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Deliverable Report

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3.	NMBP-13 RiskGONE	Yes

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1 Summary

As a consequence of the collaboration between the other two NMBP-13 projects (NANORIGO and RiskGONE) a joint Nano-Risk Governance Portal will be developed. The portal is anticipated to be the central access point to other tools and platforms. In this context, one of the aims of the Gov4Nano project is to develop a Stage-gate Nano-Risk Governance Platform, which will include state-of-the-art nanosafety governance tools to support governance activities of nanotechnologies. The main goal of T4.3 is to build a relevant case study database as well as to identify and/or develop suitable case studies for the demonstration of the Nano-Risk Governance Platform and tools included. The identified case studies will be of use for tool testing as well as to develop and generate training material (T4.5).

The task focused on the identification of two main types of case studies based on their purpose of use. The first type of case studies are those to be used for model testing of accuracy and prediction. These type of case studies are general measurement study results with contextual information focusing on a specific topic. The second type of case studies, are to be used for the development of training and/or demonstration material on the use of the Nano-Risk Governance Platform and are more detailed on a specific problem involving several actors or a broader spectrum of the life cycle.

For the first type of case studies, work in Gov4Nano has been focused on the operationalization, update and development of case study libraries. In this regard, for exposure case studies, Gov4Nano has worked on the improvement and update of an already existing database (developed during Gracious) in a common and coordinated effort towards the creation of a release and exposure Library available to the whole (nano)safety community. In regards to hazard data, Gov4Nano has updated a pre-existing (developed in caLIBRAte) database containing data on 164 dose-descriptor data on inflammation and genotoxicity based on animal inhalation studies of nanomaterials.

The second type of case studies, were identified from either previous projects, derived from existing literature and databases or were obtained as a collaborative effort between NMBP 13 sister projects and Gov4Nano partners. Three case studies have been identified and are described related to car tyre potential release of nanomaterials and public concern, use of gloves treated with a nanocoating and occupational exposure risk, and a study on exposure and release of TiO_2 during paint manufacturing, application and end-of-life. In addition, several toxicological case studies related to pristine, complex and combined nanomaterials have been identified and described.

2 Description of task

The main goal of T4.3 is to build a relevant case study database for tool testing, development of teaching materials (related to T4.5) and user training of the NRGP (T4.5). Case studies have been worked upon in previous projects (e.g. caLIBRAte as a start and further developed in an OECD project), and this database will serve as the basis for ongoing developments within T4.3.

The main objective of T4.3 can be divided in three sub-objectives, namely:

- Operationalize the database using the FAIR principles worked on in WP1, and connect it with other existing databases/ future proof the database;
- Developing an overview of existing cases- or (if needed) newly developed cases suitable for the demonstration of the NRGP;
- Updating the case study database with new/extra data;

3 Description of work & main achievements

3.1 Background of the task

The EU H2020 Gov4Nano project aims to build an inclusive, credible, science and evidence-based risk governance process for future and emerging technologies such as nanotechnology. One of the main objectives is the development of a Nano-Risk Governance Portal in collaboration with the two other NMBP-13 projects (NANORIGO and RiskGONE). The portal is anticipated as a central point to access among others tools (including governance platforms), data, and nano-risk governance guidance.

As consequence of the collaboration agreements within the three NMBP-13 projects, the anticipated Nano-Risk Governance Portal has changed character and Gov4Nano provides input to the Nano-Risk Governance Portal and develops a Stage-gate Nano-Risk Governance Platform (NRGP), which include state-of-the art nanosafety governance tools to support governance activities of research organizations, companies, service providers (consultant in risk management), regulatory authorities and other stakeholders in the governance of risks of nanotechnologies. To reach this goal, WP4 was divided in several subtasks, where task 4.3 focusses on the collection of suitable case studies and developed case study materials for demonstration of tools and the Nano-Risk Governance Processes. Existing data libraries taking into account exposure, hazard, phys-chem and environmental fate data will be operationalised and updated with (if any) new available data which can be used in the future to (further) performance test available models in the NRGP as well as being re-used.

3.2 Description of the work carried out

For this task in general, two types of "case studies" were identified. The difference between the types of case studies are driven by the purpose of these case studies. First, case studies can be used to assess individual models by comparing model outputs with measurement results as a validation exercise on the accuracy of model predictions and possible improvements needed. For this purpose, such case studies consist of a large number of datapoints which allow a comprehensive comparison between model outputs and measurement results. This can eventually also be used for the improvement of the model scope and estimations. Secondly, case studies can be used to develop training material and/or demonstration examples on using the NRGP to answer specific governance questions or problems which may be presented to the "system" in the future by users.

The first type of case studies refers in particular to measurement study which results and their contextual information focussing generally on one specific topic (e.g. exposure assessment, toxicological studies, phys-chem characterization or environmental fate studies) are reported in the literature, whereas the second type refers to a more detailed specific problem which may involve more actors or a broader spectrum of the life cycle or where multiple disciplines are involved in answering the question.

3.2.1 Development of the case study databases

Exposure data

During the last years different projects and initiatives dedicated efforts to collect and curate release and exposure data, to create different Libraries. Following the work carried out in the FP7 project MARINA, the GUIDEnano Project added data in a searchable library of exposure scenarios (http://guidenano.iom-world.co.uk/). The data contained information on "nanomaterial", "exposure scenario", "life-cycle" and "source domain" (a field included in the NECID database and added on purpose to the GUIDEnano library to be able to link data on both databases). Later, the H2020 caLIBRAte Project developed a Case study Library containing high quality data on human health and safety of NMs. The data collected in this Library was intended to be used for evaluation of nano tools and models for assessing release and exposure. The databases mentioned above were built collecting the useful parameters and data for a specific purpose (e.g., model validation in caLIBRAte Library). During the H2020 Gracious Project, there was an effort to generate a database template to allow for 'read-across' and identify similar or analogous exposure scenarios to the scenarios of interest and use this exposure information. A data collection template was generated to this aim, allowing to collect and organize all relevant information and characteristics regarding NFs (e.g., composition, size, morphology, dustiness, powder moisture, etc.), their respective processes and activities, as well as their emissions-release-exposure data. The template was filled with a preliminary set of data during the GRACIOUS project, but since different projects, including Gov4Nano, have expressed their interest in using the template, the template has undergone an updating process, in which Gov4Nano Partners were actively involved. The needs and views of additional partners allowed to improve and update the template format, and to add new data in a common and coordinated effort towards the creation of a release and exposure Library available to the whole (nano)safety community.

With regards to updating the case study library, exposure data from the library developed within caLIBRAte was transferred to the GRACIOUS database template. The GRACIOUS database template has been updated to improve the format. This was done in a coordinated effort between NRCWE, TNO and LEITAT institutes towards the creation of a database of release and exposure data linked to the eNanoMapper database available to the whole (nano)safety community. In addition, new literature not available in the previous library was added and entered in the database format. The template used to capture the exposure and release measurement data was developed within Microsoft Excel and, within eNanoMapper, a link has been made between the Excel template and the eNanoMapper database to allow upload of data to the eNanoMapper database system. This effort allows for the capturing of exposure measurements within the eNanoMapper database, which previously only contained toxicological and phys-chem data. While Excel databases have some disadvantages and can be prone to more erroneous data entering, the link with eNanoMapper and the prospect of a more general harmonised use of one exposure data library resulted in the choice for this template to capture the case studies which can be used for tool testing.

In addition to literature data NIOH-ZA has identified nine research and nine industrial settings in South Africa to conduct a multi-tiered exposure assessment in order to assess the degree of nanomaterial release during its life cycle i.e. from synthesis to application.

This multi-tiered approach includes the following:

- Tier 1: Determine the potential of nanomaterial release by assessing the laboratory lay out, processes and methodologies used, ventilation systems used etc.
- Tier 2: Assess the concentration of nanomaterial release using various direct, real-time particle measurement instruments including the Nanoscan scanning mobility particle sizer (NanoSMPS) and optical particle sizer (OPS) for area sampling and the Partector personal monitor (for sampling within the breathing zones of individuals involved with nanomaterial handling).
- Tier 3: Collect and characterize the nanomaterials released using various filter-based techniques and offline measurements including filter collection using the Naneum nanoparticulate monitor and characterization via X-ray diffraction analysis, ICP-MS etc.

Hazard data

NRCWE established a database of inhalation studies with 27 different material substances covering tubes, fibres, flakes and spherical particles associated with available exposure and physicochemical data which was initiated in the caLIBRAte project (D5.5). Toxicological endpoints in the database were lung inflammation and genotoxicity/carcinogenicity. This Excel database, was updated in the current project with quality checks, new studies and can serve to design case studies to the Nano Risk Governance Portal, as has been done for two nanomaterials described further below. The library has multiple uses including testing of tools for hazard assessment / ranking, input for quantitative hazard assessment tools, but also serve as to design case studies to the portal and NRGP. The excel hazard library will be made available as part of a scientific publication in preparation and via www.nanosafer.org.

In addition to the hazard library, a case study on in vivo hazard data (lung and gastro-intestinal) on simple to advanced solid and mesoporous silica particles, with and without copper doping, was collated based on studies in the caLIBRAte project (Hadrup et al., 2021; Cabellos et al., 2020). These data can serve to establish case studies to challenge the NRGP with advanced multiconstituent materials.

Environmental release and fate data

A generalized library for data on environmental release and fate of nanomaterials currently (to our knowledge) does not exist or at least is not publicly available. Release and fate are usually assessed by models that each have specific input requirements and are generally not data driven. Examples are SimpleBox4nano, NanoDuFLow etc.. These models have been published in the scientific literature and the input values are given either in the article, the supporting information or in input files that are provided for download. A single study from caLIBRAte has been published where environmental stack emission release of TiO_2 was measured, but near-field environmental deposition could not be measured and was therefore, not captured in any database (Fonseca et al., 2021). Although in the context of the industrial chemicals and biocides, the EUSES tool within the REACH regulation of the EU estimates the potential environmental risk. Particularly the A-tables provide emission factors (formally mentioned as 'release factors'). These factorsprovide the fraction that is emitted of a substance to each environmental compartment per specific type of process, industrial category and substance properties. Similarly, within REACH the "Environmental Release Category" ERC has been defined which, together with the industrially developed (CEFIC) specific Environmental Release Categories (spERCs), provide the environmental release factors for a number of product categories, including paints, cosmetics, construction chemicals, fuels, fertilisers etc. Nevertheless, the employability of these frameworks of estimating release factors for nanomaterials still remains uncertain to the best of our knowledge.

No new data on mass-flows relevant for environmental risk assessment were found. Moreover, regarding ecotoxicology, the case study to predict PNEC values (predicted no effect concentrations) published by caLIBRAte was identified to be still the most useful dataset that is available to compare different tools to assess environmental hazards (Sorensen et al., 2020).

3.2.2 Development of case studies for the demonstration of the NRGP

Existing detailed case studies from previous projects were evaluated for their use to demonstrate the NRGP. In addition, a new case study was developed led by the NMPB-13 sister project "NANORIGO".

The goal of these types of case studies was to allow demonstration on the general applicability of our NRG frameworks / approaches to realistic scenarios and inform on weaknesses or additional aspects for improvements or further developments in Gov4Nano. Within this task, 5 detailed case studies were developed or identified from previous projects which can be used to test or demonstrate (parts of) the NRGP. These case studies cover:

1) the release of nanoparticles from car tyres (in collaboration with EU NANORIGO),
2) exposure and release data from a grant measurement campaign set-up previously in caLIBRAte where highly detailed exposure and release measurements were conducted in paint manufacturer companies.
3) two case studies focussing on the toxicological profile of two nanomaterials were developed based on data collected in caLIBRAte, and intended to test the Nano Risk Governance Portal's capability to cope with different shapes of nanomaterials, as well as materials consisting of more than one compound/substance. The case studies are:
    a. a carbon nanotube case
    b. a case study with a combined material with copper-doped amorphous silica particle, and a silica particle that has porosity.

The case studies are described in detail below.

4 Results

4.1 Development of the case study databases

Exposure data

A total of 109 measurement studies (mostly in the form of published manuscripts) were entered in the GRACIOUS exposure database (which will be uploaded to the eNanoMapper database as soon as the link between the template and the eNanoMapper system is fully operational) representing over 946 measurements. The database contains exposure and release data measured in workplaces and covers more than different 40 contributing exposure scenarios.

The data was taken mainly from published manuscripts, but also contains unpublished data from EU funded projects such as caLIBRAte. The database consists of personal exposure measurements, measurements taken in the near-field and/or far-field, in terms of number concentration, mass concentrations and release concentrations in the air over time. It contains data for more than 24 different nanomaterials with TiO_2, CNTs, SiO_2, Al_2O_3 and ZnO representing approximately 50% of the cases (Figure 1). The database includes data for more than 40 different exposure scenarios including spraying scenarios, milling, sawing, or cleaning processes, but is dominated by pouring, testing and characterization, spray application by using a can and HVLP techniques, painting and filling of bottles. In the current state, the data entered is given mostly in particle number concentrations (69%%) and mass concentrations (22.5%) (Figure 2). A relatively small number of cases also provide particle size distribution data, lung deposited surface area (LDSA) data and microscope image analysis. A total of 274 personal exposure measurements are available, from which data is given mostly in particle number (62.5%), and mass concentrations (28%). Similarly, release data measured directly at the source (325 measurements available) and at personal or near-field (266 measurements available) is also dominated by particle number data (67% and 53.4%, respectively) followed by mass (19% and 30%, respectively).

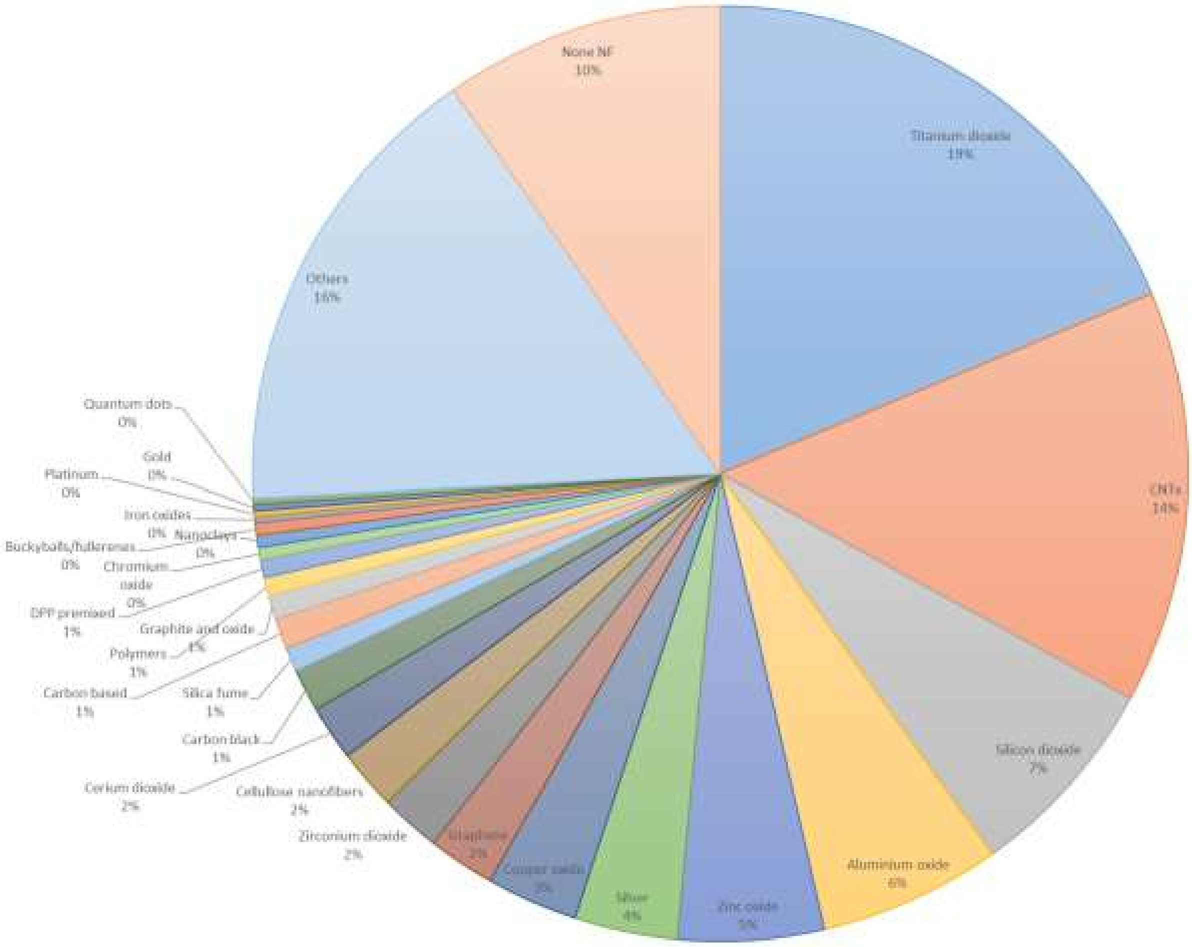


Figure 1. Distribution in percentage of materials covered in the release and exposure library.

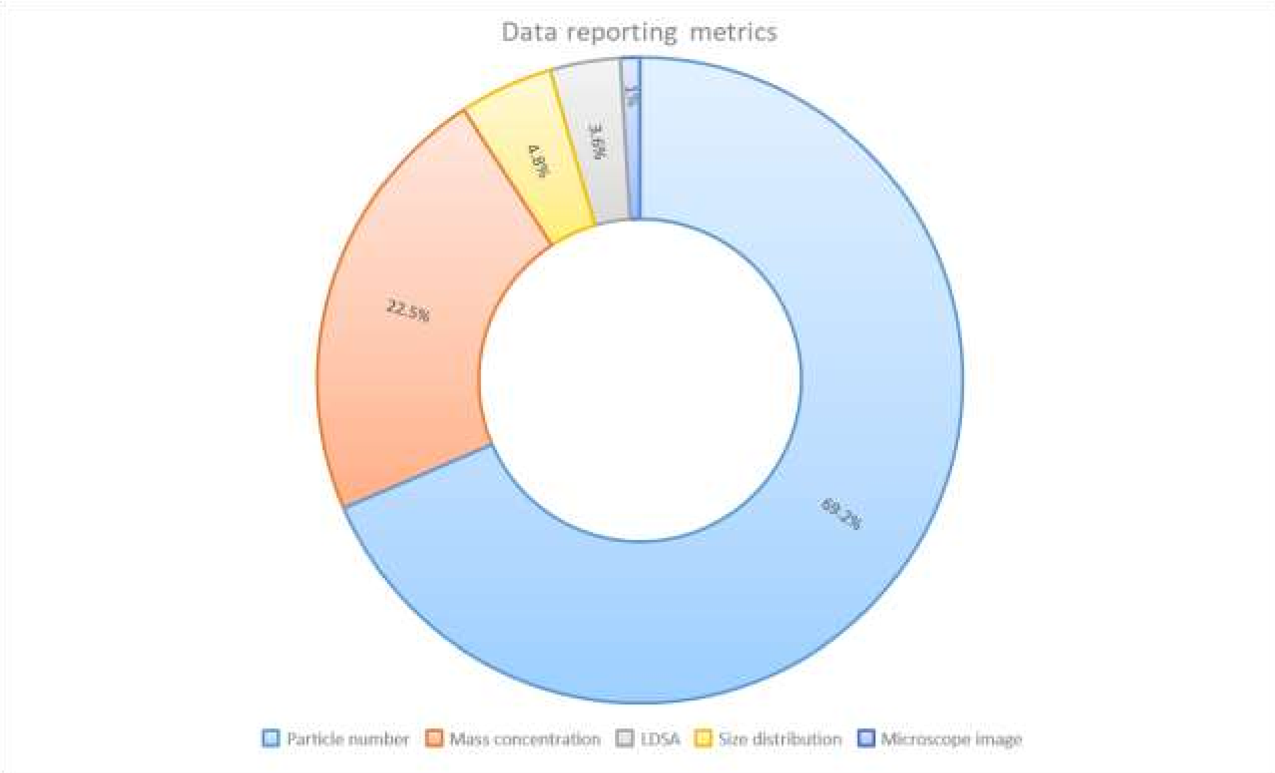


Figure 2. Overview of metrics used to report exposure data in the release and exposure library. Data is expressed as percentage of cases. LDSA: lung deposited surface area.

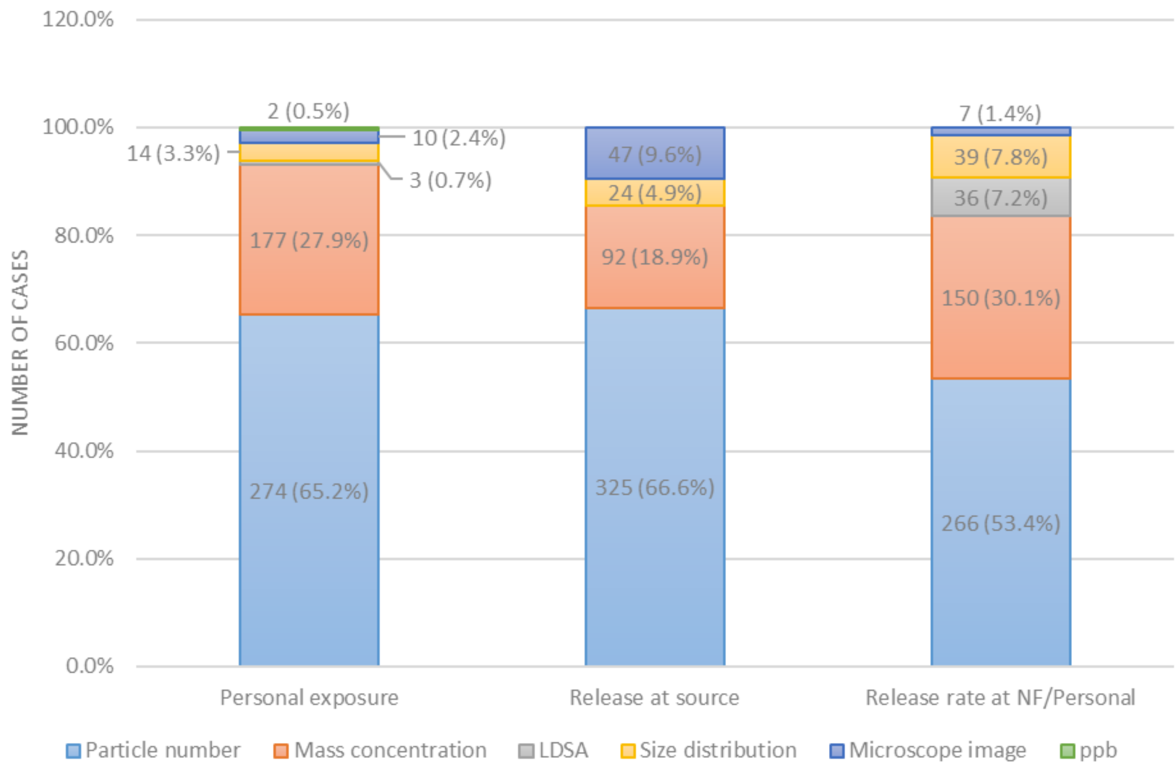


Figure 3. Number of total personal exposure and release measurements available and percentage per metric reported. LDSA: lung deposited surface area; NF: near-field.

The additional exposure assessments have been conducted at three laboratories in South-Africa to date and remaining work is delayed due to corona and will be reported in an update.

As an example at one of these laboratories (Figure 4), exposure to AuNPs and AgNPs during synthesis was assessed. AuNPs and AgNPs were monitored continuously using a scanning mobility particle sizer (SMPS). The monitoring was carried out at a height of 1.2 - 1.5 m and half a meter away from the fume hood cabinet, assuming a 30 cm breathing circumference zone. Each synthesis process was monitored three times in order to generate reliable point estimates, which will be used to assess exposure over an 8-hour duration. Furthermore, a time-weighted average concentration was calculated and compare the derived 8-hour exposure duration with the occupational exposure limit (OEL) for AgNPs (0.19 µg/m^3) and the proposed provisional nano reference value for AuNPs (20 000 particles/cm^3). The synthesis occurred over a period of 1 hour. On average, the laboratory performs only one synthesis for gold and silver nanomaterial using two different synthesis laboratories. At times the laboratory does perform two synthesis processes, with the worst case being 3 syntheses per day.

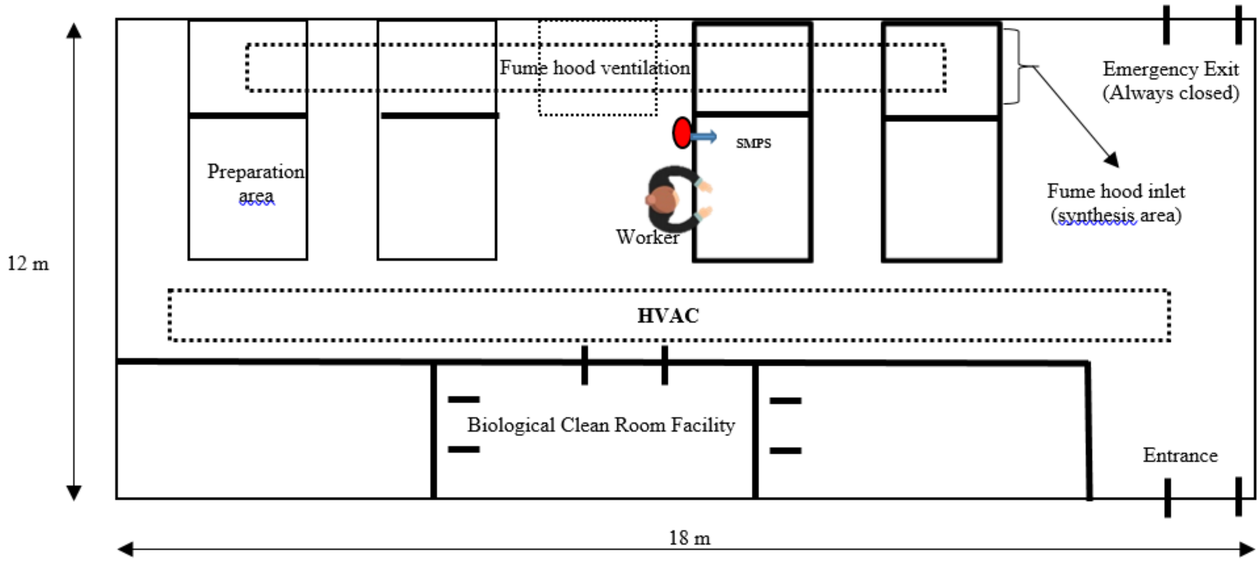


Figure 4. Schematic layout of the synthesis laboratory

Particles emitted from the synthesis process were dominant in the nuclei (79% Au & 54% Ag), followed by the Aitken (12% & 29%), with fewer particles in the accumulation mode (9.2% & 17%) (Table 1 and 2). AuNPs and AgNPs generated during the synthesis process were determined at 1617.3 ± 102 #/cm^3 and 2687 cm^3 ± 82, respectively. The particle to mass conversion resulted in AuNPs (0.046 µg/m^3) and AgNPs (0.077 µg/m^3). For the three exposure scenarios, none exceeded the OEL of both silver and gold (provisional). In conclusion, workers in the synthesis laboratory were found to be exposed to a concentration below the recommended OEL for silver and proposed provisional nano reference value for gold.

Table 1. An 8-hour equivalent exposure concentration compared against proposed provisional nano reference value for Au NPs.

Exposure scenario	Exposure duration (Minutes)	$TWA_{8equivalent}$ concentration (P/cm^3)	Proposed provisional reference (20 000 #/cm^3)
Scenario 1(minimum)	60	202.2	Below the proposed OEL
Scenario 2 (moderate)	180	614.6	Below the proposed OEL

Scenario 3 (worst case)	300	1018.9	Below the proposed OEL

Table 2. An 8-hour equivalent exposure concentration compared against the OEL for Ag NPs.

Exposure scenario	Exposure duration (Minutes)	$TWA_{8equivalent}$ concentration ($\mu g/m^3$)	OEL for silver (0.19 $\mu g/m^3$)
Scenario 1(minimum)	60	0.009	<OEL
Scenario 2 (moderate)	180	0.029	<OEL
Scenario 3 (worst case)	300	0.048	<OEL

Hazard data

An Excel database totalling 164 dose-descriptor data on inflammation and genotoxicity based on animal inhalation studies of nanomaterials of carbon black, carbon nanotubes, graphene cerium oxide, and compounds of silver, cobalt, copper, iron, nickel, silicon, titanium, zinc, and their available bulk and ionic counterparts have been collected from the scientific literature (Table 3). Based on the data different analyses can be made to understand the effect of doses, size and surface area. The work is currently being submitted for review prior to publication from which data will be available as supplemental data in an Excel format (Hadrup et al., in prep.). The data will be made available as a resource in the NanoSafer web-tool at: www.nanosafer.org. The hazard data can be used as input data for risk governance tools as well as to test tools.

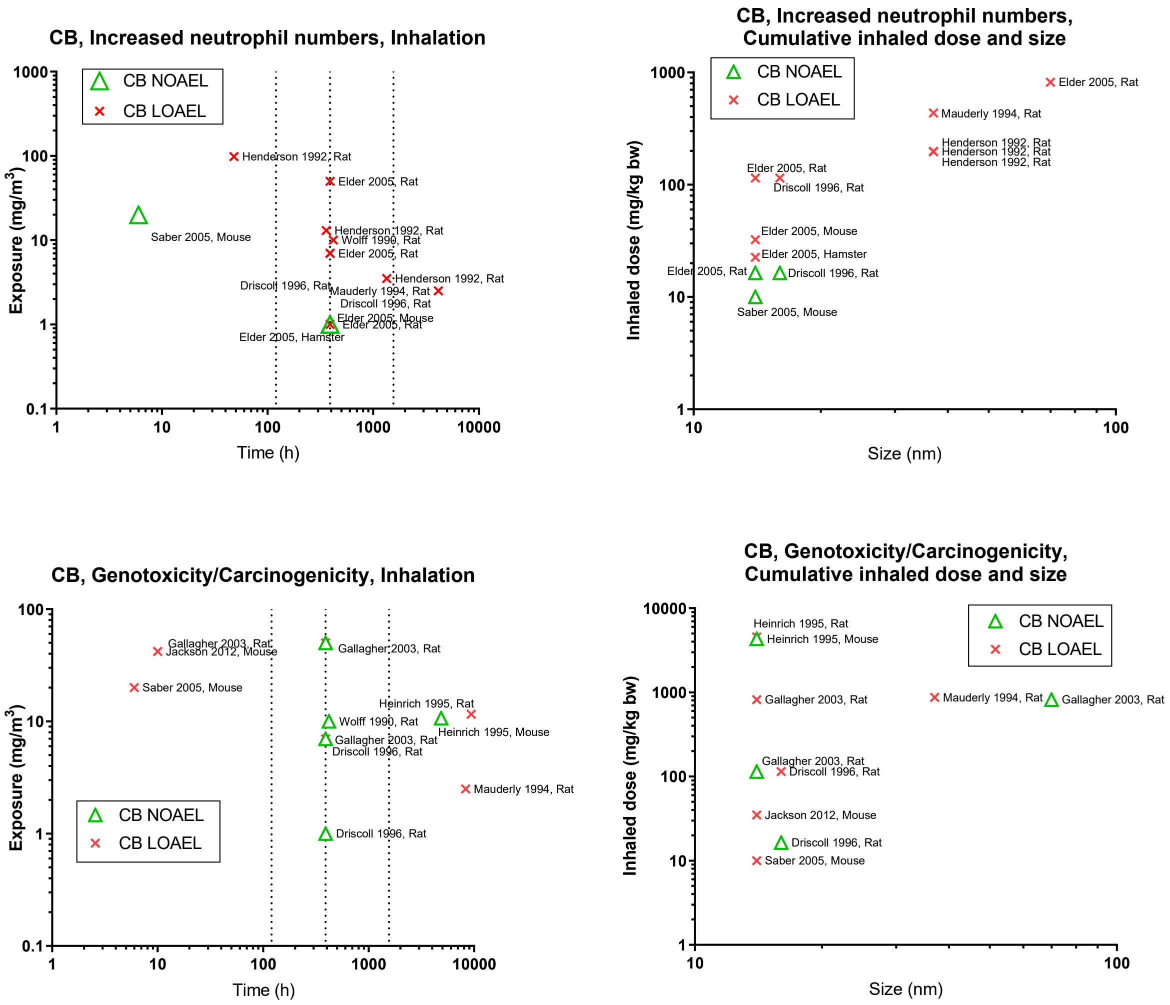
Table 3. Overview of materials in the inhalation hazard library.

Substances	Number of specific materials	Number of materials not nanomaterials	Number of materials exposed in ionic form
Ag	10	2	
CB	6		
Graphene	4		
SWCNT	1		
MWCNT	11*		
CeO_2	7	1	
Co	1		
CoO	1		
$CoCl_2$	2		2
$CoSO_4$.7H2O	1		1
Cu**	1		
$Fe_{(m)}$	1		
Fe_2O_3			
Fe_3O_4			
$Fe(CO)_5$	1	0	1
Ni(m)	1	1	
NiO	7	5	

$Ni(CO)_4$	1		1
NiCl	1		1
Amorphous silica	7	1	
Quartz	3	3	
Cristobalite	1	1	
Anatase	4	1	
Rutile	4	3 (1 substance did not report size)	
Anatase-rutile	5	1	
ZnO	10		
$Zn(NO_3).6H_2O$	1		

* Some materials may be the same, but could not be verified due to limited information on material- and physico-chemical characteristics.
** Surfaces oxidized after opening vial to CuO polymorphs.

Carbon black illustrated based on dosed mass



Carbon black data illustrated based on particle surface area

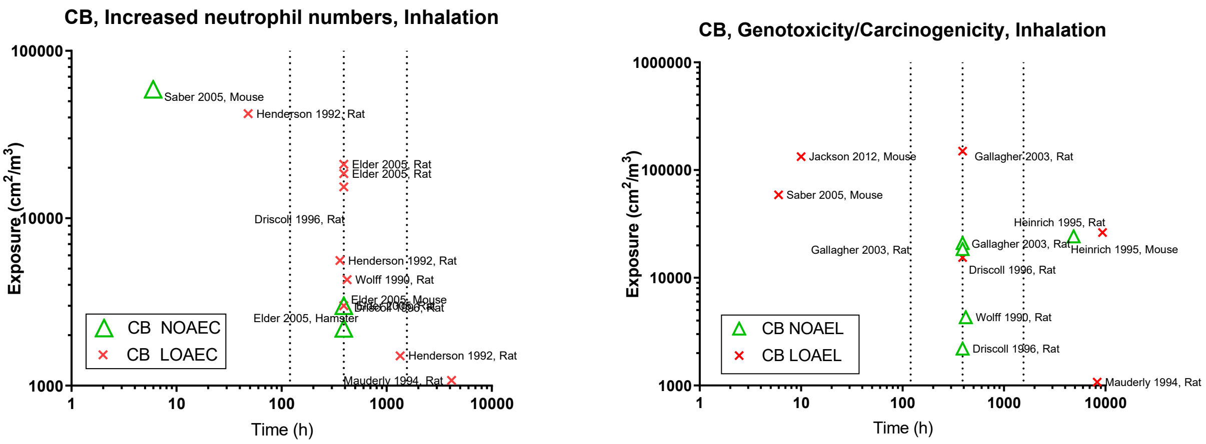


Figure 5. Carbon black (CB) hazard data given as NOAEL (No Observed Adverse Exposure Level) and LOAEL (Lowest Observed Adverse Exposure Level) for inflammation as neutrophil numbers and genotoxicity plotted against different exposure descriptors.

Environmental fate data

EMPA investigated the improvements in environmental risk assessment data and concluded that the data situation has not changed since the end of the caLIBRAte project as a database for environmental fate still does not exist. Existing environmental fate models can therefore not be compared with measurements due to the lack of environmental fate measurement data.

4.1.1 NRGP Case studies

NANORIGO Car tyre case study

- *Concise description of the case study*

This case study covers various aspects of nano-risk governance considering public concern due to the potential release of nanomaterials during in-use wear of car rubber tyres and associated risks and impacts. The case study data are reported in full in a document (van Broekhuizen and Le Blansch, 2022) developed by NANORIGO and shared within the "Joint NMBP-13 car tyre case study working group" with participation by NRCWE set up to investigate how the different risk governance approaches and supporting tools considered to support nano-risk governance can solve the questions raised in this particular case study. It is described that "The case study cooperates with stakeholders selected from the life cycle of car tyres: manufacturers and downstream sectors, industries and user organizations (and related branch organizations and trade unions)". While the NANORIGO car tyre case study covers wear emissions broadly, the joint NMBP-13 work focuses on the issues related to NM release only.

- *How it can be used to test the portal*

The case study is considered suitable to demonstrate the applicability of the nano-risk governance framework proposed in the three NMBP-13 projects to handle this real case scenario and exemplify associated risk governance processes. The data available for tools are summarized in the report by van Broekhuizen and Le Blansch, (2022) and so far appear too limited to allow quantitative nano-specific risk assessment. Further analysis will be made in the final step of developing governance case study- and training materials for the portal and NRGP.

- *Any data / results that come with the case study*

The case study report contains review data on the car tyre case-study. Most data are available as release and mass-flow information and limited exposure measurements are available. Most of the car tyre wear particles end up in the hydro- and geosphere and in a range of 0.1 to 10% are released to air as PM10. Ultrafine particles can be generated by thermomechanical processes during aggressive driving and full stop braking, but is limited. Currently mainly carbon black, silica and ZnO nanomaterials appears to be used. A number of nanomaterials (such as graphene, carbon nanotubes and nanofibers) may be emerging on the market. There is no evidence of direct release of nanomaterials from the rubber matrix. Release of nanomaterials used as fillers and for vulcanization processes may only occur in the process of aging of wear particles or extraction during specific re-use processes. However, these appear not studied.

Table 4. Examples of release and exposure data available in the car tyre case study.

Process	Material	Concentrations / release rates	Size-information
Rubber conversion	Process-generated particles – Extruder	Particle concentration >3 10^6 n/cm^3	Mean diameter 46 nm
Vulcanization	Process-generated particles - Oven	< 1.8 10^6 n/cm^3	Mean diameter 33 nm
Driving	Microplastic* emission rate by tyre abrasion (Germany 2018)	1228,5 g/person/year	Microplastics are by definition coarser than 1 µm.

Driving	Total tyre wear emissions	0,23 – 4,7 kg/person/year - 5-10% in oceans - 3-7% in $PM_{2.5}$ air	
Driving	Total tyre thread wear (Netherlands 2012)	900 t/year: airborne PM_{10} 500 t/year: surface water 2300 t/year: sewage 1000 t/year: sludge	5% as airborne PM_{10}
Driving	Total tyre wear emissions		Ultrafine particles only occur during thermomechanical processes; no $PM_{0.1}$ under controlled driving conditions. 0,1 – 10% airborne PM_{10}
Driving	Nanoplastic** emission rate by tyre abrasion	Unknown	
Driving (aggressive)	Nanoparticles / ultrafine particles	Some report an ultrafine particle (UFP) size-mode in high stress situations (racing start, cornering, full stop breaking. Peak concentrations close to source $<10^7$ n/cm^3.	UFP modes 10 - 80 nm
Nanomaterials used in car rubber tyre manufacturing	Carbon black Silica (ZnO)	A car tyre contains on the order of 30% wt.% solid fillers (Continental summer tyre, 2012) including nanomaterials.	See specific products for size information.
Nanomaterials in development and potential early phase use	Graphene Carbon nanotubes Carbon nanofibers Nanoclays Alumina Polymer nanoparticles	No data	See specific products for size information.

* Plastic particles > 1 µm in size.

** Plastic particles 1 nm to 1 µm in size and is not aligned with other nanomaterial definitions.

Implementation of the Gov4Nano Safe by Design Strategy using the car tyre case study

The approach followed was based on the NanoReg2 project and currently being tested under Gov4Nano, and represented by the SIA Implementation Platform. The SIA Implementation Platform was developed to help with the Implementation of the SIA concepts previously developed in the NanoReg2 Project. The SIA can be seen as the process to ensure that safety aspects of innovations are dealt with as early as possible during the development of an innovation. This requires improved interaction between innovators and regulators throughout the whole innovation process. The SIA concept can be divided into two sections, one which focuses more on industry (Safe by Design) and the other which was developed with regulators in mind (Regulatory Preparedness) as shown in Figure 6 below. The Tyre Case study was selected to test the Safe by Design concept.

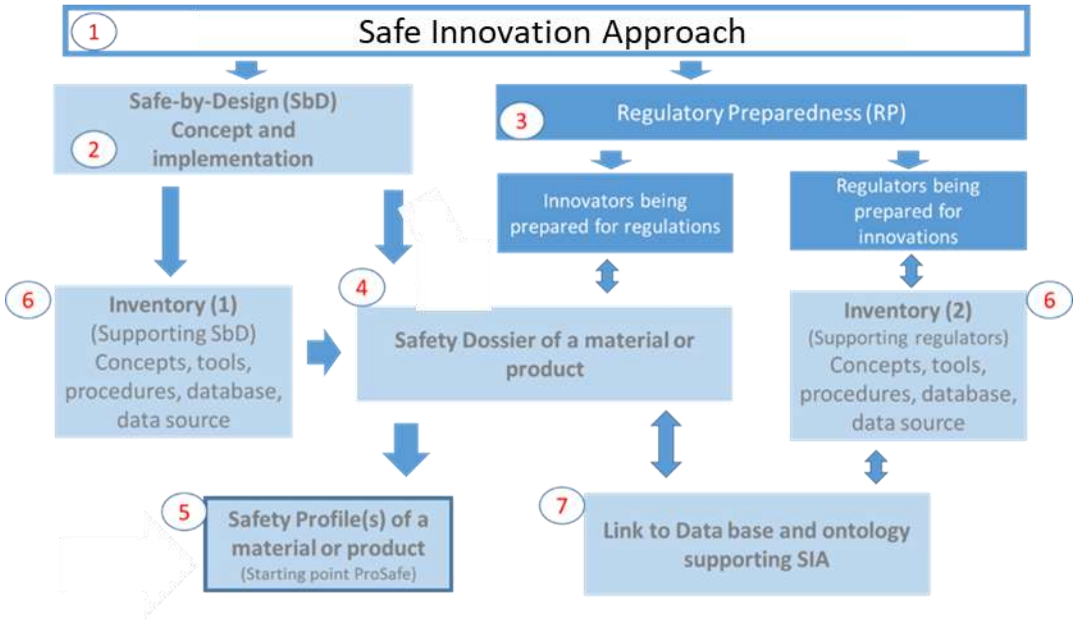


Figure 6. Overview of the Safe Innovation Approach initially developed in NanoReg2 (1). Firstly the Safe by Design concept was developed (2) composed of an Inventory of tools (6), Safety Dossier per stage (4) and Safety Profile (5) as the final output. The Regulatory Preparedness Concept (3) may also have its own inventory of tools (6) and a link to databases (7).

To assist in Safe by Design Implementation the SIA Platform was created. This Platform is composed of the three elements shown in Figure 6; the Inventory, which contains updated tools, guidelines, standards, the Safety Dossier, which contains all relevant information collected per stage and will help decision making, and the Safety Profile, which represents a final output of the Platform and can be customised depending on the intended stakeholder. This Safe by Design approach is based on the Cooper Stage Model™ on stages and gates and was previously presented in an earlier publication1.

The implementation of Safe by Design generally follows a face-to-face training session with the corresponding stakeholder (industry). The training takes two days and there, the different stages, information needs and gates are elucidated. Within the Tyre Case study such an exercise was performed internally to identify issues which could be improved while having in mind more sustainable and safer tyres. Results are shown in Figure 7 and are summarised below:

1. Design stage: NM bonding to matrix as the most important parameter
2. Production: current materials are represented by carbon black (> 1um) or a composite of amorphous silica and silane (50-300 nm). It is recommended to apply control banding tools to assess for potential hazard issues (check ISO 12901-2)
3. Use: environmental release is represented by particles of sizes 10-100 micron, hence no nanomaterial fraction is present.
4. End of life: is performed mainly through pyrolysis which, a prior, does not pose a risk neither for carbon black nor for silica, though further investigations may apply. Shredding prior to recycling accounts for 85% of tyres but as above neither silica nor carbon black may be of concern

The abovementioned exercise tried to highlight further issues to be considered while selecting novel materials for more cost effective, safer and sustainable tyres. Through this exercise the nano fraction did not seem to be of concern.

[1] Kraegeloh et al, Implementation of Safe-by-Design for Nanomaterial Development and Safe Innovation: Why We Need a Comprehensive Approach Nanomaterials. 2018 Apr 14;8(4):239.

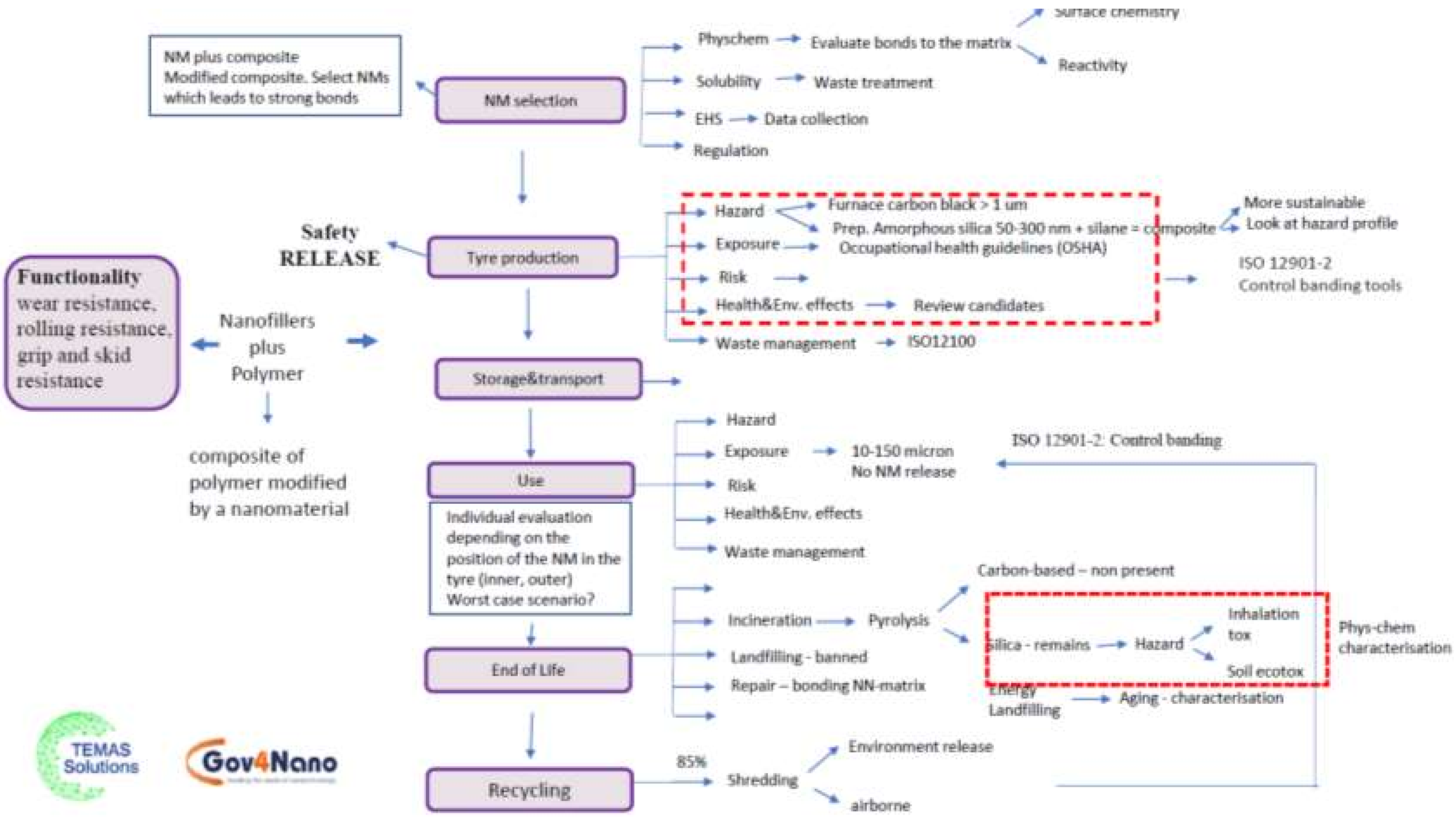


Figure 7. Outcome of the implementation of Safe by Design using the NANORIGO car case study. Following the NanoReg2 strategy the case study is broken down into stages and each stage is analysed for safety, functionality and sustainability mainly using information from the literature of from cost-efficient testing strategies.

TiO_2 exposure and release during paint manufacturing, application and end-of-life

- *Concise description of the case study*

This case study was developed in the caLIBRAte project and summarized here by NRCWE and LEITAT as key investigators of the reported work. The case study includes five different scenarios covering three life-cycle stages (LCS): 1) manufacturing, 2) use and 3) end-of-life compose.

The first scenario is the manufacturing of a paint formulation containing TiO_2 where both, release to workplace air and outdoor environment were measured. The second scenario is indoor paint application to panels where release to indoor air was measured. The third, fourth and fifth scenarios are related to the end-of-life and are the assessment of TiO_2 content in water discharge from six different paint production batches, simulation of landfill disposal and incineration of TiO_2 pigment used for paint production. The case study with high quality measurement data has potential to be suitable to demonstrate the applicability of the NRGP by exemplifying different risk governance processes either as a whole or partly.

1. Occupational exposure and environmental release during paint production

This scenario is based on the work conducted by Fonseca et al., 2021. Particle measurements were carried out at a paint manufacturer (B&J) located in the vicinity of Copenhagen (Denmark) from 29 January to 2 February 2018 during production of three different paint batches for indoor use. The plant is operated on a regular basis by ten or more workers carrying out different activities (handling, pouring, and mixing powders, paint packaging and labelling, electric forklift transport of bags, washing mixers, etc). The working environment and placement of the measurement devices and samplers are shown in Figure 8 and Figure 9. The measurement strategy adopted in this study followed the harmonized 3-tier approach for particle exposure assessment published by the Organisation for Economic Co-operation and Development (OECD 2015). It included real-time particle monitoring combined with collection of samples for gravimetric, morphological analysis during working and non-working periods simultaneously at five locations: (i) near field (NF); (ii) far field (FF); (iii) personal breathing zone (BZ); (iv) stack emitters; and (v) facility surroundings (outdoors).

In this study, occupational exposure and environmental release were monitored during handling of powder materials which included pouring of powders (pigments and fillers) performed by one worker in two different pouring lines, named hereafter as mixing station (MS) located in the ground floor and pouring station (PS) located in the basement. The amount of powders poured per paint batch (either in PS or MS) as well as the material characteristics and chemical compositions are described in Table 5.

In the PS powders were poured either from small bags (SBs; 25 kg) or big bags (BBs; 500 kg) through a quadratic opening with an area of 1.27 m^2 and conducted via tubes into a mixing tank. In the MS, SBs (25 kg) containing the pigments and fillers were opened with a knife and manually emptied by pouring directly into a mixing tank from the edge of an opening area of 0.3 m^2 and a resulting drop height of 1.4 m inside the tank.

The worker involved in the activity used working clothes, safety goggles and filtering face piece respirators (type FFP3). The facility was naturally ventilated with replacement air drawn from the outdoor air through the building and through the adjacent hall via doors which were always opened during the working hours (estimated to be 5 h^{-1}, both in PS and MS). In addition, local exhaust ventilations are incorporated in the MS and PS. In the MS LEV is located under the pouring point and attached to the funnel leading to the mixer with an exhaust air velocity of 5 m s^{-1} and a volumetric dry flow rate of 10,700 m^3 h^{-1} (Fonseca et al., 2021). The PS has a LEV at the rim along three sides of the pouring inlet (duct diameter = 125 mm; mean air velocity = 13 m s^{-1}; and volumetric flow rate = 576 m^3 h^{-1}), and the exhaust duct was connected to the pouring stack with a registered exhaust air velocity of 5 m s^{-1} and a volumetric dry flow rate of 1258 m^3 h^{-1})

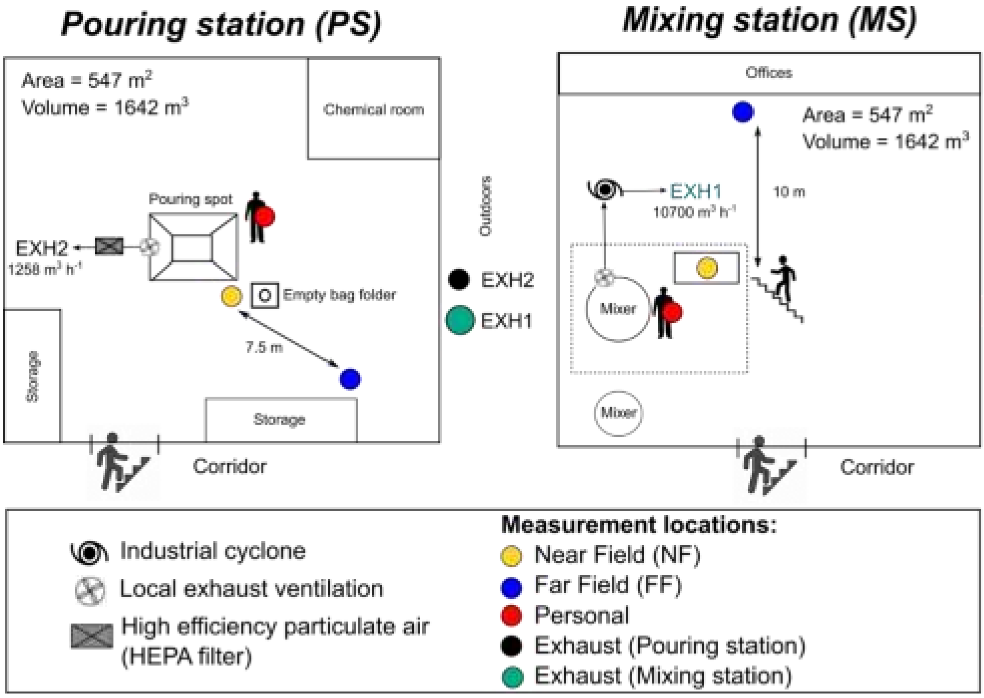


Figure 8. Layout of the working environment and location of instrumentation and samples. Source: caLIBRAte D6.4.

Figure 9. Pictures of the working environment: a) Pouring station; b) Mixing station; c) Emitters; and d) environmental exposure measurements. Source: caLIBRAte D6.4.

Table 5. Description of pouring activities and physicochemical characteristics of the materials under study. σ: standard deviation; d50: average particle size; SSA: specific surface area; VSSA: volume specific surface area; DI: dustiness index; SRD: small rotating drum method; OEL: occupational exposure limit; DI_{SRD}: small rotating drum dustiness index. Modified from Fonseca et al., 2021 and caLIBRAte D6.4.

Pouring activity description					Material characteristics								
Material Name	Measurement site	Paint batch	Amount poured [kg]	Measurement day	Bulk density [b] [g cm^{-3}]	Chemical composition [a]	d_{50} ** [µm]	Shape [a]	BET-SSA [m^2 g^{-1}]	VSSA (m^2 cm^{-3})	Moisture [%]	OEL[d] [mg m^{-3}]	DI_{SRD} ± σ (mg kg^{-1}) [c]
TiO_2 pigment (93% rutile), (Tioxid TR81; CAS-Nr. 13463-67-7)	MS	#1	2150 kg (86 SBs x 25 kg)	30.01.2018	0.94 ± 0.03	Major: Ti, O Minor: Si, Al, Zr, P, and organic coating	0.25	Sphere	N/A	53.2	0.6	6[e]	3.0 * ± 1.3
		#2	1475 kg (59 SBs x 25 kg)	31.01.2018									
		#3	2625 kg (105 SBs x 25 kg)	01.02.2018									
Functionalized $Al_2Si_2O_5$, clay-aluminosilicate mineral (OpTiMat® 2550; CAS Nr. 93763-70-3)	MS	#1	N/A	29.01.2018	0.16 ± 0.001	Major: Si, O Minor: Al, K, Na, and organic coating	25	Plate	N/A	13.6	N/A	5 [f]	149.9 ± 10.8
Microspheres (Expancel 461 WE 20 d36; CAS-Nr. 75-28-5)	MS	#1	N/A	29.01.2018	N/A	Organic (2%)	20-30	Sphere	N/A	N/A	85	5[f]	155.6 ± 66.3
		#3	N/A	01.02.2018									
Calcined clay (PoleStar™ 200P; CAS Nr. 92704-41-1)	PS	#1	925 kg (37 SBs x 25 kg)	30.01.2018	0.52 ± 0.01	Major: Si, O, Al Minor: K, Fe	2	Plate	8	27.0	0.5	2	13.3 ± 0.2
Calcined kaolin ($Al_2Si_2O_5(OH)_4$; Ultrex 96; CAS Nr. 92704-41-1)	PS	#2	500 kg (20 SBs x 25 kg)	31.01.2018	0.35 ± 0.01	Major: Si, O, Al Minor: Fe, Na, Ti	0.8	Plate	N/A	16.0	0.5	2	7.1 ± 0.4

Dolomite ($CaMg(CO_3)_2$; Microdol 1; CAS Nr. 16389-88-1)	PS	#1	1500 kg (3 BBs x 500 kg)	30.01.2018	1.09 ± 0.04	Major: Ca, O, Mg Minor: Si, S	7.5	Sphere	N/A		N/A	5[f]	23.3 ± 1.2
		#2	1500 kg (3 BBs x 500	31.01.2018						9.7			
Talc ($Mg_3Si_4O_{10}(OH)_2$; Finntalc M15; CAS Nr. 14807-96-6)	PS	#1	1186,53 (2 BBs x 500)	30.01.2018	0.46 ± 0.01	>96% Talc (Mg-Silicate with residue magnesite and chlorite); 31% MgO; 60% SiO2; 0.5% Al2O3 and 2.2% FeO)	5 (Particles <2 µm: 20%)	Plate	6	15.1	0.2	5[c]	69.1 ± 4.9
		#2	1600 kg (3 BBs x 500)	31.01.2018									
Calcite, CaCO3 (Socal® P2, Fine Grades, calcium carbonate >=98%; CAS Nr. 471-34-1)	PS	#3	331.57 kg (SBs 25 kg)	01.02.2018	0.57 ± 0.01	Major: Ca, O. Minor: Si, S, Mg	N/A	Rod	8	18.2	N/A	5[c]	0.6 * ± 0.4

[a] Determined by SEM or TEM-EDS; [b] Determined according to the procedure given in EN17199-3:2019; [c] Mass-based respirable dustiness determined by small rotating drum (SRD; EN17199-4:2019); [d] Respirable OEL according to the Danish Working Environment Authority; [e] Calculated as Ti 8-h time weighted average; [f] Respirable inert mineral dust; * Below the limit of quantification (7 mg kg^{-1}); ** Information available in the material safety data sheets provided by the manufacturer; N/A: Not available data.

A combined approach of temporal and spatial analysis for background discrimination (particles from sources other than the target process) was adopted (Kuhlbusch et al. 2011). The non-working periods were used as temporal approach and define the background concentrations by using the measurements obtained before the target process anywhere in the paint manufacturing facility. The spatial analysis assumed the measurement location in FF being representative as background concentrations.

The cumulative workers exposure for an 8-hour time weighted average (8h-TWA) was estimated as follows:

$$8\mathrm{h} - \mathrm{TWA} = \frac{t_1 C_1 + t_2 C_2 + \cdots + t_n C_n}{t_1 + t_2 + \cdots + t_n} \qquad \text{Eq. (1)}$$

where C_n is the measured background corrected particle number or mass concentration (subtracted the background concentration) during a specific operation and t_n is the time duration of the activity.

In this study, an equivalent workers exposure of a typical working shift during pouring activities was considered (total daily duration varying from 2.2 to 5.4 hours). Spatial background concentrations (measured in FF) were used to complete the 8h-TWA.

The measurement plan at the workplaces included real time particle monitoring, gravimetrical collection of particles and TEM/SEM samples collection simultaneously from NF, FF, BZ (instruments attached in the carry bag to the worker’s shoulder; Figure 9a and Figure 9b) by using a variety of gravimetrical samplers for respirable dust, total dust and >2.5,1.0–2.5, 0.5–1, and 0.2–0.5µm particle size ranges, and online instrumentation. The online instrumentation used include condensation and optical particle counters, electrical low-pressure impactors, and diffusion size classifiers. These instruments allow the non-specific determination of particle concentrations and particle size distributions.

The stack emissions (2 identified exhaust chimneys; Figure 9c) were also simultaneously measured by using specific instrumentation for exhaust characterization (Figure 9d):

- DGI Impactor (giving size determination between 0.2 and 2.5 µm, and mass concentration of Ti vs. size)
- 1 ELPI (number concentration vs. size)
- MPS systems (morphology, chemical nature by transmission electron microscopy (TEM) analysis)
- 1 CNC (total particulate concentration, monitoring of peaks)
- Quantitative Total Suspended Particles (TSP) sampling (fluxes of each emitted substance in g/hour)

For environmental exposure assessment the following instrumentation and samplers were used (Figure 2d left):

- 11 deposition passive gauges positioned on the site perimeter (1 month integrative sampling): 1 month averaged particle concentrations in atmospheric deposits (by elemental ICP/OES analysis)
- 2 high flowrate samplers DA 80 (Digitel): 1 (ideally) under the plume, the other serving as reference point, giving 720 m3 filter per day, during two weeks: 2x14 daily averaged particle concentrations in air (ICP/OES)
- MPS and Nanobadge sampling (a few minutes for MPS, 1-2 hours for Nanobadge), several samplings per day (exposed and non-exposed): morphology of particles, semi quantification of particle concentrations under the plumes.
- One portable CNC (3007, TSI), to be used as a control device for total particulate concentration.
- Meteorological station (mainly for wind direction and strength)

2. Indoor paint application

A simulation of indoor paint application to panels was conducted within EU caLIBRAte. For the experiment, three commercial paints containing TiO_2 (TIOXIDE® TR81) as white pigment and made for indoor application, were obtained from B&J. The paints were B&J Wallpaint 5 qualified as EU Eco-Label and meant for domestic houses and offices, Acrylic wall paint Gloss 7 and Pevea 5 both meant for ceilings and walls, from hereafter the three paint samples will be called paint 1, 2 and 3, respectively.

According to the manufacturer, the TiO_2 purity is 95 wt.%, it is coated with Silica, Aluminium and Zirconium and the TiO_2 crystalline phase is rutile. The TiO_2 pigment, provided also as pure material, was analysed by SEM (Merlin FE-SEM, ZEISS, Germany) for the size and shape as well as by BET (NOVA 2000e Instruments, Quantachrome Instruments, USA) to determine its surface area. The paints were analysed by ICP-MS (Agilent 7500, Agilent Technologies) to determine the TiO_2 content and by FT-IR (SHIMADZU, IRAFFINITY-1S) to investigate the presence and relative amount of organic materials. The results concluded that TiO_2 particles had a size of 173 ± 59 nm measured from SEM imaging based on 50 particles. The surface area measured by BET was 12.6 ± 1.3 m^2/g (N=2). Concentration of TiO_2 in the three paints were measured by ICP-MS and resulted to be 13.9 ± 1.3 wt.%; 14.9 ± 1.3 wt.% and 12.9 ± 0.6 wt.% for paint 1, 2 and 3, respectively.

For the paint simulation experiments, the paints were applied on pre-sanded PVC panel to have better adhesion of the paint and dried during 72 h before any future experiments. The potential transfer of TiO_2 from the paint to the skin or clothes when touching the painted surfaces was evaluated using a Crockmeter (302p, JBA instruments). A test sample, painted panel, was clamped to the instrument base and a square of standard crocking cloth, in this case cotton, was fixed to the rubbing finger. The finger rests on the sample with a pressure of 900 grams force and traverses a straight path approximately 100 mm long with each stroke of the arm, rubbing a total surface of 30.9 cm^2 per cycle. Crockmeter experiments were performed following a standardized protocol (ISO 105-X12:2016) originally designed to test the colour fastness on textiles due to rubbing. The number of rubbing cycles was set at 10, 100 and 200 and the experiments were performed using both wet and dry crocking cotton. All Crockmeter tests were conducted in triplicate. At the end of the experiments, the crocking cotton was digested with acid in an analytical microwave digestion system (MARS, CEM, 1600W) and analysed by ICP-MS (Agilent 7500, Agilent Technologies) to determine the amount of TiO_2 transferred from the painted panel surface to the crocking cotton. Moreover, some samples of the crocking cotton were also observed with FE-SEM (Merlin, Zeiss) to check the presence of TiO_2 possibly released during the Crockmeter tests.

3. Assessment of TiO_2 content in water discharge from paint production

The analysis of wastewater produced during cleaning of the reactor after six paint batch formulation was conducted at the same paint manufacturer (B&J) as in scenario 1 under caLIBRAte (detailed results are available in caLIBRAte D6.4). In specific, six different water samples from different individual large paint batches were collected and stored for determination of total concentrations of Ti, Al, Cd, Cr, Cu, Fe, Ni, and Pb using an ICP-MS methodology established at UKAS accredited Environmental Analysis Laboratory at UKRI-CEH. All the six paint batches were different and do not coincide with the same batches produced during scenario 1. The corresponding paint batch number is properly identified as Batch 568597, 568346, 568511, 568543, 568485 and 568513. As a normal procedure the paint production company usually reuses the collected water for the next batch production. Therefore, the collected water from the indicated batches was mixed with water from the cellar, which corresponds to mixed water from all the paint production facility, to create a more realistic and representative scenario of the water that is discharged by the company to the waste water treatment plant. The collection of the discharge water took place on the 14^{th} of February 2018. For every 100 L of contaminated water 50 mL sample was collected. The total amount of contaminated water collected that day was 1160 L.

4. Simulation of landfill disposal of TiO_2 pigment used for paint production

The three commercial paints used in scenario 2 (B&J Wallpaint 5, Acrylic wall paint Gloss 7 and Pevea 5 named paint 1, 2 and 3) were subjected to simulated landfill disposal under caLIBRAte (detailed results are available in caLIBRAte D6.4). For this, the dried paints were removed from the painted panels using a spatula and fragmented using a Centrifugal Mill (Retsch, ZM 200) at 18000 rpm with a 2 mm sieve size (stainless steel trapezoid holes) to obtain paint fragments with a comparable particle size distribution, and subjected to the leaching experiment. Before the leaching test, the fragments obtained were analysed by ICP-MS (Agilent 7500, Agilent Technologies), by Mastersizer3000 (Malvern, MAZ3000, UK) and by FTIR (SHIMADZU, IRAFFINITY-1S) to determine respectively the TiO_2 content in the dried paints, the fragment size distribution and the functional group present in the paint. The paint fragments were dispersed (1g/L) in pure ethanol.

To simulate the amount of TiO_2 released during leaching simulating landfilling condition, the paint fragments were subject to a TCLP test (The EPA standard Method 1311; 167 Toxicity Characteristic Leaching Procedure). Leaching medium was prepared by adding 5.7 mL glacial acetic acid to 500 mL of deionized water. Then 64.3 mL of 1M NaOH was added and diluted to a volume of 1 L (pH = 4.93 ± 0.05). For the leaching experiments, 1 g of fragment of paint was added to 20 mL of leaching media, maintaining a 1:20 solid/liquid ratio. The samples were rotated end-over-end at 40 ± 2 rpm at 23 ± 2 °C for a period of 18 h.

At the end of the leaching test, the samples were analysed by ICP-MS. The same leaching protocol was applied to the pristine nano-TiO_2 to investigate any loss by adsorption or due to aggregation of particles up to the 20µm size. Moreover, the same leaching experiments were performed by using both, TCLP solution and MilliQ water.

5. Incineration of TiO_2 pigment used for paint production

In addition, solid wastes, represented by empty bags containing TiO_2 pigments, were collected and used for experiment simulating incineration within the caLIBRAte project (detailed results are available in D6.4). Samples from three identical big bags containing TiO_2 particles with an average size of the primary particles agglomerates of about 200 nm were collected. The samples correspond to cut out pieces of the three bags. It should be noted that the samples of the big bags were in contact with various paint ingredients by direct contact with other bags containing different paint ingredients.

A simulated incineration of the collected bags samples was performed in a tubular furnace (150 cm long and inner diameter of 95 mm) analysis. The experimental set up is shown in Figure 10. Additional details of the experimental setup can be found in Ounoughene (2015).

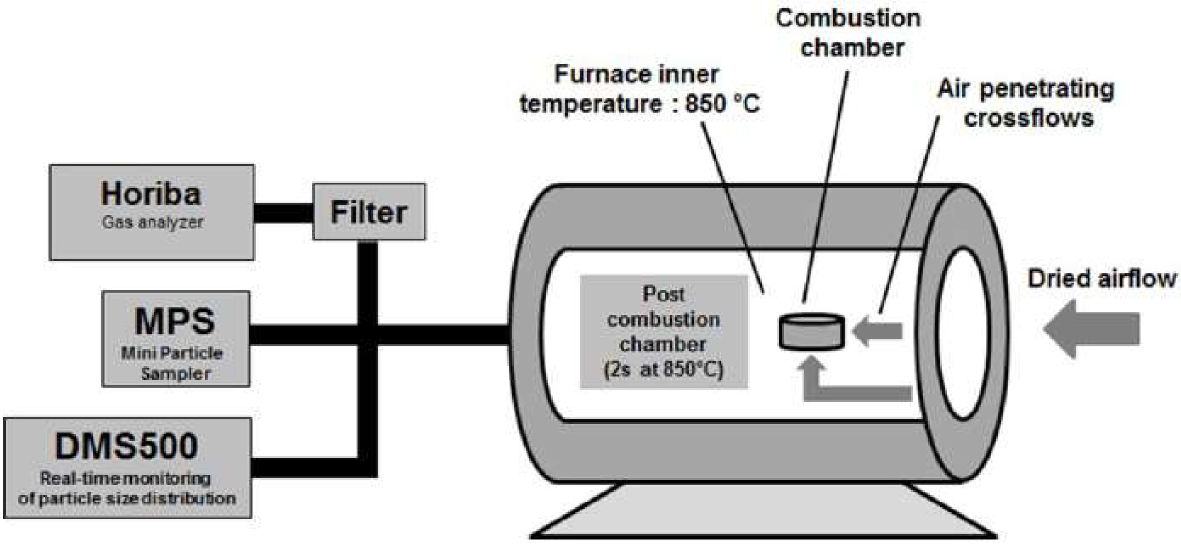


Figure 10. Experimental setup of the incineration in a tubular furnace. Source: caLIBRAte D6.4.

A sample of bag of about 200 mg was introduced in an Inconel-made meshed holder (diameter of 30 mm and 25 mm height), which allows air flow and facilitates combustion. During incineration, the sample holder is pushed through the furnace centre with a flowrate of 12 L/min to assure combustion in incineration mode. For incineration, temperature was set to 850 °C, a residence time of two seconds in the post combustion zone was assured, turbulence conditions were met in the furnace to assure good mix between combustible and oxygen. The experiment was conducted at high excess of oxygen concentrations.

These four settings and conditions were selected to simulate incineration as close as possible to the reality. These are same settings and conditions applied in in Municipal Waste Incinerator Plants.

To monitor gas and particulate emissions the following instrumentation was used:

- A gas analyser Horiba PG350, which allows monitoring of the temporal evolutions of the O_2, CO_2 and CO concentrations. And was used to identify the combustion phase.
- A particle sizer Cambustion DMS 500 fast Particle Analyzer was used for real-time particle emission monitoring. It determines particle size distributions and concentrations for sizes ranging from a few nanometers to 1 µm by combining electrical mobility measurements and a sensitive electrometer.
- A MPS (Mini Particle Sampler, Ecomesure) allowing particle sampling on TEM (Transmission Electron Microscope) grids was used (R'Mili 2013). The analyses of the collected samples were performed using a JEOL JEM 1400 Plus TEM coupled with EDX (Energy Dispersive X-ray) technique, which allowed assessment of morphology and size of particles as well as elemental analysis.

For each of the three bag samples, three test runs were conducted to ensure repeatability. The sampling time for the TEM grid was of about 15 seconds.

In addition, the fate of the particles in the aerosol and the bottom ash (residue) was examined using real-time monitoring instruments TEM. A small quantity of residue was poured in alcohol. Then, a drop of alcohol containing the mixture alcohol and residue was deposited on a TEM grid for analysis.

- *How it can be used to test the portal*

The study, composed by five scenarios, covers and can therefore be used to test the portal for three LCS, manufacturing, use and end-of-life.

Manufacturing phase

As part of this LCS, scenario 1, which deals with occupational exposure and environmental release during paint production, can be used to test the portal for worker inhalation and environmental release. The case is as an example of an industrial scenario where there is a concern of workers potential inhalation of a nanomaterial (TiO_2) during a pouring process, and how to handle the exposure by applying for example reduction control measures. On the other hand, it concerns environmental release through ventilation.

The scenario has detailed and comprehensive information on indoor workplace exposure and outdoor measurements (via exhaust air chimneys) and the facility surroundings (outdoor) during production activities of three different paint batches, using e.g. TiO_2.

The activity-based measurements inside the production plant consist of personal measurement (BZ) of one worker during powder handling and paint mixing in two different working stations (mixing (MS) and pouring (PS)), and simultaneous NF and FF measurements. Real-time (online) monitoring and mass sampling for particles from 5 nm upward was conducted for whole day shifts during five consecutive days. The dominant exposure levels were measured with respirable filter sampling for gravimetric measurements and confirmed by scanning electron microscopy (SEM) or TEM analysis.

The study provides contextual information which was collected for NECID (Nano Exposure Contextual Information Database), for use in later stages with modelling tools.

Parametrization of the study for three different tools, NanoSafer, Stoffenmanager and GUIDEnano is available in caLIBRAte D6.4, section 3.1, which will facilitate the use of the scenario for the portal and tools.

Use phase

As part of this LCS, scenario 2, deals with potential occupational and consumer dermal exposure to nanomaterials (TiO_2) through paint application containing TiO_2.

The scenario deals with the application of 3 manufactured paints of commercial indoor use on PVC panels to test the possible release of TiO_2 under simulated use condition and the dermal exposure through cleaning or touching the painted walls. To do this, the scenario provides a simulation of a mild abrasion that could be representative of the cleaning or the touching of the painted walls.

The TiO_2 released after different cycles of Crockmeter test (originally designed to test the colour fastness on textiles due to rubbing) were analysed by ICP-MS and were observed by SEM-EDX. The amount of TiO_2 released was determined after different rubbing cycles at 10, 100 and 200 and the experiments were performed using both wet and dry crocking cotton for the three paints. Data available can be used for different risk assessment tools and models.

End of life

As part of this LCS, scenarios 3, 4 and 5 deal with the assessment of TiO_2 content in water discharge from paint production, simulation of landfill disposable by leaching and incineration of TiO_2 pigments used for paint production can be used to test the portal as examples of potential environmental release (water, land and air) from industrial processes dealing with nanomaterials.

In scenario 3, the contentment on six water discharge samples from paint production was analysed and concentrations for Ti, Al, Cd, Cr, Cu, Fe, Ni, and Pb are given.

In scenario 4, the leaching of TiO_2 from paint fragments to water and media is analysed and % of release (to water) is provided.

In scenario 5, particle number, CO and CO2 concentrations are given during incineration of 3 replicates of 3 bags containing TiO_2 pigments for pain production. Aerosol released and incineration residue samples were analysed using TEM-EDX for the presence of TiO_2 particles and agglomerates.

The type of data available from these three scenarios can be used to test whether tools currently available in the NRGP are capable of predicting end of life environmental release situations as these tools. As these environmental fate models in general, don't work well "on a case study" level, these case studies may provide valuable information with regards to improvements to such models with regards to information required to run them. In addition, advanced risk assessment models may be refined/updated in the next years and may use these type data.

- *Any data / results that come with the case study*

1. Occupational exposure and environmental release during paint production

The results for measured particle number, lung deposited surface area and respirable/PM10 dust at BZ, NF and FF are summarized in Table 6. Detailed descriptions of measurements for each day and case scenario can be found in caLIBRAte D6.4 Annex I.1 and in Fonseca et al., 2021.

Stack environmental emissions are summarized in Table 3. Data corresponds to outdoor measurements in the facility surroundings during three different paint batches. Additional data can be found in Fonseca et al., 2021 and caLIBRAte D6.4 Annex I.1.

Table 6. Descriptive statistics for the measured particle number concentrations (N), and lung deposited surface area (LDSA) by using DiSCmini (DM) (size range 10–700 nm) for non-working hours and for each poured material during each batch production and respirable dust and PM10. Source: Fonseca et al., 2021.

Table 7. Emissions detected at the stack of both mixing and pouring stations in terms of the total particle number, total suspended particles (TSP), and size fraction particle mass. *DGI spectrum distribution not estimated due to saturated sample. Source: Fonseca et al., 2021.

		Amount poured [kg]	Pouring rate [kg min^{-1}]	N [cm^{-3}]						$LDSA$ [µm^2 cm^{-3}]						Respirable dust / PM$_{10}$ [µg m^{-3}]		
				BZ		NF		FF		BZ		NF		FF				
				Mean	±σ	Mean	±σ	Mean	±σ	Mean	±σ	Mean	±σ	Mean	±σ	BZ	NF	FF
Non-working hours (14h of measurements)		-	-	N/A		**4.8E+03**	**3.0E+03**	6.7E+03	3.7E+03	N/A		35.7	21.8	34.1	22.8	N/A	76/N/A	25/N/A
TiO_2 pigment	Batch #1 (55 min)	2150 kg (86 SBsx25 kg)	39.1	8.3E+03	2.6E+04	1.1E+04	2.1E+04	5.8E+03	3.4E+03	37.2	55.7	57.6	53.9	22.9	14.3	N/A	1450**	76.4/N/A
	Batch #2 (24 min)	1475 kg (59 SBs x 25 kg)	61.5	**1.9E+04**	**1.9E+04**	1.5E+04	1.2E+04	1.0E+04	2.3E+03	86.1	68.3	78.5	52.1	40.7	15.5	N/A	467.9/1150**	60.3/N/A
	Batch #3 (42 min)	2625 kg (105 SBs x 25 kg)	62.5	9.6E+03	1.1E+04	9.2E+03	1.1E+04	6.7E+03	2.0E+03	53.8	39.1	65.3	45.3	27.6	11.5	N/A/1624*	621.8/1060**	47.3 (<DL)
Clay-aluminosilicate mineral (OpTiMat® 2550)	Batch #1 (14 min)	N/A	N/A	N/A		5.3E+03	9.2E+03	6.4E+03	4.4E+04	N/A		28.0	36.3	12.2	37.3	N/A	57.6/482**	34.1 (<DL)
Microspheres (Expancel)	Batch #1 (17 min)	N/A	N/A	N/A		**3.2E+04**	**4.6E+04**	1.6E+03	6.0E+02	N/A		57.7	77.0	4.2	1.5	N/A	457.7/1050**	N/A
	Batch #3 (21 min)	N/A	N/A	**4.5E+03**	**3.1E+02**	**4.1E+03**	**5.5E+03**	2.4E+03	3.8E+02	11.6	0.4	12.4	14.3	8.2	2.52	N/A	N/A	47.3 (<DL)
Calcined clay (PoleStar™ 200P)	Batch #1 (75 min)	925 kg (37 SBs x 25 kg)	12.3	**6.3E+04**	**4.5E+05**	**1.1E+04**	**1.9E+04**	3.4E+03	9.6E+02	61.7	221.4	19.3	0.2	6.3	0.01	N/A / 1120*	N/A / 630*	N/A
Calcined kaolinite (Ultrex 96)	Batch #2 (94 min)	500 kg (20 SBs x 25 kg)	5.3	**1.7E+04**	**2.4E+04**	**6.4E+03**	**7.3E+03**	4.0E+03	5.6E+02	44.2	49.7	22.0	0.2	14.4	0.01	N/A / 2140*	N/A / 650*	N/A
Dolomite (Microdol 1)	Batch #1 (122 min)	1500 kg (3 BBs x 500 kg)	12.3	**3.6E+04**	**1.3E+05**	**2.8E+04**	**8.2E+04**	4.8E+03	2.3E+03	63.5	187.2	33.5	0.7	9.1	0.03	N/A / 1040*	N/A / 610*	N/A
	Batch #2 (117 min)	1500 kg (3 BBs x 500 kg)	12.8	**2.3E+04**	**6.4E+04**	**5.0E+04**	**2.1E+05**	3.9E+03	1.3E+03	44.2	90.5	56.5	1.7	11.2	0.03	N/A / 1750*	N/A / 1260*	N/A
Talc (Finntalc M15)	Batch #1 (64 min)	1200 (2.4 BBs x 500)	18.5	**2.7E+05**	**6.2E+05**	**4.0E+05**	**1.1E+06**	8.1E+03	6.0E+03	449.4	981.5	327.6	7.9	13.1	0.08	N/A / 5630*	N/A / 4880*	N/A
	Batch #2 (87 min)	1600 (3.2 BBs x 500)	18.4	**1.9E+05**	**5.0E+05**	**1.9E+05**	**6.3E+05**	5.9E+03	3.3E+03	313.9	810.4	185.6	4.5	16.1	0.06	N/A / 6510*	N/A / 2930*	N/A
Calcite (Socal® P2)	Batch #3 (43 min)	333 (13.3 SBs x 25 kg)	7.7	**8.4E+03**	**5.8E+03**	**6.9E+03**	**4.9E+03**	4.3E+03	6.4E+02	21.4	10.4	17.0	0.1	11.1	0.02	N/A / 300*	N/A / 299*	N/A

		ELPI	DGI sampler					TSP **(EN 14385:2004)**
		N total × 10^3 [cm^{-3}]	Total DGI [$mg\ m^{-3}$]	$PM_{2,5}$ [$mg\ m^{-3}$]	PM_1 [$mg\ m^{-3}$]	$PM_{0,5}$ [$mg\ m^{-3}$]	$PM_{0,2}$ [$mg\ m^{-3}$]	[$mg\ m^{-3}$]
Mixing station	Concentration batch #2 (7:33-12:43; TiO_2 pouring)	2.69	0.38	0.34	0.28	0.18	0.16	0.55
	Flow [$g\ h^{-1}$ / $g\ ton^{-1}$]	-	4.0/14	3.7/13	3.0/10.5	2.0/7.0	1.7/6.0	5.8/20.3
Pouring station	Concentration batch #1 (7:30-12:32)	4.78	-*	-*	-*	-*	-*	9.67
	Concentration batch #2 (13:00-14:40)	5.15	2.85	1.87	0.89	0.47	0.26	-
	Concentration batch #3 (12:13-14:57)	9.13	3.49	2.29	1.07	0.44	0.30	4.19
	Average	**6.35**	**3.17**	**2.08**	**0.98**	**0.46**	**0.28**	**6.93**
	Flow batch #1 [$g\ h^{-1}$ / $g\ ton^{-1}$]	-	-	-	-	-	-	12.0/16.7
	Flow batch #2 [$g\ h^{-1}$ / $g\ ton^{-1}$]	-	3.6/4.0	2.3/2.6	1.1/1.2	0.6/0.7	0.3/0.3	5.2/5.8
	Flow batch #3 [$g\ h^{-1}$ / $g\ ton^{-1}$]	-	4.4/23	2.9/15.2	1.3/6.8	0.6/3.1	0.4/2.1	-
	Average [$g\ h^{-1}$ / $g\ ton^{-1}$]	-	4.0/13.5	2.6/8.9	1.2/4.0	0.6/1.9	0.4/1.2	8.6/11.3

2. Indoor paint application

Table 5 reports the total release of TiO_2 after 10 rubbing cycles, from 10 to 100 rubbing cycles and from 100 to 200 rubbing cycles, under dry and wet conditions, and Figure 11 shows the cumulative release of TiO_2 after 10, 100 and 200 of dry and wet rubbing. The release rate of TiO_2 by m^2 decreased with increased number of rubbing cycles and it was higher during the first 10 rubbing cycles and decreased during the following 90 (total of 100 wet rubbing cycles) and 100 rubbing cycles (total of 200 wet rubbing cycles). Moreover, the TiO_2 released during wet rubbing was approximately two orders of magnitude higher than the one obtained with dry rubbing consistently across the three tested paints (Table 8).

Additional information and SEM images of the samples can be found in caLIBRAte D6.4 Annex I.2 and in Bossa et al., in preparation.

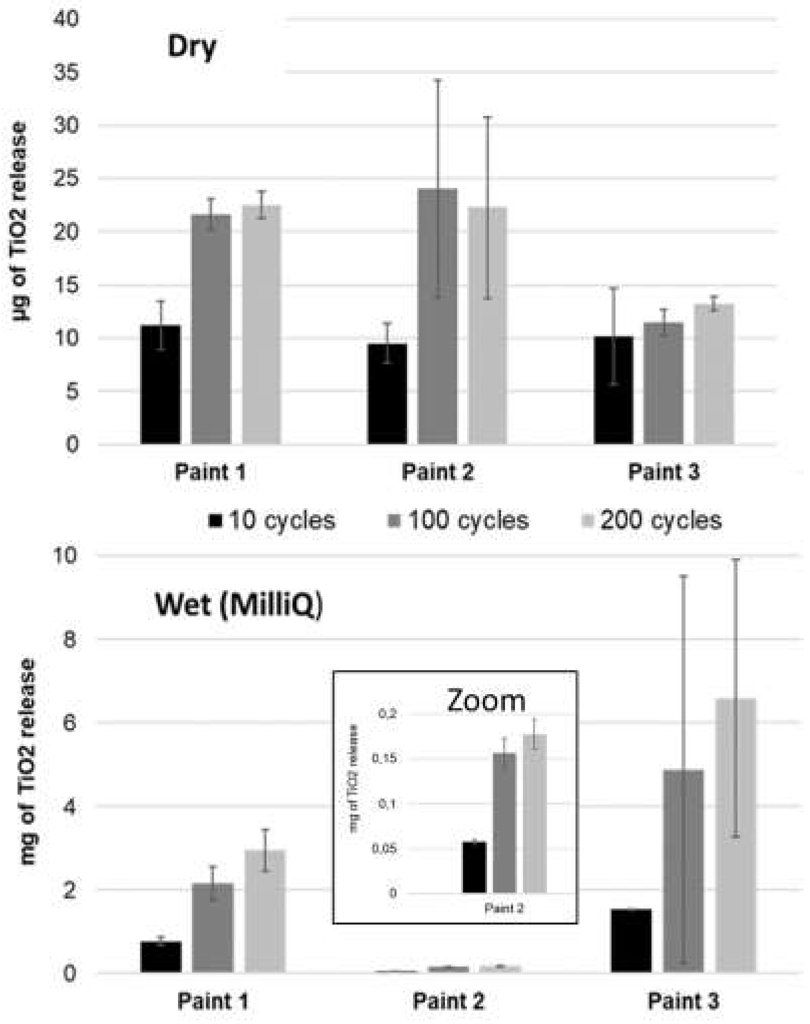


Figure 11. Cumulative TiO_2 release rate by m^2 during dry (top) and wet (bottom) rubbing after 10, 100 and 200 cycles. Source: Bossa et al., in preparation.

Table 8. Total TiO_2 released after 10, from 10 to 100 and from 100 to 200 cycles of dry and wet crockmeter tests. Source: caLIBRAte D6.4.

Paint	Crockmeter condition	TiO_2 released in 10 cycles (mg/m^2)	TiO_2 released from 10 to 100 cycles (mg/m^2)	TiO_2 released from 100 to 200 cycles (mg/m^2)
1	Dry	0,3632	0,07	0,0364
	Wet	24,9	7,0	4,8
2	Dry	0,4105	0,0372	0,0211
	Wet	49,9	15,8	10,7
3	Dry	0,3078	0,0778	0,036
	Wet	1,9	0,5	0,3

3. Assessment of TiO_2 content in water discharge from paint production

The analysis of the replicate samples from the different separate batches of processing wastewater indicated that concentrations of Cd, Cu, Fe, Ni and Pb were below the detection limits for all the analysed water, except for one sample in which Ni (24 mg/L) was present. For Cr generally (>55% of the samples) concentration were between 56-90 mg/L. For Ti, Al and Zn concentrations above detection limit were found in all samples, with Ti showing the highest concentrations as expected. Concentrations for all samples and composition of the samples in % of Ti, Al and Cr are shown in Figure 12.

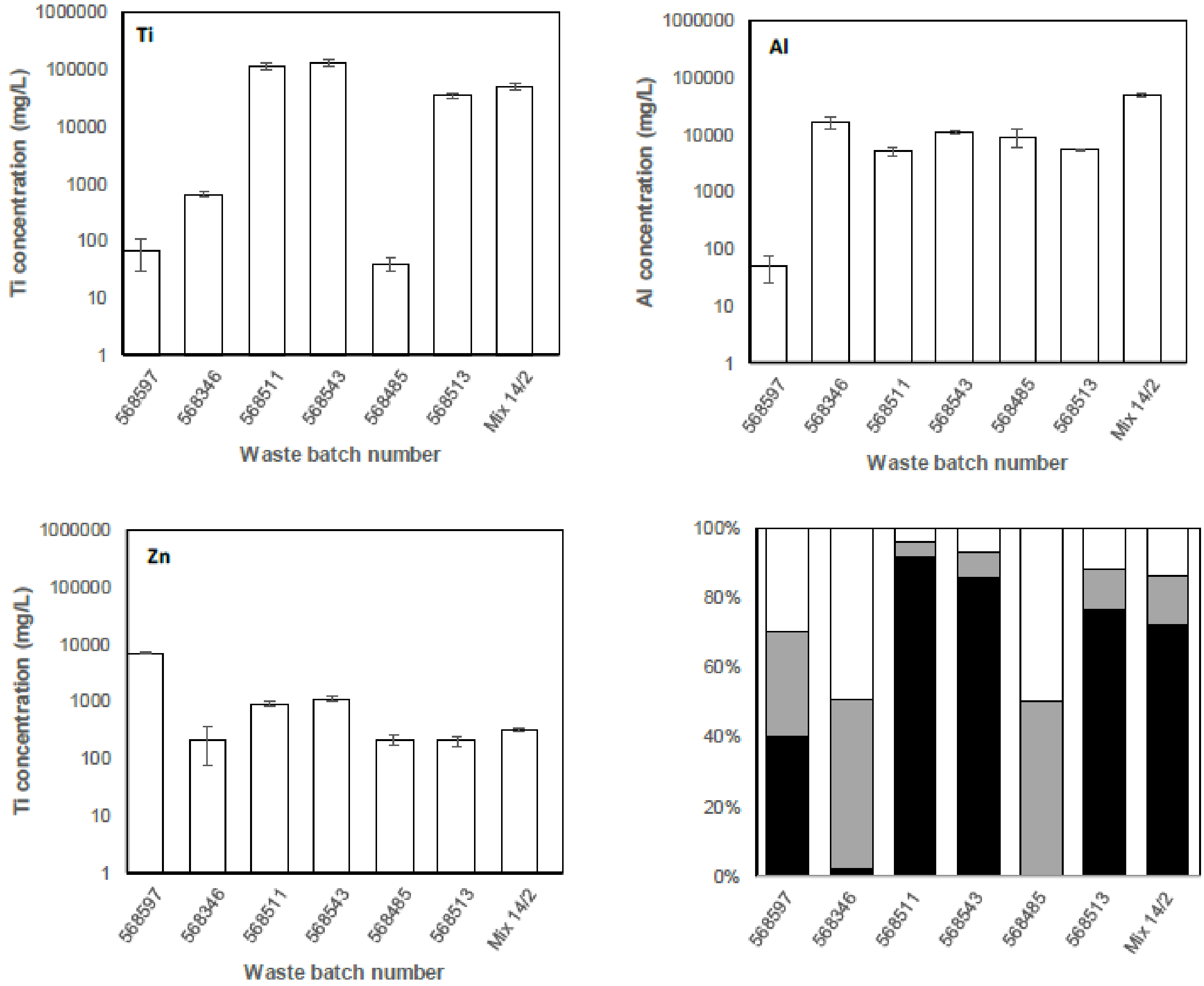


Figure 12. Measured concentrations of Ti (top left), Al (top right), Zn (bottom left) (bars are mean values for measured replicates, with error bars indicating standard deviation) and the relative proportional contribution of Ti (black), Al (grey) and Zn (white) to total concentrations of these three element measured in the sample (bottom right). Source: caLIBRAte D6.4.

Concentrations for Ti ranged from 67-127000 ppm (0.0067-12.7% w/w), 2000 fold variations between measured concentrations of different samples. Close agreement between replicates confirms that the variability observed relates to between wastewater batch differences. Concentrations of Al were in the range 5175-16,650 mg/L, except for sample 568597 where values were close to the detection limited <50 mg/L. For Zn, concentrations found between samples were also more consistent than for Ti, ranging from 215-1125 mg/L, except in sample 568597, where they were 7045 mg/L.

Characteristics of the size distributions obtained using single particle ICP-MS technique are presented in Table 9. The size distribution for Ti measured using SP-ICP-MS indicates that the average size of sample was in the range 95-265 nm across all measured wastewater batches.

Table 9. Characteristics of the size distributions obtained in each collected sample. Source: modified from caLIBRAte D6.4.

Sample	Analyte	Most frequent size (nm)	Mean size (nm)	No. of peaks	Mean intensity (counts)	Particle concentration (part/cm3)	Diss. Inten. (counts)	Diss. Conc. (ppb)
568597	Ti 47.948	128	177	1110	182	18164	0.213	0.372

568346	Ti 47.948	168	218	2361	304	38689	0.018	-0.117
568511	Ti 47.948	202	202	3377	562	55361	0.034	-0.075
568543	Ti 47.948	265	275	2774	476	45509	0.016	-0.120
568485	Ti 47.948	95	129	6363	86	104296	0.235	0.428
568513	Ti 47.948	154	211	2153	278	35279	0.022	-0.105
14/2	Ti 47.948	206	224	6678	270	109460	0.016	-0.121

4. Simulation of landfill disposal of TiO_2 pigment used for paint production

The particle size distribution of the fragments obtained from the deconstructed paint from the painted panels in scenario 2 was slightly different for each paint, with a D50 of 385, 175 and 38 µm, respectively.

The percentage of mass loss after leaching was 9, 10 and 14 wt.% in MilliQ water and 8, 6 and 9 wt.% in TCLP solution for paint 1, 2 and 3, respectively, notifying a significant mass loss in the paint fragments.

In Figure 13 the TiO_2 release (wt %) from paint fragments to the leaching media (MilliQ water and TCLP) filtered through 20 and 0.45 µm filters. For the three paints fragments, the quantity of TiO_2 released after filtration at 0.45 µm was lower than the ones after filtration at 20 µm. The amount of TiO_2 released in both leaching media follow this order, paint 3 > paint 1 > paint 2.

It should be noted that paint 3 fragments, with the smaller particles size distribution, released 33.4 wt.% of TiO_2 in the fraction filtered through 20 µm filters, while paint 1 and 2 with bigger size fragments, released respectively the 2.1 and 1.2 wt.% of the TiO_2 initially present in the fragments. When MilliQ water was used as leaching media, paint 3 showed about 5 and 15 times more TiO_2 released than paint 1 and 2, respectively, in the fraction filtered through a 0.45 µm filter. In TCLP solution, paint 3 released about 2.3 times more TiO_2 than paint 1, while almost no TiO_2 release was detected in paint 2 (0.001% of initial TiO_2 contained in the paint 2 fragments) in the fraction filtered through 0.45 µm filter. The TiO_2 primary particles size measured by SEM was 173 ± 59 nm indicating that TiO_2 particles released from paint can pass through the filter of 0.45 µm. It has been showed previously that TiO_2 released from paint had a better colloidal stability than pristine TiO_2 due to the paint matrix coating at the NMs surface, stabilizing the TiO_2. Our results support the hypothesis that in condition simulating leaching in landfilling condition, primary TiO_2 particles can be released from paints and the paint matrix highly influences the release of these particles. Additional details can be found in Bossa et al., in preparation.

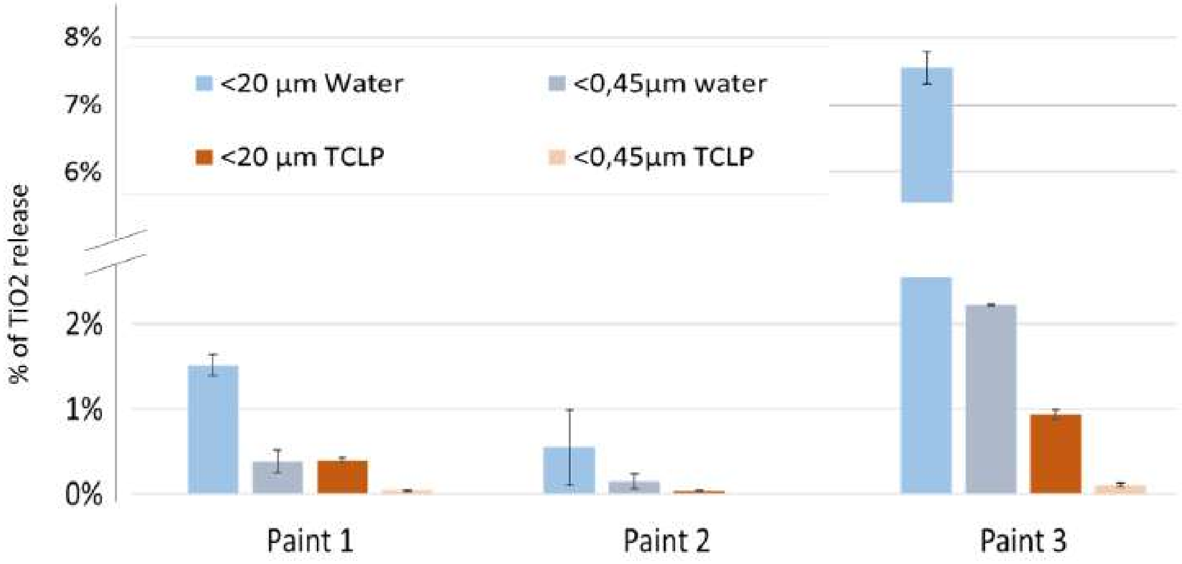


Figure 13. TiO2 released (wt.%) from paint fragments to the leaching media (MilliQ water and TCLP) filtered through 20 and 0.45 µm filters. Source: caLIBRAte D6.4.

5. Incineration of TiO_2 pigment used for paint production

Particle number concentrations from 10-100 and from 100-1000 nm for each bag and replicate are shown in together with CO (ppm) and % of CO2 in Figure 14.

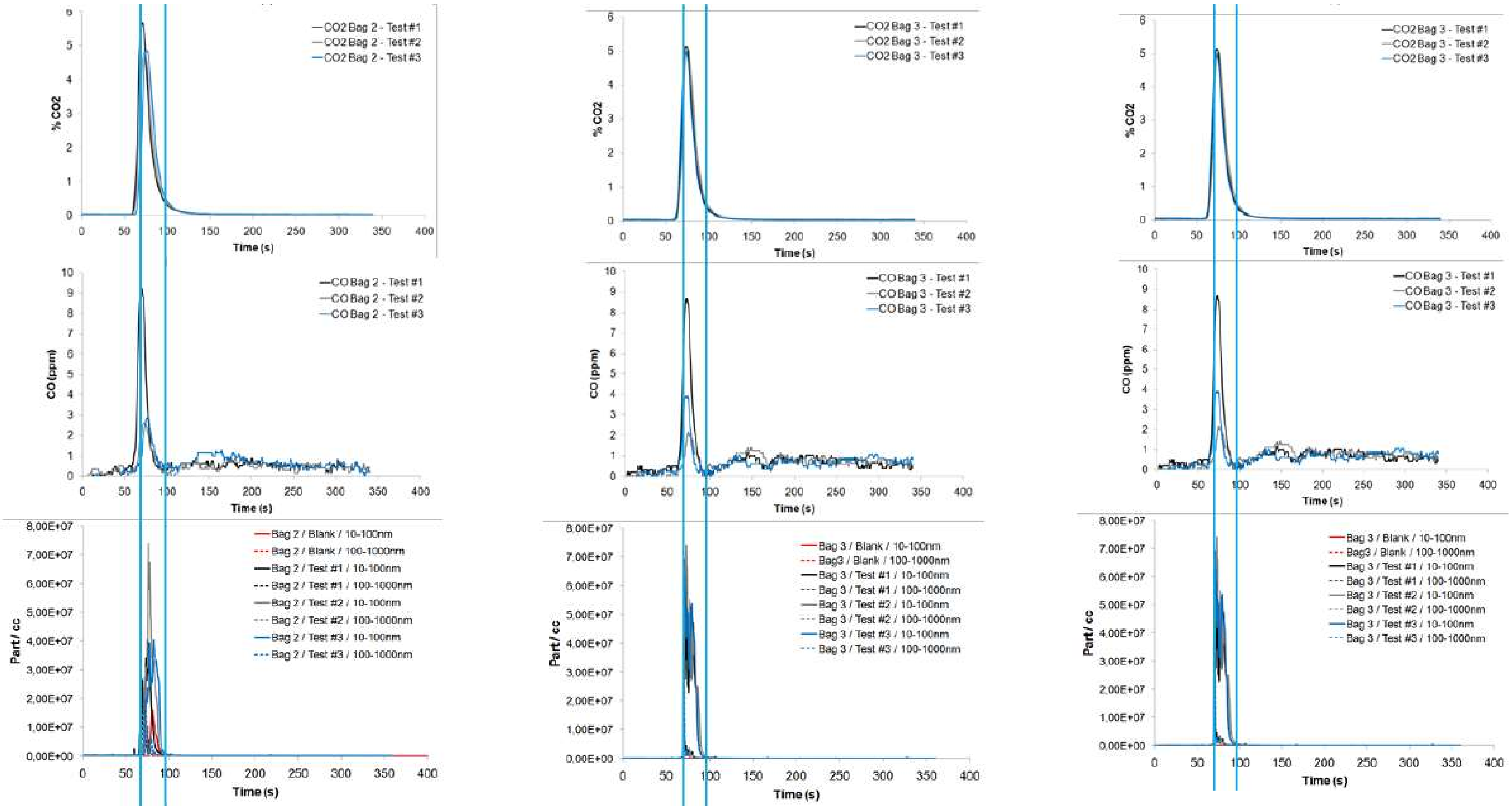


Figure 14. Temporal evolution of the different gases (CO2, CO) and of particle emission (particles/cm³) when incinerating pieces of bag 1 (left), 2 (middle), and 3 (right). Source: modified from caLIBRAte D6.4.

TEM grids samples analysis is summarized in Table 10. TiO2 particles were both in the aerosol and the residue. The observed TiO2 particles were not found to be nanometric in size neither in the aerosol nor in the residue. Only soot particles were found to be nanometric in the aerosol. These particles are generated in the course of the combustion. No agglomerates of nanoparticles were found in the residue. When incinerating pieces of bag 1, TiO2 was found to be transformed into a mix of Ti, Ca and O elements in various proportions in the residue. In that case, TiO2 is turned into another product made of the Ti, Ca and O elements. In the other cases (bag 2 & 3), titanium was found in TiO2 form in the residue. In the aerosol, titanium was always found in TiO2 form.

Table 10. Synthesis of the results obtained when analysing the particles in the aerosol and the residue using Transmission Electron Microscopy. Colours presented in the table represent the dominant chemical composition found in each bag. Source: caLIBRAte D6.4.

	Bag 1	Bag 2	Bag 3
Aerosol	TiO2 agglomerates Primary particles not nanometric	TiO2 agglomerates Primary particles not nanometric	TiO2 agglomerates Primary particles not nanometric
	Soot – carbon and silica These particles are nanometric	Soot – carbon and silica These particles are nanometric	Soot – carbon and silica These particles are nanometric
	Rod-shaped particles These particles are not nanometric Contain Na - made of NaOH most likely	Rod-shaped particles These particles are not nanometric Contain Na - made of NaOH most likely	Blot-shaped particles These particles are not nanometric Contain Na - made of NaOH most likely
	Bead-shaped particles Vast majority are not nanometric Contain Na, Zn, Si and P elements	Bead-shaped particles Vast majority are not nanometric Contain Na, Zn, Si and P elements	Bead-shaped particles Vast majority are not nanometric Contain Na, Zn, Si and P elements
Residue	Particles containing Ca Ti and O in various proportion – not nanometric	TiO2 agglomerates Primary particles not nanometric	TiO2 agglomerates Primary particles not nanometric
	CaO particles These particles are not nanometric	CaO particles These particles are not nanometric	CaO particles The Ca element is less present than in the other two cases (bag 1 & 2)

EC 4 Safe nano

- *Concise description of the case study*

  Within the EU EC4Safe Nano project, a case study was developed which describes the intended use of working gloves which have been treated with a nano coating. It is similarly suitable for the Gov4Nano project. With EC4SafeNano permission, this case study is introduced in the current deliverable by TNO.

  At one of its industrial sites, a company intends to use working gloves which have been treated with a nanocoating product. The nanocoating imparts an extremely strong and long-lasting layer on the gloves which protects the glove fabric against oil, moisture, bacteria and eventual abrasion (as claimed by the nanocoating manufacturer). However, it is unknown whether the on-site workers who wear these gloves during their 8h long work shift, are safe towards the exposure of nano-objects and their aggregates and agglomerates (NOAA) due to wear and tear of the nanocoating with time. The nanocoating used to coat the gloves contains an aqueous suspension of amorphous silica nanoparticles. The case study assesses any potential risk that these nanocoated gloves pose towards the workers.

  In such use settings, three principal routes of exposure are possible: inhalation, dermal and oral. All three exposure routes (inhalation, dermal and oral) are covered in this evaluation, which is divided in three sections: hazard in which the intrinsic toxic properties of the ingredients of the nanosilica coating are dealt, exposure in which exposure estimates are presented, and risk in which hazard and exposure are combined to assess the possible health risks of occupational exposure.

- *How it can be used to test the portal*

  Based on the critical elements constituting the case study elements, relevant nano-specific risk assessment tools from the portal can be selected to estimate the risk arising from inhalation, dermal and oral exposure routes of amorphous silica nanoparticles. The relevant aspects which can help to determine/short list the applicable tools (i.e. tools applicability) in the context of the present case study include the applicable life cycle stage to be "Use", applicable population to be "Worker" and applicable material to be "Solid reinforced/coated end products".

- *Any data / results that come with the case study*

  For a 48-week working year with 5 working days per week and 8h/day work shift in a room with volume 3x3x3 m^3 and an air exchange rate of 10/h, the case study investigation expected no adverse health effects for the workers from the use of nanosilica coated gloves based on a literature scientific evidence-based risk assessment (OECD, 2016; Reuzel et al. 1991; Stockman-Juvala et al. 2014). The evidence showed that the toxicity of synthetic amorphous silica nanoparticles via the dermal and oral routes is negligible and only adverse health risks via the inhalation route could be expected. However, to exhibit any adverse effect via inhalation route (for an OEL= 300 $\mu g/m^3$), i.e. to maintain the nanosilica concentration at the OEL, the case study evaluated required amount of released silica nanoparticles to be too substantial to get released from the gloves and far more than the total mass of nanosilica applied on the gloves could conceivably be (=155.5 g = 300 $\mu g/m^3$ x 8h/day x 10/h x 27 m^3 x 48 weeks x 5 days/week).

Toxicological case study

- *Concise description of the case study*

When testing the Nano Risk Governance Portal, it is useful to be able to challenge it in terms of pristine nanomaterials but also in terms of more elaborate materials, e.g. nanomaterials that are combinations of different elements. In Gov4Nano, we are refining a database on the pulmonary toxicity of 12 nanomaterials that was first established in the caLIBRAte project. Moreover, we obtained data in the caLIBRAte project that can be used to hazard assess nanomaterials that are modified by copper doping, and the influence of porosity. The case studies to test the Nano Risk Governance Portal encompass 1) a carbon nanotube case, 2) a case study with a combined material with copper-doped amorphous silica particle, and a silica particle that has porosity.

- *How it can be used to test the portal*

The caLIBRAte Database, and the research data from the same project; give us the opportunity to design case studies that can be used to test the Nano Risk Governance Portal. This is because we with the aforementioned data have the result of how toxic the materials are. Thus, the results of Nano Risk Governance Portal can be compare with the experimental data. One disclaimer of using the caLIBRAte Database is that it covers two endpoints only (lung inflammation and genotoxicity/carcinogenicity), and the inhalation route only. A total traditional hazard assessment of the situations would entail data from other sources than the caLIBRAte data, and in this case a more precise estimate of the hazard level would be provided. In the cases of combination materials, it is unlikely that there are many experimental data available, as modifications can come in almost infinite combinations of elements and ratios thereof. For carbon nanotubes there is a recent report from the National Research Centre for the Working Environment in Denmark that evaluated all endpoints and gave hazard levels (Poulsen et al., 2018). The values of this report is provided in the proposed end-results of the first case study below to support the findings in the caLIBRAte database.

It must be noted that the case studies are intended only for use in testing of the Gov4Nano Portal, and cannot be directly used in other hazard assessments, e.g. guidance at work places.

- *Any data / results that come with the case study*

1. Carbon nanotube development case study

A company has invented a new material that has unique properties in that it confers extraordinary strength per weight unit to such materials as bicycle frames or panels to be used in buildings airplanes or vehicles.

The material is a carbon-based tube (carbon nanotube) with a diameter of 75 nm and a typical length of 5 µm. At what air concentration does this material become toxic to humans? Is this a problem during normal handling?

Hazard data from the caLIBRAte Database to be compared with the results obtained with the Gov4Nano Portal
Cancer: seems to be the critical endpoint of the two endpoints given in the caLIBRAte Database (lung inflammation and carcinogenicity) How to derive a derived minimal effect level (DMEL) from a T25 value, as well as the use of safety factors to reach a derived no effect level (DNEL) form a no-observed-effect concentration (NOAEC) was based on the ECHA document "Guidance on information requirements and chemical safety assessment. Chapter R.8" (ECHA, 2012). The T25 value (the value at which 25% of experimental animals have tumours) is 0.011 mg/kg bw/day based on a study by Kasai and co-workers (Kasai et al., 2016). With a factor of 25.000 to reach a DMEL of 0.00044 µg/kg bw/day (1 extra case per 100.000) This is equal to 0.031 µg/person/day using a standard body weight of 70 kg (EFSA, 2012)

This translates to a very low air concentration, as the inhalation volume per workday is 10 m^3. With the rough assumption that all material in the air is deposited, this gives an air concentration of 0.0031 µg/m^3.

In addition, one can consult the aforementioned document from National Research Centre for the Working Environment (NFA) in Denmark, evaluating health effects of carbon nanotubes. NFA regarded cancer as the most critical adverse effect of CNT and gave, the expected excess lung cancer risk based on lung burden approach to be 1:1,000 at 0.03 µg/m3, 1:10,000 at 0.003 µg/m3 and 1:100,000 at 0.0003 µg/m3. (Poulsen et al., 2018)

Thus the value from the caLIBRAte database gave a 0.003 µg/m^3 compared to a value of 0.0003 µg/m^3 in the NFA report.

Thus, we find that the level at 1 extra case per 100.000 exposed is 0.0003 to 0.003 µg/m^3.

The results of the Gov4Nano Portal can now be compared to the above values.

2 Case study with a combination material and a porous silica particle

A company has invented a two new material that each has unique properties in that it confers extraordinary catalytic activity to an industrial process. The first material a) is an amorphous silica material with copper doped onto the surface. The material has a diameter of 100 nm and the content copper is 10% per weight. The second material is a silica particle of 100 nm that has substantial porosity.

At what air concentration do the materials become toxic to humans? Is this a problem during normal handling?

Hazard data from the caLIBRAte Database to be compared with the results obtained with the Gov4Nano Portal

a) Amorphous silica with copper doping:

There are no data on carcinogenicity of amorphous silica after inhalation in the caLIBRAte database (Muhle et al., 1995).

In one study in the caLIBRAte database, a 38 nm amorphous silica particle exerts pulmonary inflammation at a lowest-observed-adverse-effect concentration (LOAEC) of 24 mg/m^3 after inhalation (Chen et al., 2008).

With a safety factor of 10 to reach a NOAEC that is a NOAEC of 2.4 mg/m^3

Another study support this as there was no effect on pulmonary inflammation at highest dose of a 37 nm amorphous silica particle: 1.8 mg/m^3 (Sayes et a., 2010).

With an additional safety factor of 75 (ECHA, 2012) to reach a DNEL, the NOAEC of 2.4 mg/m^3 that gives a DNEL of 32 µg/m^3.

Turning to the copper part of the particle, there are no studies in the caLIBRAte database measuring carcinogenicity, but there is one study of pulmonary inflammation after a 16 nm particle (Adamcakova-Dodd et al., 2015).

This particle caused inflammation at a LOAEC of 3.5 mg/m^3 after inhalation.

With a safety factor of 10 to reach a NOAEC that is a NOAEC of 0.35 mg/m^3

With an additional safety factor of 75 (ECHA, 2012) to reach a DNEL that gives a DNEL of 5 µg/m^3.

Thus, silica provided a DNEL of 32 µg/m^3 and copper one of 5 µg/m^3. Copper only constitutes 10% of the combined material assessed in this case study. On the other hand, all the copper is doped onto the particle surface and thus accessible for dissolution to cause toxicity in the lungs. Based on this consideration, it could be suggested that the particle is hazard assessed based on its copper content, with a DNEL of 5 µg/m^3.

This is also supported by the experimental results from the caLIBRAte project investigating silica particles with copper doping investigated with intratracheal instillation into the lungs rather than inhalation. In this study, the toxicity of silica particles was increased by copper doping, as compared to a non-doped silica particle (Hadrup et al., 2021).

The results of the Gov4Nano Portal can now be compared to the above values.

b) Amorphous silica particle with substantial porosity

Above a DNEL for a non-porous (solid) silica particle was determined to be 32 µg/m^3.

In the caLIBRAte project with intratracheal instillation (Hadrup et al., 2021), we found that in comparison with a pristine amorphous silica nanoparticle, one with porosity was considerably more toxic in terms of pulmonary inflammation. A 100 nm solid silica nanoparticle had a NOAEL 128 µg/mouse (highest tested dose), while the porous counterpart had a NOAEL of 14 µg/mouse. Based on this we roughly estimate that in this endpoint the porous particle was 10 times more toxic than the solid one.

Thus based on this we estimate a 10 times lower DNEL of the porous silica nanoparticle to be 3 µg/m^3.

The results of the Gov4Nano Portal can now be compared to the above value.

Ecotoxicological case study

- *Concise description of the case study*

The estimation of the predicted-no-effect-concentration (PNEC) remains the foundation for the environmental risk characterization of nanomaterials. One approach to obtain the PNEC is to use Species sensitivity distributions (SSDs). This approach is feasible for substances with a sufficient amount of relevant and reliable ecotoxicity data available. The SSDs correlate the concentration of a stressor to the proportion of species being affected, based on a statistical or empirical distribution function fitted to single species toxicity data. While SSDs have been quite frequently applied in the risk assessment of various organic and inorganic substances, the SSD approach has only to a limited extent been used for ENMs. This field of application is however developing and the use of SSDs for ENM risk assessment is increasing, including models accommodating certain nano-specific properties, behavior and testing issues.

The caLIBRAte case study aimed to compare to examine how consideration of nano-specific characterization, test issues, data quality and endpoint variations affect the SSD outcome.

The case study provides:

- Two coherent high-quality datasets for nano-Ag and nano-TiO_2
- A comparison of three different tools to perform a SSD:
  - the nano-weighted n-SSWD model
  - the probabilistic PSSD+
  - the conventional SSD Generator by the US EPA.

Endpoints from freshwater ecotoxicity studies were collected for the representative nanomaterials NM-300 K (silver) and NM-105 (titanium dioxide), evaluated for regulatory reliability and scored according to the level of nano-specific characterization conducted. The two compiled datasets are unique in exclusively dealing with representative ENMs showing minimal batch-to-batch variation (Sørensen et al., 2020).

- *How it can be used to test the portal*

The datasets for NM-300 K and NM-105 were used as input to the nano-weighted n-SSWD model, the probabilistic PSSD+, and the conventional SSD Generator by the US EPA. This case study can therefore be used to test any tool for ecological risk assessment that is based on determining PNEC values for nanomaterials from ecotoxicological data using the SSD-approach. As the case study is evaluating ecotoxicological data used within the environmental hazard assessment of nanomaterials, the approach is independent from the life cycle stage the nanomaterial is used in, as it describes a general property of a material.

- *Any data / results that come with the case study*

An overview of the datasets for nano-Ag and nano-TiO_2 is shown in the table below (Table 11).

Table 11. Overview of the collected and grouped freshwater toxicity data for the representative nanomaterials NM-300K (silver) and NM-105 (titanium dioxide). The sub-datasets include: "All data", "Reliable data" including only data from studies evaluated as regulatory reliable according to the nanoCRED framework, and "NOECs only" including only experimentally derived NOEC values from long-term tests (excluding data transformed from acute and/or short-term data by use of extrapolation factors). Table taken from Sörensen et al. (2020).

	Ag (NM-300 K)			TiO_2 (NM-105)		
Number of:	All data	Reliable data	NOECs only	All data	Reliable data	NOECs only
Publications/studies[a]	31	27	12	26	16	3
Total entries[b]	40	36	15	55	31	3
Biological species	16	15	8	23	12	3
Taxonomic classes	8	8	6	8	5	3
Ecotoxicological endpoints	13	12	8	5	5	3
HONEC values	1	0	–	19	11	–
NOEC values	15	15	15	3	2	3
LOEC values	1	1	–	7	2	–
EC_{50} or LC_{50} values	20	17	–	11	6	–
EC_{10-13} values[c]	3	3	–	15	10	–
Most sensitive species	C. sphaericus	C. sphaericus	D. magna	S. obliquus	D. magna	–

-: data no available/relevant; HONEC: highest-observed-no-effect concentration.
[a] Studies in the OECD dossiers not published as scientific papers are referenced as studies rather than publications.
[b] There are more entries than publications/studies as some publications include more than one test and/or endpoint.
[c] Effect concentrations in the range 10 – 13%.

Results presented in the paper by Sørensen et al. (2020) include:

- Comparison of HC5 values (5th percentile of the SSD distribution) obtained by the three tools.
- Comparison of the full SSD-curves obtained for both datasets by the three different tools.
- Influence of data reliability of the generated HC5 values
- Influence of data transformation on the generated HC5.

All data is available in the caLIBRAte database at the eNanoMapper inventory, https://search.data.enanomapper.net/calibrate; Search term: DTU.

5 Evaluation and conclusions

Based on the results, the body of measurement results with regards to occupational exposure measurements as well as toxicological information with respect to nanomaterials has increased in the past years. Important efforts have been made among different projects and Gov4Nano partners to bring all gathered information during last years into one common template, and to make this available for all the (nano)safety community. This information is very valuable for individual performance testing / validation studies for available risk assessment models / tools which are becoming more and more available (thus increasing the need for validated tools). The data is of high importance as it contains information on release rates of different activities, which can be used as input for modelling. On the contrary, environmental fate data remains scarce and the current data available is limited in their usability for performance tests/ validation studies of environmental fate models and tools.

In addition, more detailed case studies were identified from either previous projects, derived from existing literature and databases or were obtained as a collaborative effort between NMBP 13 sister projects and Gov4Nano partners. The detailed descriptions of the case studies contained in this deliverable and the accompanying data can be used to test the NRGP over several life cycle stages, risk (both exposure and hazard) and occupational, consumer and environmental compartments. A car tyre case study is available which can serve as example of a public concern case due to potential release of nanomaterials during in-use wear of car rubber tyres and associated risks and impacts. This case can also be of use for the implementation of the Gov4Nano Safe by Design Strategy. On the other hand, a case study on exposure and release of TiO_2 during paint manufacturing, application and end-of-life can be used to test the NRGP concerning occupational (during production and application) and consumer (application) inhalation and dermal exposures, as well as environmental release to water (cleaning), land (leaching) and air (ventilation and incineration). Moreover, a study is available on occupational exposure linked to the use of gloves treated with a nanocoating which serves as an ideal case demonstrating the employability of the NRGP to estimate the occupational risks for the workers. In addition, this document contains several toxicological case studies related to pristine nanomaterials, but also more challenging cases including more elaborate materials such as carbon nanotubes and a case with a combined material with copper-doped amorphous silica particle, and a silica particle that has porosity.

The wide range of applicability of the case studies will lead to valuable insights on whether the NRGP and its tools and methods covers all these areas sufficiently or whether there are domains which require more improvement.

6 Data management

For exposure, data was entered in a pre-defined format with the use of Excel. This template was developed as a read-in template which can be imported in the eNanoMapper database. Therefore, while at the moment of writing only the excel templates are filled, the curated data will ultimately be imported within the eNanoMapper database.

For the car tyre case study, data generated by NANORIGO and we refer to NANORIGO and the Data Management plan for their work.

For toxicological studies, a previously existing format was used for further elaboration of the data library and additional literature data and exposure information added. Published toxicological results was used for derivation of the two case studies demonstrating how results in the data library could be used. The data library will be made accessible from www.nanosafer.org and as an excel file associated with scientific publication.

With regards to environmental fate data, no direct data management is relevant for this deliverable.

7 Literature

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8 Deviations from the work plan

Submission of the report is delayed from original planned submission on Month 40; April 2022. The main reason for the delay was the timing of the finalisation of the GRACIOUS exposure template and the time it took to convert the exposure data from the calibrate case study library to the new template as well as adding new literature studies to the template.

9 Performance of the partners

The partners contributed greatly in providing an update on new measurements available for specific expertise domains (exposure, hazard, phys-chem and the environment). In addition to this update, the partners NRCWE and EMPA contributed to the writing and data were provided by input from LEITAT and NIOH-ZA. TNO, EMPA and NRCWE all contributed specifically to the specific description of the individual case studies.

10 Annex

No annexes are included.