging methods developed in NanoBioQuant to the quantitative detection of metal and mixed oxide NMs in tissue sections to single particle detection, subsequent SOP development, supported by ToF-SIMS for organic particle detection.
REFINE (H2020 NMBP-14) [**BIOMS**, IBE, Tascon, RIVM]: MACRAMÉ will utilised the imaging technologies for the label-free detection of organic nanoparticles, and advance these to the study of AdMas; it will develop SOPs to use human macrophages for AdMa research.
PlasticsFatE (H2020-SC1-BHC) [GAIKER]: The Project will provide characterisation and *in-vitro* hazard assessment methodologies to MACRAMÉ, as well as protocols for the integration of data for a new risk assessment (RA) strategy into the assessment of Use-Cases. The verification of methods to determine health & environmental effects of micro- and nanoplastics established in PlasticsFatE will be further developed into the predictive and preventative elements along the life-cycle of AdMa Use-Cases.
POLYRISK (H2020-SC1-BHC) [BAuA]: Results of the examination of human exposure to micro- and nanoplastics and their potential toxic effects (with an emphasis on potential adverse effects on the immune system) will enable the development of correlative microscopic techniques for identification and quantification of nanoparticles in complex media, and test the ability of macrophages to take up polymer nanofibres.
CompSafeNano (H2020 MSCA-RISE) [LIST, UoB, MyB]: The exchange and networking around assessment of engineered NMs, developed in ComSafeNano, will be widened to include the industrial network of the MACRAMÉ Use-Cases; the collaborative development of a model for exposure, hazard and risk of NMs and AdMas is planned.
DIAGONAL (H2020 NMBP-16) [LIST]: Experience in inhalation toxicity studies of NMs (especially high aspect ratio nanomaterials (HARNs)) will be utilised in the advancement of MACRAMÉ Methods towards validation and TG-/GD- and standards developments; detailed knowledge of NMs offers a link for the exploitation of toxicological methods to sectors other than AdMa/ENMs.
Flagship Graphene (H2020) [EMPA, TEMASOL, LNE, MEDICA]: MACRAMÉ will build on the established experience and results of the Flagship, through namely (a) validation of reference characterisation methods within the Graphene Flagship Validation Service (GFVS), (b) the transfer of characterisation methods to standardisation bodies (ISO/TC 229) through the Graphene Flagship Standardisation Committee (GFSC)
European partnership for the assessment of risks from chemicals (PARC) [UoB, LIST, EMPA, RIVM]: Strong links with PARC provide additional exploitation means for the technologies and solution developed (for both inhalation toxicology and characterisation) by MACRAMÉ, and provide guidance on FAIR data, and integration with IPChem and the emerging DG chemicals data portal.
CEN NOAA in the workplace [BAuA]: Work on two European Standards (CEN) for air sampling at the workplace and for exposure assessment of nanoobjects and their agglomerates and aggregates (NOAA), incl. high aspect ratio particles, based on counting rules for sample evaluation with electron microscopy will provide MACRAMÉ with a foundation for further development of the technology platform for the sampling and evaluation of aerosols.
HARMLESS (H2020 NMBP-16) [BAuA, TEMASOL]: The development of a novel, multifaceted Safe Innovation Approach to complex multi-component, hybrid nanomaterials (MCNM) as well as high aspect ratio materials (HARM) by integrating a toolbox of New Approach Methodologies will be further developed in MACRAMÉ to establish novel risk assessment strategies for HARM based on property banding.
PATROLS (H2020 NMBP-29) [RIVM, BASF]: PATROLS' development of a human bronchial epithelial cell model (co-culture with macrophages) for exposure to nanomaterials at the air-liquid interface will be further developed to aid the *in-vitro* exposure to MACRAMÉ materials at the air liquid interface
InnoMat.Life (German BMBF) [BAuA, IBE, BASF]: Experience gained in InnoMat.Life will support the development of a risk banding and grouping approach for HARM based on property banding, as well as the testing of macrophage ability to take up fibres at the air liquid interface and on fibre-laden tissue dishes.
French National Metrology Networks [**LNE**]: The French NanoPesticide Network provides tools and methodologies for the controlled aerosol generation of liquid samples to be compared to others generation systems and adapted to the MACRAMÉ Use-Cases. Other Networks provide validated methods to generate aerosol from powder samples.
EMPIR- **ISO-G-Scope** [LNE]: This EMPIR project will provide reference methodologies for the size-characterisation of few-layer graphene (FLG) and graphene oxide (GO) particles for the MACRAMÉ Use-Cases.
NANODetox (French ADEME) [**LNE**]: The project will provide MACRAMÉ with a test bench (calorimeter cone) to simulate the incineration of nanocomposites on a pilot scale, as well as its resources and expertise in the sampling and physical-chemical characterisation of gas and particles emitted during the thermal degradation process
LIFE REMEMBRANCE LIFE20 ENV/IT/001001 [**MEDICA**]: This key EU sustainability project will be the starting point of further recycling studies in MACRAMÉ; Use-Case 1 will lay the foundation for similar considerations in the other four Use-Cases.
NanoCommons (H2020-INFRARIA-02-2017) [**UoB**, 7P9-SI, 7P9-DE]: The NanoCommons project will provide tools to support safety data management across the data lifecycle, as well as a knowledge infrastructure; MACRAMÉ will advance the project's data shepherd concept, metadata tools, electronic lab notebooks, and instance maps.

Project name (*Project call*) [Coordinator, partner]. Value-Add to and by MACRAMÉ
NanoSolveIT (H2020-NMBP-14) [UoB]: MACRAMÉ will utilise the NanoSolveIT computational-ready database with API accessibility, as well as its cloud platform and user-friendly models for occupational and environmental exposure, PBPK, biomolecule corona prediction, AOP development, and risk assessment approaches.

1.2.2. Interdisciplinarity

MACRAMÉ's Objectives are pursued by a highly interdisciplinary team of leading research institutes, regulatory agencies and innovative large and small industries. **AIST**, an experienced R&I management provider, project coordinator, and science communication- and dissemination expert with an established track record in policy-assessment and -development, as well as standardisation, will enable the MACRAMÉ Project and its results through a centralised, streamlined approach. The initial and ultimately strengthened bridge between the scientific disciplines and regulatory silos pertaining to the R&I and use of AdMas will be provided by **BioMS** - a regional cluster of companies providing a central communication and information platform for scientists, entrepreneurs, investors and members of the public who are interested and engaged stakeholders in bioanalytics and nanomedicine. The five market-relevant MACRAMÉ Use-Cases are organised and have their AdMa@CMs LCA and LCC assessed by **TEMASOL** – a Swiss based SME dealing with regulatory affairs, toxicology, risk assessment and sustainability of nanomaterials and AdMas, and conventional products. The MACRAMÉ Use-Cases are provided by SME industrial players, who are currently commercialising the AdMas in market-relevant applications, or have reached advanced stages of negotiation market approval: (a) **MEDICA**: UC1 - Graphene oxide (GO) flakes in drinking-water filters; (b) **Carbon Waters**: UC2 - Few-layer graphene (FLG) in battery management systems (BMS); (c) **FILK**: UC4 - Carbon nanotubes (CNTs) in Polymer Foils for thermal Applications; (d) **MyB**: UC5 - Inhalable Antibiotics. All Use-Case-leading SMEs are providing insights into their products' life-cycle, and AdMa and AdMa@CMs samples taken from their industrial life-cycles; all SMEs are interested in detailed insights into both the safety of their products, as well as the potential for and improvement of their sustainability. The national metrology laboratory and notified body **LNE** will provide state-of-the-art characterisation and measurement techniques, and – through their role in the relevant public bodies – drive and support the dissemination of the resulting MACRAMÉ Methods into harmonisation and standardisation projects. The translation of the resulting methods and protocols into (proposals for) TG-, GD- and standard-developments will be orchestrated by **BAuA**, Germany's Federal Institute for Occupational Safety and Health, the current coordinator of the NanoHarmony (2020-2023) Project, which supports the Malta-Initiative. BAuA is a leading player in the regulation, risk assessment and characterisation of fibres. The advancement of characterisation, detection and imaging methods will furthermore be advanced beyond the state-of-the-art by BAuA and the highly specialised SMEs **IBE** and **TASCON**: TASCON is a service provider for high-end surface analytical services. IBE is a private non-profit organisation with a longstanding expertise in the field of inhalable (nano)particles and (nano)fibres whose biologic activities in the lung are described by *in-vitro* and *in-vivo* methods, which led to the development of the well-accepted alveolar macrophage assay (AMA) that can predict acute inflammatory reactions of the lung to particles. The further development and validation of the AMA (in communication with the US EPA) is supported by **BASF**, a large, international chemical company that has – for a number of years - played a leading role in the development of TGs, GDs and standards pertaining to the regulatory assessment of nanomaterials. The exploratory conduct, further development and advancement of inhalation toxicity models towards their validation is organised by **LIST**, a public research and technology organisation (RTO) active in the domains of environmental sciences, material sciences, information security. **EMPA**, the Swiss Federal Laboratories for Materials Science and Technology, **RIVM**, the Dutch National Institute for Public Health and the Environment, and **EPITHELIX**, a Swiss biotech focussing on the *in-vitro* assessment of drug efficacy and toxicity of aerosolised xenobiotics on human respiratory tract, will work with LIST on the establishment of the MACRAMÉ Tiered Lung-Models, as well as *in-vivo/ex-vivo* correlations. The LCA-Department at EMPA will furthermore support TEMASOL in the assessment of the Use-Case life-cycles. The latter is supported by strategic eco-toxicity studies, conducted by **GAIKER**, a private non-profit organisation with an established track record on the development of novel *in-vitro* methodologies both for human and environmental hazard, risk and sustainability assessment to industries, and **UoB**, whose team integrates ecotoxicity assessment and characterisation of the materials transformations under the exposure conditions, to facilitate development of quantitative structure-property relationships correlating material properties with adverse impacts. **7P9-SI** and **7P9-DE** are Slovenia- and Germany-based SMEs strongly collaborating and specialising in the development and implementation of data-management and -sharing solutions, protocol and (meta)data reporting workflows and templates, and collaboration infrastructures and platforms, and will together provide the central data- and information processing innovation (MACRAMÉ Information Hub) at the heart of the Project.

1.2.3. Gender Dimensions in the MACRAMÉ Project

The MACRAMÉ Project Partners are aware of the gender aspects associated with the acceptance of new technologies, processes and products, specifically those that promise high environmental and socio-economic impacts. Gender- and minority-relevant aspects will be considered following the EU policy and the suggested

activities on gender equality within it. This entails ensuring that gender concerns and other relevant factors of diversity (e.g. age, user/consumer preferences and needs) are integrated at the earliest possible stage, and avoiding biases in activities, where they could result in the distortion of results (e.g. consumer engagement, product specifications). MACRAMÉ will contribute to overcoming barriers for females and other underrepresented groups in the technical sector and in leadership positions, aiming to increase the female labour-market participation and the equal economic independence of women and men. This will contribute to the efforts in advancing gender-balance in decision making processes, leading to a higher uptake of safety- and sustainability measures (OECD (2021)):[1]

- Wider support of sustainability and the UN Sustainable Development Goals (SDGs) will be targeted through the measures described by the OECD study on *'Gender and the Environment : Building Evidence and Policies to Achieve the SDGs'*: (a) *SDG 4 – Quality Education* and *SDG 9 – Industry, Innovation and Infrastructure: Build resilient infrastructure, promote inclusive and sustainable industrialisation and foster innovation*: encouraging more gender diversity in STEM (science, technology, engineering and mathematics) subjects is a key driver to counteracting the existing gender imbalance in decision making, processes and innovation in these disciplines.
- A higher level of female participating supports the reaching of the following two SDGs: (b) *SDG12 – Responsible Production and Consumption*: The promotion of gender diversity in decision making roles forms an important step to innovate industrial processes and composite in the medical and maritime sector to reach higher safety and sustainability standards. Industrial Partners will be supported in the implementation of specific precautions to encourage proactive gender-diversity in the new attractive, value-add jobs resulting from the MACRAMÉ Methods; this can be achieved through gender-consideration in both job-advertising (Evidence that gendered wording in job advertisements exists and sustains gender inequality) and job-description practices.
- Special emphasis will be given to supporting the *'Women's New European Bauhaus'*, and the *'YesWePlan! Promoting Women in Architecture and Civil Engineering'*-initiative, in order to both attract more female engagement into the feedback- and engagement-seeking activities of the MACRAMÉ Project, and to disseminate its results to potential user-groups with high female ratios, due to the propensity of women to be more sensitive to ecological, environmental and health concerns and thus stronger supporters of new sustainability measures.
- LCAs and sustainability-studies will be conducted following the OECD *Gender Policy Platform: Accelerating Gender Mainstreaming*, and using the *OECD Toolkit for Mainstreaming and Implementing Gender Equality*).
- Special precautions will be taken during the conduct of research into the biological toxicity models: (a) The gender component of the lung cell response will be considered: the donor samples will be sourced from 50/50 male/female donors; the relevant literature will be surveyed within the framework and such differences will be considered in testing phases. (b) Upper airway models will be reconstituted with pool primary cells with balanced male/female donors. Primary alveolar models will be made from male and female donors separately, the corresponding study will include gender equality consideration. (c) Ecotox experiments with Daphnia will be performed using both males and female equally. MACRAMÉ aims to overcome gender-effects in the following cases: (i) NR8383 cell lines used for AMA are derived from male Sprague-Dawley rats with the help of conditioned media prepared from male gerbil cells (Helmke *et al.* (1987)). (ii) the efficacy of oral broad-spectrum antibiotics (incl. ciprofloxacin) varies due to gender-related determinants, such as microbial and inflammatory responses.

A **MACRAMÉ Privacy, Ethics and Gender Committee (MACRAMÉ PEGC)** will be formed as part of the Project governance; it will be led by the Project Coordinator (PC) and include the Project Secretary (PS) and members of the External Advisory Board (EAB), as well as WP-Leaders. It will oversee the development of the **Project-internal MACRAMÉ Ethical Guideline** (Task 6.3, M06), and regularly review and update these, if deemed necessary; WP-Leaders will be responsible for its implementation within their WPs. PEGC meetings will be scheduled to take place at every in-person Project Meeting; additional, extraordinary meetings may be arranged, if necessary. The MACRAMÉ Ethical Guideline will outline the underlying activities that the Project team must consider at all steps of their R&I activities, and describe processes for reviews of privacy-, ethics- and gender-issues as and when these become necessary; these activities and processes include review of occupational safety & health (OSH) in the market sectors impacted by the MACRAMÉ Project (e.g. personal protective equipment) (OSHA)).

1.2.4. Open Science in the MACRAMÉ Project

MACRAMÉ is fully committed to follow Open Data and Open Science policies, as well as a proactive strategy for FAIR knowledge management and sharing of (meta)data (incl. materials and sampling plans, study designs, method specifications including the measurement principles, protocols and SOPs), and data (raw, transformed and robust summary) using community data documentation standards (CHADA, NanoFASE/NIKC (NIKC: CEINT NanoInformatics Knowledge Commons, Duke University)) and their extensions. To fully implement the transition from the Open Data initiative in H2020 to the Open Science initiative of HE, MACRAMÉ will further extend this approach to all other research output including software, guidelines, reports, training materials and publications. As

[1] Although not asked for here, the MACRAMÉ Consortium is proud to surpassed gender parity in both the participation of all researchers, as well as female leaders at the Partners (10/19), and female WP-Leadership (4/6).

open access (OA) is a core strategy by the EC to improve knowledge circulation and reusability, all MACRAMÉ publications will be OA, following EU guidelines for OA to peer reviewed scientific publications to adopt a combined OA strategy, allowing maximum impact without increased costs. Green OA channels allowing free OA will be preferred but if institutional regulations or embargo periods impact accessibility of Project results, partners have reserved internal budgets for providing public access to their papers through the gold OA. The Project will use self-archiving (or green OA) services like ResearchGate and Academia and institutional repositories, allowing a balance between traditional publications and OA. Articles and deliverables will be made available in suitable repositories, adhering to possible time restrictions (embargo period). Following the HE guidelines, all Project publications will be made available *via* OpenAIRE and its open research repository Zenodo; training material and tutorials are protected by copyright and by using an appropriate CreativeCommons licenses.

1.2.5. MACRAMÉ Data Management & Management of other Research Outputs

As a part of the open science philosophy, high quality data management will be implemented using state-of-the-art data sharing concepts, approaches and tools and fully implementing the FAIR principles. A research output management plan (ROMP), an extension of data management (DMP), will be generated at the start of the Project and continuously updated. The ROMP will consider all output of the Project. According to the EC recommendation, as part of making data findable, accessible, interoperable and re-usable (FAIR), the ROMP will include information on: 1) handling of research outputs (RO) during & after the end of the Project; 2) what RO will be collected, processed and/or generated; 3) which methodology and standards will be applied; 4) whether RO will be shared/made OA; 5) how RO will be curated and preserved (including after Project end). A key set of outputs from the Project will be (meta)data standards for circular materials, which will be given a priority treatment with respect to public and open sharing to achieve global uptake by the community and adoption into European and international standards. Personal data as part of the scientific data will only be collected in the form of information on the data generator (names, institution, addresses including email required according to the FAIR principle R1.2) and we will take particular care of applying state-of-the-art security measures and treating such sensitive information according to GDPR regulations as outlined in the Ethics section in Part A. Other situations, in which personal data will be collected, include user management for data access (name and email) and participation in training events, workshops, conferences organised by MACRAMÉ, and expert groups (name, institution, type of institution and countries). This information will again be handled according to GDPR, only be visible to the provider of the service or organiser of the event and will under no circumstances be shared with third parties or even with others within the consortium. The use of artificial intelligence approaches will be limited to literature mining and material property prediction. Development and implementation of the ROMP will be coordinated and monitored by 7P9-DE as part of the data shepherd services and supported by FAIRification tools developed and provided by 7P9-SI in coordination with all partners. MACRAMÉ will make use of existing and emerging open and FAIR repositories, fostered by the early implementation of improved data reporting guidelines and high-quality data management practices that will allow for the direct upload of the outputs to public repositories for method descriptions (in the form of CHADA repositories) and community-endorsed public data warehouses NanoCommons and eNanoMapper. MACRAMÉ will seek collaboration with the providers of these resources to integrate extensions needed to cover nanomaterials in complex environments. A license (Creative Commons, Open Data Commons) will be associated with each research output to clearly defined options for data sharing and re-use.

To protect commercial exploitation, the ROMP will balance openness and protection of information, commercialisation and IPR, privacy concerns, security as well as data management, fostering protection through patenting prior of publication. Confidential data will be kept restricted unless permission is granted by the data owner. However, metadata describing the data will be made open available at the earliest stage to allow others to get information on the data even if it is not yet publicly released or is not meant to be because of commercial interest. Access can then still be provided to interested parties based on individual agreements.

2. Impact

2.1. MACRAMÉ's Pathway to Impact

The following section explains how MACRAMÉ will (a) achieve the specific IMPACTS arising from the (expected) outcomes of the topic (IMPACTS 1 to 6), and (b) provide stepping-stones to reaching higher targets (e.g. Key Strategic Orientations (KSOs)) of the underlying **Destination of the Horizon Europe** work programme (IMPACTS 7 to 9). The narrative follows the assignment of specific MACRAMÉ High-Level Objectives (see Section 1.1), in that each one corresponds to a strategic Project activity towards the following impacts (cf. Summary Table, Section 2.3). To ease contextual reading, each IMPACT listed below also discusses its achievement-indicative target values (i.e. 'KPIs'), MACRAMÉ Actions to reach these targets (i.e. 'Action'), barriers and obstacles to achieve the Impact (i.e. 'Barriers/Obstacles'), and mitigation actions (i.e. 'Mitigation') taken by MACRAMÉ to overcome the latter.

IMPACT 1: Develop high-resolution imaging methods for quantification and characterisation of nanomaterials and advanced materials (AdMas) in complex matrices and determinations of their transformations in such environments.

MACRAMÉ's focus on **inhalable carbon-based materials**, representative of **five market-relevant, industrial Use-Cases**, aims to address the challenge of detecting, characterising and quantifying such AdMas within product-relevant, biological, and environmental complex matrices for toxicity/ecotoxicity studies, representative of relevant exposure points. Scientific impact: The results obtained will allow the **development and validation** of the **applicability and limitations** of complementary **high resolution imaging methods**, and their correlation towards **advanced automated image analyses** beyond classical materials (i.e. inorganic spherical particles). Their advance for the characterisation and quantification of inhalable carbon-based materials in relevant matrices will provide the scientific community, industry and regulators, with guidance and means to reach a fundamental understanding of the key attributes of inhalable carbon-based materials, to determine their dynamic changes in polymeric composites at different life-stages and in relevant biological environments, in relation with their toxicological assessment.

IMPACT 2: Increase availability of validated protocols to advance both safety studies of advanced materials (including nanomaterials) and material characterisation.

MACRAMÉ Methods will be developed/advanced and **validated** (with respect to robustness, repeatability and reproducibility); the strengths and weaknesses and details of constraints and limits of detection (both material- and particle-size-specific) will be identified. Scientific impact: The results obtained by MACRAMÉ will increase the availability of **SOPs**, which will be **proposed for regulatory validation**, in the following key areas: (i) detection & characterisation of AdMa@CMs, including physical-chemical stabilities & (bio)transformation; (ii) characterisation of potential human hazard upon inhalation, including protocol for generation and characterisation of aerosols; and (iii) assessment of environmental impact and eco-toxicological potential. To support these activities, a materials library, the **MACRAMÉ Control Material Library (CML)**, will be established to provide benchmark materials and Use-Case materials for the R&I activities. The CML will be offered to the community to support future studies on safety and characterisation of AdMa(@CM)s. All methods and assays developed will be subjected to laboratory validation to assess their **validity, reliability and robustness** prior to initiating standardisations activities (IMPACT 5), and include methods for sampling, toxicology, ecotoxicology, and data-handling.

IMPACT 3: Ensure appropriate Control Experiments and more realistic *in-vitro* Models to address current Gaps in the (Eco)Toxicity Testing of AdMas and Nanomaterials

A **MACRAMÉ Control Material Library (CML),** which will comprise materials with different physical-chemical properties and known biological effects, will be created; the CML will be made available as **a new service to collaborating and future projects**, as well as the standardisation community. The Project's **biological systems are based on existing technologies** at different TRLs, with some being **commercially available, and validated on chemicals**. All models and methods will be benchmarked against the CML, which will contain well-characterised reference materials, materials of known toxicological effect, and AdMa(@CM)s, collected from the **Use-Cases; it** will allow the **benchmarking and testing of the characterisation and human/eco-tox hazard assessment methods**, thus ensuring optimal experimental controls.

IMPACT 4: Deliver reliable Data and improved Data-Reporting Guidelines supporting direct Use in computational Modelling for Grouping and Read-Across Methods. Make Use of Open Access Database, using Standards for Data Documentation (e.g. CHADA).

Data-driven knowledge transfer between technology developers, data providers, modellers and data users is essential to provide traceability, integrity and interoperability and in this way, **generate confidence and trust in industries** demanding these technologies. This will be fostered in MACRAMÉ by **adopting highest-quality method specification and data reporting standards** and providing a **centralised data and knowledge integration and exchange framework**; a state-of-the-art **data repository** and **digital data transfer technology (API)** will allow direct integration of the data in computational approaches profiting from the better predictive power of the new characterisation techniques. Training and support actions will provide the workforce with the required skills to use the new digital technologies in industrial setting, and to subsequently act as multipliers for re- and upskilling.

IMPACT 5: Increase the Efficiency and Effectiveness of Materials and Product Development by reducing Costs and Time for Product Design, Time-to-Market and regulatory Compliance.

The founding of the MACRAMÉ R&I Strategy on **five market-relevant, industrial MACRAMÉ Use-Cases** proves the Project's focus to develop and advance those characterisation and testing methods that **support industrial R&I and manufacturing processes** (especially for SMEs), because they offer either **cost reductions** to the regulatory approval process (e.g. the MACRAMÉ Tiered Lung-Model approach), or **predictive capabilities** that can be deployed early in the design and R&I stages (e.g. the modelling-supported approaches for grouping and read-across, the **MACRAMÉ Decision Tree for environmental health assessment**, and the **MACRAMÉ Safety & Sustainability Matrix**). This Project will provide access to validated methodologies in an industrial setting to

optimise the characterisation and regulatory assessment of AdMas, and thus support the de-risking of R&D activities. The validity of this claim will be demonstrated by **dedicated life-cycle costing activities** for each **Use-Case**, including the evaluation of cost for characterisation and testing using conventional and MACRAMÉ methodologies.

IMPACT 6: Develop novel and advance existing Methods & Methodologies towards harmonised/standardised Test Methods that can be used in a regulatory Framework (incl. Hazard & Sustainability Assessments for AdMas).

To ensure the maximum impact and engagement with industrial actors, policy-informing and –making, as well as standardising bodies, a strategic **MACRAMÉ Engagement Roadmap** will be defined to enhance the chances of acceptance and advancement of the developed methods toward standardisation and harmonisation; the latter is supported by the **needs assessment of policy frameworks**, in connection with **two Risk Assessors Summits**. **MACRAMÉ Methods** are advanced for harmonisation & standardisation by the preparation of the strategic **MACRAMÉ Summary Roadmap** (consisting of different methods, and thus addressing different harmonisation/standardisation destinations) showing possible implementation of the methods in risk assessment and testing for regulatory purposes. Early in the Project we will connect and engage relevant working groups, such as VAMAS, OECD, CEN TC 352, ASTM E56 and ISO TC 229, to which we will submit the methods and assays developed within MACRAMÉ to start the process that leads to regulatory validation.

2.1.1. Pathways towards wider impact.

The MACRAMÉ Project furthermore makes some unique contributions towards a number of Key Strategic Orientations (KSOs) of the underlying Workprogramme Destination:

IMPACT 7: Leading the Development of key digital, enabling and emerging Technologies, Sectors and Value-Chains to accelerate and steer the digital and green Transitions through Human-Centred Technologies and Innovations

MACRAMÉ's technologies help to avoid health and environmental risks of AdMas and will make important contributions to transform Europe into the **first digitally led circular, climate-neutral and sustainable economy** (**KSO A and C**). The new characterisation- and test-methods will enable highly accurate and cost-efficient assessment of health and environmental effects considering all life-cycle stages of the materials. In combination with the digitisation of the processes with respect to data handling but especially also replacing animal-based methods by integrated *in-vitro*/*in silico* approaches, this will lead to a more effective and efficient materials design process, reduce costs and time-to-market and aid regulatory compliance. Corresponding **new business models**, like the '**Materials as a Service'**-concept **promoting collaboration and alliances (academia/SME/industry)** and **trust-building** through digital service, will support the building of a dynamic innovation ecosystem, where experts can work together, based on global and secure data-driven knowledge exchange. Applying this system to the design of AdMas, nanomaterials and their products creates **opportunities across industry sectors**, sustaining and enforcing European **industry leadership across the digital supply chain**. MACRAMÉ's centralised (meta)data-handling approach provides an important stepping-stone towards this wider impact, but the community of contributors and users and the acceptance of the concept must grow significantly, for the digital and green transition to have a lasting impact.

IMPACT 8: Creating a more resilient, inclusive and democratic European Society

Due to the digitisation and harmonisation of the MACRAMÉ technologies, they can become part of **Industry Commons** and, in this way, be integrated into the **European (digital) ecosystem and manufacturing landscape**. New collaborative, multi-stakeholder business models can be implemented **starting with MACRAMÉ concepts** (e.g. Data Stewardship, Information Hub, Safety & Sustainability Matrix) and **continuously adding other services addressing high environmental and socio-economic impacts**. Integration of expertise and data is not limited to modelling and characterisation techniques for safety assessment, but can extend to many other areas like SSbD, LCA and ultimately become central to supporting the **green and digital twin transition**, enhancing innovation and growth potential, fostering economic and social resilience and ensuring quality employment and social inclusion (addressing both *Destination 6 - A human-centred and ethical development of digital and industrial technologies* and *Destination 3 - World leading data and computing technologies*). Especially the **Project's foundation on market-relevant, industrial Use-Cases** supports the integration of industrial processes into innovative, future-oriented value creation, thus serving the philosophy of IndustryCommons and especially the New European Bauhaus situated at the crossroads between art, culture, social inclusion, science and technology.

IMPACT 9: **Graphene: Europe in the lead** - strengthen and accelerate the Technology Developments that support a strong European supply and value chain in graphene and related materials.

Most importantly, the MACRAMÉ Consortium decided to work with graphene (and GRMs) as one of its AdMas of focus, in order both (a) to build on and support the Graphene Flagship and its excellent results beyond the end of its funding period, and (b) anticipate the concern of 2D materials as an upcoming regulatory issue of advanced materials (ECHA (2021)). Through its **focus on market-relevant graphene-related materials**, the MACRAMÉ Project aims to make an initial contribution to secure the safe and sustainable development of graphene-based technologies, in order to now pursue and accelerate the achieved technology advances into *'concrete innovation opportunities and*

into production capabilities in many industrial sectors (e.g. aviation, automotive, electronics, batteries, healthcare), [and to thus] strengthen and accelerate the technology developments that support a strong European supply and value chain in graphene and related materials and provide first-mover market advantages of scale,' as outlined in *Cluster 4, Destination 4 'Digital and emerging technologies for competitiveness and fit for the green deal'*.

2.2. Summary of the Project's Pathway towards Impact

SPECIFIC NEEDS	EXPECTED RESULTS (see also Error! Reference source not found.)	D & E & C MEASURES (see also Error! Reference source not found.)
N1: Characterisation, detection and imaging technologies for AdMas in complex matrices **are needed to keep up with the rapid increasing demand for new materials. For showing their reliability, appropriate control experiments are required and data must be made available, supported by improved data reporting guidelines for their use in** grouping and read-across**.**	**R1:** (a) at least 5 sampling protocols to collect, transport and store AdMas or AdMa-containing materials: (b) SOPs developed by WP2; *Reports:* Performances & regulatory Readiness of SOPs for Aerosol Generation; Performances of High-Resolution Imaging methods for the Quantification of AdMa@CM; Characterisation and Hazard Assessment in 5 MACRAMÉ Use-Cases **R2:** At least eight methods evaluated for their applicability in R&D & assessment tools **R3**: (a) Information Hub and 'Data Exchange Format'-Specifications and initial ROMP; (b) MACRAMÉ Data-Reporting Guidelines (incl. Reporting Templates) *Reports:* Data Provision for Method Optimisation and Use-Case Completion; Data Preparation & Quality Control for Grouping & Read-Across	Dissemination to other national and international projects, as well as industries, consultancies, CROs and academic scientists.
In addition to N1 **N2:** The technologies have to be made **industry-ready** by providing applicable and validated protocol for the safety testing and translated into **time- and cost-efficient** regulatory safety-assessment tools and methods, in order to support industrial materials R&I and manufacture.	**R4:** (a) sampling protocols of R1, (b) Life-Cycle-based Evaluation of AdMas in complex Matrices; (c) Life-Cycle-Inventory Data-Set and Characterisation Factors of the AdMas in market-relevant Use-Cases; *Reports:* Ecotoxicity (Hazard) & Risk Assessment of Use-Cases (incl. Decision Tree); Environmental & economic Sustainability of Use-Cases; Consumer-friendly 'MACRAMÉ Safety & Sustainability Matrix' **R5:** (a) Scientific document to support the development of a Guidance Document on inhalation toxicology; (b) Recommendations on future Needs for Test Guideline- & Standards Development	• Dissemination to NGOs & CSOs • Dissemination to industries, consultancies, CROs and academic scientists
In addition to N1+N2 **N3:** The advanced characterisation-/ detection-/ imaging- and (eco)tox-methods must be **harmonised / standardised for use in regulatory Frameworks.** **N4:** This needs to be fostered by enabling the scientific community to **better understand the process** and engage and adopt more.	**R6:** (a) Needs Assessment Report of Regulation & Policy Frameworks; (b) MACRAMÉ Harmonisation & Standardisation Roadmap Summary Report for MACRAMÉ Methods and Models; *Reports and scientific doc.*: Performance & regulatory Readiness of characterisation methods (multiple); Development of a GD for in-vitro Hazard Assessment; Ecotoxicity (Hazard) & Risk Assessment of Use-Cases (incl. Decision Tree)	• Submission of the scientific documents and recommendations to harmonisation (OECD) and standardisation (VAMAS, CEN/ISO) bodies. • Dissemination to international organisations (policy-informing), as well as policy-makers and regulatory bodies.

TARGET GROUPS (see also Error! Reference source not found. and Error! Reference source not found. & Error! Reference source not found.)	OUTCOMES	IMPACTS
G1: National and international R&I projects **G2:** (environmental) consultancies, CROs, academic scientists	• Sampling and Sample-Provision Procedures of **representative inhalable Materials** from the five Use-Cases established • **Life-Cycle-Inventory & Characterisation Factors** (CFs) of the AdMas in Use-Cases established • In-vitro & ex-vivo models for **toxicity assessment** established and qualified at respective premises • Draft version of SOPs for exposure and characterisation measurements available • **Data Stewardship & Quality Control** established within the MACRAMÉ Consortium and embedded *via* ongoing data management training and support • Human- and machine-readable **data-exchange formats** defined and fully integrated into data reporting workflows at partner institutions	**I1:** Develop **high-resolution imaging methods** for quantification and characterization of nanomaterials and advanced materials (AdMas) in **complex matrices and determinations of their transformations in such environments** **I3:** Ensure appropriate **Control Experiments** and more realistic in-vitro Models to address current Gaps in the (Eco)Toxicity Testing of AdMas and Nanomaterials **I4:** Deliver reliable data and **improved data reporting guidelines** supporting direct use in computational modelling for grouping and read across methods. Make use of open access database and using standards for data documentation (e.g. CHADA).
In addition to G1+G2 **G3:** Industries commercialising AdMas & AdMa-containing products & processes	• MACRAMÉ Information Hub fully operational • MACRAMÉ **SOPs and data available** in public repositories and data warehouses • **Life-Cycle-Inventory dataset** & Development of CFs of the AdMas in Use-Cases established • At least **five MACRAMÉ Methods and Methodologies** have been identified **for TG/Standards Development** and strategic development of their topics mapped	**I2:** Increase **availability of validated protocols** to advance both safety studies of advanced materials (including nanomaterials) and material characterisation. **I5:** Increase the **efficiency & effectiveness of materials and product development** by reducing costs and time for product design, time-to-market and regulatory compliance.
In addition to G1-G3 **G4:** Standardisation communities (national, EU, international) **G5:** International organisations (policy-informing) **G6:** Policy-Makers (national & EU)	• At least five MACRAMÉ Methods and Methodologies have been identified for TG/Standards Development and strategic development of their topics mapped	**I6:** Develop novel and advance existing Methods & Methodologies towards **harmonised/standardised Test Methods** that can be used in a **regulatory Framework** (incl. Hazard & Sustainability Assessments for AdMas).

3. Quality and Efficiency of the Implementation

Resilience in Project Implementation: SARS-COV2 pandemic protection measures have highlighted the difficulties in efficient and productive collaborations when conduced in distributed infrastructures. The MACRAMÉ Project will minimise the risks of potential disruption to the Project's workflow by gathering all Partners in WP1 in the first two Project months, where the **MACRAMÉ R&I Strategy**, outlined in this proposal, will be collaboratively re-confirmed and further detailed (and benchmarked), beyond what is possible in this proposal.

The work that is to be conducted within the MACRAMÉ Project over the duration of **36 months** has been organised into six work packages (WPs) that are interconnected through a time- and content-dependent workflow. The PERT-Chart in Figure 8 below provides an outline of the MACRAMÉ Project structure, indicating the workflow between the individual WPs.

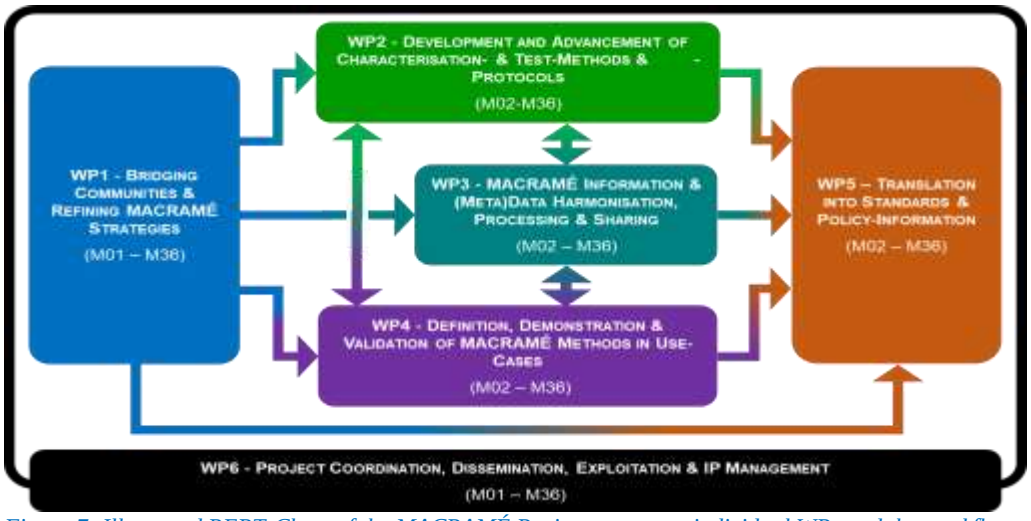

Figure 7: Illustrated PERT-Chart of the MACRAMÉ Project structure, individual WPs and the workflow and dependencies between them.

3.1. Workplan and Resources

Figure 9 below provides a Gantt-chart, indicating the start- and end-months of each WP and subordinate Tasks within the WPs, as well as the Project Milestones (blue boxes).

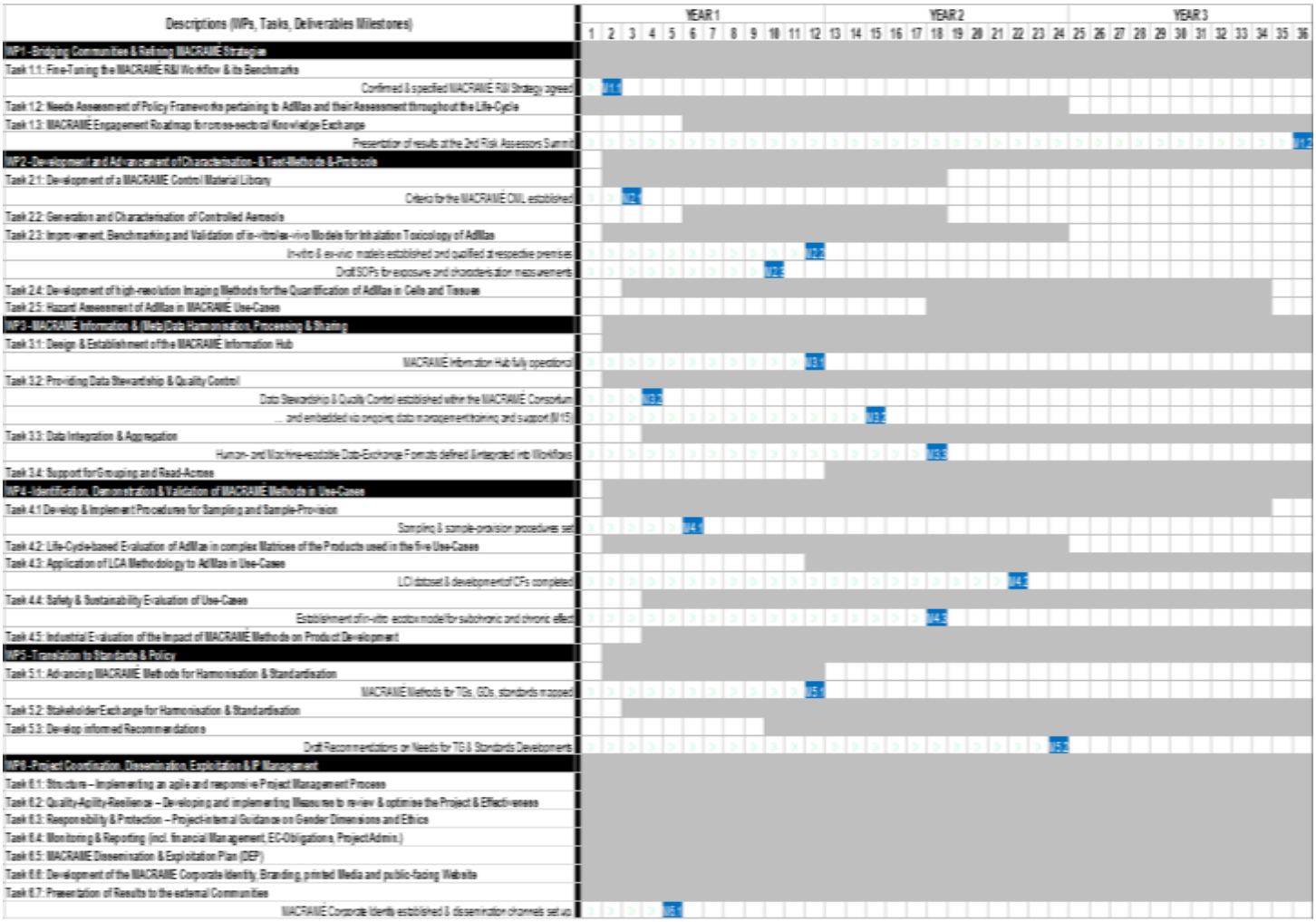

Figure 8: Gantt-Chart of the MACRAMÉ Project.

3.2. Capacity of Participants & Consortium as a whole and Roles in the Context of the Project

Figure 10 outlines the workflow between the MACRAMÉ Partners, highlighting their main roles and the Project milestones; the table below describes the Partner roles, as well as their exploitation/dissemination interests.

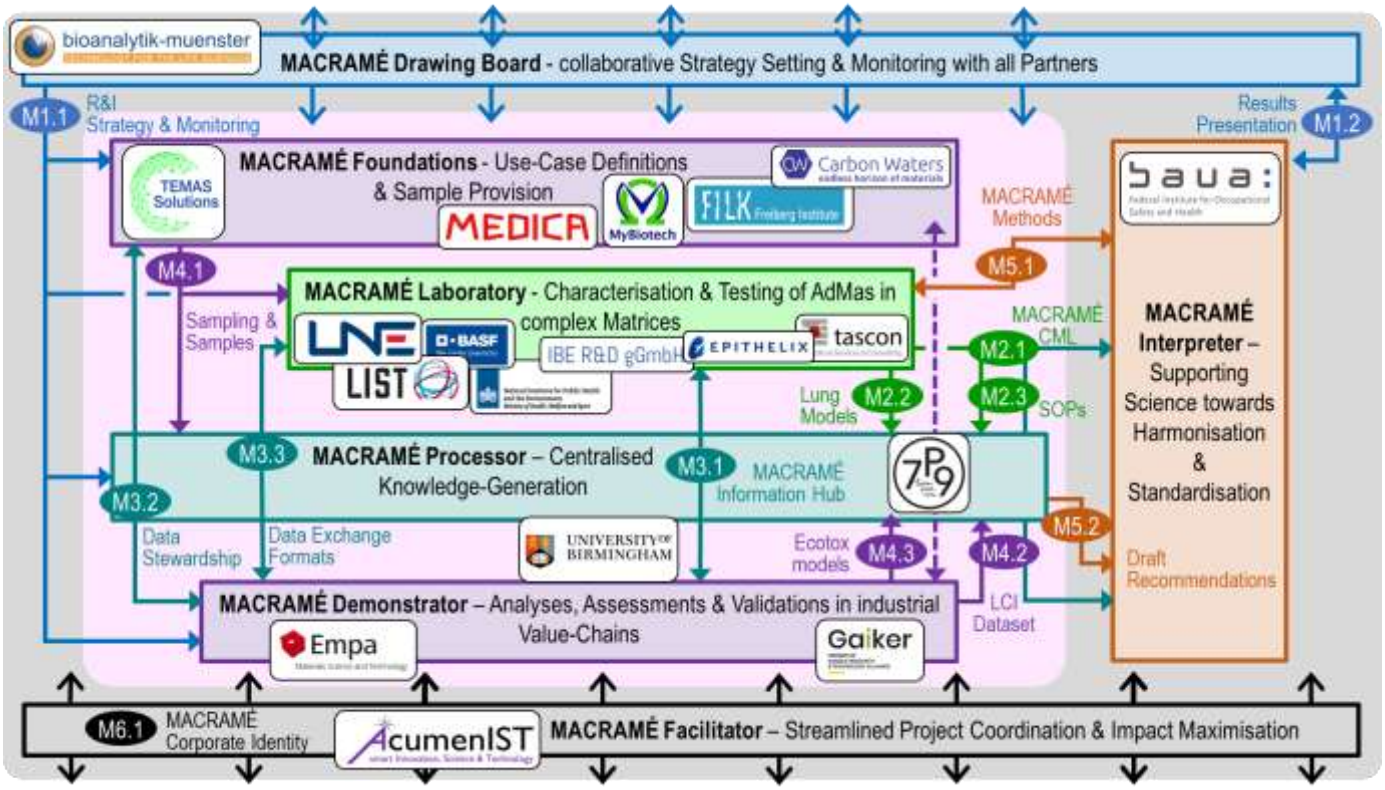

Figure 9: Schematic overview of the MACRAMÉ Project (in WPs, Deliverables and Milestones) and the main Partner Roles.

Partner (Short Name) & Description & main Role in the MACRAMÉ Consortium
AIST has a strong track record in materials-science and R&I-management, public and regulatory affairs, harmonisation & standardisation, and policy-assessment/-development, and provides MACRAMÉ with streamlined scientific coordination, administration and targeted dissemination-, communication- and exploitation- management, thus widening the collaborative network of both the AIST concept consultancy and the new NanoFabNet Hub.
BioMS provides state-of-the-art scientific, technical, regulatory, and political background for the external alignment, finetuning and guidance of the work in all WP, to establish interfaces between communities and regulatory frameworks for the complete life-cycle of AdMa and AdMa-bearing products (WP1). BioMS will thus widen its network and business-support portfolio to include services for the next generation of materials
LNE leads the advanced characterisation approaches of AdMa(@CM)s, based on its expertise in nanoparticles aerosol generation, airborne particles characterisation, and simulation of end-of-life scenarios. As France's NMI and Notified Body, LNE will use its strong links to standardisation, harmonisation and policy-making & -informing bodies. LNE' role in MACRAMÉ strengthens its NanoMesureFrance Centre in connecting industry and regulatory bodies.
LIST provides advanced *in-vitro* models and expertise for inhalation toxicology, physical-chemical characterisation of AdMa(@CM)s, regulatory support, and standardisation & harmonisation activities. Based on a fully equipped infrastructure, LIST developed several *in-vitro* alternative models (with 3 under validation) and will exploit the MACRAMÉ models in projects, and to provide services and support companies through spin-offs and licenses.
7P9-SI and 7P9-DE designs (meta)data formats, data warehouses, web applications for R&I in life science, toxicology, and chem-, bio- and nano-informatics. The 7P9-Collective will guide MACRAMÉ in community and regulatory reporting standards, through its data shepherd consultancy services, which – in turn – will be widened to include AdMas and novel ontologies for their complex matrices, thus allowing exploitation in future research and commercial projects.
TEMASOL specialises in LCA, toxicology and human and environmental risk assessment. Having developed a Safe-by-Design and leading the corresponding standard under CEN/TC 352, and the SafeGraph Project. Through its strong links to various industries, TEMASOL leads the Use-Cases and aims to exploit the Project results in consultancy services regarding SSbD and regulatory affairs to industry in the chemical, cosmetic and medical device sector.
BAuA coordinates MACRAMÉ's harmonisation of characterisation and test methods, as the secretariat of the Malta-Initiative, and drives the Project's study of the fibre-paradigm and corresponding aerosol generation method. Through close contact with scientific and industry partners throughout Europe and international regulatory bodies (incl. US, South Africa and Korea), BAuA disseminates the Project to international decision-makers.

Partner (Short Name) & Description & main Role in the MACRAMÉ Consortium
RIVM contributes to benchmarking and demonstration of cell-line-based *in-vitro* assays for initial screening of inhalable materials. RIVM will broaden its activities and expertise in transregulatory activities on nanosafety, nanomedicine (i.e. Gov4Nano, REFINE) to include AdMa(@CM)s, thus strengthening its lead of the OECD WPMN.
EMPA provides advanced *in-vitro* models and expertise for inhalation toxicology, hazard assessment, RA and SSbD, and standardisation and harmonisation, integrating state-of-the-art infrastructures and platforms to assess abrasion and thermal decomposition, and parallel 'emission/acute lung'-cytotoxicity. Through long experience in the exploitation of models and tools (NanoBioMat, LICARA), EMPA will support SMEs with the MACRAMÉ results.
IBE conducts and intends to validate the AMA, prepares biological samples for quantitative imaging with LA-ICP-MS, conducts DFM/HSI/Raman bioimaging, and pre-evaluate biological samples for ToF-SIMS analyses, and develops innovative and cost-effective testing strategies for aerosolised fibres deposited in µ-dishes. The latter technique will be exploited through appropriate IPR. IBE owns all necessary equipment.
TASCON develops and performs ToF-SIMS on raw materials, develops and performs ToF-SIMS imaging on biological samples pre-characterised by IBE. It owns three ToF-SIMS 5 and uses one Hybrid-SIMS instrument. Knowledge gained in the project will improve TASCON's services to academic and industrial partners. Exploitation of results will be performed by peer-reviewed publications and contributions to scientific conferences.
GAIKER leads the development of novel ecotox tests at relevant Exposure Points for the Use-Cases. Owning all necessary infrastructure, GAIKER's aims to transfer technologies and offer technological services to its Foundation members and industrial clients. The assays and methods developed and ready for standardisation will be incorporated the service portfolio, thus helping industries to reduce costs, and time to market in regulatory compliance.
UoB provides data shepherding to all partners, including dataset curation, annotation and compilation for modelling / ML (NanoCommons), and conducts ecotox testing on the Use-Case AdMa@CMs, based on fully equipped labs for cytotoxicity and materials characterisation. As coordinator of the NanoSafetyCluster, and partner in PARC, UoB will disseminate the results to both the academic and regulatory (nano)materials- and chemical safety communities.
MyB provides ciprofloxacin loaded PLGA NPs and know-how for inhalation therapy, produced by following ICH guidelines under GMP like conditions (Use-Case 5), physical-chemical characterisation of those particles and preparation of surrogate (controle) particles. A successful biotech-SME with international presence, MyB plans to use the Project results to transfer its ciprofloxacin-loaded PLGA NPs to GMP-environments for human trials.
Epithelix provides standardised and commercially globally available *in-vitro* models based on primary human cells and performs *in-vitro* evaluation of impact airborne substances on human respiratory tract models. The SME aims to achieve validation as part of a tiered approach, offering efficiency and cost-reduction to the industries.
BASF conducts the AMA and validates the assay in a round robin together with IBE and LIST, while its analytical department performs quantitative imaging with LA-ICP-MS of the exposed samples to establish dosimetry in the macrophage assay. BASF is highly interested in establishing and validate *in-vitro* methods, to support its own material-development work, as well as the practices and standards at its suppliers and customers.
MEDICA provides market-relevant AdMas and AdMa@CMs for water filters to soon be applied to end-user markets (e.g. building developers) (Use-Case 1), by grating Partners access to the production area for sampling of process- and waste-water, in accordance with hydraulic and recirculation plants' designs. MEDICA exploits the results to 'close-the-circle' of the product's sustainability, in line with its Medica Water Division business plan.
Carbon Waters provides Use-Case 2 (i.e. FLG in BMS) through the production of FLG dispersions and samples of epoxy resin (with FLG), following in-house quality-control through Raman, UV Visible as well as rheological measurements of the formulations; The Project results on the (eco)tox and LCAs will be exploited in proprietary Graph'Up-enhanced epoxy resins for thermal management systems and presented at carbon-based materials conferences.
FILK provides Use-Case 4 (i.e. CNTs in thermoplastic polymeric systems for heating applications), by providing samples at every stage of the life-cycle of the AdMa(@CM)s, created through a roll-milling process to manufacturing of conductive polymer composite films. FILK will pass the resulting safety- and LCA-information to its customers, and publish them in peer-reviewed journals, and its annual report, and present them at international conferences.
JRC will be invited to accession (in case of Project funding), to continue the established collaboration with the JRC's expertise both in (a) read-across / grouping, (b) dissemination to high-level groups, (c) information exchange and collaboration with ongoing initiatives, and (d) support of methods development and validation. [NOTE: failing the accession, MACRAMÉ will involve two JRC representatives in its EAB (see below, Section 3.2.1).] [Update 22.08.2022: In agreement with the JRC representatives Susanne Bremer-Hoffmann and Juan Riego Sintes, it was decided not to pursue a formal accession of the JRC (i.e. JRC's formal entry as 'Associated Partner' into the grant agreement), and to instead invite the JRC to join the MACRAMÉ Consortium through a collaboration agreement (i.e. JRC becoming party to and signatory of the MACRAMÉ Consortium Agreement); until the latter comes into force, and in case it is not possible for the JRC to join the MACRAMÉ Consortium in this way, Susanne Bremer-Hoffmann and Juan Riego Sintes will remain members of the MACRAMÉ External Advisory Board (EAB).]

3.2.1. MACRAMÉ External Advisory Board (EAB)

An External Advisory Board (EAB) has been established to support the Project during its duration. Table 3 lists the EAB Members, who provided written agreement to the corresponding ‚Experience & Role'-description. The EAB will participate in meetings (i.e. invited presence at the in-person events), stakeholder workshops and Risk Assessors Summits, if appropriate. [2] The EAB is responsible for (a) evaluating the overall progress of the Project, (b) providing input on specific matters of their expertise, (c) advising the Project of any noteworthy, external developments with regard to its aims and objectives, and (d) supporting the dissemination of Project results to their networks.[3]

Table 3: Members of the MACRAMÉ External Advisory Board (EAB) and their expertise and roles in the Project.

Name (Affiliation): Experience &Role in the MACRAMÉ EAB
Dr Anke Jesse (German Federal Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection): Chair and initiator of the Malta-Initiative (MI), Dr Jesse will enrich the EAB through direct advice on the Project's objective not only provide a seamless continuation of the MI beyond the end of the NanoHarmony project (March 2023), but to also widen its demonstrated applicability to market-relevant AdMa@CMs.
Dr Annie Jarabek (United States Environmental Protection Agency (US EPA), CPHEA at HEEAD): An inhalation toxicology with an international regulatory view-point, Ms. Jarabek will provide expert advice to the Project's activity on (a) further developing inhalation toxicology models including IATAs to for efficient industrial compliance, and (b) quantitative (Q)IVIVE investigations to support translation across experimental platforms.
Dr Jose Maria Navas (INIA-CSIS): Dr José M. Navas is a Spanish representative at the OECD-WPMN and at the ECHA NM-WG and has also been named member of the ECVAM Scientific Advisory Committee (ESAC). He will support the application of the ecotoxicity *in-vitro* methodologies and models.
Dr Fernando Castro (Versailles Project on Advanced Materials and Standards (VAMAS) & National Physical Laboratory (NPL)): Chair of VAMAS, Dr Castro will provide dissemination-support to the Project on matters pertaining to (pre-)standardisation and harmonisation of results; Head of Science for Materials at NPL, a Core3 partner of the Graphene Flagship, he knows the AdMas and their products studied in the MACRAMÉ Use-Cases.
Eckhard Schwenner (Healthtech Translation Advisory Board (EIC): Based on his profound management experience in international leadership positions within the healthcare industry, and his strong track record in programme management and R&I in pharmaceuticals and diagnostics business groups, Mr Schwenner will guide the healthcare- and (nano)medicine-related and work in MACRAMÉ with respect to industrial requirements.
Xavier Chaucherie (SARPI - VEOLIA): Representing the European Leader for the treatment and recovery of hazardous waste, Mr Chaucherie will guide the Project with regard to concerns associated with the incineration of AdMa-bearing products, and provide technical feedback on the characterisation of aerosols for GRM Use Cases.
Carolina Brantes (Hexcel): Representing a global leader in advanced composites technology, a leading producer of carbon fibre reinforcements and resin systems, and the world leader in honeycomb manufacturing for the commercial aerospace industry, Carolina Brantes will provide end-user expectation information and support the re-confirmation of exposure points in the MACRAMÉ Use-Cases.
Dr Juan Riego Sintes and **Dr Susanne Bremer-Hoffmann** (EU Joint Research Centre (Ispra)): As the EU's lead delegate to the OECD WNT and delegate to the OECD WPMN, Dr Sintes will provide strong connections and information flow between the activities at the OECD and the Project, enabling strategic alignment with / support of ongoing and/or planned OECD work items, as well as dissemination of the Project results. Based on her work in formal validation of toxicological *in-vitro* tests and their regulatory acceptance at EURL-ECVAM, Dr Bremer-Hoffmann will support the advancement of *in-vitro* methods and models. Following her role in REFINE, she will provide liaison with regulatory bodies and help to identify regulatory challenges in the field of (nano)medicine.

[2] Travel costs will be reimbursed according to the EU Commissions reimbursement rules for experts: https://ec.europa.eu/research/participants/data/ref/h2020/other/experts_manual/regl_experts_en.pdf).

[3] One EAB seat is kept empty; the relevant Member will be agreed by all and appointed at the Kick-Off Meeting.