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The JRC Nanomaterials Repository

Safe handling of nanomaterials in the sub-sampling facility

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Title The JRC Nanomaterials Repository - Safe handling of nanomaterials in the sub-sampling facility

Abstract
The JRC Repository distributes representative nanomaterials worldwide, for the harmonisation of test methods and for a better comparability of scientific data, supporting research and regulatory projects.
This report describes the new sub-sampling facility with particular emphasis on safety aspects.

Contents

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Abstract

The JRC Nanomaterials Repository has been established to respond to an increasing demand for representative nanomaterials (NMs) for testing. The facility serves the scientific community active in nanotechnology, environmental-health-and-safety and regulatory research, by distributing subsamples of test nanomaterials.

The service provided by the JRC Nanomaterials Repository has underpinned the Testing Programme of the OECD Working Party on Manufactured Nanomaterials, as well as several EU-funded research projects. It contributes to the harmonisation of test methods and enhances the comparability of scientific results.

Recently, the JRC Nanomaterials Repository has extended its original range of operation by launching a novel sub-sampling facility. Due to the potential hazards of the handled NMs, this laboratory has been designed to ensure the highest safety levels for the operators and for the environment.

The present report describes the set-up of this novel facility, with emphasis on Occupational Health & Safety aspects. It illustrates the complete workflow that leads to the production of those vials that are distributed worldwide as benchmark nanomaterials.

1 Introduction

In the field of nanomaterials safety, one of the main issues that researchers and regulators have to address is the difficulty in comparing test results generated by different laboratories. Among the factors influencing the outcome of an experiment, the actual studied nanomaterial (NM) may be an obstacle to the understanding and the interpretation of test results, when comparing data from tests with different NMs of the same chemical composition. As a matter of fact, the general lack of thorough characterisation data, especially in earlier scientific literature, constitutes an important knowledge gap when accounting for the variability in the results. Small differences in the nanomaterial's physicochemical characteristics can have a relevant impact, for instance, on the functional or (eco)toxicological properties of the NM, thus influencing the outcome of a nanotoxicology study. Therefore, despite the large amount of published data and studies on nanomaterials, at present it is still a challenge to derive general conclusions on aspects such as the (eco)toxicity of NMs based on the evaluation and comparison of what can be found in literature data.

Furthermore, as stressed by H. Krug, the lack of both reference and control samples, as well as a substantial lack of harmonisation of the procedures used to generate the data, represent two of the main issues limiting the full use of nanotechnology and environmental health and safety (nanoEHS) studies [1] and, consequently, the full exploitation of nanotechnology benefits.

Another point under discussion concerns the appropriateness of using data, obtained by testing one nanoform of a substance, to infer the toxicity of another nanoform with the same chemical identity [2] [3]. The European Chemicals Agency's (ECHA) Nanomaterials Working Group (NMWG) works on creating a framework for the identification, grouping and read-across for nanomaterials [3]. Moreover, it is still debated within the nanoEHS community to which degree NMs with the same chemical identity, but from different sources (e.g. manufacturers, batches, lots, processes) can be considered exactly the "same" material, or just "similar" ones [4] .

In this context, the European Commission's Directorate General Joint Research Centre (JRC) has established the JRC Nanomaterials Repository to host industrially manufactured nanomaterials with the aim of providing the scientific and regulatory communities with NMs for safety testing. Over the years, the JRC Repository has evolved from a "*storage-and-shipping*" service to a complete and independent sampling facility that distributes representative test materials worldwide.

The scope of the present report is to describe, from a technical point of view, the set-up of this novel laboratory facility equipped with state-of-the-art instrumentation, with emphasis on Occupational Health & Safety (OH&S) aspects. The following chapters will present the general concept and history of the JRC Repository, as well as the safe design of the sub-sampling facility and its complete range of activities. Finally, the current list of the hosted nanomaterials and the importance of representative materials for the harmonisation of test methods will be illustrated, together with some general conclusions.

Note: Hereafter the term *Nanomaterial(s) (NM)* is preferentially used and considered as synonymous of *Manufactured Nanomaterial(s)* (MNM) and *Engineered Nanomaterial(s)* (ENM). The *JRC Nanomaterials Repository* may be referred to simply as the *JRC Repository,* or just the *Repository*.

2 The JRC Nanomaterials Repository

In 2006 the OECD Working Party on Manufactured Nanomaterials (WPMN) was established to provide a global forum for the promotion of international cooperation in human health and environmental safety related aspects of manufactured nanomaterials, in order to assist in the development of rigorous safety evaluation of NMs by exchanging information and sharing work on nanosafety, with particular attention to the regulatory context [5]. In 2007 the WPMN launched a Testing Programme to generate datasets for agreed lists of nanomaterials and endpoints. This information was used, via subsequent analysis of the methods applied and experimental experience gained, to propose nanomaterial-relevant updates of the existing OECD Test Guidelines for regulatory testing of chemicals and to develop Guidance Documents for testing and assessment [6].

In this framework, in 2009 the JRC created the JRC Nanomaterials Repository to host the first set of nanomaterials to be used within this Testing Programme, as benchmarks for research and regulatory testing methods development [7]. The NM samples originated from single batches of commercially available nanomaterials and were sub-sampled following standard operation procedures. The scope was to minimise a very important source of variability associated with the NMs tested [4], thus facilitating the comparability of the results obtained by different laboratories and across research projects. However, at that time, the sub-sampling operation was not part of the activities performed by the JRC Repository, the role of which was to store the acquired NM vials and to coordinate their distribution to the research partners. Over the years, the JRC Nanomaterials Repository has been involved in many national and international research projects, including those funded by the EU framework programmes for research, FP7 and H2020, the Life programme, as well as non-EU funded initiatives. The distributed NMs have been often tested using harmonised protocols (e.g. MARINA [8], NANOGENOTOX [9], NANoREG [10]).

Thus, the NMs of the JRC Repository have been extensively tested and characterised. Nowadays their widespread use is, *de facto*, converting them into global benchmark materials, not only for research and methods development, but also for regulatory (eco)toxicological testing.

Finally, in 2015, due to the increasing demand for representative test materials, the JRC Repository widened its range of activity by launching the new sub-sampling facility with the aim of in-house processing the purchased batches of NMs. The scope was to sub-sample the acquired large volumes of nanomaterials into representative small samples, under controlled conditions. Due to the potential hazards of some of the handled NMs, the laboratory was designed to ensure the highest occupational safety levels for the operators.

The following chapters describe in detail the design of this facility and the whole process for NM vials production and distribution.

3 Design of the sub-sampling facility

The scope of the sub-sampling facility is to reduce the large volumes of material, either purchased from industrial producers or sent by project partners, into small amounts, stored in small glass vials that can be easily handled by research laboratories. In practical terms, the facility splits tens of kilograms from a single batch of material into vials, whose content ranges from milligrams to grams, depending on the material density and the customer's requests. The whole process is performed in such a way that the final content is representative of the starting batch.

Since the incoming nanomaterial is a commercial product, it is received in its original packaging, which varies in:

- **Size:** ranging from tens of grams to tens of kilograms;
- **Type:** bags, sacks, bottles, drums;
- **Material:** plastic, paper, glass.

This aspect has had a major influence in shaping the entire operational workflow.

As a matter of fact, dealing with such a broad range of containers, has led to splitting the sub-sampling activity into two-steps. A **primary sampling** transfers the nanomaterials from their original containers into a selection of amber glass bottles (Thermo Fisher Scientific, Ulm, Germany) that are used as **intermediate containers**. In a **secondary sampling**, the bottles from the previous step feed an automatic dosing device, which produces the final vials. The whole process is completed by the third and last step: the management of orders and the **shipment** of the requested vials to the customers.

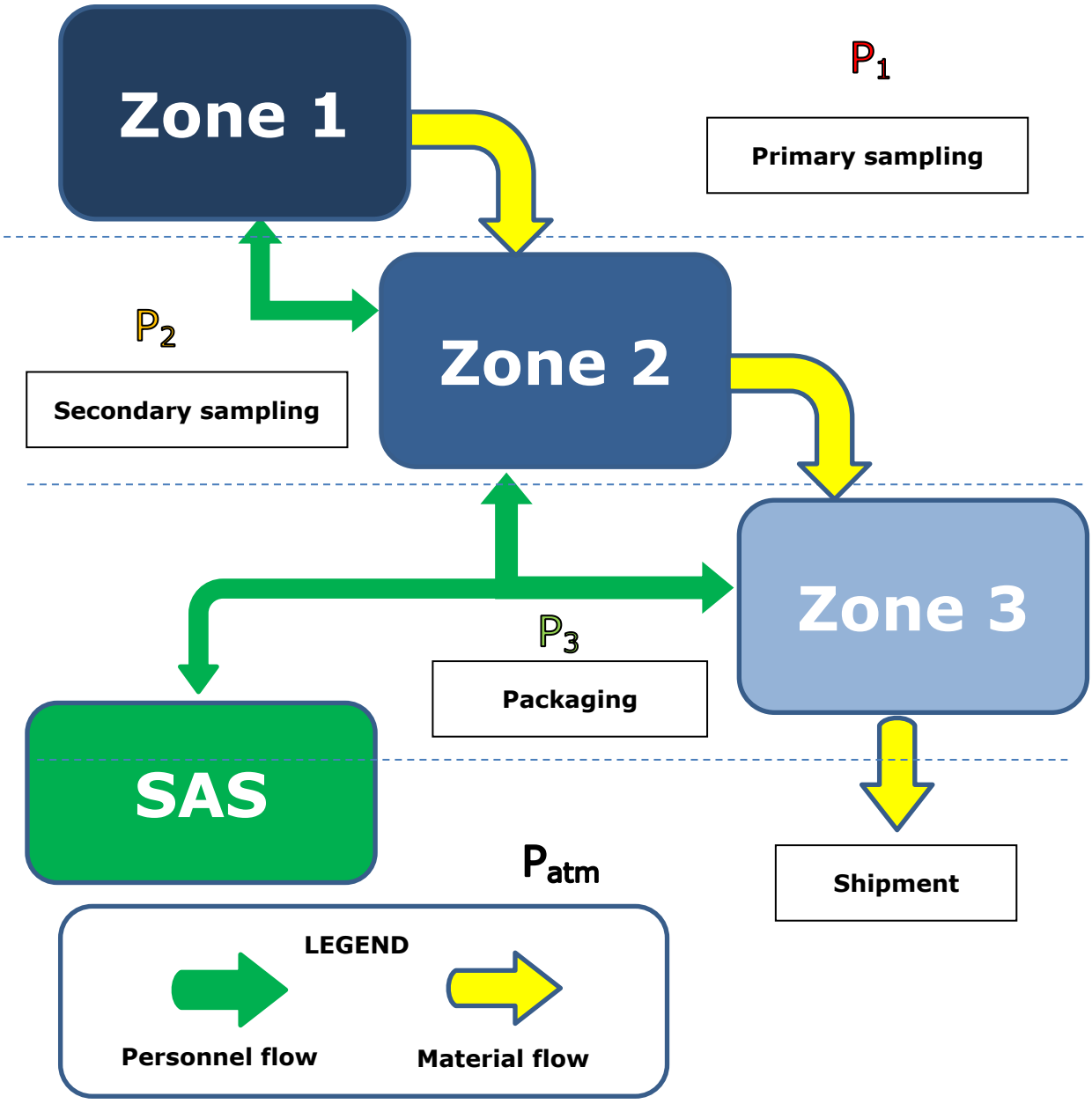
The operations that are performed are illustrated in Figure 1. The laboratory is physically subdivided into three **zones**, devoted to the three different activities. The separation among the three areas reflects the different levels of potential exposure associated to each operation. The higher the zone number, the lower the quantity of handled material and the level of potential exposure.

The different zones correspond to different sub-sampling and operational phases. Each zone corresponds to a specific room of the laboratory, physically separated from the others by closed doors. Each Zone features an air pressure level, whose transition is represented by the horizontal dotted lines. The access to the laboratory is possible via a **Safety Access System** (**SAS**), where the operators get ready to enter the work area by wearing or changing their personal protective equipment, as shown in Figure 1.

The following paragraphs present the general safety principles applied to the design of the facility and describe the ventilation system. Chapter 4 explains the features and structural set up of the JRC Nanomaterials Repository, detailing the material flow, the handling operations and the equipment used during these different operations, as follows:

1. From the material reception to the primary sampling: Zone 1(see §4.1)
2. From the intermediate containers to the secondary sampling: Zone 2 (see §4.2)
3. From the vials to their packaging and shipping: Zone 3 (see §4.3)

Figure 1: Schematic drawing of the JRC Nanomaterials Repository with the detail of the different zones, activities and pressure levels. The personnel and material flows are represented by the green and yellow arrows, respectively.



Air pressure levels in the facility: $P_1 < P_2 < P_3 < P_{atm}$

3.1 Safety principles and measures

When planning the set-up of the sub-sampling facility, no regulations or guidelines for the safe handling of nanoparticles as dry powder could be identified to support the design phase. Today, a few documents have been produced detailing occupational protection and safety of workers, specifically focusing on nanomaterials [11] [12] [13]. Amongst these publications it is worth mentioning the guidelines published in 2015 by the EU-funded project NanoValid [14]. Notably, what was recommended by the NanoValid group, had already been implemented for the set-up of the sub-sampling laboratory, thereby confirming the efficacy, effectiveness and correctness of the safety measures in place for the JRC Repository.

The guidance gap caused by the lack of a specific regulation to be followed has been filled with the implementation of some fundamental safety principles, usually employed in other fields, such as radiation protection. This has led to the creation of a facility, which could be rightfully considered as a model for the correct and safe handling of large volumes of nanomaterial powders.

The three safety principles are:

(a) Conservative safety approach;

(b) Reduction of exposure;

(c) Redundancy of protections.

Regarding the **conservative safety approach,** since all the hazards of the hosted materials are still unknown, it was decided to equally treat all of them as potentially harmful, without any exceptions. In other words, exactly the same procedures are followed, for instance, during the sub-sampling of silicon dioxide (SiO_2) NM as for the handling of single-walled carbon nanotubes (SWCNT).

From a risk management perspective, risk is the combination of *hazard* and *exposure*. Since *hazard* is an intrinsic property of the handled material, the **reduction of exposure** is the only possible solution for the minimization of the risk. As will be described in chapter 4, this goal has been achieved by a thorough planning of all the operations that are carried out in the facility and by a careful design of the work environment. In particular, during the normal operation the personnel is not exposed to the sampled NM substance because throughout the whole workflow there is no direct contact between the dry NM powder and the laboratory atmosphere. This is guaranteed by the use of several solutions, such as gloveboxes, glovebags, air-tight bags, vials etc.

However, the implementation of principles *(a)* and *(b)* alone is not enough to guarantee the safety of workers and environment as the system must ensure their safety at all times, even in case of an accident. The worst-case scenario shall always be considered. Only through the **redundancy of protections** it is possible to create a fail-safe system.

The translation of these principles into practical safety measures becomes evident considering the sets of mandatory Personal Protective Equipment (PPE) that are required in the three different zones.

Zone 1 PPE:

- Laboratory protective shoes;
- Disposable shoe covers;
- Disposable non-textile laboratory complete coverall (e.g. Tyvek®);
- One pair of nitrile gloves taped to the coverall;
- One extra pair of nitrile gloves to be worn inside the glovebox/glovebag (over the device ones), or when cleaning the intermediate container (see § 4.1);
- Full-face mask equipped with FFP3 filters (in alternative, hermetic goggles combined with FFP3 half mask).

Zone 2 PPE:

- Laboratory protective shoes;
- Disposable shoe covers;
- Disposable non-textile laboratory coat (e.g. Tyvek®);
- One pair of nitrile gloves;
- One extra pair of nitrile gloves to be worn inside the glovebox (see § 4.2);
- Safety goggles combined with FFP3 half mask, when handling NM outside the glovebox.

Zone 3 PPE:

- Laboratory protective shoes;
- Disposable shoe covers;
- Disposable non-textile laboratory coat (e.g. Tyvek®);
- One pair of nitrile gloves;
- Safety goggles combined with FFP3 half mask, before the packaging of the vials (see § 4.3).

Another fundamental point for the effective implementation of safety measures is the **awareness** of the personnel. The JRC Repository workers have received specific training on the risks related to the handling of nanomaterials, as well as a course on the techniques to correctly use, wear and dispose of their PPE, either during the normal activity or in case of contamination. The standard operating procedures in place and the safety data sheets of all the hosted NMs are available within the laboratory.

In case of an accidental release of NM powder, the personnel is trained to intervene to decontaminate the area with a dedicated set of cleaning instruments, that includes a vacuum cleaner equipped with H13 HEPA filters. The waste water and the other cleaning materials are collected once the decontamination procedure is completed and treated as hazardous chemical waste.

Figure 2: Background: the stainless-steel glovebox, where the secondary sampling takes place. Foreground: the control panel of the Quantos™ is on the left, while the Model 3007 portable CPC is visible behind the amber glass vials.

Finally, the level of particulate matter in the laboratory atmosphere is constantly monitored using a portable Condensation Particle Counter (CPC) (TSI Incorporated, Shoreview, USA), visible in Figure 2. Even if the values measured by the CPC may vary significantly over time, due to the influence of many environmental factors, such as atmospheric conditions, a CPC can be meaningfully employed, provided that its measurements are evaluated in relative terms. If the measurement is taken before, during and after a specific operation, it is possible to assess whether that activity has had a direct influence on the amount of indoor particulate matter. A significant increase in the CPC value gives a strong warning to the operators that an accidental release of nanoparticles may have occurred. In this way, they are induced to carefully check the integrity of the powder containing system (i.e. glovebox, glovebag, plastic bags, etc.) and to act accordingly.

3.2 The ventilation system

In case of an accidental release of NM powder inside the JRC Repository, the inhalation of airborne nanoparticles is the foreseeable route of exposure for laboratory operators. Therefore great care has been taken in designing the Repository ventilation system. It allows the workers to operate in a safe environment, while preventing the pollution of the surrounding premises. In particular, in case of an accidental release of NMs, the spontaneous flow of the contamination towards the "cleanest" zone of the laboratory (i.e. towards the entrance) or, even worse, towards the outside shall be prevented at all times.

In order to achieve this goal, a low-pressure air gradient has been set up in the three zones of the Repository. Among the three areas, the air pressure has its lowest value, P_1 in Zone 1. It increases to an intermediate value (P_2) in Zone 2 and finally reaches the highest value (the one closest to the atmospheric pressure P_{atm}) in Zone 3. Each step of the pressure gradient equals few tens of pascals. The pressure values are reversely proportional to the amount of NMs handled in the rooms. The lowest pressure is set where the largest amount of NMs is handled (Zone 1), whereas P increases towards P_{atm} where smaller amounts of NMs are handled (Zone 2 and Zone 3). In this way, in case of an accidental spill of nanomaterial, the released powder is conveyed towards Zone 1 (where the risk is normally considered to be the highest, and the use of the complete set of personal protective equipment is mandatory for the personnel), instead of flowing towards the SAS, where the contamination could escape and spread outside the facility.

Clearly, in order to avoid any emission of airborne nanoparticles in the outside environment, the air extraction system is equipped with HEPA filters at the exhaust, whose efficiency is constantly monitored. The personnel and the JRC Site Response and Support Team are immediately warned about any malfunctioning of the ventilation system by means of optical and acoustic alarms installed both locally and remotely.

Finally, in terms of dosimetry for the workers, the level of airborne particulate is continuously monitored before, during and after each operation by means of the portable CPC instrument (as explained in § 3.1). In case of accident the operators can assess whether there has been a significant release of nanoparticles in the atmosphere and they can opt to leave the facility, following the safety procedures in place. They, thus, have the opportunity to call for assistance and to wait until either the particles have deposited onto the ground or the ventilation system has cleaned the indoor atmosphere by filtration, while introducing fresh air from the outside. Since the contamination is kept confined inside the laboratory area, the cleaning procedure can be delayed until the air quality conditions do not pose a threat to the workers, anymore.

4 The nanomaterials workflow

The workflow of the JRC Repository nanomaterials begins with the reception of a material batch and ends with the shipment of the sampled vials of NM to the customers.

Whenever a new batch of material is acquired, it is duly registered and labelled. Depending on the nature and size of its original container it is inserted in a plastic bag of convenient dimensions and sealed with a heat-sealer or with tie-wraps and duct tape. The bag is then stored into the ventilated cupboards of Zone 1, which are dedicated to host incoming nanomaterials in their original containers.

4.1 From the material reception to the primary sampling: Zone 1

As briefly described in chapter 3, the primary sampling has the purpose to transfer and divide the content of the incoming NM batch into intermediate containers.

Whenever a sampling of a new nanomaterial is needed, e.g. because the JRC Repository stock is depleted or because a new NM has to be added to the list of the offered materials [15], a primary container is picked up from the cupboards of Zone 1, with the scope of transferring its content into intermediate containers of 3 possible sizes:

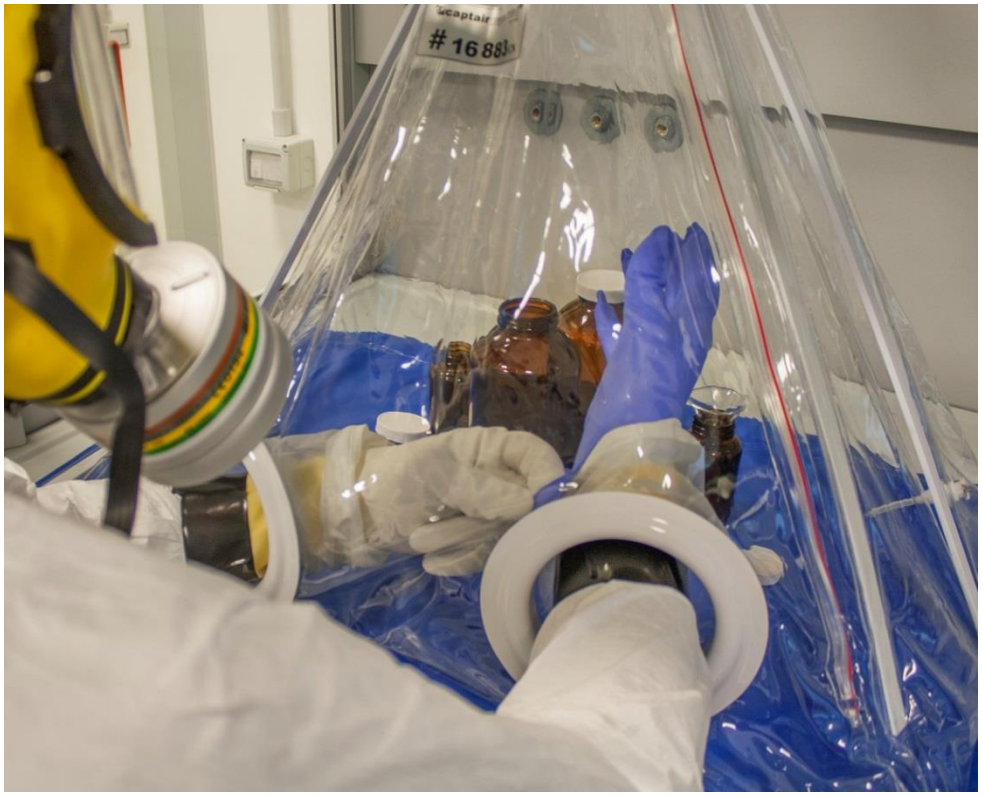
— 120ml bottles;

— 500ml bottles;

— 1000ml bottles.

The 120 ml bottles are those eventually mounted onto the Quantos™ system dispensing heads (see description in § 4.2) and used as material reservoirs during the secondary sampling phase. The larger bottles (500ml or 1000ml) need a further primary sampling step to be subsequently split into 120ml ones, as shown in Figure 3.

Figure 3: Preparation of the Pyramid™ portable glovebag for the primary sampling of nanoparticles from 500ml intermediate bottles to 120ml ones.



Depending on the size of the original container, two different devices can be employed for the primary sampling:

— The Pyramid™ portable glovebag by Erlab DFS S.a.s. (Val de Reuil, France), safely mounted into a chemical hood, can be used for small containers (e.g. bottles or small bags) (see Figure 3)

— The ProClean™ Expendable-Powder-Sampling (EPS) system by Hecht Technologie GmbH (Pfaffenhofen, Germany) is used for large containers (e.g. paper bags, drums, etc.) (see Figure 4)

Figure 4: Preparation of the ProClean™ disposable glovebag for the primary sampling of a plastic drum containing nanoparticles.

Identical operations are performed during the primary sampling process, regardless of the type of glovebag (GB) used.

First, the GB chamber is prepared with all the necessary materials and instruments, since nothing else can be introduced in the GB, once it has been sealed and the sampling has begun. The intermediate containers are individually inspected and weighted before being introduced in the GB.

Then, when everything is set and the GB is sealed, the chamber is filled with argon (Ar) gas in order to make the sampling environment more inert, for a better future storage of the sub-sampled NM. The GB is now ready and the primary sampling operations may start.

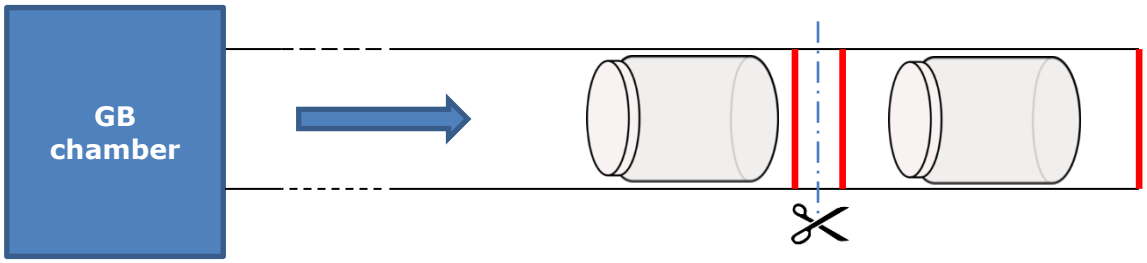
The first operation, performed after the opening of the original container, is the **homogenisation** of its content. This operation is critical to ensure that the nanomaterial powder in each intermediate container is representative of the original batch. This operation is performed with great care. The operator mixes and stirs the material in order to counteract any possible sedimentation effects (e.g. size selective stratification) of the NM. Given the heterogeneous range of original containers to be treated, this step has to be performed manually by the operators.

Then, the transfer of the NM into the intermediate containers takes place. The operator fills the amber glass bottles by means of glass funnels and spatulas. The bottles are closed and externally pre-cleaned with a wet cloth, before being introduced in a polyethylene (PE) tube that is attached to the GB chamber (visible in Figure 4). The PE tube is heat-sealed and cut in bags as shown in Figure 5. The intermediate containers, protected by the plastic bags, are then introduced in a second plastic bag for safety redundancy. They are labelled and stored in the ventilated cupboard of Zone 1.

When needed, they are cleaned under a chemical hood. This operation is carried out by cutting a small portion of the plastic bag and by spraying water and cleanser inside the bag. When all the free dry powder inside the bag has been wetted, the bag is completely opened and the wet bottle is carefully extracted. Its cap and surface are cleaned with water and dried with paper tissues. The cleaned bottle is finally weighted and relabelled with the material code, the date of sampling and the net weight, before being transferred to Zone 2, where it is stored in a dedicated safety cupboard. During this operation a two digits incremental and unique code is assigned to each 120ml bottle. It will be subsequently used as part of the vial coding system, to identify all those vials produced from that specific intermediate container (see § 4.3).

At the end of the primary sampling the GB is disposed of, together with its content, as laboratory hazardous waste. In order to ease its disposal, it is deflated by connecting its gas line connector to a filtered (liquid trap + HEPA filter) inlet port of an air pump, whose exhaust goes to the air extraction system of the facility.

Figure 5: The transfer of the intermediate container from the GB chamber into the polyethylene tube. The red lines represent the seals obtained by a heat sealer. The tube is cut (illustrated by the blue dashed line) between the two seals (red), forming a protective bag around the bottle.



4.2 From the intermediate containers to the secondary sampling: Zone 2

The purpose of the secondary sampling is the production of the final NM samples stored in vials. The automated powder and liquid dosing system Quantos™ by Mettler Toledo (Greifensee, Switzerland) sits at the core of this operation. The system is able to automatically fill 30 vials per run with a maximum measurement resolution as low as 0.1 mg (the weighing capacities are those of the Mettler Toledo XP 504 balance).

The Quantos™ system is installed inside a 4-handed stainless steel glovebox by Iteco Engineering (Castel Bolognese, Italy), fitted with H13 class HEPA filters and a nanoparticle water trap. As shown in Figure 6, the Quantos™ requires a 120ml intermediate bottle as powder reservoir for the dosing head. The dosing head consists of a mechanical dispensing device equipped with a RFID chip that stores important data about the NM, such as the ID code, the name of the substance, the filling date of the head and the remaining quantity of material. Each NM is always associated with a specific dosing head. The combination head+container thus remains the same and avoids any cross-contamination of NMs between different sampling campaigns, together with the scheduled decontamination of the work environment. The heads are stored in hermetically closed containers in ventilated cupboards in Zone 2 for the time between each sampling run. During sub-sampling, the relevant head is identified and introduced into the stainless steel glovebox and mounted on the Quantos™ system. An important

feature offered by this solution is the possibility to choose the type of head from a range of different models, in order to better match its dispensing properties with the powder characteristics (e.g. level of dustiness, compacting tendency, powder flowability, hydrophobicity, etc.).

Figure 6: Preparation of the Quantos™ device for the production of JRC Nanomaterials Repository vials. The 120ml intermediate bottle is connected to the interchangeable dosing head to fill the 30 vials of the auto-sampler.

Once the Quantos™ device is ready for dosing, and the glovebox atmosphere has been filled and purged 5 times with the injection of argon gas, the secondary sampling is started via the machine software installed on the computer connected to the sampling station. For each 30 vials run, the job data files give the possibility to individually control the vial ID, the content tolerance and the filling amount. During the dispensing phase the system transfers the weighing data to the controlling computer and to the label printer, installed outside the glovebox. This information is fed to the electronic inventory system that accounts for the JRC Repository vial stocks.

At the end of the dispensing phase, the operator manually closes the 30 pre-cleaned certified 40ml vials (Thermo Fisher Scientific, Ulm, Germany) while a steady non-turbulent stream of argon gas fills the glovebox atmosphere. The sealed vials are arranged in a numbered rack and are individually cleaned with sprayed water and paper tissue. Finally, the cleaned rack can exit the glovebox and the vials are labelled (see Figure 7) before being stored in the ventilated cupboards located inside the Zone 2. The numbering of the rack is important for identifying the vials before their labelling, thus avoiding any accidental vial substitution and mismatch.

Considering the average dispensing time, the maximum number of vials that can be produced during the secondary sampling is about 600 per week.

A further development that could be implemented in the future is the possibility to dispense liquid suspensions of NMs, thanks to the system flexibility. At the moment the only nanomaterial that is offered in suspension, is the JRCNM03300a gold (see also Table 1 in chapter 5). For comparison purposes the JRC Repository hosts the dispersant of the gold nanoparticles, JRCPD03301a, as well. However, JRCNM03300a and JRCPD03301a have not been sub-sampled yet, because they are still available on stock as 20 ml vials, which were acquired as such by the JRC Repository, before the establishment of the sub-sampling facility.

Figure 7: Close-up of four amber glass vials with their content labels. The linear barcode encodes the Sample ID value, which identifies each vial in the inventory database. The barcode is used during the order preparation in Zone 3.

4.3 From the vials to their packaging and shipping: Zone 3

Zone 3 is dedicated to packaging and shipment preparation. These activities are part of the order management, for which the administrative part is performed outside the facility. Thanks to the use of data sharing and automation these tasks are easily and efficiently coordinated.

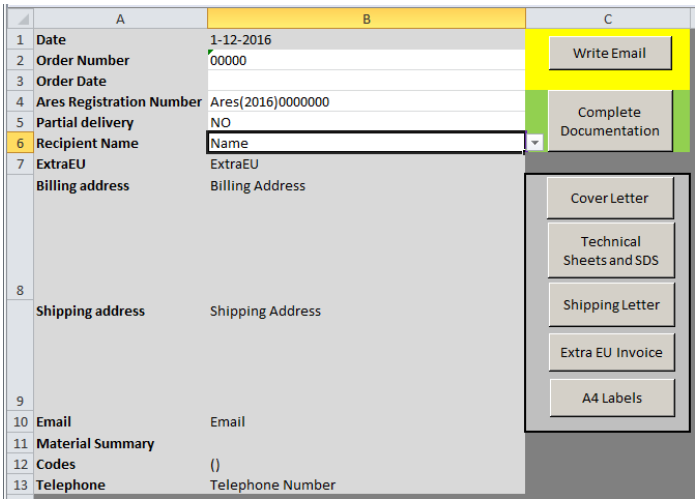
Any order request is linked to an email received in the JRC Nanomaterials Repository functional mailbox:

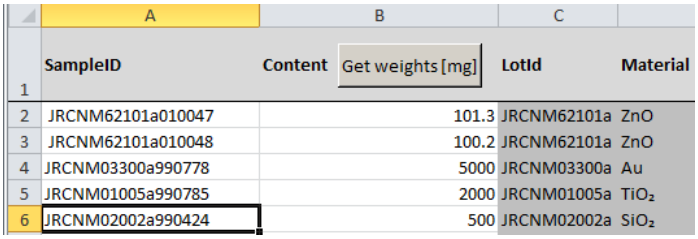
JRC-NANOMATERIALS-REPOSITORY@ec.europa.eu

Every order is evaluated with the aim of verifying the requested amounts of NMs, the planned testing activities and the stock availability of those NMs. After its approval, the order is registered in ARES, the European Commission official document recording system. It is then executed by the JRC Nanomaterials Repository Support Team. When needed, a new sub-sampling is scheduled in collaboration with the operators and the customer is informed about the planned delivery date. When the vials are available, the operator prepares them together with the documentation for the shipment. For this purpose, an Excel® template file has been programmed with Visual Basic for Applications (VBA®) *macros* to automatically process the orders (see Figure 8). The first visible worksheet contains the customer data and the request details. The white cells are the only ones to be manually filled in, while the customer field is a dropdown list linked to an address book. The grey cells are the results of text formulas. The second sheet consists of the list of the selected vials, which is populated by scanning their label barcodes and by automatically importing the weights from the material inventory. By doing so, the system also marks the selected vials as no more available for distribution and it records the Ares code of the order for each vial entry. Moreover, on the first sheet a set of buttons, underpinned by *macros*, automatically generates the order documentation:

- The cover letter;
- The technical sheets;
- The material safety data sheets (MSDS);
- The shipping letter for the logistic;
- The *pro-forma* invoice for customers outside the European Union;

Figure 8: Two screenshots of the Excel® workbook for the management of incoming orders.





This automation relies on the way the vial ID has been designed. As a matter of fact, the code contains information about both the nanomaterial and the sub-sampling process. To easily understand the ID structure, one can consider the following example:

JRCNM62001a030052

This vial code means that:

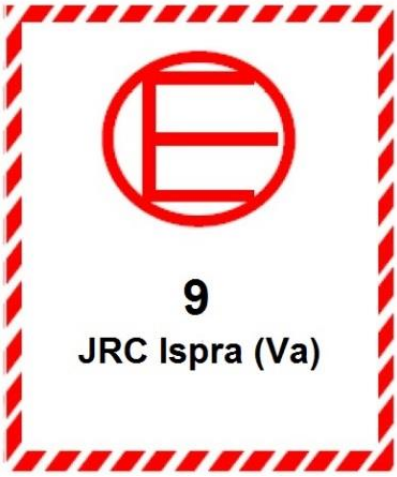
- It is a nanomaterial distributed by the JRC :**JRCNM**
- It contains titanium dioxide (TiO_2):**62001**
- Its content originates from the first acquired batch of material: **a**
- It has been sampled from the 120ml intermediate bottle number 3: **03**
- It is the 52nd vial produced from that container: **0052**

The shipment preparation is performed in Zone 3, where only small amounts of NMs in sealed vials are handled. The vials are individually covered with a protective plastic tubular net (as shown in Figure 9) and introduced in bubble wrap bags, which are closed inside grip-seal plastic bags. The materials, together with the documentation, are shipped in foam-filled boxes, in order to prevent any damage and accidental release of NMs during the transport. The packaging operation takes place in the chemical hood installed in Zone 3.

Moreover, in compliance with the UNECE ADR European Agreement [16], for those materials that are classified as hazardous goods, the JRC Nanomaterials Repository has performed an internal certification of the shipment containers, as required by chapter 3.5 of the ADR European Agreement to assess and certify their degree of protection. The selected boxes have been tested with the ADR protocol and an internal report has been issued with the results. At present the only classified Repository NM is zinc oxide (ZnO).The packages containing ZnO vials are labelled according to ADR prescription, as shown in Figure 10.

Figure 9: Labelled vials of nano-silica ready for the final packaging and shipment. The one on the right has the protective plastic tubular net already on.

Figure 10: One of the employed shock-absorbent boxes that have been certified for transports according to the UNECE ADR European Agreement. On the right, the ADR label for excepted quantities (ADR 3.5) as it is applied to all the parcels containing ZnO vials.



5 The nanomaterials in the JRC Repository

As explained before, the JRC Repository hosts a selection of nanomaterials that are either commercially available or synthesised *ad hoc* for a specific research project. In the first case the starting NM batches are usually purchased by the JRC. In the second case, the NMs are provided by research partners in the framework of EU-funded project consortia.

In general terms, the hosted NM are meant to be 'representative' of a relevant part of the NMs global market, as their commercial application covers a wide range of fields, such as cosmetics, pigments, food additives, photo-catalytic materials, chemical polishing and fuel additives. The sampled vials are eventually distributed free of charge to projects partners or to any customer whose work is of strategic relevance for nanoEHS research. In this case, the requested amount shall be clearly justified, for instance on the basis of the customer's test planning and investigation techniques.

Over the years, the JRC Repository has shipped around 10.000 vials to about 100 different organisations worldwide. Most of the NMs have been tested in the OECD WPMN Testing Programme and in several EU-funded projects, such as ENPRA, MARINA, NANOGENOTOX and NANoREG. Moreover, with the supply of test materials, the JRC Repository has supported several national programmes, university activities, as well as industry-led initiatives.

The JRC Nanomaterials Repository expects from the customers to be informed about any kind of publication that is produced using the distributed NMs and to be acknowledged in the publication text as the NMs supplier, indicating, when appropriate, the vial codes.

Through this wide distribution of NMs, the JRC has contributed to their thorough physicochemical characterisation. The main results have been included in five JRC Scientific Reports [17] - [21]. Moreover, the testing activities performed under the umbrella of the OECD Testing Programme have enabled the publication of OECD dossiers available online [6]. The scientific community has employed the same NMs to thoroughly assess several (eco)toxicological endpoints (e.g. [22] - [32]).

The current list of NMs available at the JRC Repository is presented in Table 1. Updates of the list and detailed information about the NM physicochemical characteristics can be found at the JRC Nanomaterials Repository webpage [15] (Figure 11).

Figure 11: Screenshot of the JRC Nanomaterials Repository webpage and the URL QR code.

The stock includes vials from the batches that were used for the OECD WPMN and are still available for distribution (the starred entries in the table). When some of these batches got depleted, they were replaced by new ones from the same producers. These new batches, which have received new JRCNM codes, have been basically characterised by means of techniques such as electronic microscopy, centrifugal liquid sedimentation and x-ray diffractometry. The main purpose of this investigation is to verify and confirm the technical specifications provided by the producers. In some cases, customers themselves have collaborated to verify and complete the characterisation data, by sharing the results of their physicochemical analysis.

After the launch of the sub-sampling-facility, 17 new materials have been made available and the facility has reached the peak production of about 600 vials per week.

The NMs currently hosted belong to 7 different 'chemistry classes':

- Titanium dioxide (TiO_2);
- Silicon dioxide (SiO_2);
- Cerium dioxide (CeO_2);
- Zinc oxide (ZnO);
- Gold suspension (Au);
- Nanoclay (Bentonite);
- Carbon-based materials (multi-walled carbon nanotubes MWCNT, single-walled carbon nanotubes SWCNT, and graphene).

For each chemistry class, there are several types of NMs, which differ by one or more of their physicochemical properties. These differences generally refer to properties such as particle size, surface functionalization (i.e. coating) or type of crystalline phase. For some of the classes, a 'non-nano' sized material (according to the EC Recommendation on the definition of nanomaterial, EC 2011/696/EU [33]) is also available, thus allowing the comparison with the nanoform of the same material in order to infer the effects of the nano-size factor for a given endpoint. For example, for TiO_2 7 different codes are available in the Repository, with sizes ranging from 5 to 115 nm [15] [21]. Still for TiO_2, different crystalline phases are also available: pure anatase, pure rutile, or a mix of both.

As mentioned in section 4.2, the sub-sampling facility has not yet been used to produce NM suspensions. Since it is fully equipped to properly handle suspensions, this future development is envisaged.

Table 1: List of the representative NMs hosted in the JRC Nanomaterials Repository. A more detailed and updated list is available at: http://europa.eu/!CR83vD

JRC ID	Substance
JRCNM01001a *	Titanium Dioxide
JRCNM01005a *	
JRCNM10200a	
JRCNM10202a	
JRCNM62001a	
JRCNM62002a	
JRCNM02101a *	Cerium Dioxide
JRCNM01101a *	Zinc Oxide
JRCNM62101a	
JRCNM06000a *	Nanoclay
JRCNM03300a	Gold dispersion
JRCPD03301a	Gold dispersant
JRCNM02000a *	Silicon Dioxide
JRCNM02001a *	
JRCNM02002a *	
JRCNM02004a *	
JRCNM10404a	

JRC ID	Substance
JRCNM04000a *	MWCNT
JRCNM04001a *	
JRCNM04002a *	
JRCNM04003a *	
JRCNM40001a	
JRCNM40002a	
JRCNM40003a	
JRCNM40004a	
JRCNM40005a	
JRCNM40006a	
JRCNM40007a	
JRCNM40008a	
JRCNM40009a	
JRCNM40010a	
JRCNM46000a	SWCNT
JRCNM48001a	Graphene

(*) NMs that have been used in the OECD WPMN Testing Programme

Source: [15]

6 The importance of representative test materials and the impact of the JRC Nanomaterials Repository

Regarding the general lack of harmonisation mentioned in chapter 1, in 2014 Krug [1] underlined that the unification of criteria and standardisation of methods are absolutely necessary in order to arrive at a situation where investigations may effectively be compared and provide reliable data.

As a matter of fact, it has been demonstrated that procedures, such as sonication, when employed to create a liquid-based dispersion of nanoparticles, may influence the outcome of toxicity studies. Therefore, the harmonisation of the test methods would enhance the consistency of the scientific data. Unfortunately the tendency of many laboratories to adhere to in-house developed protocols, instead of applying harmonised test procedures is a limiting factor for inter-laboratory comparability of the results and for the extrapolation of general conclusions for regulatory purposes [34]. For this reason, in recent years, several projects have attempted to maximize the harmonisation of procedures, such as the dispersion protocols, thus paving the way to the subsequent data comparability.

The OECD, with the Test Guidelines Programme has worked on the same topic by starting a long process of evaluation of the available regulatory test guidelines (TGs). The scope was to understand to which extent these TGs, developed for the regulatory testing of chemicals in general, would be applicable to NMs.

In this framework, as stressed by Krug, the availability of reference and control sample is a *condicio sine qua non* for the correct evaluation of test methods. Due to the considerable amount of resources and time needed to produce reference materials (RMs) and certified RMs (CRMs), their availability today is still limited to a few nano-sized (C)RMs. To overcome this shortage, an alternative to RMs is represented by representative test materials (RTMs). As a matter of fact, RTMs can actually act as benchmark materials to enable pre-normative work and to develop new or updated test methods, thus promoting faster innovation.

According to Roebben at al.'s [35] definition, a "Representative Test Material (RTM) is a material from a single batch, which is sufficiently homogeneous and stable with respect to one or more specified properties, and which implicitly is assumed to be fit for its intended use in the development of test methods which target properties other than the properties for which homogeneity and stability have been demonstrated"

The selection and the sampling methodology of the NMs distributed by the JRC Repository has been conceived so that the final NM vial complies with this definition of RTM. As a matter of fact, since each set of sub-sampled RTM originates from the same starting batch, all the subsamples can be rightfully assumed to be identical.

The above mentioned OECD Test Guidelines Programme has employed the NMs distributed by the JRC Repository to carry out the experimental work that has resulted in the initiation of nano-specific adaptation of some existing OECD TGs (e.g. the Test Guidelines for Inhalation Toxicity), in the development of new specific guidelines and in the identification of a number of new environmentally relevant end-points.

Thus, through the distribution of its representative nanomaterials, the JRC Repository keeps contributing to the method harmonisation and to the creation and consolidation of knowledge in the nanoEHS field by constituting a common and unique NM source for all the stakeholders.

7 Conclusions

In order to respond to the global demand for NMs for testing, the European Commission's Joint Research Centre has established a repository for representative test nanomaterials. It is a unique facility that serves the scientific community active in the nanoEHS and regulatory research. The distributed nanomaterials represent a relevant part of the global NM market typology and, given the scarcity of (certified) reference nanomaterials, their use as RTM is a prerequisite of paramount importance for the generation of comparable and reliable experimental results and datasets in support to regulatory research.

Over the years, due to the increasing demand for NMs from the scientific community, the JRC Repository has been a key test NM supplier in many EU-funded projects, national programmes, university activities, as well as industry-led initiatives. The JRC had to adapt the structure of the laboratory, upgrading it from its original 'storage-and-shipment' configuration to the present 'standalone' sub-sampling facility. Full operation was reached in 2015. The selling points of the present-day JRC Repository are manifold:

— Full control over the operational parameters of the sub-sampling,

— Possibility to replace the depleted stocks of materials with new batches,

— Possibility to customise the sample sizes (NM amount in a single vial set with high accuracy) to better meet customer needs,

— Broad and expanding range of offered nanomaterials, up to the distribution of next generation NMs.

The JRC has developed internally the new laboratory from the very design stage to the operational phase by:

— Selecting the most suitable devices, capable of processing nanomaterial quantities ranging from few grams to tens of kilograms,

— Designing a safe work environment and obtaining the local safety authorisations, needed to start the activity,

— Designing and implementing standard operation procedures, allowing the safe handling of nanomaterials from their reception to their final shipment.

One of the main innovations of the facility consists in the set of health-and-safety measures that have been put in place, in order to allow the handling of significant volumes of potentially hazardous nanomaterials powders. The conservative safety approach has been adopted, without any discrimination on the type of NM actually processed.

This JRC technical report provides information on considerations and actions undertaken by the JRC to guarantee the safety of the operators and environmental protection, during each phase of NM handling. It also describes the automation of the subsampling procedure, which offers an unrivalled degree of flexibility and precision in the preparation of NM vials for customers. Moreover, the efficiency and effectiveness of the Repository, as well as the traceability of materials, have been increased by streamlining the sample distribution process with the use of unique vial IDs and barcodes, together with an in-house developed system for the management of stocks and orders.

The positive results of the Repository have been confirmed by many acknowledgements received from the customers, such as the OECD Environment Health and Safety Division. In conclusion, since the further development and use of the RTMs could eventually lead to the generation of additional high-quality scientific results that can help to consolidate the nanoEHS-related knowledge, with the ultimate and very important purposes of supporting regulation and promoting safe and sustainable nanotechnology innovation, the JRC Nanomaterials Repository will aim at strengthening its role as a global distributor of representative tests materials.

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List of abbreviations and definitions

ADR	the European Agreement concerning the International Carriage of Dangerous Goods by Road (from the French "***A**ccord européen relatif au transport international des marchandises **D**angereuses par **R**oute*")
CPC	Condensation Particle Counter
CRM	Certified Reference Material
ECHA	European Chemicals Agency
ENM	Engineered NanoMaterial
EU	European Union
GB	Glovebag or Glovebox
HEPA	High Efficiency Particulate Air filter
JRC	European Commission's Directorate General Joint Research Centre
MSDS	Material Safety Data Sheet
MWCNT	Multi-Walled Carbon Nanotube
nanoEHS	nanotechnology Environmental-Health-and-Safety
NM	NanoMaterial
OECD	Organisation for Economic Co-operation and Development
OHS	Occupational Health and Safety
PE	Polyethylene
RM	Reference Material
RTM	Representative Test Materials
SAS	Safety Access System
SOP	Standard Operating Procedure
SWCNT	Single-Walled Carbon Nanotube
TG	Testing Guideline
UNECE	United Nations Economic Commission for Europe
VBA	Visual Basic for Applications
WPMN	Working Party on Manufactured Nanomaterials

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