SAbyNA – D2.2 – Recommended testing and non-testing methods for human and environmental release, fate and exposure data generation

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SAbyNA

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Simple, robust and cost-effective approaches to guide industry in the development of safer nanomaterials and nano-enabled products

Start date of the project: 01/03/2020

Duration 48 months

WP2 D2.2 – Recommended testing and non-testing methods for human and environmental release, fate and exposure data generation

WP	2	Streamlined methodologies, models and tools to facilitate release, fate & exposure assessment of NFs/NEPS for SbD purposes		
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[1] Dissemination level: **PU** = Public, **PP** = Restricted to other programme participants (including the JU), **RE** = Restricted to a group specified by the consortium (including the JU), **CO** = Confidential, only for members of the consortium (including the JU)

[2] Nature of the deliverable: **R** = Report, **P** = Prototype, **D** = Demonstrator, **O** = Other

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Deliverable abstract

Based on the resources analysis carried out in Task 2.1, key methods were selected and simplified to facilitate their implementation at the early stages of the innovation process. Methods and tests for data collection were selected from key parameters such as release rates, dustiness, release forms, transformation rates and exposure concentrations. From this activity, sector-specific methods that allows human and environmental release, fate and exposure data generation for the two SAbyNA targeted industrial sectors (i.e. additive manufacturing and paints) were developed or adapted and are reported in the present deliverable. The methods reported in the present deliverable D2.2 for human and environmental release, fate and exposure data generation are currently being tested on the two SAbyNA case studies in collaboration with WP7 to establish the relevance and effectiveness of SbD approaches. Provisional results were already reported as D2.9 and 2.10 and those two interim deliverables will be further elaborated by the end of the project gathering the whole experimental activities conducted along the project to assess release and exposure of nanomaterials for the two case studies.

Table of Content

Abbreviations

CEN	The European Committee for Standardisation
CPC	Condensation Particle Counter
DoW	Description of Work
EHS	Environment, Health and Safety
ENM	Engineered Nanomaterial
FF	Far field
FMPS	Fast Mobility Particle Sizer
GC	Gas Chromatography
HEPA	High Efficiency Particulate Arrestance
ICP	Inductively Coupled Plasma
ISO	International Standardization Organization
MS	Mass Spectrometry
NEP	Nano-Enabled Product
NEM	Nano-Enabled Material
NF	Nanoform
NIOSH	The National Institute for Occupational Safety and Health
NM	Nanomaterial
NOAA	Nano-objects and their aggregates and agglomerates > 100 nm
NP	Nanoparticle
OECD	Organisation for Economic Co-operation and Development
OEL	Occupational Exposure Limit
OPC	Optical Particle Counter
RA	Risk Assessment
RH	Relative Humidity
RMM	Risk Mitigation Measure
SbD	Safer-by-Design
SEM	Scanning Electron Microscopy
SME	Small and Medium Enterprise
SOP	Standard Operation Procedure
SSbD	Safe- and Sustainable-by-Design
TEM	Transmission Electron Microscopy
VS	Vortex Shaker
WP	Work Package

1. Introduction

The SAbyNA project aims at providing new approaches for the development of safe(r) nanoforms (NF) and nano-enabled products (NEP). This approach rests for a large part on two aspects:

1. Larger and improved use of existing data which involves identifying available data sources and adequate model to process such data. This part of the work is already documented in other deliverables (such as Deliverables 2.1, 4.1 and 5.1).

2. The general SAbyNA approach with regard to the improvement of the experimental work in order to eventually allow the assessment of the efficacy for Safe by Design (SbD) strategies. The improvement of experimental work can be achieved by modifying experimental protocols and/or by applying/developing novel ways of data treatment.

The aim of WP2 is to identify and optimise methodologies, models and tools for estimating release and exposure of NFs and NEPs throughout the life cycle for SbD purposes. The first step in the SAbyNA project was the mapping and establishment of context and purpose of the most relevant existing resources that can support SbD of nanotechnology. WP2 have performed a review of current available resources (tools, models, methods and data sources) for estimating release and exposure of NFs and NEPs and identified the most suitable resources which could be used for SbD purposes. This is described in Deliverable 2.1 with the criteria used for the selection described in Milestone 2.1. Identified methods from D2.1 were classified under testing and non-testing methods in regard with their practical implementation by industry (SMEs in particular) in the early stage of development of NEPs. Based on the analysis carried out in Task 2.1, key methods were selected and simplified to facilitate their implementation at the early stages of the innovation process. Methods and tests for data collection were selected from key parameters such as release rates, dustiness, release forms, transformation rates and exposure concentrations. From this activity, sector-specific methods that allows human and environmental release, fate and exposure data generation for the two SAbyNA targeted industrial sectors (i.e. additive manufacturing and paints) were developed or adapted and are reported in the present deliverable. The reported methods are being tested in collaboration with WP7 to implement and assess the effectiveness of SbD approaches for both sector related (AM and paints) case studies.

2. Description of the tasks

2.1 Classification of testing and non-testing methods identified in D2.1

As part of D2.1, testing and non-testing methods to generate input data for SbD for environmental release and fate and human exposure were identified, distilled and shortlisted. For further information on this process, please see D2.1.
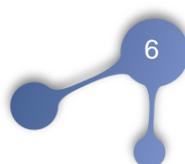
These methods can be categorised into testing methods and non-testing methods which is discussed in the following sections.

2.1.1 Testing methods

- **Human exposure**

For the shortlisted human exposure testing methods these are divided into dustiness methods and release & exposure and RMM testing methods.

Five dustiness testing methods are relevant. These are:

- Measurement of dustiness of bulk materials that contain or release respirable NOAA and other respirable particles - Part 1: Requirements and choice of test methods (EN 17199-1:2019)

- Measurement of dustiness of bulk materials that contain or release respirable NOAA or other respirable particles - Part 2: Rotating drum method (EN 17199-2:2019)

- Measurement of dustiness of bulk materials that contain or release respirable NOAA or other respirable particles - Part 3: Continuous drop method (EN 17199-3:2019)

- Measurement of dustiness of bulk materials that contain or release respirable NOAA or other respirable particles - Part 4: Small rotating drum method (EN 17199-4:2019)

- Measurement of dustiness of bulk materials that contain or release respirable NOAA or other respirable particles - Part 5: Vortex shaker method (EN 17199-5:2019)

The outputs of these dustiness methods are dustiness ratings in either mg/kg for the mass-based dustiness index and/or particle/mg for a number-based dustiness index. Input parameters required for these methods include dustiness index, density, size, shape, diameter, particle number, particle concentration, particle size distribution, amount used (for testing), room temperature, humidity, duration (testing duration of the data collection), mass percent and physical state. These input parameters are also linked to human exposure models and tools inputs which is further discussed in Deliverable 2.1.

For the Release & Exposure Assessments and RMM testing methods, seven methods are relevant. These are:

- Nanotechnologies – Method to quantify air concentrations of carbon black and amorphous silica in the nanoparticle size range in a mixed dust manufacturing environment (ISO/TS 201361:2019)

- Workplace exposure – Assessment of exposure by inhalation of nano-objects and their aggregates and agglomerates (EN 17058:2018)

- Assessment of dermal exposure to nano-objects and their aggregates and agglomerates (NOAA) (CEN ISO/TS 21623:2018)

- Nanomaterials. Quantification of nano-object release from powders by generation of aerosols (CEN ISO/TS 12025:2015)

- Characterisation of ultrafine aerosols/nanoaerosols- Determination of the size distribution and number concentration using differential electrical mobility analysing systems (ISO 28439:2011)

- Workplace exposure - Measurement of exposure by inhalation of nano-objects and their aggregates and agglomerates - Metrics to be used such as number concentration, surface area concentration and mass concentration (EN 16966:2018)

- Workplace atmospheres — Ultrafine, nanoparticle and nano-structured aerosols — Inhalation exposure characterization and assessment (ISO/TR 27628:2007).

For these methods, there are various required input parameters and the outputs of the methods. These are summarised in the following table.

Table 1. Input parameters and method outputs for testing methods

Method	Input parameters	Method Outputs
Nanotechnologies — Method to quantify air concentrations of carbon black and amorphous silica in the nanoparticle size range in a mixed dust manufacturing environment (ISO/DTS 21361:2019)	Size, particle size distribution, particle concentration	Air concentration (number of particles/cm^3) Carbon black Amorphous SiO$_2$ amorphous
Workplace exposure – Assessment of exposure by inhalation of nano-objects and their aggregates and agglomerates (EN 17058:2018)	Size, particle number, particle concentration, amount used, amount handled, amount in product, air exchange rate, frequency, spray duration, OEL, exposure controls	Direct reading count and particle size
Assessment of dermal exposure to nano-objects and their aggregates and agglomerates (NOAA) (CEN ISO/TS 21623:2018)	Size, state ('high hazard' NOAA, flexible/non-rigid NOAA, liquid Nano-scale droplets and all other NOAA))	Risk assessment evaluation
Nanomaterials. Quantification of nano-object release from powders by generation of aerosols (CEN ISO/TS 12025:2015)	Dustiness, density, size, shape, particle number, amount used	Nano-object number release, nano-object release rate, nano-object aerosol number concentration, mass specific nano-object number release
Characterisation of ultrafine aerosols/nanoaerosols- Determination of the size distribution and number concentration using differential electrical mobility analysing systems (ISO 28439:2011)	Surface area, particle number	Aerosol size distribution (number concentration distribution)
Workplace exposure - Measurement of exposure by inhalation of nano-objects and their aggregates and agglomerates - Metrics to be used such as number concentration, surface area concentration and mass concentration (EN 16966:2018)	Surface area, particle number, amount used, amount handled, physical state of matrix	Aerosol mass, number concentration

Workplace atmospheres — Ultrafine, nanoparticle and nano-structured aerosols — Inhalation exposure characterization and assessment (ISO/TR 27628:2007)	Surface area, size, shape, particle number, amount used, amount handled,	NOAA metric (calculated or estimated)

2.1.2 Non-testing methods

- **Human exposure**

A number of non-testing methods (Release & Exposure Assessment and RMMs) were distilled and shortlisted as part of D2.1 for human exposure. These are:

- Nanotechnologies – Overview of available frameworks for the development of occupational exposure limits and bands for nano-objects and their aggregates and agglomerates (NOAAs) (ISO/TR 18637:2016)
- Nanotechnologies – Occupational risk management applied to engineered nanomaterials - Part 1: Principles and approaches (ISO/TS 12901-1:2012)
- Nanotechnologies – Occupational risk management applied to engineered nanomaterials – Part 2: Use of the control banding approach (ISO/TS 12901-2:2012)
- Nanotechnologies – Health and safety practices in occupational settings (ISO/TR 12885:2018)
- Nanotechnologies - Nanomaterial risk evaluation (ISO/TR 13121:2011)
- Occupational Exposure to Carbon Nanotubes and Nanofibres (NIOSH bulletin 65)
- Occupational Exposure to Titanium Dioxide (NIOSH bulletin 63)

Methods ISO/TR 18637:2016, ISO/TS 12901-1:2012 and ISO/TS 12901-2:2012 discuss the use of control banding. This is relevant to those models and tools that use control banding which will be discussed in Deliverable 2.4 on improving the usability of models which will be submitted in October 2022.

Method 13121/2011 provides a description of health and safety practices relevant to nanotechnologies. Methods NIOSH bulletin 65 and 63 provide recommended exposure levels in the workplace for carbon nanotubes and nanofibres and nano TiO_2. References to these three methods will be provided within the SAbyNA platform.

Further information on the input parameters required and the method outputs are summarised in the following table.

Table 2. Input parameters and method outputs for non-testing methods

Method	Input parameters	Method Outputs
Nanotechnologies – Overview of available frameworks for the development of occupational exposure limits and bands for nano-objects and their aggregates and agglomerates (NOAAs) (ISO/TR 18637:2016)	Size, duration, OEL, ventilation type, ventilation rate	Control banding guidance

Nanotechnologies – Occupational risk management applied to engineered nanomaterials - Part 1: Principles and approaches (ISO/TS 12901-1:2012)	Density, surface area, size, shape, particle number, particle concentration, particle size distribution, air exchange rate, duration, frequency, employees exposed, OEL, exposure controls	Size and density for deriving exposure limits. Guidance on occupational health and safety for engineered nanomaterials
Nanotechnologies – Occupational risk management applied to engineered nanomaterials – Part 2: Use of the control banding approach (ISO/TS 12901-2:2012)	Dustiness, size, particle size concentration, amount used, amount handled, room characteristics, ventilation rate, local controls	Control banding for inhalation exposure
Nanotechnologies – Health and safety practices in occupational settings (ISO/TR 12885:2018)	Dustiness, density, surface area, size, shape, particle number, particle concentration, particle size distribution, amount used, amount handles, amount in products, air exchange rate, OEL, exposure controls	Description of health and safety practices in occupational settings. Focus on occupational manufacture and use of manufactured NMs
Occupational Exposure to Carbon Nanotubes and Nanofibres (NIOSH bulletin 65)	Worker respirable sample, OEL	Recommended exposure limit, worker exposure level
Occupational Exposure to Titanium Dioxide (NIOSH bulletin 63)	Personal sample, OEL	Recommended exposure limit, worker exposure level

2.2 Simplification and adaptation of standardized methods for data generation

2.2.1 Generic methods

- **Human exposure**

The rapidly advancing field of nanotechnologies and concerns on its potential impact on occupational health and safety has initiated efforts by Standardization bodies to provide guidance how health and safety issues can be appropriately addressed. CEN has published a document (EN 17058:2018) that focuses on the assessment of occupational exposure by inhalation of nano-objects and their aggregates and agglomerates (NOAA). In general the objectives of an exposure assessment can vary widely and can include exposure exploration and determination, evaluation of the effectiveness of exposure control measures, check for compliance with any occupational exposure limit or other benchmark level, and can contribute to risk assessment and epidemiological studies. The measurement strategy used for the assessment will depend amongst other factors on the objective of the assessment. The EN 17058:2018 document concerns the elements of exposure assessment and provides guidance for various applications. Since this document was used as such to conduct occupational exposure assessment during the course of SAbyNA project, a simplified version was not added to the present deliverable.

The release of airborne NOAA from nano-enabled powders is an important information to manage the risks of potentially hazardous NOAA during the handling and transport of bulk materials, and is an important consideration for the design of safe powders, processes and operations. OECD, REACH

and CLP recommend the use of dustiness data to help develop strategies to reduce the risk of exposure. In addition, dustiness data can be used along SbD and SSbD strategies to select the less emissive products or processes. The proposed dustiness tests rely on different dust generating benches that use a specific amount of powder, energy/power for a specific generation time, from which the dust characteristics are analysed quantitatively. These methods simulate various types of powder handling processes used in industry that could generate dust. As described in the EN 17199 series of standards, several methods applicable to nanomaterials are proposed to quantitatively assess the dustiness indices of manufactured nanomaterials. We proposed in SAbyNA to investigate specifically the Vortex Shaker (VS) Method (EN 17199-5:2019). This method allows the determination of mass-based and number-based dustiness indexes with a limited sample volume of about 0.5 cm^3 which is very convenient for SbD and SSbD research purposes. The Annex 5.1 on page 20 described a simplified protocol.

- **Release studies**

CNRS and CEA built on developments from SERENADE project (French funded LABoratory of EXcellence) to implement relevant weathering conditions through an optimized aging protocol applied to paints in climate chamber. LEITAT built on different methodologies to determine release quantities and release forms for processes that take place during the use phase and end of life stages of NEP. Protocols for simulating processes such as weathering of solid matrices under indoor/outdoor conditions, mechanical stress processes (abrasion, rubbing, shredding…), leaching (simulating landfill conditions) were optimized from their original source (e.g. ISO 16474-3 for artificial weathering, ISO 7784-2 for Taber-abrasion, ISO 11998 for wet abrasion…). Detailed descriptions on the analytical methodology to monitor release materials (quantities and forms) are provided.

- **Fate and environmental exposure in freshwater mesocosms**

Mesocosm testing offers a means of providing meaningful data to inform environmental risk assessment of complex systems.[4,5] Among the many definitions for a mesocosm, a more general one describes a mesocosm as an enclosed and essentially self-sufficient (but not necessarily isolated) experimental environment or ecosystem with a number of interdependent system parameters. The SAbyNA mesocosm experiment was set up under to mimic the contamination of a freshwater lentic ecosystem under a Mediterranean climate to nano-enabled product (NEP). The main contamination scenario involved the chronic addition of fragmented acrylic paint incorporating $ZnAl_2O_4$ nanospinels.

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[4] Auffan, M. *et al.* (2014) 'An adaptable mesocosm platform for performing integrated assessments of nanomaterial risk in complex environmental systems', *Scientific reports*, 4, p. 5608.

[5] Auffan, M. *et al.* (2019) 'Contribution of mesocosm testing to a single-step and exposure-driven environmental risk assessment of engineered nanomaterials', *NanoImpact*, 13, pp. 66–69.

Hence, the main research question aimed at understanding the fate and effect of such NEP in realistic environmental conditions during the use or end of life stages of the product lifecycle.

CNRS optimized the mesocosm testing that was previously developed as Standardized Operational Procedure for pre-regulatory purpose under the frame of the NANoREG project. It addresses hazard and exposure issues in a single experiment. The objective within SAbyNA project was to develop a sampling strategy to reduce the analytical workload associated with a mesocosm experiment thereby improving time and cost efficiency. The aged NFs will be provided to WP3 to optimize/ validate the hazard methods.

2.2.2 Appropriate methods for the paint case study

As presented in Figure 1 and Figure 2, an experimental design will be applied and adapted for each activity to determine the release rate and form and when possible, the release mechanisms and parameter controlling it to provide useful information for the NFs and NEP product design. When simulating cascade activity, the degraded NEP and the NEP residue of degradation characterization will be used as input for the following activity.
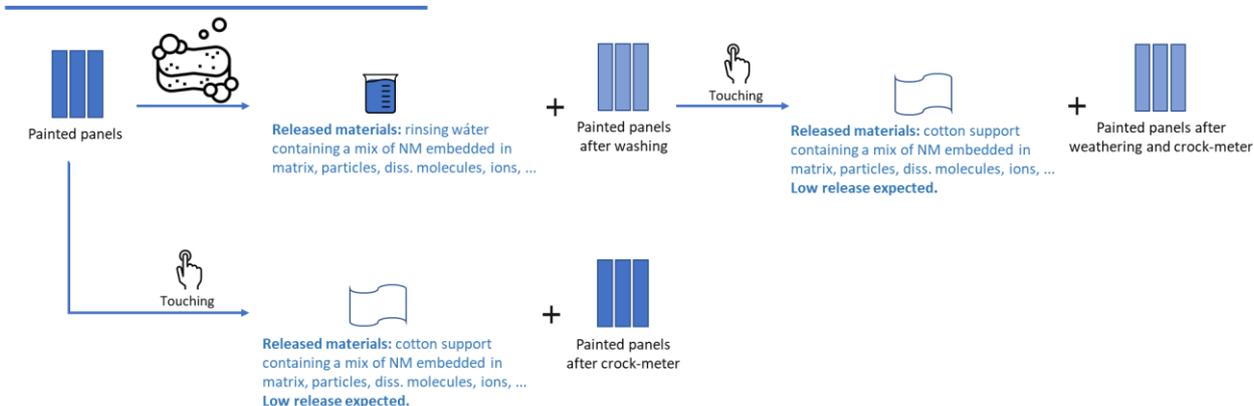
Figure 1. Overview of release experiments and released materials for anti-soiling paint case study (indoor use scenario).
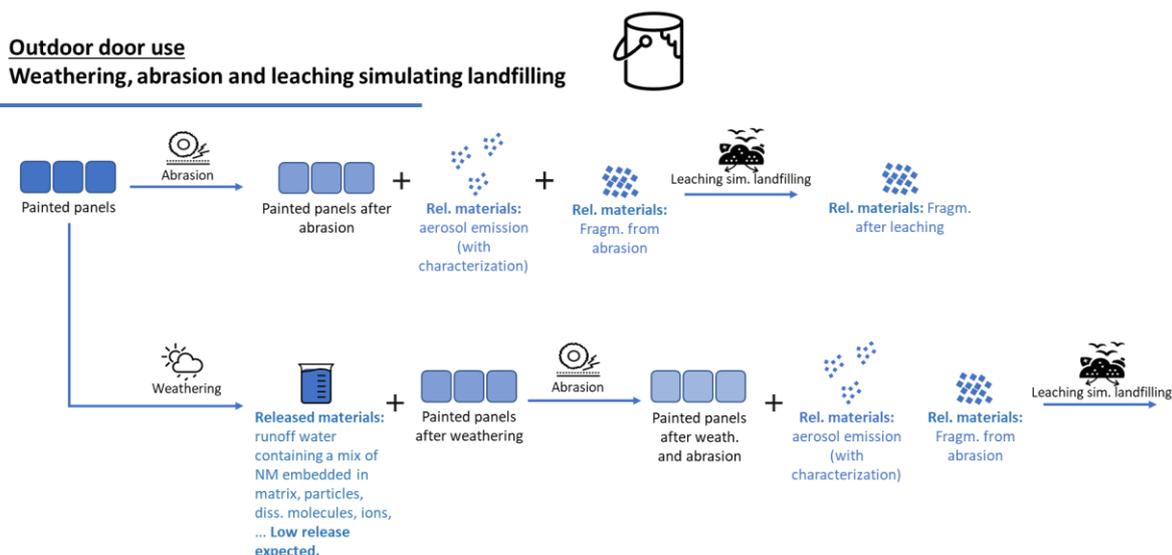
Figure 2. Overview of release experiments and released materials for anti-soiling paint case study (outdoor use scenario).

Two types of scenarios will be investigated to determine the release of NFs during the paint service life i.e. indoor or outdoor use of the paint. According to the manufacturer, the produced paint design is versatile and can be used for both indoor (i.e. in humid and dirty conditions such as a bathroom, kitchen, kids bedroom) and outdoor conditions (i.e. wall façade). In that sense, two relevant cascades of activity will be tested, for indoor, the painted panel will undergo washing and dermal contact. For outdoor conditions, paint panels will be submitted to weathering, then abrasion to simulate a paint removal, and then landfilling to assess the end of life of abraded paint debris. In the following section, each method's scope and justification is described to provide a deeper understanding and the scientific context. Details of each method are then fully described in the annexes.

- Paint washing:

The washability of a coating and the ability to withstand cleaning processes can be easily tested to assess the comparative resistance of coatings to wear and damage in normal service. These tests allow coatings to be evaluated in repeatable and accelerated conditions so that coatings can be formulated and approved for best performance in the service conditions that are to be expected for the coating. For indoor use, the resistance of paint to washing is critical, indoor paint can be classified according to its resistance to this process. We suspect a potential high release of NFs from low resistive paint during washing. For this specific test, we will use an Elcometer following ISO 11998:2006 to determine the wet-scrub resistance and have indications on the cleanability of indoor coatings. This involves 200 wash cycles with sponge and soapy water and the evaluation of the gloss, mass loss and thickness removal layer to classify them into the specified categories according to DIN EN 13300.

- Dermal contact:

Crockmeter experiment to assess nanomaterials for potential ingestion through hand-to-mouth contact using surrogate dermal transfer methods developed previously for evaluating CCA lumber for arsenic. Originally designed to simulate the action of a human finger and forearm, the Crockmeter uses a standard pressure and rubbing motion to provide reliable and reproducible test results. Specimens are positioned on the base of the Crockmeter and held in place with the sample holder. To prevent the specimen from shifting during testing, a sandpaper pad is provided to place under the specimen. A hand crank moves a reciprocating arm a distance of approximately 100 mm. The rubbing action is provided by a 16mm diameter acrylic "finger" which moves back and forth in a straight line with each complete turn of the crank. The reciprocating load arm is weighted to provide a constant 9N load on the sample at all times and a mechanical counter keeps track of completed cycles. A dry and wet crock meter test following ISO method can be used to simulate the release of NFs during rubbing. The Consumer Product Safety Commission (CPSC) and the United States Environmental Protection (EPA) agency develop and validate a method to simulated pollutant dermal transfer from the wood product using a similar setup as the Crockmeter.[6,7] Their homemade setup applies a weight of 1.1 kg resulting from a low-downward force of 10.8 N. In our experiment, Crockmeter experiment involves a similar range of force (i.e. 9 N) making our experiment reasonably realistic to simulate dermal exposure. To extrapolate to real-life events, the energy involved in human movement such as a poke, press, pull, or play a piano note is 45.5, 43.1, 60.1, and 5 N, respectively.[8] The crock meter experiment corresponds to the energy of a smooth rubbing.

- Weathering:

Coatings from paints, varnishes and similar materials are often used outdoors or in indoor locations where they are exposed to solar radiation or to solar radiation behind glass for long periods. It is therefore very important to determine the effects of solar radiation, heat, moisture and other climatic stresses on the color and other properties of polymers. Weathering may alter its physicochemical properties potentially leading to release nanomaterials, which can be in the form of pristine, dissolved or embedded in the matrix. Rainwater in contact with the weathered material may transport the released NF fragments to cleaning water (indoor environment) or surface water drainage systems and from there to the environment, either discharged directly to other water bodies (e.g. rivers, sea) or via wastewater treatment plants. Thus, it is important to evaluate the effect that weathering has on nano-enabled products to determine if release occurs, and in the case of release to determine in what

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[6] Clar J. G. *et al.* (2019) 'Transformation and release of nanoparticle additives & byproducts from commercially available surface coatings on pressure treated lumber via dermal contact', *Science of the Total Environment* 694, 133669.

[7] Platten W.E. *et al.* (2016) 'Estimating dermal transfer of copper particles from the surfaces of pressure-treated lumber and implications for exposure', *Science of the Total Environment* 548, 441-449.

[8] Astin, n.d.; Flückiger et al., 2016

forms and which amounts. Therefore, it can be interesting and time-saving to perform artificial accelerated weathering or artificial accelerated irradiation exposures that use specific laboratory light sources in order to assess the effects of UV-light, heat and moisture on the physical, chemical and optical properties of coatings.

- Abrasion:

Abrasion of various materials (e.g. bulks, NEPs) is a common process that takes place at different scales, under diverse conditions, and in numerous activities. It can be the main or part of a more complex process, and it is also used a test method to determine a material´s abrasion resistance. Moreover, abrasion can be used to simulate another process (e.g. wear, attrition) occurring during the use or end-of-life of NEPs. Therefore, abrasion has been selected as a representative process by which to study emissions and release of nanoforms. However, along with Engineered Nanomaterials (ENMs) process generated NPs (incidental/non-engineered NMs) are also expected to be emitted, making it challenging to discriminate the particle origin. Hence, a combination of online instruments (e.g. particle counters/sizers) and offline chemical characterization (ICP/MS) and electron microscopy (SEM-TEM/EDX) can provide highly resolved data, which may facilitate the quantitative determination of released nanoforms.

- Landfilling (end of life):

The fate and behaviour of NFs incorporated in NEP at the end-of-life stage have received significantly less attention than others process. Since NEP and other consumer goods will be finally disposed in landfills, the understanding of the potential for NFs to be released and transported through landfill leachate is required. In addition, the difference between ionic and particulate released is required under relevant leaching conditions representative of physical-chemical conditions present in the landfill. The results can be used to assess the need to consider the NEP as hazardous waste. We use toxicity characteristic leaching procedure (TCLP) to simulate landfilling. TCLP is a soil sample extraction method for chemical analysis employed as an analytical method to simulate leaching through a landfill. NEP undergoes the TCLP test (The EPA standard Method 1311; 167 Toxicity Characteristic Leaching Procedure) to determine the leaching behaviour from this procedure alone.

2.2.3 Appropriate methods for the additive manufacturing case study

For the sector of the Additive Manufacturing (AM) two different cases have been selected to be studied over their whole life cycle, throughout their different life-stages (synthesis, formulation, manufacturing, use phase/service life, end-of-life). These case studies were based on the applications of the two different NFs (SWCNTs and Ag nanoparticles) and their respective NEPs, more specific information regarding the case-study materials is given in the D 2.10. The applications selected for the case studies were an antistatic tool for vacuum cleaners (SWCNTs in polymer matrix) and an

antibacterial/antimicrobial orthopaedical splint (nano-Ag in polymer matrix). These objects are conventionally composed of plane polymers, but the specific NF provide to the final NEP its respective advanced functionality to amplify their utility. The filaments for both applications were evaluated in an air-controlled dedicated furnace tube to identify and quantify the release of airborne particles and volatile organic compounds upon heating.

Overview of release experiments & released materials for the case study of splints

Exposure scenarios (ES) selected		ES 1: Formulation and production of nano-enabled filaments	ES 2: 3D printing process		ES 3: Touching printed objects ES 5: Touching printed obj. after UV-weathering	ES 4: Weathering of printed objects	ES 6: Recycling of printed objects ES 7: Disposal of printed objects
LCS	Manufacturing	Formulation	Use at Industrial site	Professional Use	Consumer Use	Service Life	End of Life
NMs & NEPs	NM: Ag (TF=antibacterial properties)		NEP: filaments and 3D printed antibacterial door handlers				
Process & Activity	Synthesis of Ag	Formulation and production of nano-enabled filaments	3D Printing of demonstrators (= printed objects)		Simulated touching of printed objects		Recycling & Disposal of printed objects
Compartment(s)		Indoor air	Indoor air		Bio-compartment (skin)		Indoor air & Waters, soil
Exp. population(s)		Workers	Workers		Consumers (end-user)		Workers & Env. Species
Exp. route(s)		Inhalation	Inhalation		Dermal		Inhalation & Dermal, Oral

Figure 3. Overview of release experiments and released materials for AM case study of splints.

On Figure 3, the different exposure scenarios during the whole life cycle for the 3D printing of antistatic NEPs are presented (ES#). The respective processes and activities are linked with the potential release compartments, exposure populations and routes. At the Figure 4 an overview of the methodological studies are presented, where release products are produced via experiments that simulate real-world processes that refer to the exposure scenarios identified above (Figure 3).
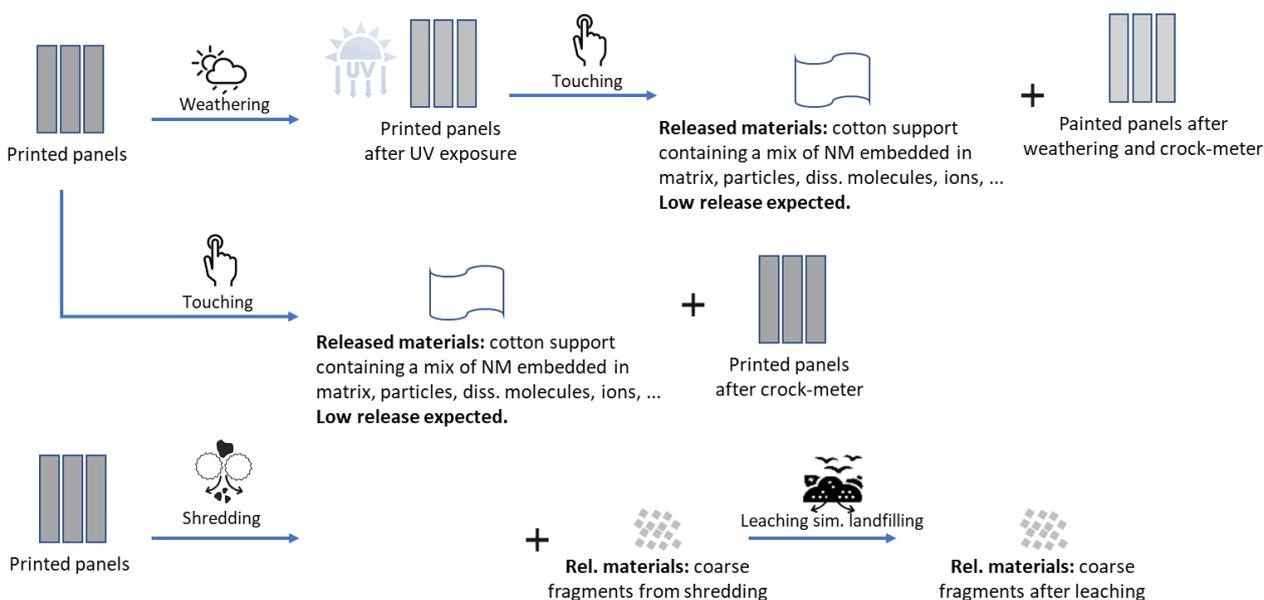
Figure 4. Overview of the methodological studies to assess release for AM case study of splints.

Filaments developed by LATI (conventional and nano-Ag loaded) are used to 3D-print panel-samples that are then solicited mechanically to investigate release. An example of the 3D-printed panels (samples) of the polypropylene-based samples is shown in Figure 5a, while the abraded sample is shown in Figure 5b where the released powders (debris) are collected for further use in hazard studies and characterization. The experimental protocols for the abrasion, dermal contact (Crockmeter) and landfilling (leaching) are common with the ones described in section 1.2.2. Regarding the weathering studies there is a difference in the experimental protocol in comparison with the paint case-studies. Here, only UV exposure of the samples will be considered since it is more relevant for the defined exposure scenario and the subsequent Crockmeter experiments. Scenarios related to the end-of-life will be investigated through shredding studies. Further technical details regarding the experimental protocols for data generation may be found in the Annexes section (page 20 and the followings).

a   **b**

Figure 5. Example of 3D-printed panels before (a) and during Taber abrasion (b).

Overview of release experiments & released materials for the case study of Antistatic tools

Similarly, to the case study of the splints, the exposure scenarios for the case study of the antistatic tools that are 3D-printed using polycarbonate-based filaments (conventional and SWCNTs loaded), are identified in Figure 6 and the respective release experiments are depicted in Figure 7.

					ES 5: Recycling of printed objects
Exposure scenarios (ES) selected		ES 1: Formulation and production of nano-enabled filaments	ES 2: 3D printing process	ES 3: Touching printed objects	ES 4 : Abrasion of printed objects	ES 6: Disposal of printed objects
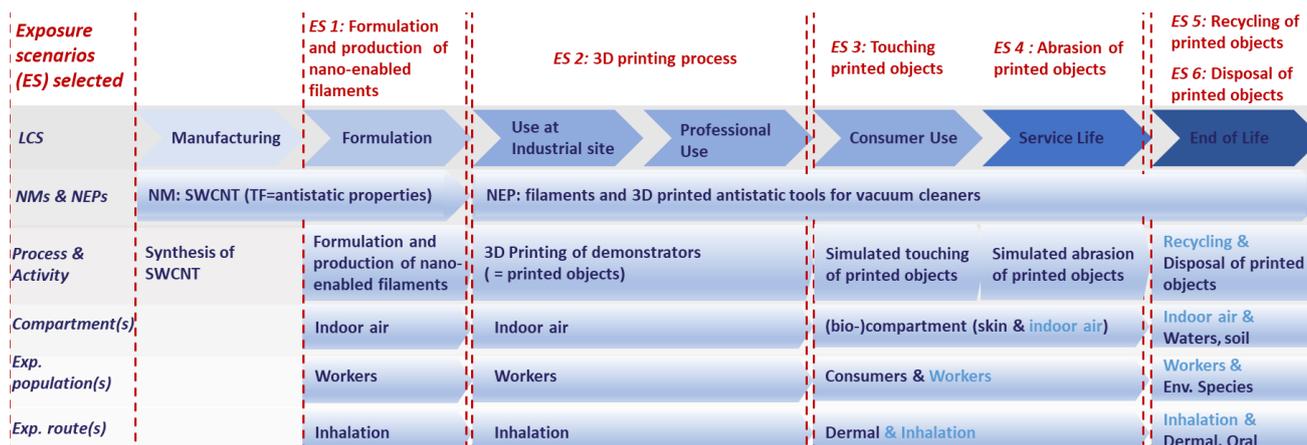
Figure 6. Overview of release experiments and released materials for AM case study of antistatic tools.
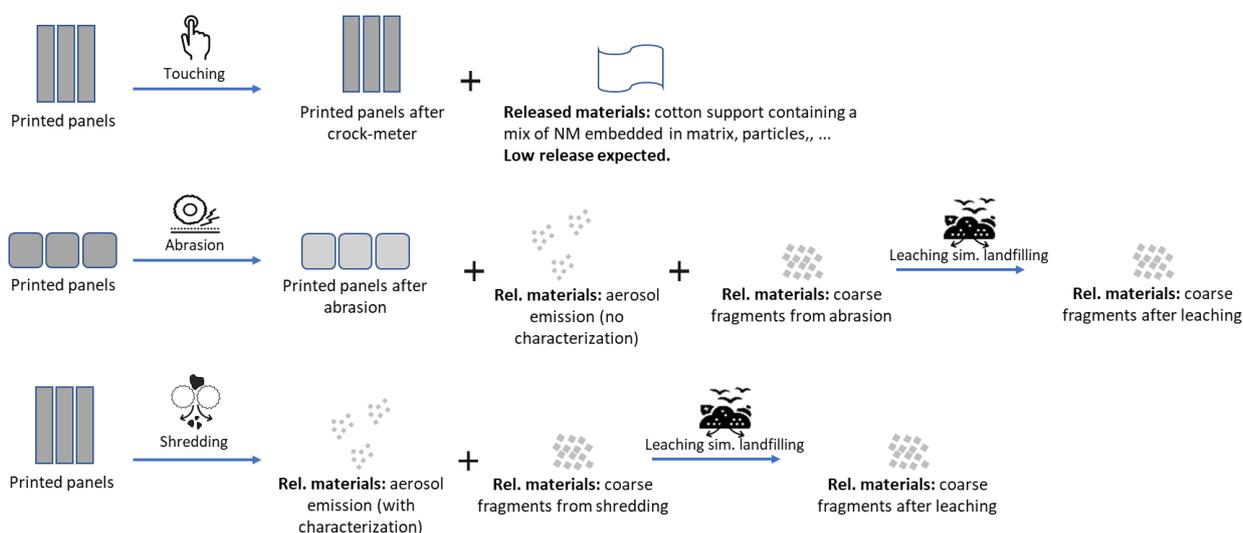
Figure 7. Overview of the methodological studies to assess release for AM case study of antistatic tools.

Further technical details regarding the experimental protocols for data generation may be found in the Annexes section (page 20 and the followings).

3. Deviations from the workplan

No deviations to be reported. The outputs of this task will be further expanded and updated over the course of the SAbyNA project.

4. Summary of work and recommendations for the experimental activities (WP2 & WP7)

Methods and tests for data collection were selected from key parameters such as release rates, dustiness, release forms, transformation rates and exposure concentrations. From this activity,

sector-specific methods that allows human and environmental release, fate and exposure data generation for the two SAbyNA targeted industrial sectors (i.e. additive manufacturing and paints) were developed or adapted and are reported in the present deliverable. Standardized key methods were simplified to facilitate their implementation at the early stages of the innovation process and are detailed in the following annexes:

- Dustiness testing protocol (Vortex Shaker method)
- Weathering and aging protocols
- Mechanical solicitations on paint samples (Taber abrasion, cryomilling and crockmeter protocols)
- Mechanical solicitations on 3D-printed samples (Taber abrasion, crockmeter and shredding protocols)
- Thermal degassing protocol on AM polymer spools
- Mesocosm testing protocol

The recommended methods reported in the present deliverable for human and environmental release, fate and exposure data generation are currently being tested on the two SAbyNA case studies in collaboration with WP7 to establish the relevance and effectiveness of SbD approaches. Provisional results were already reported as D2.9 and 2.10 and those two interim deliverables will be further elaborated by the end of the project gathering the whole experimental activities conducted along the project to assess release and exposure of nanomaterials for the two case studies.

5. Annexes – Simplified protocols for data generation

5.1 Dustiness testing protocol : Vortex shaker method

As described in the EN 17199 series of standards, several methods applicable to nanomaterials are proposed to quantitatively assess the dustiness indices of manufactured nanomaterials. CEA proposed in SAbyNA to investigate specifically the Vortex Shaker (VS) Method (EN 17199-5:2019). This method allows the determination of mass-based and number-based dustiness indexes with a limited sample volume of about 0.5 cm^3 of bulk materials that is adapted for a variety of (nano)powders. This test can be used to classify bulk materials according to their propensity to emit dust and thus aid occupational hygienists and process engineers to evaluate and control the health risk of airborne dust, but also to produce less dusty bulk materials by implementing SbD approaches.

Data reporting templates are being harmonized and the protocols robustness checked through a running intercomparison under the frame of the OECD.

- **Scope and required operating condition**

The vortex shaker method described below is used to characterize nanomaterial powders in order to determine their dustiness indices. The vortex shaker method (VS) consists of a centrifuge stainless tube agitated by a vortex in which the test powdered material is placed. HEPA filtered air, controlled at 50± 5% RH and 21±3°C, passes through the tube in order to transfer the released aerosol to the sampling and measurement section. Using such method allows determining number-based and mass-based dustiness indexes of NOOA powders in the respirable fraction. In addition to the dustiness indexes, the size distributions are also measured. Electronic microscopy analysis of particles aerosolized through Vortex Shaking is optional. The procedure to follow is indicated in the EN 17199-5 standard. The present document reports the key aspects to operate the tests in the best conditions.

- **Process description**

Tests are conducted with VS method using approximately 0.5 cm3 of powder in an 1.5 mL Eppendorf tube. The sample is conditioned (vial remain open) for at least 24 hours in a closed chamber swept by an airflow at 50± 5% RH and 21±3°C. The preparation of NM samples for VS testing requires to take a series of minimum 3 samples of 0.5 cm^3 of the vial containing the nanomaterial received and to accurately weigh the samples. Weighing of the NM samples and of the 37-mm filters from the respirable sampler is performed at CEA with a XPE26 analytical balance (1 µg readability, Mettler Toledo) located in the same room with the VS set-up.

The dimensions of the Vortex tube are indicated in standard EN 17199-5 and the characteristics of the inlet and outlet inner and outer diameter are indicated in the CEN amendment document (outer diameter 8mm, inner diameter 6 mm). All the elements of the setup must be electrically grounded, and this includes: the vortex tube, the cyclone, and the flow splitter. The rotation speed of the vortex tube should be set to 1800 rpm (contrary to what is indicated in EN 17119-5 2019). It is recommended to carry out number-based tests prior to mass-based tests in order to detect possible difficulties

Number-based dustiness index:

The experimental set up corresponding to the determination of number-based dustiness (with size distribution measurements) is presented in Figure 8.
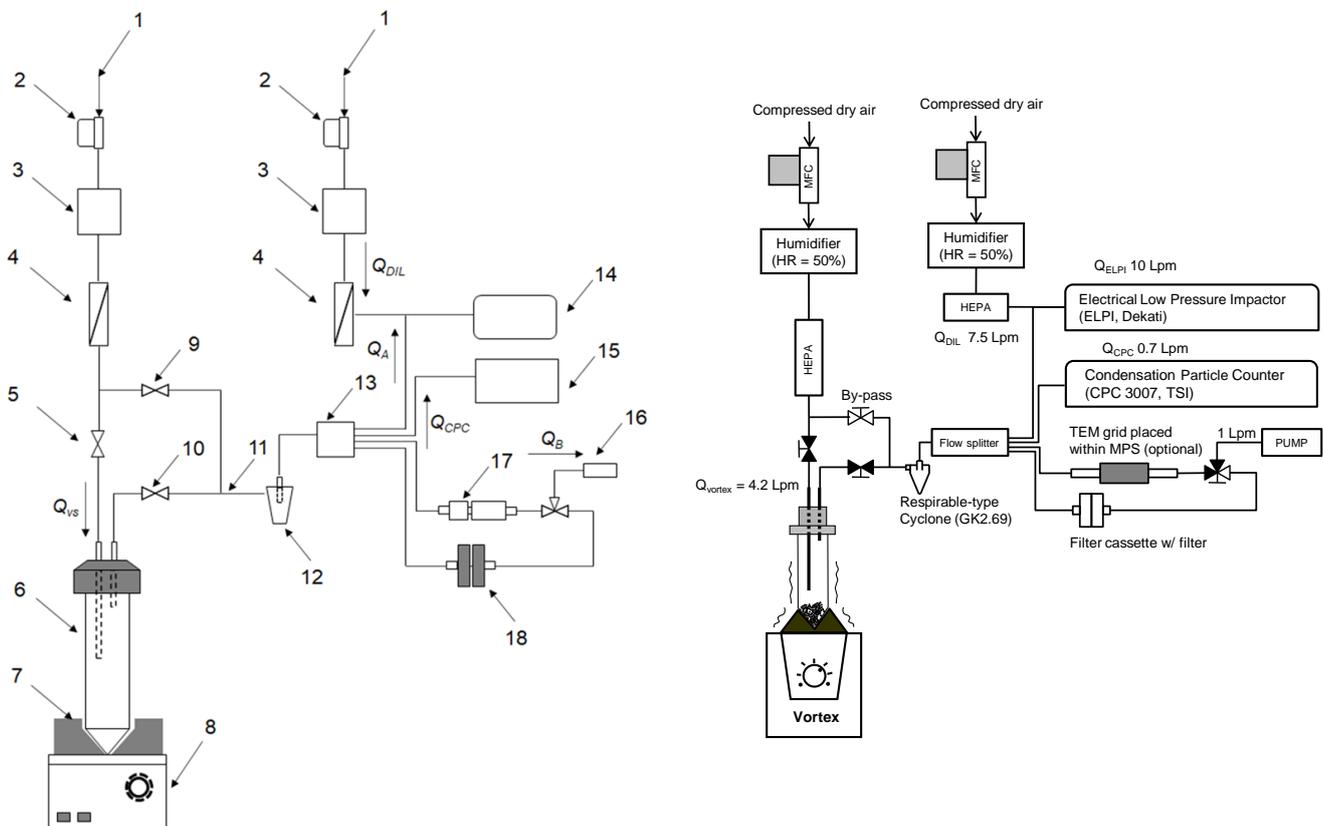
Figure 8. Experimental set-up of the vortex shaker method for measuring number concentrations and particle-size distributions, and for collecting airborne particles for subsequent EM observations.

The different elements this experimental setup is made of are indicated below:

1. Compressed dry air
2. Mass flow controller
3. Humidification system to deliver Qvs = 4.2 lpm at 50 ± 5 % RH and 21 ± 3°C
4. High efficiency particle arrestance (HEPA) filter cartridge
5. Valve to direct incoming air flow through the cylindrical tube
6. Cylindrical container, in which the test sample is poured

7.   Attachement rubber piece adapted to the design of the bottom of the container

8.   Vortex-shaker apparatus producing a circular orbital motion

9.   Valve to direct incoming air flow bypass the cylindrical tube

10. Valve to direct outflow to the sampling and measurement section

11. Tube to the sampling and measurement section

12. GK 2.69 Cyclone

13. Aerosol flow splitter

14. Time- and size-resolving aerosol instrument (ELPI™ Classic 10 Lpm without filter stage and with sintered plates, Dekati)

15. Condensation particle counter (Condensation Particle Counter - Model 3007 CPC, TSI)

16. Sampling pump

17. TEM-grid holder (Mini Particles Sampler developed by INERIS) including a TEM grid (Quantifoil R1.2/1.3 Cu 400 x10).

18. 37 or 25 mm air sampling cassette containing a filter

The different steps to be followed are indicated below:

- Install all the elements of the setup according to scheme of Figure 8.
- Check that the following flowrates are applied: Q_{VS} = 4.2 lpm; Q_{CPC} = 0.7 lpm; Q_A = 2.5 lpm; Q_B = 1 lpm and Q_{DIL} = 7.5 lpm.
- Plug an HEPA filter with a calibrated flowmeter on the particle counter and check the flow rate, the particle concentration (it should be zero) and connect the CPC to the experimental setup according to scheme of Figure 8.
- Check that CPC counts less than one part / cc over 60 s. Start CPC recording at t = 0 s
- Between t = 0 s and t = 300 s
  a.   Open bypass line (valve 9) and close main line (valve 5 and 10)
  b.   Load the test sample in the cylindrical vortex tube
  c.   Place the O-ring
  d.   Screw the vortex tube head
- At t = 300 s, perform quickly the following sequence
  a.   Start agitation of the Vortex
  b.   Open valve 5
  c.   At the same time close valve 9 and open valve 10
  d.   Check that rotation speed is 1800 rpm.
  e.   Stop recording with the particle counter at t = 900 s

     f.   [Optional] Between 300 s and 365 s, activate the valve allowing sampling on the TEM grid. Set sampling time to about 10 s. Close the valve to stop sampling before the end of the agitation.

- Stop the agitation at t = 365 s
- At t = 900 s
  - a.   At the same time close valve 5 and open valve 9
  - b.   Close valve 10
  - c.   Stop the recording
- Remove all the elements positioned between the vortex tube and the CPC for cleaning and this includes:
  - a.   The vortex tube
  - b.   Valve 10
  - c.   The cyclone
  - d.   The flow splitter
  - e.   The cyclone of the CPC
  - f.   And all the tubing from the vortex tube to the CPC
- Clean all the elements using ultrapure water then alcohol and finally dry all the inner parts of the different elements using compressed clean air
- Put back all the elements where they belong according to scheme of Figure 8.
- Restart the whole procedure. Carry out 3 successive test runs following this procedure. Should the relative standard deviation of dustiness indices exceed 30%, increase the number of runs to improve repeatability

The number-based dustiness index is calculated according to the following formula:

$$I_{VS} = \frac{1}{m_{t_1}} \times \sum_{t_1}^{t_1+60} C_{CPC}(t) \times Q_{VS} \times \Delta t_{CPC} \times \frac{10^3}{60}$$

Where

I_{VS} is the number-based dustiness index obtained with the vortex shaker method, in number of particles per milligram (1/mg)

m_{t1} is the mass of powder placed in the test apparatus in milligrams (mg)

$C_{CPC}(t)$ is the particle number concentration from CPC at the time t, in number of particles per cubic centimetre (1/cm³)

Δt_{CPC} is the time step of the CPC in seconds (s)

Q_{VS} is the flowrate within the cylindrical tube during the test, in litres per minute (l/min)

The second version of the setup (Figure 9) is used for collecting respirable mass fraction of the emitted aerosol. The respirable mass fraction is obtained by sampling with a GK 2.69 cyclone (BGI, UK) with a cut-off size d_{50} at 4 µm. The filters are pre-weighed and post-weighed following the recommendations of the ISO 15767:2009 on the same analytical balance.
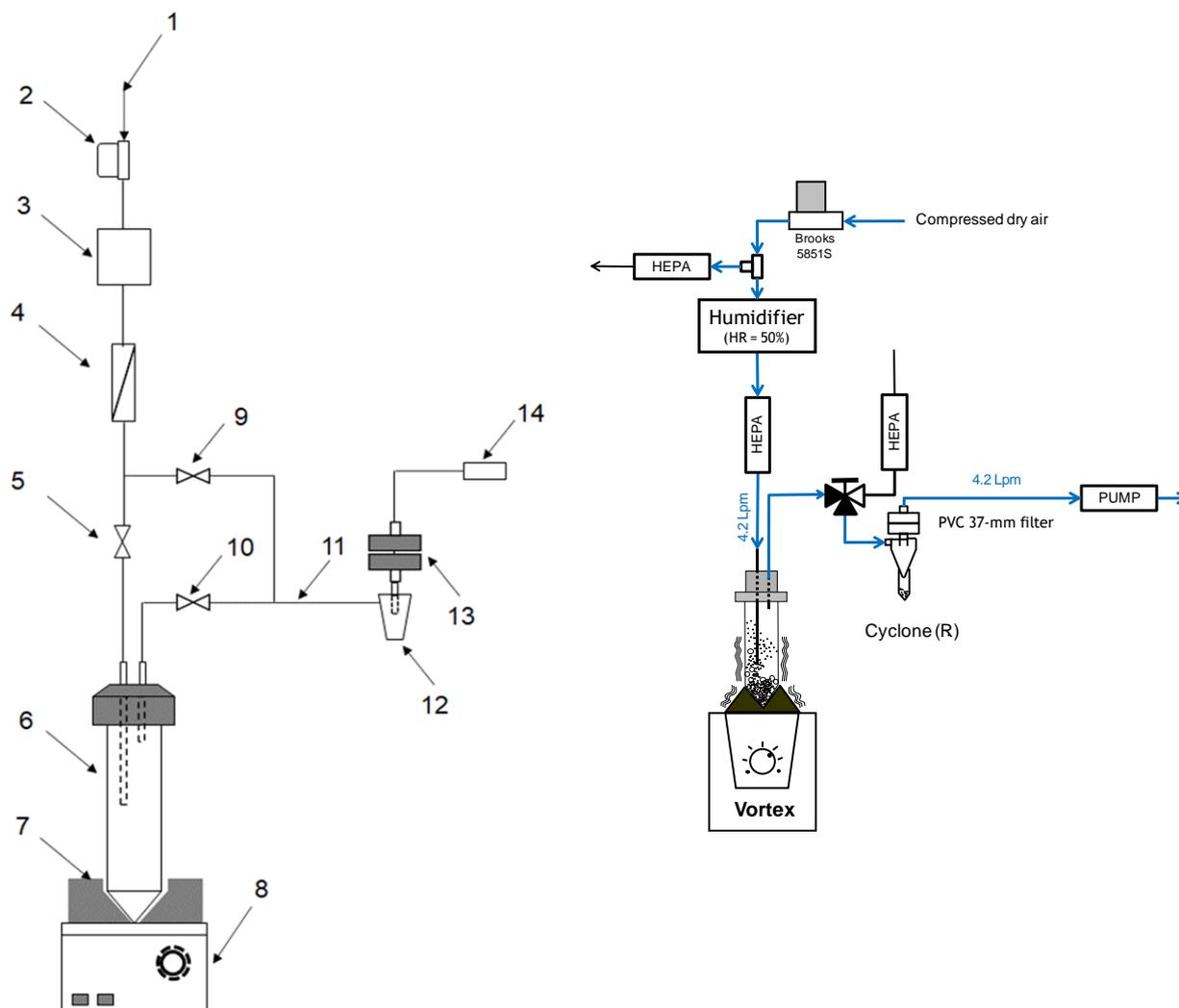
Figure 9: Experimental set-up of the vortex shaker method for collecting respirable mass fraction of the emitted aerosol and determining mass-based dustiness index.

The filter preparation for each powder and for each 37 mm PTFE filter is the following:

- Condition each filter in a petri dish, the box being open at 50 ± 5 % RH and 21 ± 3°C for at least 24h
- Prepare three or more filters for sampling
- [Optional] Prepare at least one lab blank to be taken to the laboratory where dustiness tests take place. These blanks undergo the same handling as the filters used for sampling except that no sampling ever takes place using these, as described in ISO 15767.
- [Optional] Before weighing, neutralize the filters using a neutralizer

- Perform three successive weighings for all the filters and calculate an average value over three measurements for all the filters
- Close the petri dishes for the filters, to be taken to the vortex shaker apparatus namely the filters that undergo sampling and one or several lab blanks

The limit of detection of the mass-based dustiness index (LOD (DI_{RM})) corresponds to the ratio of the gravimetric detection limit to the mass of the test sample, thus the LOD (DI_{RM}) was determined for each experiment. The limit of detection associated with the gravimetric analysis was obtained from the reproducibility of the blank PTFE membrane weights as measured before and after their assembly within a 37 mm sampling cassette used with the respirable cyclone. The blank weight measurements took place over a half-month period and were performed on 3 series of 3 virgin PTFE membranes from the same batch. The gravimetric limit of detection finally obtained was 4 µg (3 times the standard deviation of a blank measurement). Assuming a mass M_0 of 51.67mg, this correspond to a LOD (DI_{RM}) of 80.1 mg/kg.

The test procedure for one run is the following:

- Install all the elements of the setup according to scheme of **Figure 9**.
- Put the filters dedicated to sampling in the cassettes
- The lab blanks undergo all the same steps as filters used for sampling except that sampling never takes place with these
- Check the flowrates. The flow meter is positioned between the filter and the pump to check that 4.2 lpm flows through it.
- Connect the cassette containing the filter to the cyclone
- At t = 0 s, open bypass line (valve 9) and close valve 5 and 10
- Between t = 0 s and t = 300 s
  - a. Load the test sample in the cylindrical tube
  - b. Place the O-ring
  - c. Screw the tube head
- At t = 300 s, perform quickly the following sequence
  - a. Start agitation of the Vortex
  - b. Open valve 5
  - c. At the same time close valve 9 and open valve 10
  - d. Check that rotation speed is 1800 rpm.
- Stop the agitation at t = 365 s
- At t = 900 s

- a.   At the same time close valve 5 and open valve 9
- b.   Close valve 10
- Remove the filter from its cassette
- Put the filter in a petri dish and put the cap on the petri dish
- Remove all the elements positioned between the tube and the CPC for cleaning and this includes:
  - a.   The vortex tube
  - b.   Valve 10
  - c.   The cyclone
  - d.   And all the tubing from the vortex tube to the cyclone
- Clean all the elements using ultrapure water then alcohol and finally dry all the inner parts of the different elements using compressed clean air
- Put back all the elements where they belong
- Restart the whole procedure. Carry out 3 successive test runs following this procedure. Should the relative standard deviation of dustiness indices exceed 30%, increase the number of runs to improve repeatability

Particular attention is given to the experimental device cleaning between successive tests. All pipes and other connections are systematically cleaned with water and/or ethanol and dried in an oven, or changed when needed. The checking of the airflows is performed using a primary flow bubble calibrator (Gillian® Gillibrator 2). Prior to each test, the cleanliness of the air is assessed on the basis of measurements made using the CNC. In the case of a non-compliant result, the whole procedure is repeated from the beginning.

The enriched filters are weighed as follows:
- Remove the cap of the petri dishes and let the filters in the conditioning chamber for at least 24 hours (for stabilization)
- [Optional] Before the weighing, neutralize the filters using a neutralizer
- Perform three successive weighings of each of the enriched filters and each of the lab blanks (at least one) and calculate the average value of the mass over these three measurements for each filter
- Calculate the sampled aerosol mass Δm for each filter by subtracting the average mass (average over three measurements) measured before sampling from the average mass measured after sampling
- [Optional] Calculate a possible mass variation ΔT of the lab blanks by subtracting the average mass of each of the lab blanks (at least one) measured before the Vortex Shaker experiments

from the average mass measured after the Vortex Shaker experiments (note that ΔT could be positive or negative)

- The final mass M is obtained as follows: M = Δm – (1/n)*(ΔT1+…+ΔTn) (n= 1, 2 or 3)
- Report the obtained mass for dustiness index calculation
- Carry out 3 successive test runs following this procedure. Should the relative standard deviation of dustiness indices exceed 30%, increase the number of runs to improve repeatability

The mass-based dustiness index is calculated according to the following formula:

$$W_{r,VS} = \frac{\Delta m_f}{m_0} \times 10^6$$

Where

$W_{r,VS}$ is the mass-based dustiness index with the vortex shaker method given in mg/kg

Δm_f is the mass collected by the filter in the respirable cyclone, in milligrams (mg)

m_0 is the mass of the test sample, in milligrams (mg)

The validation of a test depends on several factors such as: 1) the stability of the parameters during the test, 2) a good reproducibility of measured number concentrations, 3) a good sequence of steps for the respirable aerosol sampling etc. At CEA, the entire set-up is located inside a fume hood to prevent exposure of the operator (see Figure 10).

Figure 10 : Overview of the vortex shaker set-up positioned in the variable flow fume hood at CEA.

- **Dustiness data gathering**

Test reports shall contain at least the following information:

1. a reference to the standard (EN 17199 series);
2. name and address of test laboratory and test personnel;
3. date of testing;
4. batch identification of the bulk material tested if applicable;
5. a reference to EN 17199-5, to specify the VS test method that was used;
6. volume of each sample taken from the bulk material in cubic centimetres (cm^3);
7. sample conditioning conditions (relative humidity, in per cent (%) temperature, in degree Celsius (°C), and conditioning duration in hours (hr))
8. moisture content of the bulk material, given as mass ratio in per cent (%), with specification of analytical method;
9. bulk density in kilograms per cubic metre (kg/m^3) with specification of analytical method;
10. testing environmental conditions such as relative humidity, in per cent (%), and temperature, in degree Celsius (°C), at the time of testing;
11. respirable dustiness mass fraction, given in mg/kg, for each replicate test, plus the mean and the standard deviation of all test results;
12. number-based dustiness index, given in 1/mg, for each replicate test, plus the mean and the standard deviation of all test results;
13. number-based emission rate, given in in 1/mg·s, for each replicate test, plus the mean and the standard deviation of all test results;
14. number of modes of the time-averaged number-based particle size distribution as dN/dlogD i;
15. values of the modal aerodynamic equivalent diameters corresponding to the highest mode and to the second highest mode of the time-averaged number-based particle size distribution as dN/dlogD i for each replicate test, plus the mean and the standard deviation of all test results.

The test report may contain the following additional information for non-mandatory testing (optional):

16. dustiness mass fractions (thoracic, inhalable), given in mg/kg, for each replicate test, plus the mean and the standard deviation of all test results;
17. number of modes of the time-averaged mass-based particle size distribution as dM/dlogD i;
18. values of the modal aerodynamic equivalent diameters corresponding to the highest mode and to the second highest mode of the of the time-averaged mass-based particle size distribution as dM/dlogD i, plus the mean and the standard deviation of all test results;
19. morphological and chemical characterization of the sampled particles;
20. data (e.g. dustiness indices) from benchmark material for comparison and ranking purpose.

The OECD working group on Dustiness testing developed a specific Excel sheet to compile the above mentioned parameters.

5.2 Weathering and aging protocols

CEA, CNRS and LEITAT adapted protocols to conduct artificial weathering studies on paint samples and printed items using climatic chambers.

Materials and equipment needed

➢ Materials

For paints, the coating needs to be applied on test panels. It is really important to achieve a uniform coating to perform these tests. Different panels are commercially available (e.g. steel S-16, wood S-17, aluminum S-18, glass S-31, Leneta black scrub test panels…). It has been decided to apply the Allios-formulated paints thanks to a bar-coater (manual film applicator) on the following types of panels:

- Aluminum (S-18) - Produced out of #19 gauges 5052 aluminum. 100 mm squares with rounded corners and a 6.35 mm-diameter center hole. (Taber industries, USA).
- LENETA black scrub test panels (165 mm x 432mm) (black plastic-vinyl chloride/acetate copolymer with a smooth matt surface, Leneta company, USA.

Figure 11. Left Taber aluminium substrate (up coated, down uncoated), right leneta substrate

➢ Equipment

Leneta and Taber substrates coated with the different paint formulations are subjected to artificial weathering in an accelerated weathering chamber. The following instruments are complying with ISO 11507, ISO 16474 and ISO 4892, respectively:

- Accelerated weathering tester (Q-UV from Q-LAB) that allows UV irradiation, heat and water spray. The irradiance spectrum is increased in the UV region to provide accelerated degradation of the samples

- Xenon test chamber (Q-SUN from Q-LAB) that allows light irradiation, heat, moisture and water spray. The irradiance is close to the solar spectrum with enhanced energy
- Suntest XXL, Atlas. The models is adapted to collect the runoff water. The chambers is divided in 4 compartments

Figure 12: Suntest XXL, Atlas, Accelerated weathering tester

Figure 13: Q-UV from Q-LAB, Accelerated weathering tester

Figure 14: Q-SUN from Q-LAB, Xenon test chamber

To study the effects induced by the climatic testers on the surface structure and chemistry, several types of characterizations can be performed using the following instruments:

- X-ray Photoelectron Spectroscopy (XPS)
- Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)
- Scanning Electron Microscopy with Energy Dispersive Spectrometer (SEM-EDS).
- Hydrophobicity by contact angle measurement (goniometer)
- Surface roughness
- Potential color testing

It has been decided to age the Leneta and Taber coated substrates in an accelerated weathering chamber (model QUV accelerated weathering, Q-Lab, USA - Figure 13) at CEA, according to the ISO 16474-3 standard (method C, cycle 5). The paints were exposed to UVB-313 lamps for 5 hours under the following conditions: an irradiance of 0.71 W m^{-2} at 310 nm, temperature set at 50 ± 3 °C and a relative humidity of 50%. A second step was applied for 1 hour under wet conditions where water was sprayed at a flow rate of 7 L min^{-1} to reach a specific relative humidity of 100% and a temperature of 25 ± 3 °C without irradiation. The paints underwent sequentially those cycles for 1000 hours.

The surface was characterized along the aging with a BYK-Gardner Color-Guide 45/0 spectrophotometer by steps of 200 hours.

The LEITAT proposed to follow a variant for the aging of plastics following ISO 4892:2013 (Plastics – Methods of exposure to laboratory light sources – Part 2: Xenon-arc lamps). The printed panels are exposed to UVB lamps for 102 min under the following conditions: an irradiance of 0.5 W m^{-2} at 340 nm, temperature set at 65 °C. A second step is applied for 18 min under wet conditions where water is sprayed to reach a specific relative humidity of 100% and a temperature of 25°C without irradiation. The paints underwent sequentially those cycles for 1004 hours.

➢ Procedure for an experiment using the Q-SUN or Q-UV devices:

- Put on protective equipment (gloves, glasses and laboratory coat)
- Clean the plate where samples will settle using wipes and deionized water
- Start a cleaning procedure (see below) and empty the drain water collector
- Fix the sample on the main plate and check if the correct side is oriented towards the lamps
- Close the door of the apparatus
- Turn on the equipment and program the weathering cycles to be applied (temperature, moisture, irradiance, water spray, time …)
- Start the program
- Once the program end, take the samples out and collect the drain water if needed
- Start a cleaning procedure (see below)
- Turn off the apparatus

➢ Cleaning procedure:

- Check the irradiation chamber is empty
- Start the cleaning program (water spray for 24h, lamps off, room temperature)
- empty the drain water collector and rinse the drain water collector

To study the leaching and to evaluate the subsequent emissions to the environmental compartments, the waste water from the experiments could be analyzed using the following instruments:

- Dynamic light scattering (DLS, Zetasizer Nano ZSP+ from Malvern) could be used to characterize the hydrodynamic diameter distribution and the zeta potential of the released (nano)particles.
- Field flow fractionation coupled to a multi angle light scattering detector (AF4-MALS from Wyatt Technologies) and to an inductively coupled plasma-mass spectrometer (ICPMS 7900 from Agilent) could be used to characterize the size distribution, the chemical composition (of metal-based particles only) and the concentration of the released (nano)particles.

- Transmission electron microscopy (TEM Tecnai Osiris from FEI) could also be used with or without a cryogenic mode to corroborate the information regarding the size distribution and the chemical composition of the particles and their state (primary particles, aggregates or agglomerates, particle bounded to chunks of matrix …).
- Centrifugal filtration at 3KDa to quantify the ionic and particulate release fraction

> ➢ Adapted procedure for nanospinels paints (CEREGE)

The paint was tested on fibro-cement blocks with dimensions of 25x25x5 mm each (material used by partner Allios to test the paint aging). A total of 100 samples was prepared. First, all the samples received one layer of nanospinel-free paint (on all 6 sides of the blocks), and were let to dry overnight. This layer was meant to prevent the direct exposure of the fibrocement during the experiment. Once dried, the samples were randomly assigned to either "control" or "nanospinel" treatment. In the "nanospinel treatment", one 25x25 mm side of the samples was painted with three layers of $ZnAl_2O_4$-doped paint (2% weight), with a 2 hour drying time between each layer. The $ZnAl_2O_4$ nanospinel were synthesized at 1000 °C and coated with citrate. For the control samples, this procedure was repeated with the nanospinel-free paint. All samples are weighed before and after each paint layer in order to estimate the amount of paint.

The aging of the nanospinel-doped paint was carried out with the procedure described by Scifo et al. (2018)[9], viz. a 12 weeks incubation performed in a climate chamber (Suntest XLS+, Atlas Material testing Solutions, Germany). The experiment was performed according to the aging standard NF EN 927-6, 2006, designed for wood stains. Here, we applied this protocol with minor modifications for studying the aging of paint coated fibro-cement samples. Briefly, the weathering program consisted of weekly cycles which included condensation, irradiation and water spraying steps (Figure 15).
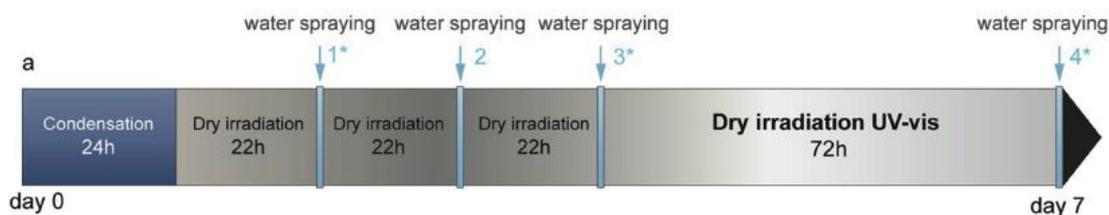
Figure 15. Weekly cycle applied for aging of the paint. Cycles were repeated 12 times.

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[9] Scifo L., Chaurand P., Bossa N., Avellan A., Auffan M., Masion A., Angeletti B., Kieffer I., Labille J., Bottero J.Y. and Rose J. (2018) Non-linear release dynamics for a CeO_2 nanomaterial embedded in a protective wood stain, due to matrix photo-degradation *Environ. Pollut.*: 241; 182-193.

At the beginning of each cycle, a 24 h condensation step was performed using a double-wall reactor system outside the climate chamber (Figure 16). The samples were placed into the reactor, which was constantly fed with ultrapure water (UPW) vapor from a heated reservoir. A refrigeration system allowed the steam condensation onto the reactor walls, and a peristaltic pump recirculated the condensed water back to the reservoir. Two separate reactors were used for the control and nanospinel treatments. After the condensation step, the samples were placed into 800 ml glass beaker, and transferred into the climate chamber. The chamber held 5 beakers: 3 containing nanospinel samples and 2 containing control samples. In each beaker, 6 samples were placed onto a raised and slanted PTFE holder in order to prevent soaking during the water spraying.

In the climate chamber, the weathering combined UV irradiation (Xe UV lamp) and "rain" (viz. UPW spraying), in order to simulate long-term "realistic" scenario. The samples underwent three sequences of 22 hour irradiation periods followed by 30 min water spraying. The last sequence of the weekly cycle consisted in a 72 hour irradiation followed by 30 min water spraying (Figure 16). Each spraying step consisted of approx. 300 mL UPW.
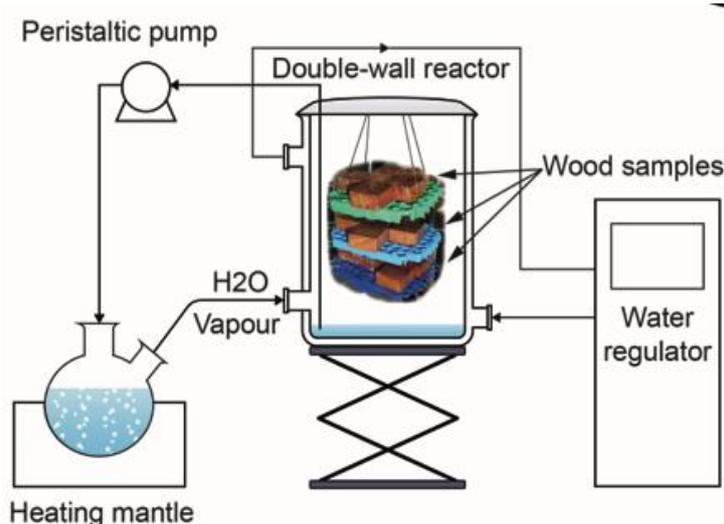
Figure 16. Setup used for condensation phases in Suntest weathering (outside climate chamber).

In order to monitor the Zn and Al release during aging, ICP-MS measurements were performed on the condensation and sprayed water samples: 10 mL water were sampled for total metal determination. Additional 10 mL underwent ultrafiltration at 10 kDa before the analysis, in order to determine the dissolved metal fraction. After sampling, conductivity and pH measurements were carried out in the beakers.

5.3 Mechanical solicitations (dry and wet abrasion protocols)

CEA also adapted protocols to conduct mechanical solicitations following dry and wet abrasion protocols. The "dry" abrasion protocol will be referred to the Taber abrasion protocol while the "wet" protocol refers to the wet-scrub resistance test. Both protocols could be applied to paints and printed panels.

- **Scope and required operating conditions of the Taber abrasion protocol**

This procedure describes a method to determine the potential emission level of NOAA from different coatings and composites. This procedure could also be used to determine personal exposure level of practitioner when performing abrasion tests on different products. To perform accelerated wear testing, the TABER® Rotary Platform Abrasion Tester (Model 5135 or 5155) is commonly used. The Taber Abraser (Abrader) rapidly became the world standard for evaluating abrasion resistance. This precision-built instrument is capable of providing reliable data in a matter of minutes compared to the years that may be required by in-use testing.

The aim of this SOP is also the development and implementation of "resistance" tests for materials incorporating nanoparticles. Thanks to the Taber Abrader, it is possible to deduce the effect of an aging process on the material resistance. The mass loss deduced by the difference of specimen weight before and after abrasion is one of the possible measured parameters with the Taber device. Another possible measurement is the Taber Wear Index. It indicates the rate of wear and is calculated by measuring the sample weight loss per thousand cycles of abrasion. The lower the wear index, the better the abrasion resistance.

Two different sets of abrasion experiments are described in the following section. During the first set of abrasion experiments, the weight loss of each sample is monitored, and the release rates is determined by weighing the NEP after abrasion every minute for total duration of 10 min. The steps of the first experimental protocol are:

    a) firstly, the weight of untreated sample is measured (beforehand)

    b) then one minute of abrasion is performed

    c) the powder (fragments) on the top of the sample is removed

    d) the sample is weighted again (note # minutes)

The second set of experiments is the real-time monitoring of aerosol emissions with the use of direct-reading (online) instruments. For the second experimental protocol three different types of measurements are performed online before the abrasion of the selected NEP:

- Background, which is the online monitoring of air inside the emission chamber without any activity.

- Blank (abrasion), which is the online monitoring of air inside the emission chamber with the motor of the Taber Abraser running but without any contact with the substrate (no abrasion).
- Reference, which is the online monitoring of an abrasion process of the conventional material (matrix polymer or paint) of the respective NEP without any nanoform in their matrix.

This test consisted of mechanical solicitation by abrasion performed on a Taber rotary abraser (model Abraser 5135, Taber industries, USA), according to the ISO 7784-2 standard. Experiments are performed in triplicates for each sample (3 replicates). For each repetition, two sets of new S42 abrasive sticker paper were used on CS-0 type wheels, and an untreated surface of the sample is abraded for at least 200 cycles at a load from 500 g to 1250 g for a rotation speed of 60 rpm during at least 200 cycles.

Sampling of airborne particles is done using a pre-weighted filter. A portable pump (GiliAir and LELAND SKC) is connected to the filter cassette (SKC). One filter sample is taken during all the replicates of the same experiment (one NEP sample) to obtain sufficient mass of potential released material for further analysis. The cellulose filter samples are digested and analysed chemically by ICP-MS. A second portable pump is connected to a sampling cassette which is housing an electron microscopy grid, for offline TEM/EDX analysis. The sampling is performed simultaneously to ensure harmonization.

Alternative protocols:

For nanocomposite materials like tires, a specific International Standard was published with the number ISO 5470-1. This standard is entitled "Rubber or plastic coated fabrics – Determination of abrasion resistance – Part 1: Taber abrader" and specifies the abrasion conditions with a Taber. Another possibility for specific scenarios of use is to use a custom-made apparatus for the abrasion. The proposed setup is composed of a weighing scale, an abrasive paper stuck with a double-face scotch tape, a circular support connected to an electric drill on which the sample is stuck with a double-face scotch tape. With this experimental apparatus, it is possible to control parameters like rotation speed, pressure (weight) on the sample to reproduce different abrasion conditions (Figure 17). In the case of nanocomposites, the sample is abraded keeping a relative speed between abrading material and sample of 0.5 m/s or 1.8 m/s. Then, the sampler collects the aerosol and the particles are analyzed by particle counters and granulometers. Particles are also collected on a TEM grid thanks to a specific device. ELPI membranes might be advantageously analysed by SEM/EDX.
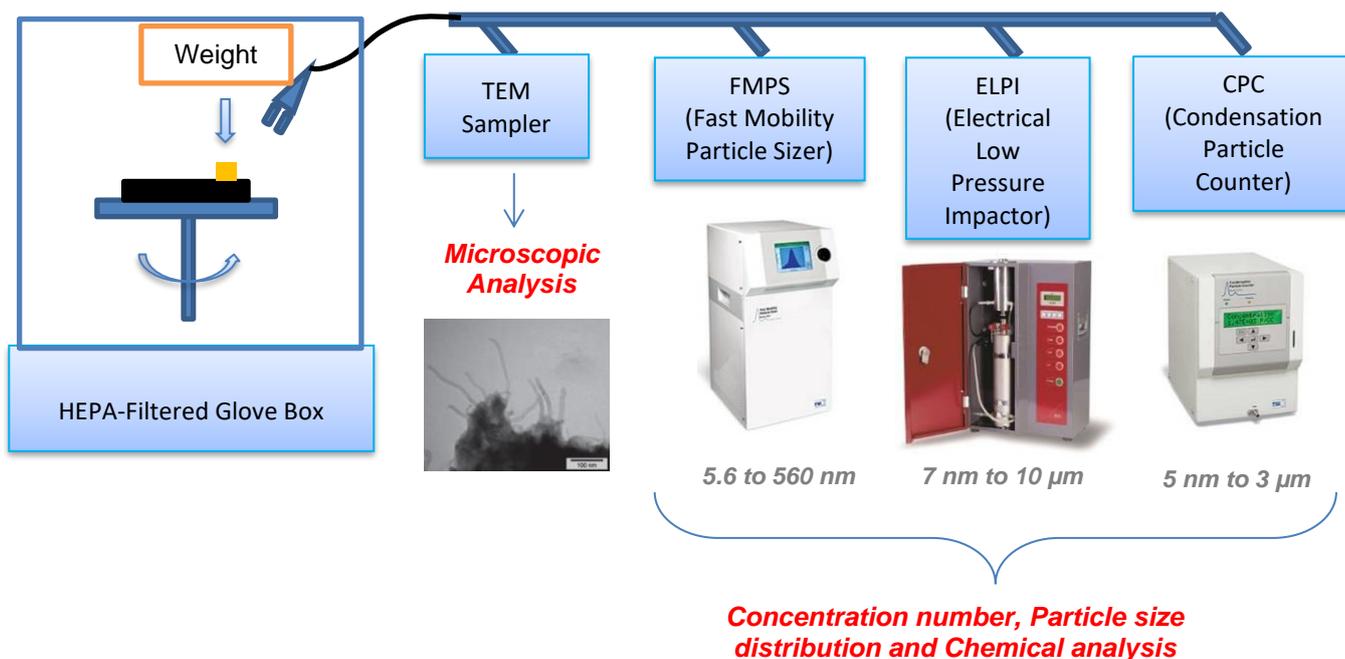
Figure 17: Experimental scheme of a customized sanding setup for nanocomposites

The potential release of metal oxides such as TiO_2, SiO_2 from paint during smooth mechanical stress can be assessed by tailored abrasion tests using an Abrader (Taber linear abraser 5750) with controlled pressure and various available tools to evaluate its resistance to scratch and mar. For instance, ISO 105-x12 (initially designed to determine the resistance of the colour of textiles, two tests may be made, one with a dry rubbing cloth and one with a wet rubbing cloth) can be adapted. The maximum total rubbed area is between 15 and 25 cm², depending on the tool. Both wet and dry abrasion can be performed. The Abrader is mounted on a measurement bench located in a glove box with very low particle concentration level, so that potential airborne particle release during the abrasion process can be measured. Transfer of material to the abrading tool (cloth for instance) can be analyzed quantitatively by acid digestion in a µ-wave oven (Multiwave 3000, Anton Paar) and determined by ICP-MS (Agilent 7700, Agilent Technologies).

- **Materials and equipment needed**

  ➢ Nano-enabled paints and coatings

As indicated in the weathering section, different panels are commercially available (e.g. steel S-16, wood S-17, aluminum S-18, glass S-31, Leneta black scrub test panels…). It has been decided to conduct the Taber abrasion testing on aluminum coated panels (S-18) (Taber industries, USA).

  ➢ Equipment

To perform sanding experiments on painted panels or printed nanocomposites, the following instruments are used within a glove box having a low nanoparticle background:

Paints and coatings	Nanocomposites
ISO 7784	ISO 23794:2015 and/or NF EN ISO 5470
- TABER® Rotary Platform Abrasion Tester (Model 5135 or 5155, Taber industries, USA) - S42 Taber abrasive sticker paper (180 ANSI-CAMI)	- TABER® Rotary Platform Abrasion Tester (Model 5135 or 5155, Taber industries, USA) - Abrasive wheel from gentle to very coarse abrasive action (80 to 1420 grit size) - [optional] Abrasive powder for Frick Taber abrasion test to simulate wear of flooring

The experiments were performed in a 0.25 m^3 glove box equipped with a HEPA filter reducing the particle concentration background below 5 particles per cm^3. The glove box air was extracted upwards (HEPA filter at the bottom and extraction at the top) at a rate of 150 L min^{-1}. The glove box is equipped with transparent organic glass walls (charge dissipative material). The volume of the cubic chamber is 0.2 m^3. An airtight small hole was drilled on the wall to allow sampling.

The Taber Abraser will be placed in the glove box and the sampling line is located at the closest point where the abrasion occurs between the sample and the tool (Figure 18).

Figure 18 : Taber abrader placed inside a low background glove box with the associated instrumentation (CEA setup)

The particles released by the abrasion process were characterized in real time in terms of particle size distribution and concentration using respectively a fast mobility particle sizer (FMPS, model 3091, TSI Incorporated, USA) and a condensation particle counter (CPC, model 3775, TSI Incorporated, USA). Optionally, an ELPI might be used to determine the particle number size distribution in real time while airborne particles are deposited on filters put on impaction plates. These filters (hydrophilic polycarbonate membrane) could then be analyzed by Scanning Electron Microscopy (SEM) to determine the shape/morphology and size of the collected particles, their aggregation state, and whether they are free or embedded in the matrix. SEM/EDX analysis can also be performed to determine the particle elemental composition. In addition, a collection device installed on a sampling

rod allowed the released particles to be trapped on a hydrophilic polycarbonate membrane (pore size 0.4 μm, Millipore, USA). The collected samples were characterized by SEM-EDX to determine the particle morphology, size and elemental chemical composition.

At LEITAT, particle size distributions and number concentrations are monitored using a NanoScan-SMPS (model 3910, TSI, USA) for particles in the size range 10-420 nm, and an Optical Particle Counter (model 3330, TSI, USA) to monitor particle emissions in the size range 0.3-10 μm. The inlets of the instruments were positioned above the abraser. Tygon® conducting tubes are used as sampling tubes both at CEA and LEITAT.

- **Generic procedure for an experiment:**

  - Put on protective equipment (gloves, glasses and laboratory coat).
  - Clean the glove box and check the filter.
  - Ensure that the ventilation of the glove box is turned on at the correct rate.
  - Fix the sample on the Taber abrader or on the circular support according to the above description.
  - Fix the appropriate abrasive wheels, or the abrasive paper on the wheels of the Taber or on the specific support according to the above description.
  - Close the door of the glove box.
  - Turn on the ELPI and its pump. The equipment has to be cleaned with a pure air flow during 15 min (before each test, all the filters of the column of the ELPI have to be changed).
  - Turn on the CPC. This device must warm up for 10 min so that the detector is operational.
  - Turn on the FMPS. This device must warm up for 10 min.
  - Make the baseline of the ELPI.
  - Stop the cleaning of the equipment and connect the ELPI to the aspiration system.
  - Synchronize the starting time of all tools and start data acquisition.
  - Reduce the ventilation flow to 30 L/min.
  - Start the abrasion process. Note down the starting time point. This process lasts approximately 4 minutes.

- **Cleaning procedure:**
  - Turn on high level ventilation.
  - Use a special vacuum cleaner equipped with a HEPA filter to clean the box.
  - Remove the abraded sample.

- **Scope and required operating conditions of the wet-scrub resistance test**

For this specific test, an Elcometer (Figure 19) following ISO 11998:2006 is used to determine the wet-scrub resistance and have indications on the cleanability of indoor coatings. This involves 200 wash cycles with sponge and soapy water and the evaluation of the gloss, mass loss and thickness removal layer to classify them into the specified categories according to DIN EN 13300.

As indicated in the weathering section, it has been decided to conduct the wet-scrub resistance test on LENETA black scrub test panels (165 mm x 432mm) (black plastic-vinyl chloride/acetate copolymer with a smooth matt surface, Leneta company, USA.

Figure 19. Elcometer 1720 model (wet scrub resistance apparatus)

5.4 Crockmeter protocol

- Process description

The potential release of Nanomaterials from paint during smooth mechanical stress was assessed by performing rub abrasion tests, following a standardized protocol (ISO 105-X12:2016), originally designed to test the color fastness on textiles due to rubbing. The test consisted of a cotton wipe used as abrasion material and a Crockmeter (302, JBA instruments), which applied a controlled pressure and rubbing motion to the paint surface to provide reliable and reproducible results. One cycle corresponds to two passages on the surface (go and back) with a total area of 30.9 cm^2. Rubbing tests with both wet (i.e. milliQ, simulated saliva and sweat) and dry cotton were performed. Once the total number of cycles was reached, the cotton used as abrasion material was digested with acid in an analytical microwave digestion system (MARS, CEM, 1600W) and the amount of NMs transferred from the paper to the cotton was determined by ICP-MS (Agilent 7500, Agilent Technologies). All the experiments were conducted in triplicate. For TiO_2 release rates calculations, the release was assumed to be uniquely caused by rubbing (no airborne emissions).

- Materials and equipment needed

Figure 20. Crockmeter (302, JBA instruments).

Nano-enabled paints and coatings: without further treatment the painted panel or 3D printed plastics plates of at least 10 cm length and 5 cm large (to be able to performed triplicate) will be tested.

- Generic procedure for an experiment:
- Clean the crockmeter surfaces
- Fix the nanocomposite plate with a double tape
- If dry rubbing, fix the cotton on the support
- If wet rubbing, soaked the cotton with 2 mL of the appropriate solution and left overnight
- Set the number cycle to perform
- Run the crockmeter
- Collect the coton
- Move the nanocomposite panel and repeat the process to have triplicate
- Digest the coton

5.5 Landfilling simulating protocol

Toxicity Characteristic Leaching Procedure (TCLP) is designed to determine the mobility of both organic and inorganic compounds present in liquid, solid as well as multiphasic samples. It is a method that simulates what happens when a sample is disposed in a landfill and is utilized to determine whether the contaminated soil meet the EPD landfill disposal guideline.

Summary: The solid phase is extracted with an amount of extraction fluid equal to 20 times the weight of the solid phase. The extraction fluid employed is a function of the alkalinity of the solid phase of the waste.

Perform preliminary TCLP evaluations on a aliquot of waste: This aliquot may not actually undergo TCLP extraction. These preliminary evaluations include: (1) determination of the percent solids; (2) determination of whether the waste contains insignificant solids and is, therefore, its own extract after filtration; (3) determination of whether the solid portion of the waste requires particle size reduction; and (4) determination of which of the two extraction fluids are to be used for the nonvolatile TCLP extraction of the waste.

In the case of paint / plastics NEP, all product are 100% solid, and the focus of is on the NFs release, and the NEP contains negligible amount of volatile organic compound, Point 1 and 2 are not relevant in our case.

Point 3, <u>The size reduction of waste</u>. Particle size reduction is required, unless the solid has a surface area per gram of material equal to or greater than 3.1 cm^2, or is smaller than 1 cm in its narrowest dimension (i.e., is capable of passing through a 9.5 mm (0.375 inch) standard sieve). If the surface area is smaller or the particle size larger than described above, prepare the solid portion of the waste for extraction by crushing, cutting, or grinding the waste to a surface area or particle size as described above. For paint and 3D printed plastics we use powder cryo-milling powder from a 80 microns sieving and powder from abrasion process. In both case the powder size is below 9.5 mm.

Point 4, <u>Determination of the right extraction fluid:</u>

Weigh out a small subsample of the solid phase of the waste, reduce the solid (if necessary) to a particle size of approximately 1 mm in diameter or less, and transfer 5.0 grams of the solid phase of the waste to a 500 mL beaker or Erlenmeyer flask. Add 96.5 mL of reagent water to the beaker, cover with a watch glass, and stir vigorously for 5 minutes using a magnetic stirrer. Measure and record the pH. If the pH is <5.0, use extraction fluid #1.

If the pH from Section is >5.0, add 3.5 mL 1N HCl, slurry briefly, cover with a watch glass, heat to 50 EC, and hold at 50 EC for 10 minutes. Let the solution cool to room temperature and record the pH. If the pH is <5.0, use extraction fluid #1. If the pH is >5.0, use extraction fluid #2. Proceed to Section 7.2.

Extraction fluid # 1: Add 5.7 mL glacial CH_3CH_2OOH to 500 mL of reagent water, add 64.3 mL of 1N NaOH, and dilute to a volume of 1 liter. When correctly prepared, the pH of this fluid will be 4.93 + 0.05.

Extraction fluid # 2: Dilute 5.7 mL glacial CH_3CH_2OOH with reagent water (See Section 5.2) to a volume of 1 liter. When correctly prepared, the pH of this fluid will be 2.88 + 0.05

1 g of NEP abrasion residue was added to 20 ml of extraction fluid to fix the 1:20 solid/liquid ratio. The sample was rotated end-over-end at 40 ± 2 rpm at 23 ± 2 °C for a period of 18 h, pH and conductivity were checked at the end of the test.

Release rate: particles were removed from the extraction fluid by filtering through a glass filter (20 µm), a fraction up to 20 µm was dried and weighted (to quantify any NEP mass loss) saved for acid digestion to determine total NFs remaining on NEP and determined any preferential release of NFs over the product matrix.

Release form: An aliquot of the extraction fluid was processed through a 3 kDa centrifuge filter, another aliquot was saved for total metals analysis. Both were digested for further analysis by ICP-MS and the last aliquot was used to analyze the particle size distribution by the NanoSight (Malvern, NTA300). TEM analysis will be carried on the different leached fraction.

5.6 Cryomilling protocol

To be performed using a NEP pieces less than 0.5 cm size.

Material:

- Retsch Ultra Centrifugal Mill ZM 200
- Liquid nitrogen N_2
- Sample to mill *(more than needed; a small but considerable % of the sample is lost during the process)*
- Vessel to store the generated particles
- Protection face shield and protective gloves
- 2 plastic jars for the sample + liquid nitrogen
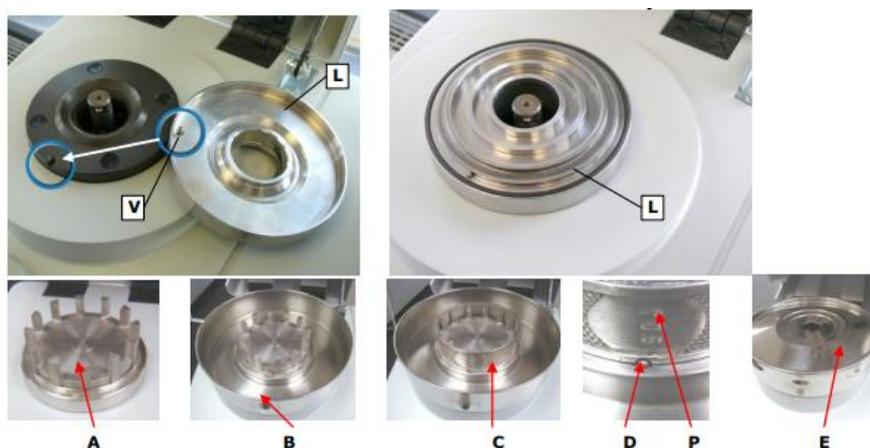
No se precisa ninguna herramienta para introducir los dispositivos de molienda.

- Abrir la tapa de la caja de la cámara de molienda.
- Introducir el disco de laberinto **L** observando el seguro contra giro **V**.
- Insertar el rotor **A**.
- Introducir el recipiente colector (cajetín) **B** observando el seguro contra giro.
- Introducir la criba anular **C** observando el seguro contra giro.
- Observar el asiento correcto, es decir, la abertura del seguro contra giro **D** debe estar abajo y la flecha **P** a la derecha.
- Colocar la tapa del cajetín **E**.
- Cerrar la tapa de la caja **F**.

Situar las aberturas para el seguro contra giro en el recipiente colector y en la criba anular encima del pasador del seguro contra giro.
De lo contrario, el ZM200 no se puede arrancar.

En la molienda en frío no hay que extraer a la fuerza el rotor embridado, que por razones físicas se atasca ligeramente, sino que se debe esperar a la compensación de temperaturas.
La extracción por la fuerza produce desperfectos en el eje motor y el rotor.

Jamás se olvide de poner la tapa del cajetín.
Sin tapa del cajetín, se bloquea el motor debido a las piezas de presión en la tapa de la caja.

Saque el rotor del eje si no se utiliza. **Con el rotor montado se puede producir corrosión por fisuras.**

1) Introduce the parts of the system in order (see protocol images before). **Remember both lids!! Make sure it is correctly closed.**

2) Turn on the instrument and stablish fixed the rom at 16000

3) Make sure the lid is put! and try it without any sample inside. If it correctly rotates, continue with the process.

4) Put the temperature-safety blue gloves, the safety glasses and the **face shield!**

5) Put N_2 in a plastic jar and some sample (as much amount as we want/need to be milled) in the other one, also with N_2.

6) Start the cryomill and make the next steps relatively fast, but very carefully:

    a. Pour N_2 into the instrument.

    b. Pour N_2 + sample into the instrument.

    c. Pour N_2 into the instrument.

    d. Pour N_2 + sample into the instrument.

    e. Continue until all sample has been poured (use a metal spatula, if needed).

* Tip!: if the plastic or material is very hard to break, add it to the cryomill piece by piece (if not, it can melt and/or generate bigger particles that it should).

7) Turn off the instrument.

8) Collect the milled product (e.g., paint) in a vessel/receptable (e.g., falcon tube or Petri dish). Label it, especially the size of the filter used!

9) Clean it very well.

When finalizing:

- Clean it very well and organize materials.
- Go to the mastersizer 3000 to measure the size of the generated particles.

5.7 Mechanical solicitations 3D printer parts

5.7.1 Abrasion

Mechanical solicitation through abrasion tests will be performed using a Taber abrader and a crockmeter following the description respectively on page 34 and page 40.

Dermal contact (crockmeter) will be investigated for the vacuum cleaner nozzle since it appears to be a relevant scenario especially for commercial use. Wiping and mild abrasion (Taber® abrader) might also be considered for the vacuum cleaner nozzle as the eroded surfaces might release airborne particles that are downstream trapped by the waste bag or cylinder. Leaching will also be investigated to simulate sweat in combination with dermal contact testing for these products along with artificial weathering where applicable. Leaching simulating landfilling will also be studied after adequate material aging following the described protocol on page 41.

5.7.2 Shredding

The end-of-life of printed parts will be investigated along mechanical recycling pathways and leaching tests to simulate landfilling. For that purpose, exposure experiments will be conducted during shredding processes and will be performed for two demonstrators (biocidal and antistatic surfaces) to monitor airborne particle emissions in WP2 and to collect the debris for hazard assessment as part as WP3 activities. The airborne particle emissions induced by the shredder depicted in the picture below will be monitored according to EN 17058:2018 mentioned in the generic methods section using direct reading instruments (CPC, FMPS and OPC) and particle sampler. The purpose is to monitor the whole emission of intentionally added NOAA and the incidental emissions during shredding operations. The extrusion of shredded materials to produce secondary materials is not planned within SAbyNA project which focuses on primary materials.
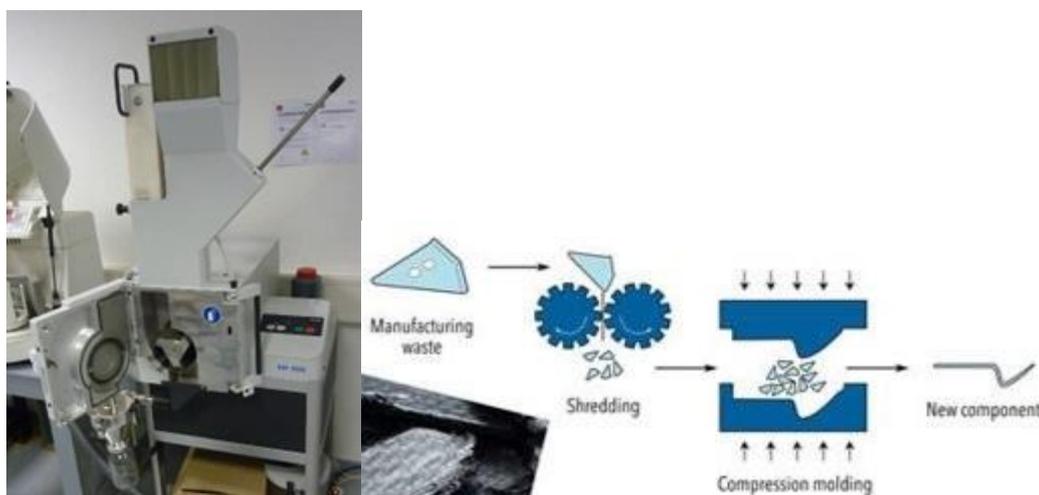
Figure 21. Lab scale shredder to investigate the airborne emission induced along mechanical recycling pathways (End of life scenarios).

5.8 Thermal degassing protocols on 3D printing polymer spools

CEA and LEITAT have also adapted protocols to investigate thermal degassing of 3D printing polymer spools.

The test bench simulate materials behaviour at their work temperatures. It provides assess the emission of airborne particles during their uses. This bench consists of a furnace capable of reaching temperatures of the order of 1400°C and is equipped with a series of measuring devices which include particle counters and filter samplers. The sample is placed in a crucible which is introduced into the furnace. The material is degraded in a few seconds and generates a cloud of particles which are transferred towards the measuring devices by the carrier gas. The quantity of particles and their size distribution are measured online while their morphology and chemical nature are determined by electron microscopy and chemical analysis from the deposits recovered on the filters of the samplers.
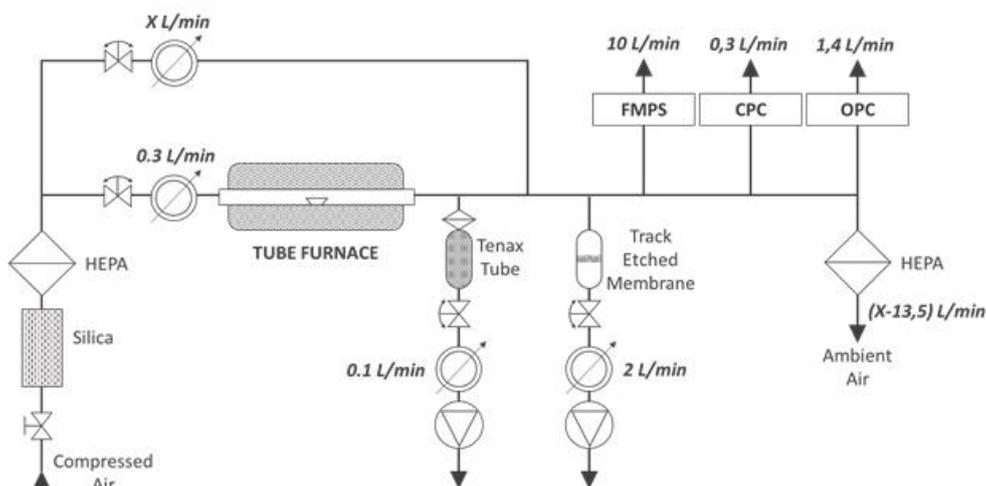
Figure 22. Tube furnace set-up (ERALY model AMC.H 1400 supplied with HEPA-filtered dry air).

Figure 20. Picture of the tube furnace. From left to right (air flow): introduction tube (room temperature), main furnace (set temperature), auxiliary furnace (180°C).

Prior to an experiment, the sample is introduced in the transparent quartz tube on the side the tube furnace. The tube is then flushed with HEPA-filtered dry air and the tube furnace is set to the working temperature (i.e. 230°C for PP materials and 300°C for PC materials). Once the set temperature is reached, the sample is moved while maintaining the clean air flowrate from room temperature to the interior of the tube furnace at the set temperature. The figure below describe the temperature monitored in the different zone of the furnace. Working temperatures are fully controlled in the center of the tuber furnace (where the sample studied is placed) as illustrated in the figure below. One can observe that after the "hot zone", the temperature is maintained above 100°C to minimize the condensation of VOCs in the tube and the Tenax sorbent tube is connected few centimeters after the furnace outlet (room temperature) as depicted in Figure 23.

PP filament material
Set temp. 230°C
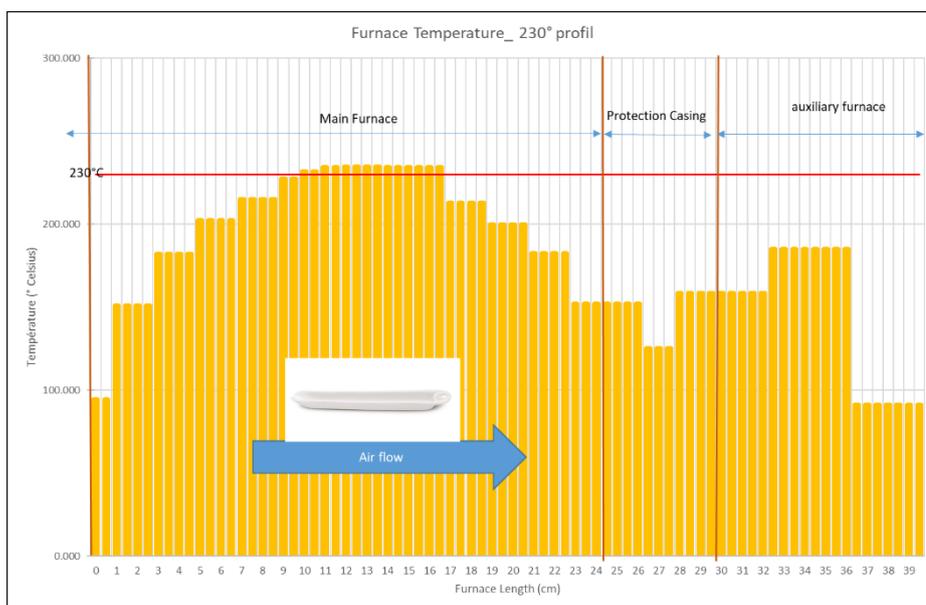
PC filament materials
Set temp. 300°C
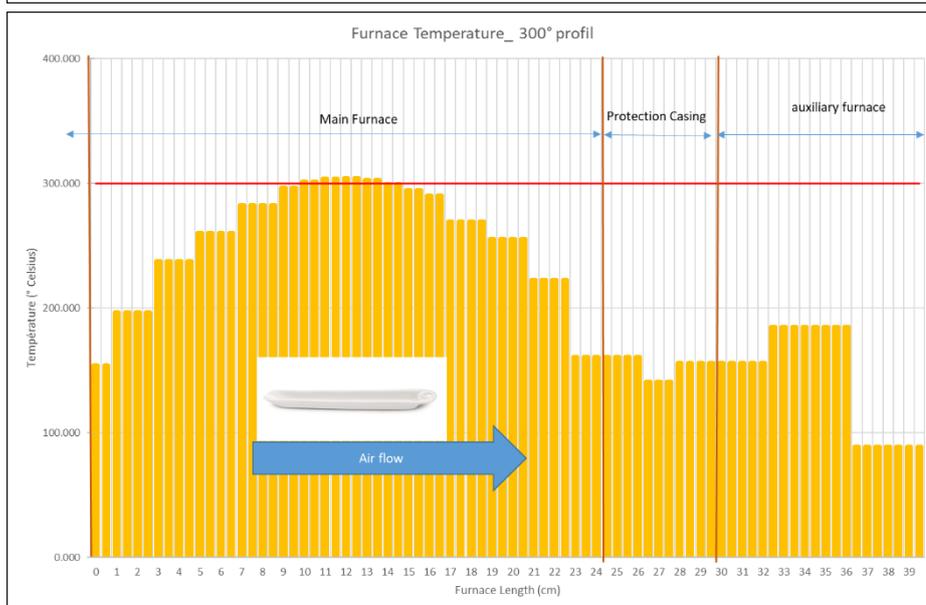
Figure 23. Temperature map of the tubular furnace for the two types of materials.

- Air Sample sweep rate: 0.3L/min for 2s residence at set temperature

- Two crucible blanks (cleaning+control) are performed before each test in order to avoid the contribution of background noise.
- Time real and offline measurements :
  o FMPS (TSI© Model 3091): the Fast Mobility Particle Sizer spectrometer measures particle sizes in the range between 5.6 and 560 nm, offering a total of 32 channels of resolution (16 channels per size decade). This produces particle-size-distribution measurements with one-second resolution, providing the ability to visualize particle events and changes in particle size distribution in real time. It operates at a high flow rate (10 L/min) to minimize diffusion losses of ultrafine and nanoparticles. It operates at ambient pressure to prevent evaporation of volatile particles and it requires no consumables.
  o CPC (Grimm© model 5416): the Condensation Particle Counter is a instrument that detects airborne particles in the range between 4.0 nm to greater than 3 µm to provide a concentration in number of particles per volume ($\#.cm^{-3}$). It measures concentrations range between 0 and 10^7 particles/cm^3
  o OPC (PALAS Fidas© Mobil): the Optical Particle Counter is a instrument that detects airborne particles in the range between 0.18nm to 18µm to provide a concentration in number of particles per volume ($\#.cm^{-3}$). It measures concentrations range between 0 and 2.10^4 particles/cm^3
  o Particles Sampling for SEM/EDX: one part of released particles are collected on Track-Etched membrane (polycarbonate 0.4µm Millipore©). Scanning Electron Microscopy provide us about particles some morphological informations and Energy Dispersive X-ray Spectroscopy (EDS), the elemental composition.
- COV Sampling for GC/MS: Volatile Organic Compounds (VOCs) were collected on Tenax sorbent tubes. Their analysis following collection was performed using TD-GC-MS/FID (Thermal Desorption- Gas Chromatography-Mass Spectrometry/Flame Ionization Detector) to identify and quantify the collected VOC molecules. The method used corresponds to the US-EPA Method TO-17 (Determination of VOC in ambient air using active sampling onto sorbent tubes); VOC sampling was carried out by pumping an air flow (0.3 l/min; 15min) directly through an sorbent tube (Markes Air Toxics Analyser® tubes) at ambient temperature. Using this sampling method, organic compounds containing 2 to 12 carbon atoms (excluding methane, ethane, acetylene, ethylene but including methanol, ethanol, acetaldehyde) are then trapped and concentrated on the sampler. Before use, Tenax sorbent tubes were previously thermally conditioned under inert gas and their cleanliness was controlled by TD-GC-MS/FID analysis. Since emission sources of VOC are to be characterized, a relatively low air sampling volume was required (4.5 L) in order to avoid the analysis of too concentrated specimens. Sampling tubes were then thermally desorbed in order to release trapped compounds for

analysis by GC-MS/FID used for species identification and quantification, respectively. The equipment used are a Thermal Desorber (Ultra-xr Unity series 2) from Markes and gas chromatograph 5975C VL MSD from Agilent Technologies. The FID calibration is performed using toluene as are the quantification of individual VOC and Total VOC (obtained from the sum of all chromatographic peaks) is given in toluene equivalent amounts.

- Two crucible blanks (cleaning+control) are performed before each test in order to minimize the contribution of background noise.
- Some COV sampling on the crucible/oven blank are performed to get an idea of their levels and types produced as some Particles sampling are also performed on the crucible/oven blank to get an idea of their morphological types and chemical composition.

5.9 Mesocosm testing

A mesocosm experiment was set up to mimic the contamination of a freshwater lentic ecosystem under a Mediterranean climate to nano-enabled product (NEP). The main contamination scenario involved the chronic addition of fragmented acrylic paint incorporating $ZnAl_2O_4$ nanospinels. Hence, the main research question aimed at understanding the fate and effect of such NEP in realistic environmental conditions during the use or end of life stages of the product lifecycle.

Mesocosm testing offers a means of providing meaningful data to inform environmental risk assessment of complex systems.[10,11] Among the many definitions for a mesocosm, a more general one describes a mesocosm as an enclosed and essentially self-sufficient (but not necessarily isolated) experimental environment or ecosystem with a number of interdependent system parameters.

The mesocosms used herein were developed by CNRS-CEREGE as a Standard Operational Procedure for pre-regulatory purposes in environmental nanosafety during the NANoREG EU project (FP7 Grant Agreement n.310584). This method consists of monitoring the evolution of a re-created miniature ecosystem following nanoparticle contamination and environmental aging.[12] The only decision in this risk assessment strategy is the definition of an environmentally relevant exposure scenario (incl. dose). Such a robust testing strategy [13] bears clear advantages for the determination of both (nano)particle/tritium exposure and hazard in a single experiment[14,15,16,17,18] and for producing dependable and intercomparable data[19,20].

An experiment performed in these mesocosms involves 3 phases: the implementation of the mesocosms, their acclimatization and equilibration, and the contamination phase of the experiment *per se*. Briefly, a total of 16 mesocosms were set up including:

- Artificial sediment composed of quartz sand, $CaCO_3$ and a natural inoculum,

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[10] Auffan, M. *et al.* (2014) 'An adaptable mesocosm platform for performing integrated assessments of nanomaterial risk in complex environmental systems', *Scientific reports*, 4, p. 5608.

[11] Auffan, M. *et al.* (2019) 'Contribution of mesocosm testing to a single-step and exposure-driven environmental risk assessment of engineered nanomaterials', *NanoImpact*, 13, pp. 66–69.

[12] Masion, A., Auffan, M. and Rose, J. (2019) 'Monitoring the Environmental Aging of Nanomaterials: An Opportunity for Mesocosm Testing?', *Materials*, 12(15), p. 2447.

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- Water column composed of mineral water and a natural inoculum,
- Aquatic plants (*Chara Vulgaris*),
- Benthic mollusks (*Planorbes sp.*).

In addition, a natural planktonic community developed in the aquaria during the experiment, which included *Daphnia sp., Copepodes and Ostracodes*.

The mesocosms (namely M_01 to M_12) were randomly assigned to different treatments. In general, treatment I served as a non-amended control across the experiment. Treatment II addressed the effects of fragmented paint matrix alone (no nanospinel), to discern the role of nanospinels and paint matrix. Treatment III focused on the fate and effects of $ZnAl_2O_4$ nanospinel coated with citrate (synthesis temperature 1000°C) i.e. nanomaterials that are not incorporated in a matrix. Treatment IV was the main subject of the experiment, i.e. addressing the fate and effect of the paint doped with nanospinel. Finally, given the issues in detecting Al in naturally high backgrounds, two additional treatments were set up addressing fate endpoints only. These two treatments were carried out in duplicate incorporating Gallium in the nanospinel to be traced in the natural matrices.

- **Mesocosm implementation**

The mesocosms set up correspond to 350 × 200 × 400 mm glass aquaria adapted from the protocol reported by Auffan et al. (2014)[4] that used 60 L tanks. The mesocosms consisted of two main compartments, a sediment layer and an overlying water column, of approximately 3 cm and 28 cm, respectively. The artificial sediments were obtained by mixing 2.05 kg of quartz sand, 23 g of $CaCO_3$, 0.5 L Volvic water and 120 g of natural inoculum. It must be noted that, compared to the original protocol reported in Auffan et al. (2014)[4], no kaolinite was added in order to avoid high Al and Zn input from the clay. The artificial sediment was then mechanically homogenized by stirring for 2 min at 200 rpm (Figure 24a) and homogeneously spread at the bottom of the aquaria. Then, 1 L of Volvic water was gently added and the system was left unaltered for 24 hrs to allow for the settling of the suspended particles (Figure 24b).

Figure 24. Artificial sediments preparation. A) Homogenisation of the artificial sediments components. B) The resulting sediments layer after addition of the mixed matrix and 1 L Volvic water. C) The aquaria after 5 days preparation and gradual addition of Volvic water.

In the following days, the tanks were gradually filled with additional Volvic water (Figure 24c), for a final volume of 16 L, and the aquatic macrophytes were introduced (Figure 2). *Chara sp.* macrophytes were sampled at the targeted natural ecosystem, and transferred into the aquaria within 24 hrs as following. Groups of 4-6 specimen (approx. 0.5 m length) were separated from the natural bundle, rinsed with Volvic water, and transferred into a 3 L glass container. At this stage the plants were cleaned of insect larvae, eggs and natural sediments. Then, 35 g wet weight (water content 83.1±0.5 %) were transferred into the aquaria and homogeneously distributed in the water column (Figure 25). The plants deposited at the sediment surface and gradually re-established a vertical position in the following days.

Figure 25. *Chara sp.* immediately after addition into the aquaria.

21 aquatic snails (*P. Corneus*) of approximately 1.2 cm in diameter, collected within targeted natural ecosystem, were introduced in each aquarium in the following three days. The selected organisms are involved in real food webs and have different habitats and ecological functions in the ecosystems. The density of organisms was adjusted as a function of their natural environment.

- **Mesocosm acclimation and equilibration**

P. Corneus introduction marked the beginning of an equilibration time of 3.5 weeks needed for the acclimatization/development of the (micro)biotic communities and individuals, settling of suspended particles and equilibration of the physical-chemical parameters.

Each tank was equipped with a set of probes (Odeon® Open X) a mid-height in the water column for the continuous measurement of physical-chemical parameters (pH, temperature, redox potential, conductivity, dissolved O_2) for the duration of the experiment. The duration of this phase depends on the target values (and variations around them) defined for each key parameter (e.g. ΔpH, ΔT, turbidity, ammonia). The duration of the acclimation also depends on biological features of the species as growth rate, metabolism activity, life cycle duration.

At the end of the equilibration phase, the mesocosms were ready for contamination.

- **Mesocosm contamination**

All treatments were carried out in triplicate (exposure and effects endpoints) except for the Ga-doped nanospinel that were duplicated (exposure endpoints only) (Figure 26).

I.   Control: no amendment

II.   Paint (P): chronic addition of fragmented acrylic paint (non-doped)

III.   Nanospinel (N): chronic addition of $ZnAl_2O_4$-1000citrate nanoparticle

IV.   Paint + Nanospinel (P+N): chronic addition of $ZnAl_2O_4$-1000citrate nanoparticle-doped fragmented paint (acrylic).

V.   Ga-doped nanospinel (GaN): chronic addition of $ZnAl_2O_4$-1000citrate nanoparticle where 5% of Al was substituted with Ga.

VI.   Paint + Ga-nanospinel (P+GaN): chronic addition of fragmented paint doped with $ZnAl_2O_4$-1000citrate nanoparticle where 5% of Al was substituted with Ga.
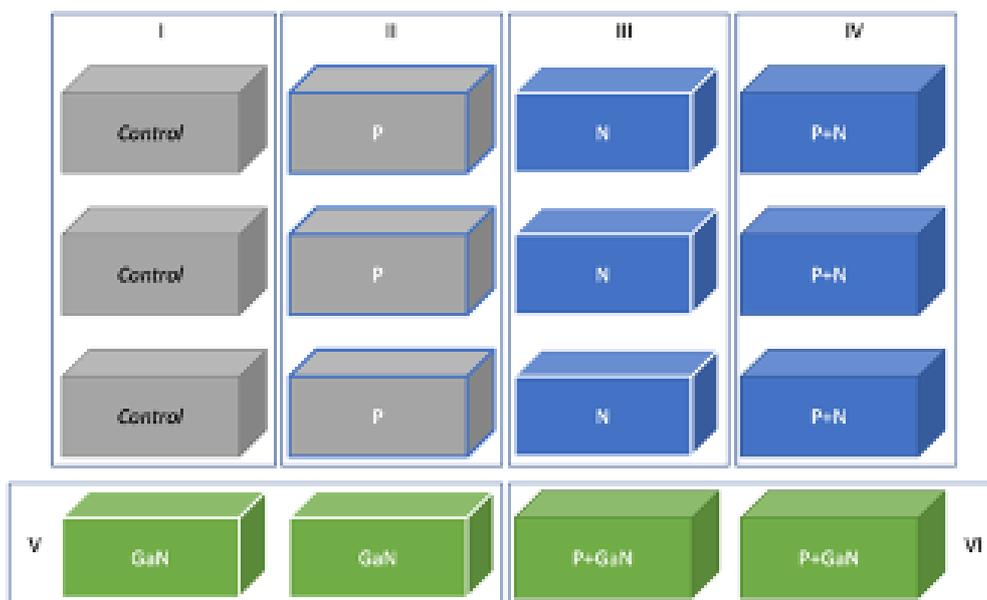
Figure 26. Treatment distribution in the 16 mesocosms.

For mesocosm contamination, nanospinel or fragmented paint powders were transferred into 9 mL milli-Q water, kept under constant agitation and spiked at the top water immediately after preparation. The fragmented paints were previously sieved at 100 µm in order to facilitate a homogeneous dispersion in water.

In order to mimic a chronic exposure scenario, the mesocosms were contaminated three times per week over a period of 28 days (12 contamination in total). The final nominal concentration reaches 1 mg/L nanospinel. In the paint contaminated treatments (II, IV, VI) this corresponded to a final concentration of 35.9±0.16 mg/L of fragmented paint. In these treatments, larger paint fragments (referred as "paint-sheet", 0.5x0.5 cm, Figure 27) were placed at the mesocosm corners (n=4).

Figure 27. The paint-sheet consisting of squared 0.5x0.5 cm paint fragments placed at the corners of the paint-contaminated mesocosms.

- **Mesocosm sampling**

The sampling of the mesocosms components (water, sediments, biota) took place after 7, 14, 21 and 28 days. The samples were stored at 4°C in the dark, unless specified otherwise.

With regard to the water column, in each aquarium the samples were taken at approximately 10 cm surface depth and processed as following. 5 ml samples were directly analyzed with a particle counter for the estimation of suspended particles size and size distribution. Bacteria counting was performed on 10 ml samples after addition of 200 µL formaldehyde. For total organic carbon analysis (TOC), 10 ml water was acidified with 20 µL HNO_3 and transferred into a 15 mL plastic syringe. Then, filtration was performed with a 0.45 µm polyethylene sulfonate filter previously rinsed twice with milli-Q water. For the determination of the elemental under investigation (Al, Zn, Ga), 10 ml samples were acidified with 200 µL HNO_3 and analyzed with an Inductively Coupled Plasma Mass Spectrometer (ICP-MS). For the estimation of free species in the water column, additional 10 ml samples were analyzed after ultrafiltration, performed with Millipore 10 KDa tubes centrifuged at 4000 rpm for 45 min.

Sediment samples were collected at the water-sediment interface, viz. at the 0-3 mm layer. In each aquarium, approximately 100 mL sediments (wet weight) were collected into plastic bottles, thoroughly homogenized and redistributed as following. 10 mL were dedicated to bacterial community analysis. Two samples of 10 mL and 50 mL were dried at 56°C and dedicated to metals speciation and content analysis, respectively. Prior the analysis, the latter underwent alkaline fusion with the protocol reported above (see section 2).

Finally, in the paint contaminated treatments (II, IV, VI), the larger "paint sheets" were sampled at all the time intervals, paying attention to keep the orientation (towards the water or sediment column) unchanged.

The algae *Chara sp.* were sampled and separated in two different portions, namely a "planktonic" part (greener, more recent, vertically oriented) and a "benthic" part (pale color, older biomass, horizontally oriented at the sediments surface). For each of these, 3 specimens were sampled and dried over 72

hrs at 56°C for elemental determination. Additional samples were stored at 5% formaldehyde for imaging analysis.

The snails *P. corneus* were sampled at each time interval for elemental composition (n=2) and imaging (n=1) analysis. At day 14 and day 28, additional snails (n=3) were sampled for further analysis as described below. In the mesocosms contaminated with Ga-doped nanospinel and paint (treatments V and VI), we investigated the elements partitioning within the organisms. For this purpose, the snails were transferred into 100 ml glass dishes filled with 60 ml Volvic water and left for 48 hrs. Then, the excretion accumulated in the dishes were collected and the specimens were sectioned in order to separately analyse the shells, the digestive glands and the rest of the body. In the remaining mesocosms (treatments I, II, III and IV), the snails were dissected in order to collect digestive glands and remaining body, which underwent biomarker analysis. The behaviour of the snails was monitored over time with regard to two specific endpoints, snail distribution and snails burrowing. In both cases, the measurements (number of snails) were done in the morning (within the first hour light) and in the afternoon (within two hours before the light turned off). For snail distribution, five categories were monitored based on mollusks position in the aquaria: a) sediment, b) plants (benthic grazing), c) plants (water column), d) mesocosm walls and probes and e) air/water interface. For burrowing trait, the sediment surface was divided in 20 sub-sections, as shown in the scheme of Figure 5. This helped discerning active/inactive snails as well as identifying dead specimen. In general, a snail was considered as "burrowed" if the whole body and > 50% of the shell were beneath the sediments (Figure 28). A snail burrowed at the same location for two or more sampling time (i.e. > 24 hrs), was considered inactive.
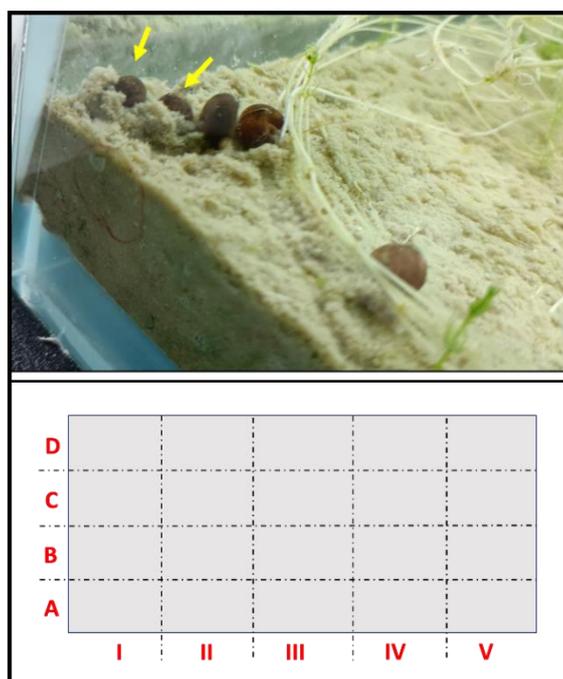
Figure 28. Top, an example of snails in the process of burrowing (arrows). Bottom, the division scheme of the mesocosms for monitoring mollusks burrowing, activity and mortality.

A large number of egg clutches were laid in all the aquaria during the equilibration phase, resulting in a large juveniles population at the beginning of the experiment. These were sampled, similar to adults, at day 28 only. Novel egg clutches were observed during week 3 after contamination. In treatments with Gallium (V and VI), the clutches were sampled at day 28 for laser ablation ICP MS analysis. In the other treatments, the egg clutches were monitored with regard to their size, number of eggs and number of embryos successfully developed within the clutch (Figure 29). Given the difficulty to observe and analyse the eggs onto plants, mollusk and probes, only those present on the mesocosm walls were examined.

Figure 29. An example of egg clutches observed on the mesocosm walls. Left, egg clutch at the early development stage (< 1 week). All the eggs present an embryo within (yellow dots). Right, an egg clutch at an advanced development stage (~ 2 weeks). In this case, one of the egg (at the bottom of the clutch) did not developed an embryo.

- **Alkaline fusion prior chemical analysis**

Alkaline fusion was selected as the most efficient protocol for elemental determination in the complex paint matrices. All the samples were processed following the program proposed in Table 3. This fusion program uses a ratio sample / flux (lithium tetraborate with lithium metaborate fused, anhydrous) 1/5.

Table 3. High refractory materials program in fusion alkaline process.

	STEP 1	STEP 2	STEP 3	STEP 4	STEP 5	STEP 6	STEP 7
TYPE	Heat	Heat	Heat	Heat	Pour	Cool	Cool
DURATION	06:00	06:00	04:00	01:00	-	01:15	03:30
HEAT	1065	1065	1065	1065	-	-	-
SPEED	0	10	30	20	-	-	-
ANGLE	0	10	35	45	-	-	-
FAN	-	-	-	-	-	50	100