MACRAMÉ

Deliverable Report D2.1

Report on the MACRAMÉ Control Material Library

-

first annual report

Project & Programme Information	
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Project & Programme Information	
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[1] R= Document, report (excluding the periodic and final reports); DEM = Demonstrator, pilot, prototype, plan designs; DEC = Websites, patents filing, press & media actions, videos, etc.; DATA =Data sets, microdata, etc.; DMP = Data management plan; ETHICS = Deliverables related to ethics issues; SECURITY = Deliverables related to security issues; OTHER = Software, technical diagram, algorithms, models, etc.

[2] PU = Public, fully open, e.g. web (Deliverables flagged as public will be automatically published in CORDIS project's page); SEN = Sensitive, limited under the conditions of the Grant Agreement; Classified R-UE/EU-R = EU RESTRICTED under the Commission Decision No2015/444; Classified C-UE/EU-C = EU CONFIDENTIAL under the Commission Decision No2015/444; Classified S-UE/EU-S = EU SECRET under the Commission Decision No2015/444

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Acronyms Listed in this Document	
AdMas	Advanced Materials
AMA	Alveolar Macrophage Assay
CML	Control Material Library
CNTs	Carbon nanotubes
EC	European Commission
FLG	Few Layer Graphene
GO	Graphene Oxide
LA-ICP-MS	Laser Ablation-ICP-MS
NPs	Nanoparticles
PCL	Polycaprolactone
PLGA	poly-lactic-co-glycolic acid
SOPs	Standard Operating Procedures
UCs	Use Cases
WP	Work Package

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Executive Summary

Setting up the Control Material Library (CML) is the objective of the MACRAMÉ Project's Task 2.1. The library contains representative materials with largely known properties impacting the *in vitro* test development and of materials to be investigated in the use cases (UCs). Data gaps will be filled, and measurement protocols will be developed to identify and describe critical attributes impacting the toxicological profiles of CML materials, which fall into three main categories:

i. Materials of known properties to develop, harmonise and validate the generation of aerosols and the development of toxicological *in vitro* models;
ii. Advanced Materials (AdMas) in their pristine status (e.g. Few Layer Graphene-FLG, Graphene Oxide-GO, Carbon Nanotubes-CNTs, poly(lactic-co-glycolic acid)-PLGA nanoparticles and Polycaprolactone-PCL nanoparticles), representing materials and products investigated in MACRAMÉ's use cases;
iii. Materials to study the properties of UC products at their "end of life", i.e. after controlled abrasion or incineration.

The aim of setting up a specially tailored CML is many-fold; the selected materials shall be used for the development, harmonisation and standardisation of the methods included in all MACRAMÉ's R&I activities. Besides the controlled aerosol generation and the harmonisation of the tiered in-vitro model approach for a toxicological risk assessment, materials for establishing high-resolution imaging methods were to be included, allowing a quantification and characterisation of nanomaterials and AdMas in complex matrices. Eventually, a well selected sub-group of CML materials shall be made available for the scientific community to support studies on safety and characterisation of AdMas beyond the duration of the MACRAMÉ Project.

In the first seven months of the Project, all the partners involved in WP2, in collaboration with WP1 and WP4 have taken the following measures:

a. selected the materials to be included in the CML,
b. identified a list of the critical properties to be characterised, and
c. included the different materials to the R&I strategy, identifying the most appropriate candidates for the development, optimisation and harmonisation of the methods included in *Task 2.2 - generation and characterisation of controlled aerosols*, *Task 2.3 - Improvement, benchmarking and validation of in vitro / ex vivo models for inhalation toxicology of AdMas*, and *Task 2.4 - Development of high-resolution imaging methods for the quantification of AdMas in cells and tissues*.

Those progress steps are described below in the first annual report (D2.1).

1 Development of the MACRAMÉ' Control Material Library

WP2 participants, including LIST, BAuA, RIVM, IBE, TASCON, GAIKER, MyB, BASF, MEDICA, Carbon Waters, FILK, TEMASOL, EMPA, UoB, GAIKER and EPITHELIX, with the support of AIST, BioMS, 7P9-SI and TEMASOL, and under the coordination of the LNE, have started the discussion on the materials to be selected for the CML. The first meeting took place during the 1st (virtual) Project Meeting of the MACRAMÉ Project the 1st of December 2022. The list of materials to be included in the CML was presented and discussed at the in-person Kick-Off Meeting (and 1st Annual General Meeting (AGM)), held on the 27th and 28th February 2023 in Brussels, as described in the milestone M2.1, delivered in project month M03 (February 2023). The list of materials was further refined during the 2nd Project Meeting held online the 11th of May 2023 and then during a series of online follow up meetings.

In particular, the list of commercial materials and of representative testing materials from the JRC repository to be used for the validation of *in vitro / ex vivo* Models for Inhalation Toxicology was finalised during the WP2 meeting held the 7th of June 2023. Materials to be included in the CML and that are provided by the use cases (UCs), were defined as part of the R&I strategy planning during the online meetings co-organised by WP1, WP2 and WP4 held in May 2023 (i.e. 11th of May (UC2), 24th of May (UC4), 26th of May (UC3), 30th of May (UC1 & UC5)). All WP2 participants, WP4 (including the UCs responsible, partners participating in the ecotoxicity studies and TEMASOL as WP4-Lead) and BioMS as WP1-Lead contributed to the discussion for the definition of the criteria of the CML.

Protocols for material sampling have been discussed by the consortium during the WP meetings and at the MACRAMÉ Kick-Off Meeting in Brussels. Sampling of the materials provided by the use cases will be performed by the use cases responsible persons according to their internal SOPs. Purchasing and sampling of commercial products for Tasks 2.2 and 2.3 is being performed by the partners involved in the respective tasks (IBE, LIST, BAuA, LNE, EMPA), with a centralised purchase/production and material distribution for each material, with the aim to use the same batch for multiple experiments.

Standard Operating Procedures (SOPs) are under development for the production and sampling of complex materials (e.g. the incinerated and abraded particles) starting from the protocols available at LNE and EMPA on similar composites.

Sample exchanges have been initiated including the shipment of AdMas provided by the UCs. The purchase of commercial materials and the ordering of representative materials from the JRC repository have been initiated as described below.

2 Commercial Materials and Materials from the JRC Repository

2.1 Materials with known Toxicity, as Benchmark for *in vitro / ex vivo* Models for Inhalation Toxicology

Reference testing materials (RTMs) available in the JRC repository have been considered as part of the CML, specifically for the validation of the *in vitro* assay cascade as described in Task 2.3, including: ZnO NM-111, TiO2 NM-105, TiO_2 NM-101, SiO_2 NM-203, SiO_2 NM-201, CeO_2 NM-212, $BaSO_4$ NM-220, Bentonite NM-600 and Graphene-JRC NM-48001.

In addition, five commercially available materials (corundum, Quartz DQ12, Mn2O3, tungsten carbide-Cobalt (WC-Co), and SiC Nanowires) from known commercial sources were included in the list.

All the materials in the JRC repository and the commercial materials will be used for the validation of the Alveolar Macrophage Assay (NR8383 Cells). A subset of materials from the same list will be used for the validation of the other *in vitro* assays included in Task 2.3, as defined in a meeting held online the 7th of June 2023 and as reported in the Table 1.

The material needs of each partner for all the materials listed in Table 1 were collected by IBE in collaboration with all the partners involved in Task 2.3, including LIST, BioMS, LNE, LIST, BAuA, TASCON, EMPA, Epithelix and BASF.

The materials were ordered from the JRC repository the 14th of June 2023 by IBE and already shipped by JRC to IBE, LIST, BioMS, LNE, LIST, BAuA, TASCON, EMPA, Epithelix and BASF. The materials provided by IBE and commercial sources (corundum, quartz DQ12, Mn_2O_3, SiC nanowires), have been purchased by IBE and were shipped in June 2023. LIST will ship tungsten carbide/cobalt to the partners in September 2023.

The aim of the materials selected and reported in Table 1 is to validate the *in vitro* assay cascade of Task 2.3 and to prepare particle-loaded alveolar macrophages for quantitative uptake studies (BASF/IBE, Task 2.4). Respirable materials with different size (nano-to-micro), morphology (spheres, fibres), chemical composition, and solubility have been selected to validate the *in vitro* tests for a broad spectrum of material properties before they are applied to the more complex UCs. Well-characterised materials in terms of their physical-chemical properties (e.g. JRC repository) have been prioritised, in order to save resources and reduce the physical-chemical characterisation to a minimum. Wherever the characterisation of a material appears to lack information on key physical-chemical properties, analyses will be performed by the MACRAMÉ consortium partners. In particular, LNE will perform elemental analysis and detect impurities of the Quartz DQ12, SiC nanowires and Mn_2O_3 nanoparticles by ICP-MS. BAuA will perform the analyses on particle size, morphology and chemical composition by SEM/EDX and, together with IBE, Raman spectroscopy.

Finally, in-depth characterisation is planned for JRC's graphene sample NM-48001 as JRC is not providing any characterisation data on this important CML material. For this graphene sample, the ratio of structurally graphitic and defective carbon will be assessed by Raman together with an estimate of the number of graphene layers. XPS will be used to measure the elemental composition and chemical bonding states of the graphene, whereas electron microscopy will be used to study the particle size, morphology and exfoliation structures. The results will be included in the CML and reported in the next annual report.

Table 1: Materials of the CML ordered from the JRC repository and commercial materials selected for the validation of the in vitro / ex vivo Models for Inhalation Toxicology.

Assay	Partner	Quartz DQ12	Corundum ERM-FD066	ZnO NM-111	TiO2 NM-105	TiO2 NM-101	SiO2 NM-203	SiO2 NM-201	CeO2 NM-212	BaSO4 NM-220	Mn2O3	SiC Nano-wires	NM-401	Bentonite NM-600	Tungsten carbide-Co (WC-Co)
Source		IBE*	JRC	JRC	JRC	JRC	JRC	JRC	JRC	JRC	US Research Nano-materials**	ACS Nano-materials	JRC	JRC	LIST
Supporting PCC	LNE	0.25 g									0.25 g				
AMA (=Alveolar Macrophage model with NR8383 Cells)	IBE	0.5 g	0.5 g	2 g	2 g	2 g	2 g	2 g	2 g	1 g	1 g	0.1 mg	0.1 mg	0.5 g	2 g
AMA , LA-ICP-MS Detection	BASF	0.5 g	1 g	3 g	3 g	3 g	2 g	2 g	3 g	1 g	1 g	0.1 mg	0.1 mg	0.5 g	2 g
AMA, AliSens Alveolar Model	LIST	1 g/2 g	1 g/2 g	1 g/2 g	1 g/2 g	1 g/2 g	1 g/2 g	1 g/2 g	1 g/2 g	1 g/2 g	1 g/2 g	0.5g/1 g	0.5 g/1g	1 g/2 g	1 g/2 g
Bronchial Model, Aersolisation	RIVM	2 g/4 g	2 g/4 g	2 g/4 g	2 g/4 g	2 g/4 g	2 g/4 g	2 g/4 g	2 g/4 g	2 g/4 g	2 g/4 g	1 g	1 g	2 g/4 g	2 g/4 g
MuclairTM, AlveolAirTM, (OR) Ex vivo bronchial, Ex vivo Alveolar	Empa	0,75					8 g					4 g	2 g		
MuclairTM, AlveolAirTM	Epi-thelix	0.25 g					4 g					2 g	2 g		
Raman and SEM/EDS Characterisation	BAuA	0.1 g	0.1 g	2 g	2 g	2 g	1 g	2 g	2 g	1 g	0.1 g			0.1 g	0.1 g

* ultrafine Fraction, original material from "Quarzwerke Frechen"; **source: https://www.us-nano.com/nanopowders.

2.2 Fibres for the Generation and Characterisation of Controlled Aerosols

In order to support the development and harmonisation of the methodologies for the controlled generation of aerosols with respect to the dose (quantitative parameter) and morphological composition of the particles, BAuA, as Task 2.2 leader, has proposed a list of commercial fibres and graphene, as reported in Table 2. Those materials are included in the CML as representatives of the most challenging scenario for aerosol generation (non-polar, i.e. poorly wettable nanoscale objects in powder form). In addition, SiC nanowires and NM-48001 graphene already included in the list of materials for the validation of *in vitro / ex vivo* Models for Inhalation Toxicology, may be included for the development and validation of methods to generate aerosols of controlled concentration and morphological composition, in order to link the methodological approaches developed in Tasks 2.2 and in Task 2.3.

The list of materials selected by BAuA and the Task 2.2 participants to be included in the CML are summarised in Table 2. The final material selection will be refined in the next weeks.

2.3 Biological Matrices

Commercial biological matrices relevant for particle dispersion in liquid media relevant for cell exposure in the *in vitro* models, including culture media (MucilAir, SmallAir or AlveolAir). A pulmonary lung surfactant preparation (Curosurf™) may be included in the CML and may be ordered in the next months (practical details for the purchase are under evaluation). Additional media for eco-toxicology assays may also be selected.

Table 2: Materials of the CML selected for the development and validation of controlled aerosol generation (Task 2.2).

	Name	Supplier	Link to Product	Criteria for the selection	Interesting Features	Also used in other projects
Nanotubes						
MWCNT test material	ARIGM001	BAuA Repository		Serves as default testing material for method development, available in large quantities	High dustiness, medium degree of entanglement, mean diameter ~35 nm, mean length ~1-2µm	CarboLifeCycle,
Graphitised MWCNT	NM401	OECD Repository		Positive control for fibre paradigm (rigid), test with µ-Dishes	Rigid and long fibres, easy to disperse, >20% WHO fraction	NanoGRAVUR, InnoMat.Life, HARMLESS, NanoHarmony
MWCNT	Baytubes C150P	BAuA Repository		Negative control for fibre paradigm (NM400 not a real one), test with µ-Dishes		older BAuA projects
Aligned flexible MWCNT	NG01AM0102	nanografi	Link	Thin commerical CNTs marketed as beeing produced in such way that they are aligned and bundled, test with µ-Dishes	Bundles are very long up to 95 µm.	not yet
MWCNT 30-50 nm	NG01MW0501	nanografi	Link	Presumed to be a mixture of more flexible and less rigid MWCNTs (proportions), test with µ-Dishes		not yet
MWCNT 50-90 nm	SA 901019	Merck	Link	Presumed to be a mixture of less flexible and more rigid MWCNTs (proportions), test with µ-Dishes		not yet
SWCNT	Tuball	OSCial	Link	Test with µ-Dishes	Aerosol particles are mainly HAR-bundles with varying diameters that match WHO criteria	nanoGRAVUR, InnoMat.Life BAuA i.p. study

	Name	Supplier	Link to Product	Criteria for the selection	Interesting Features	Also used in other projects
Aligned rigid MWCNT	Hanos CM	Hanwha	Link	Commerical MWCNT bundles available with different bundle diameters and lengths, test with µ-Dishes	LINK to method of bundle diameter control	not yet
Graphene						
Single Layer Graphene - Suspension	Single Layer Graphene in water	ACS	Link	Use-case relevant, testing with CLOUD		not yet
Single Layer Graphene - Powder	Single Layer Graphene	ACS	Link	Use-case relevant, testing with µ-Dishes		not yet
Graphene Oxide	Industrial-Grade Graphene Oxide	ACS	Link	Use-case relevant, testing with µ-Dishes		not yet

3 Advanced Materials Representatives of the MACRAMÉ Use-Cases

3.1 Materials Benchmarks for *in vitro / ex vivo* Models for Inhalation Toxicology and for the Development of High-Resolution Imaging Methods

Several types of poly(lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL) biodegradable polyester nanoparticles will be included in the CML. All these particles are provided by MyB (UC5) and are listed together with their intended purposes in Table 3. Besides unloaded PLGA and PCL particles there will be those loaded with either ciprofloxacin, the fluorescent dye Lumogen Red, or gold nanoparticles (20 nm, 50 nm). Pristine gold nanoparticles of the same size (20 nm, 50 nm) will be provided as well. Subsets of particles will be used by several partners with aim to further test the influence of particle size *in-vitro* models for inhalation toxicology (LIST, IBE, BASF), to develop the high-resolution imaging methodologies included in Task 2.4 (BASF), and to study polymer-dependent uptake efficacy (BASF/IBE).

Furthermore, IBE will carry out time-lapse video imaging, enhanced dark field microscopy (DFM), hyperspectral imaging (HSI), and confocal Raman microscopy (CRM) to qualitatively describe the particle uptake in conjunction with TASCON who contributes a ToF-SIMS study on the possibilities for a subcellular detection of PCL particles in cells. To quantitatively describe the particle uptake and its dependency on polymer coating, BASF will investigate pristine as well as PLGA- and PCL-enclosed gold NP in alveolar macrophages not only with LA-ICP-MS but also with sp-ICP-MS methodologies. To this end, the most versatile types of polymers (PLGA & PCL), the size of the gold nanoparticles (20 and 50 nm), and the necessary concentration of gold NPs (320 ug/mL) were discussed and defined by the partners LIST, BioMS, LNE, LIST, BAuA, TASCON, Epithelix and BASF, under the coordination of IBE and MyB.

In addition to the materials provided by JRC and commercial materials, PLGA and PCL biodegradable polyester nanoparticles (empty, loaded with ciprofloxacin and loaded with the Lumogen Red dye), naked gold nanoparticles (Au-NPs) of different sizes (20 nm, 50 nm) and PCL/PLGA doped with gold nanoparticles (20 nm, 50 nm) provided by MyB (UC5) will be included in the CML.

The detailed study design for the application of Polycaprolactone (PCL) biodegradable polyester nanoparticles (blank, loaded with ciprofloxacin and loaded with the Lumogen Red dye), naked gold nanoparticles of different sizes (20 nm, 50 nm) and PCL doped with gold nanoparticles (20 nm, 50 nm) was discussed and finalised the 30th of May 2023 as part of the R&I planning strategy (see Figure 1 and Figure 2). The instance map linking the characterisation to be performed on those materials, the associated *in vitro / ex vivo* models and the high resolution imaging methods have been drafted by 7P9-SI (https://instance-maps.stage.sevenpastnine.com/app/instance-map/29d78032-cab5-4219-bcf5-8fc05f7650fe/).

The development of the protocols for high resolution imaging of those samples have been initiated; for example, the measurements of PLGA and PCL naked particles by TOF-SIMS was performed by TASCON in April 2023, showing, as preliminary results a higher sensitivity for the detection of PCL *vs* PLGA. The protocols and the final results for the characterisation of those materials will be reported in the second annual deliverable of the CML.

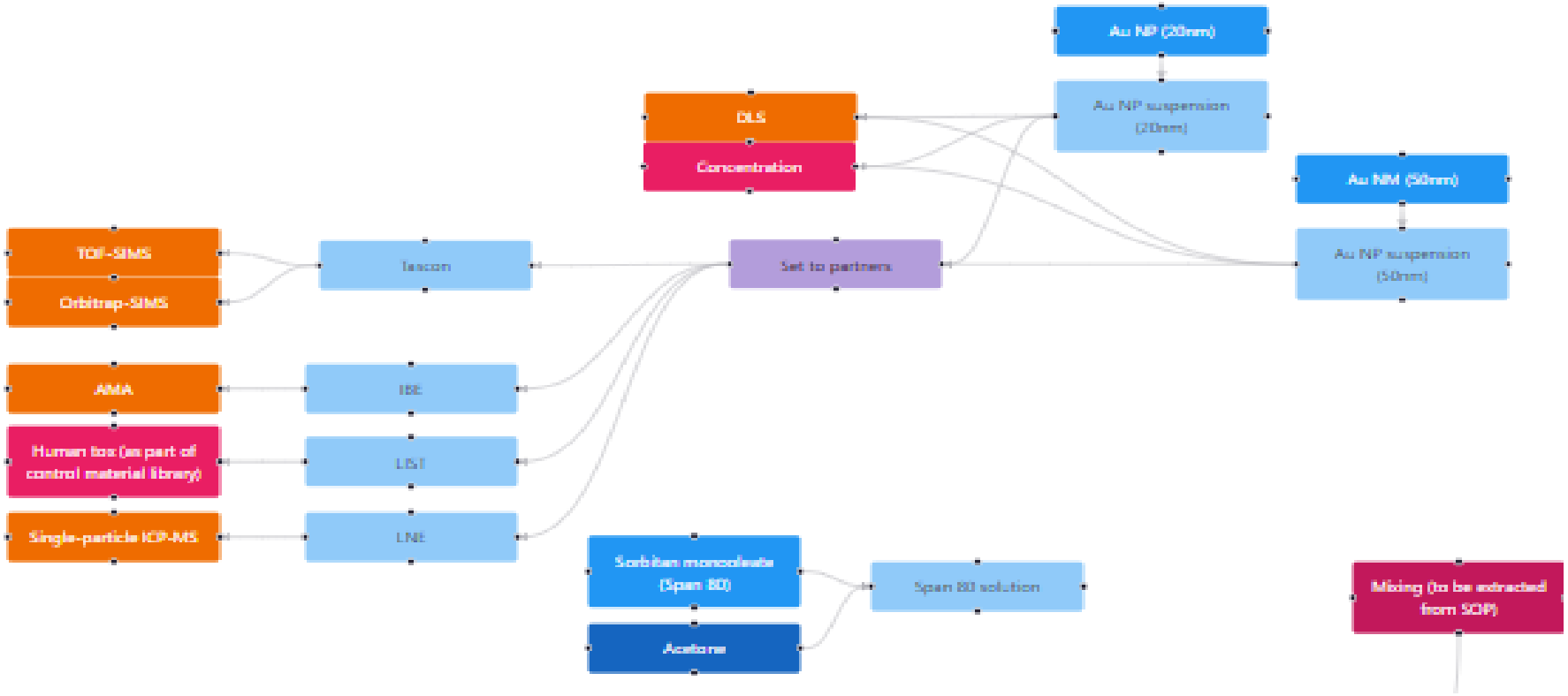

Figure 1: Instance map representing an example of the study design for Au NPs of 20 nm.

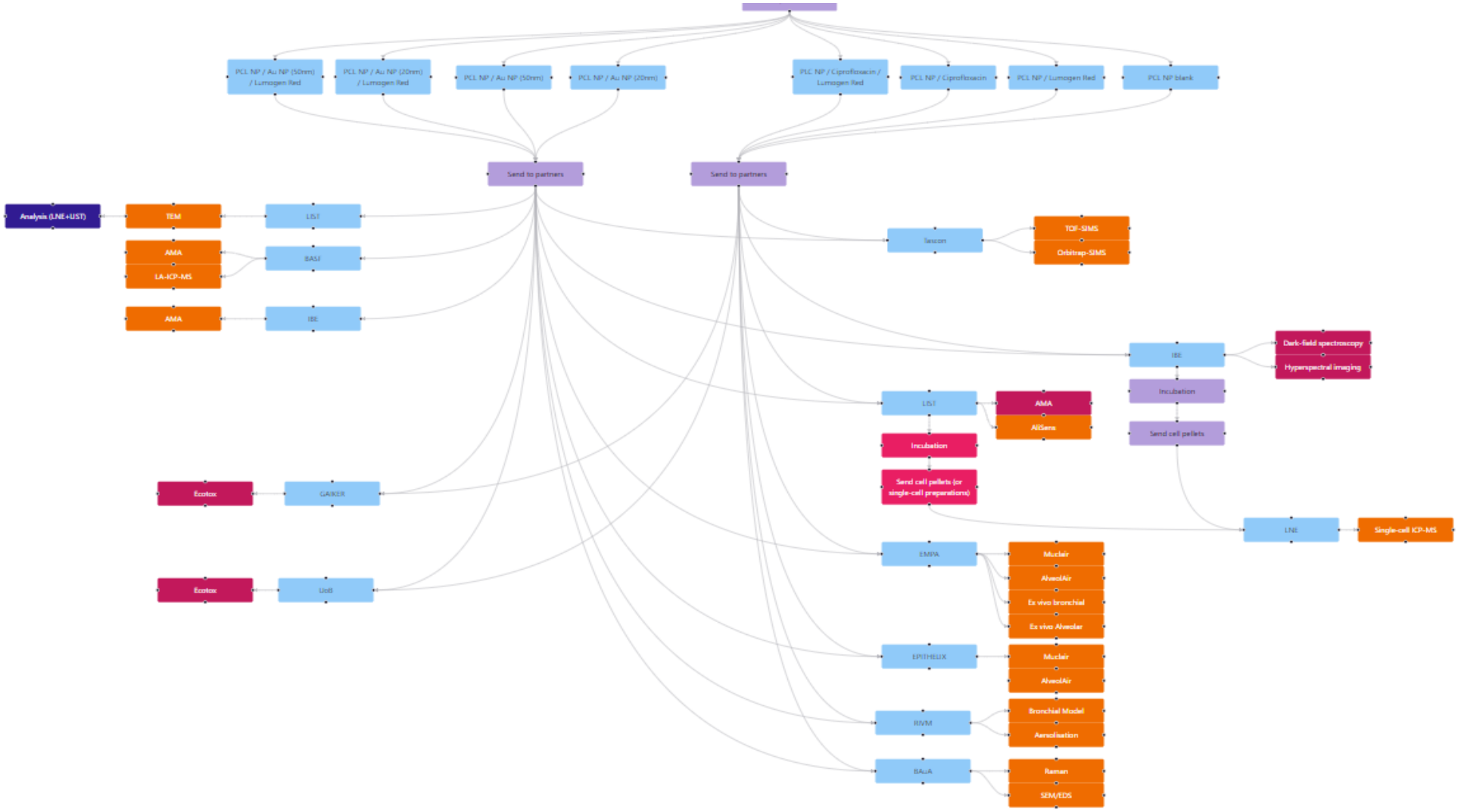

Figure 2: Instance map representing an example of the study design for PCL NPs, including naked NPs, NPs loaded with Au NPs, NPs loaded with ciprofloxacin and with the Lumogen Red dye.

Table 3: Materials of the CML provided by MyBiotech and selected for the validation of the in vitro / ex vivo Models for Inhalation Toxicology in Task 2.3, and for the development of high-resolution imaging techniques in Task 2.4.

Assay	Partner	Au-NP (50 nm)	Au-NP (20 nm)	PLGA NP blank	PLGA NP with Lumogen red dye	PLGA NP with cipro-floxacin	PLGA NP with cipro-floxacin and Lumogen red dye	PLGA-Au 50 NP blank	PCL NP blank	PCL NP with Lumogen red dye	PCL NP with cipro-floxacin	PCL NP with ciprofloxacin and Lumogen red dye	PCL-Au 50 NP blank
AMA imaging (HIS, DFM, CRM) *	IBE	25 mL (320 µg/mL)	25 mL (320 µg/mL)	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g
AMA , LA-ICP-MS Detection	BASF	25 mL (320 µg/mL)	25 mL (320 µg/mL)					0.5 g					0.5 g
AMA, AliSens	LIST	25 mL (320 µg/mL)	25 mL (320 µg/mL)						1 -2 g	1 -2 g	1 -2 g	1 -2 g	1 -2 g
Bronchial Model, Aersolisation	RIVM	25 mL (320 µg/mL)	25 mL (320 µg/mL)						2 -4 g	2 -4 g	2 -4 g	2 -4 g	2 -4 g
MuclairTM, AlveolAirTM	Epi-thelix												
Raman and SEM/EDS Characterisation	BAuA	1 mL (320 µg/mL)	1 mL (320 µg/mL)	0.5 g	0.5 g			0.5 g	0.5 g	0.5 g			0.5 g
SC ICP-MS	LNE	1 mL (320 µg/mL)	1 mL (320 µg/mL)					0.5 g					0.5 g
LA-ICP-MS	BASF	√	√					√					√
ToF-SIMS	TASCON			0.1 g	0.1 g	0.1 g	0.1 g		0.1 g	0.1 g	0.1 g	0.1 g	

* AMA = Alveolar Macrophage model with NR8383 Cells

3.2 Graphene Material Family and Carbon Nanotubes

3.2.1 Pristine Materials

AdMas representative of the MACRAMÉ Use-Cases as selected by the Project partners, to complement the list of the commercial materials described above includes the following pristine materials (see XXX):

a. Few layer graphene (FLG) in water-based suspension at 0.1 mg/mL provided by Carbon Waters;
b. Graphene oxide, dry powder from Layer One (product number 18002), provided by Medica; and
c. NanoCyl NC7000™ series, thin multiwall carbon nanotubes.

Those materials will be used for testing the methods developed in Tasks 2.2 and 2.3 after the method validation. The attributes to be characterised, and the information already available on the materials have been mapped by the Project partners as summarised in table 4. Some key properties will be measured by the MACRAMÉ consortium and the information will be included in the CML. For example, the ratio of structurally graphitic and defective carbon will be assessed by Raman together with an estimate of the number of graphene layers, elemental composition and oxygen content will be measured by XPS, and electron microscopy (TEM or SEM) will be used to determine the particle shape, layer of carbon and the particle lateral dimensions. The results will be included in the CML and reported in the next annual report.

Table 4: Summary of analytical results of the pristine materials from the MACRAMÉ Use-Cases and associated properties for the FLG and for the GO.

Properties	Measurand	Few layer graphene (FLG)	Graphene Oxide
Physical-structural	SP2 Bonded Carbon	na	na
	Structural Defects	na	na
	Z-Axis Dimensions	na	na
	Layers of Carbon Atoms	1 to 8 (AFM)	many (SEM)
	Particle Shape	Graphene sheets suspended in water	GO dried flakes (SEM)
	Lateral Particle Dimensions	0.3-2 µm (TEM)	To be measured by SEM
	Particle Aspect Ratio		90 % 300 – 3000 nm (AFM)
	Specific Surface Area	500 m2/g	
	Crystallinity	002 (XRD)	001 reflection about 50 x Bg (XRD), >99 % Graphene Oxide, About 60 % of area oxidised (TEM)

Properties	Measurand	Few layer graphene (FLG)	Graphene Oxide
Chemical	Elemental Composition	C: 64% O: 25% K: 11% (XPS)	
	Oxygen Content	25.2% (XPS)	O: 33 – 34 %, C: 64 – 65 %, (XPS)
	Impurities	Na (<1%) from graphite (XPS)	Sulfur: 1-2%, Cl, N<1% (XPS)
	Functionalisation		
	Surface Particle Charge	-30mV (zeta potential)	-54 mV
	Dispersibility	Stable dispersion in water and polar solvents.	Stable dispersion in de-ionised water. In other polar solvents slightly dispersible

3.2.2 AdMas and finished Products subjected to controlled Incineration and Abrasion

Abrasion and incineration of the MACRAMÉ use cases will be performed by EMPA and LNE, and the materials will be included in the CML as representative of "end of life" products. For this purpose the following materials have been selected by the Project partners:

a. Reticulated (solid samples) of Epoxy-resins with/without few-layers graphene (FLG) produced by EMPA and Carbon Waters, as finished product will be abraded by EMPA or incinerated by LNE;
b. hollow fibres contained in water filters made from polysulfone and graphene oxide (5% w/w) will be incinerated by LNE; and
c. polyurethane (PU) thin layer nanocomposites containing multi-walled (MWCNT) will be incinerated by LNE.

As part of the CML, the resulting powder materials will be collected and characterised with respect to chemical composition, presence of FLG, GO or CNTs, and particle size of the abraded/incinerated powder. A protocol to disperse the powder material in the relevant biological matrices (cell media, media for ecotoxicology experiments) will be developed based on the experience accumulated in previous projects, and then provided to the partners performing *in vitro / ex vivo* experiments.

The study designs for those three materials have been discussed in a series of online meetings held on the 11th, the 24th and the 30th of May 2023. The three instance maps linking the characterisation to be performed on the materials to be included in the CML, the associated *in vitro / ex vivo* models and ecotoxicological experiments have been drafted by 7P9-SI.

The first experiments to design the conditions for the controlled incineration and abrasion of the materials are currently ongoing at EMPA and LNE.

EMPA has established the protocol for and performed the abrasion of pure Epoxy and Epoxy-FLG materials as well as started with initial characterisation of abraded particles (Figure 3). The steps illustrated in Figure 3 are:

- Step 1: Epoxy alone or epoxy-FLG (2%) composite was mixed, and plates of fixed dimensions were prepared by moulding and curing for two consecutive cycles of 12h and 4h at indicated temperatures.

- Step 2: The prepared plates were abraded at indicated conditions using a taber abrader placed in a fume hood. The abraded particles passed through a metallic probe and accumulated in a 0.22 µm polycarbonate filter.
- Step 3: The abraded particles from the filters were taken off by scraping using a sterile spatula and collected in a sterile falcon tube. For characterisation, the abraded particles were dispersed in endotoxin-free BSA (0.1%)-Water followed by bath sonication for 45 minutes.

For the characterisation, primary particle size was determined by online measurement of airborne abrasion particles (DMS500), revealing that particles were in the respirable size (80-100 nm for abraded particles from pure epoxy, FLG-epoxy measurements pending). For cell experiments, the abraded particles were dispersed in 0.1% BSA-Water by bath sonication for 45 minutes and then characterised for size distribution using dynamic light scattering, and endotoxin contamination was assessed by performing LAL test (ThermoFisherScientific). The average hydrodynamic size of abraded pure epoxy and epoxy-FLG particles in water was 1164 ± 292 nm and 1673 ± 243 nm, respectively. The endotoxin content in pure epoxy and FLG-epoxy particles was 0.002 ± 0.001 EU/mL, and 0.013 ± 0.008 EU/mL, respectively which corresponds to both abraded particles being endotoxin-free. The detailed protocols and full characterisation results will be included in the CML and reported in the next annual report.

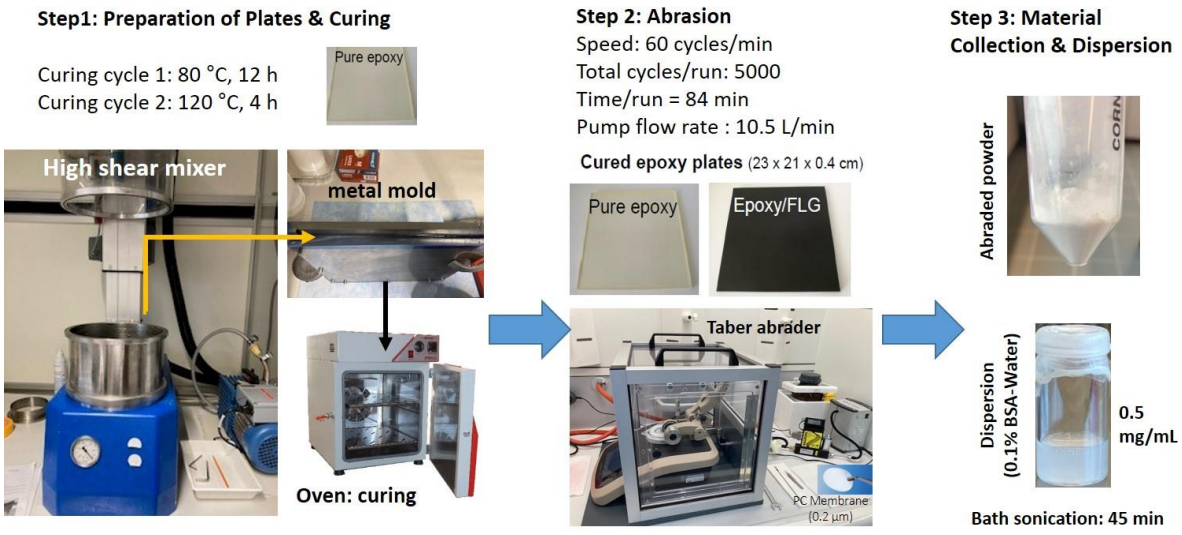

Figure 3: Abrasion of pure epoxy and epoxy-FLG composite.

4 Conclusions

In the first 7 months the consortium successfully identified and selected the materials to be included in the CML. Furthermore, their use (measurements, scope) was defined within the planning of the R&I MACRAMÉ strategy, e.g. for the development of the methods for controlled aerosol generation, for the validation of the of the tiered in-vitro testing approach for the toxicological risk assessment and for the development of the high-resolution imaging methods for quantification and characterisation of nanomaterials and advanced materials (AdMas) in complex matrices. The consortium considers the first steps for building up the basis of the CML to be complete.

The steps for developing the CML until M18 will be:

1. to complete the distribution of the materials,
2. to perform their characterisation, and
3. to exploit them for the validation of the methods included in the MACRAMÉ R&I strategy.

In the second half of the Project, we aim to select the most promising materials, and consider building a roadmap to provide them to the community for future studies beyond the MACRAMÉ Project. All those steps will be reported in the next two annual D2.1 reports of the CML.