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1. Executive Summary

NanoPack will demonstrate a solution for extending food shelf life by using novel smart antimicrobial surfaces, applied in active food packaging products. It will run pilot lines in operational industrial environments to manufacture commercially feasible antimicrobial polymer films. The project will employ natural halloysite nanotubes (HNTs) as carriers of bio-active compounds. From the very beginning of the project, safety issues will be an integrated part of the work considering the potential risk of impact on human and environment when handling HNTs both in laboratory and pilot scale settings.

The present report is the first out of four which will provide recommendations and guidance on risk management measures related to the use processes of HNTs within NanoPack. Further, it will serve as a knowledge base on relevant regulation related to nanosafety throughout the project. Thus, it will be a living document which will keep the project partners updated with regulation on safety and improved safety recommendations.

As a starting point for the safety assessment we sent out a questionnaire on exposure and handling of HNTs to NanoPack partners with activities involving handling of or contact with material containing HNTs. Our aim was to identify processes with a high risk of exposure to HNTs in order to provide recommendations to ensure safe handling of HNTs. In parallel we initiated preliminary human and environmental hazard assessments of HNTs. The hazard assessments were based on the today very limited results from toxicology and ecotoxicology studies on HNTs and supplemented with results from studies on clays minerals and carbon nanotubes (CNTs). Further, occupational exposure limits (OEL) for analogue bulk materials, kaolinite, were considered.

Knowledges on safety regulation is highly relevant for NanoPack. This applies to the development and the implementation of the production processes as well as bringing the final product – the food packing material (FCM) – to the market. Both the production processes and the application of the FCM itself are covered by the general EU regulations. This includes the EU chemical regulation REACH, the EU Council Directive on safety and health of works at work, the EU environmental legislation and additional regulations directed specifically to food contact materials. There is no separate legislation for nanomaterials, but rather specific guidelines for nanomaterials requirements that have been developed in some instances.

The Code of Conduct (CoC) for responsible nanoscience and nanotechnologies adopted by the European Commission in 2007 supplement the regulation. When the human and environmental risk related to a nano-related product or nanotechnology is limited or unknown, a precautionary approach should be applied to regulate the potential risk of nanomaterials.

From our Nanopack survey we identified ten processes of use with possible risk of exposure from airborne HNTs from the NanoPack partners with activities involving handling of HNTs in either laboratory or pilot scale. The processes covered among others: reception and storage of HNTs; weighing, partitioning, fillings and emptying of HNTs; mixing; transferring from one process to another; spraying and aerosolizing.

HNTs belong to the category of high aspect ratio nanomaterials (HARN) and should be regarded as inhalable, insoluble fibres. They are not expected to hold asbestos-like properties but will very likely be potent inducers of inflammation in the lungs. Results of the few *in-vitro* cell studies of HNTs presented in the literature indicate that HNTs induce inflammation. Prediction of exposure for use of HNTs in small laboratory settings, using the control-banding tool NanoSafer, showed that functionalised HNTs constituted the worst case scenario. This was mainly due to applying precautionary risk management for unknown toxicological effects of HNTs.

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The risk management measures for occupational safety, as described in the survey, included mostly safety measures such as fume hoods, face mask, gloves and transport of NMs in double containers, while glove exhaust box and double layer of gloves were not commonly used. Based on the available information and the results of the survey among NanoPack partners, it can be concluded that the overall risk management for the identified processes has to focus on avoiding inhalation of HNTs. Guidance on suitable risk management are given for: small-scale use of HNTs; handling of HNTs in large rooms; handling of HNTs in suspension and for disposal. Furthermore, a range of safety measures are described in more details.

The environmental risk management measures for handling of wastes with a possible content of HNTs were mainly: treatment of exhaust effluent air by HEPA filtering; wrapping of waste in sealed plastic bags or plastic containers and collection of wastewater as chemical waste.

Two available studies of the environmental effects of HNTs showed lack or low ecotoxicity of HNTs. Assessment of results of ecotoxicological studies in accordance to OECD TG (Technical Guidelines of the Organisation for Economic Co-operation and Development) on platy nanoclays and CNTs led to the suggestion that nanoclays including pristine HNTs in general are less toxic than CNTs. However, studies are needed to test this hypothesis.

Due to the unknown impact on the environment and population of HNTs we proposed a set of precautionary risk management measures for handling of the HNTs-wastes.

The occupational and environmental exposure and hazard assessment of HNTs will be improved throughout the duration of the NanoPack project as new results will be collected either within the NanoPack (WP 6 tasks 6.1 to 6.4) or the public literature. This again will be reflected in the recommendation of risk managements.

2. Introduction

This report provides guidelines on safe handling of halloysite nanotubes (HNTs) and materials containing HNTs. It also informs on legislation related to safety of nanomaterials such as the European chemical regulation REACH (Registration, Evaluation and Authorization of Chemicals) [1] and the workplace framework Directive 89/391/EEC [2]. The guidelines focus on the actual working conditions and processes applied in the NanoPack project and recommends procedures for the NanoPack activities with HNTs to comply with the ethics requirements in accordance with the European Commission recommendation of 07/02/2008 on a code of conduct for responsible nanoscience and nanotechnology research [3].

The report will be a living document during the NanoPack project. Any new knowledge on physical-chemical properties, toxicity, ecotoxicity etc. will be considered to improve the safety guidelines. We also aim to stay updated with the regulatory requirements on safety related to engineered nanomaterials and –products.

3. Regulation

The EU chemical regulation REACH

Definition of a nanomaterial in REACH

EU adopted the current definition of a nanomaterial in 2011 [4]. The definition is:

"'Nanomaterial' means a natural incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm-100 nm."

Further, the recommendation states that by derogation from the definition above substances such as carbon nanotubes with one or more external dimension below 1 nm should be considered as nanomaterials.

An assessment of the definition in 2014 based on feedback from stakeholders during 2013 and early 2014 showed no need for revision of the definition. However, there is a need for clarification of some terms used in the definition and additional implementation guidance would be useful. In addition, the role of the volume specific surface area deserves clarification [5].

Registration of chemicals

Nanomaterials meet the substance definition in the chemical regulation REACH. The general obligations of REACH are therefore, applicable to nanomaterials as well. A company manufacturing or importing a substance in a volume of 1 ton per year or more is obliged to register the substance in accordance with REACH. If the same company manufactures or imports a substance both in its nano form and non-nano form it is the total volume, i.e. the sum of the two forms that has to be considered, when assessing obligations related to REACH.

A registrant of both nano forms and non-nano forms of the same substance identity [6] has to consider other parameters in addition to chemical composition in order to determine their impact on properties relevant for the hazard profile. If there are several nano forms, the properties of each nano form has to be included. A registrant has to consider at least the particle size, the particle shape and the surface chemistry. Furthermore, the registrant has to address the obligation to provide data in accordance with the REACH Annex VII-XI and demonstrate that the hazard profile is meaningful [7]. The European Chemical Agency (ECHA) has developed specific guidance on requirements for nanomaterials [8].

A company manufacturing or importing a substance in a volume of 10 ton per year or more is obliged to make a chemical safety assessment as part of the registration dossier. A chemical safety assessment has to cover assessment of safety for professionals, consumers and the environment during manufacturing and use. The safety of food contact materials is, however, covered by other regulations as explained in the section "Food Contact Materials" below.

If the pristine HNTs are purchased from a European manufacturer or importer, the producer of chemically-modified HNTs would then be a downstream user and is not obliged to register. However, it presupposes that the registrant has included the chemically-modified HNTs and all its uses i.e. the chemical modification itself, mixing of HNTs with polymers, granulation, extrusion etc. in his registration dossier. If your use is not included in his registration, you may contact the registrant with the aim to make a use known and included in the registration dossier. Alternatively, you may make a downstream user chemical safety report [9]. The downstream user is, however, not required to make a chemical safety report if he use less than one tonne per year of the substance (REACH Regulation Art. 37.4(c)).

According to the REACH register of registered substances, there are registrations of both multiwall carbon nanotube (MWCNT) and single wall carbon nanotube (SWCNT) [10]. However, there are currently no registrations of HNTs.

Classification, Labelling and Packaging (CLP) Regulation

The CLP regulation includes all substances regardless of the tonnage. The manufacturers, importers and downstream users shall consider the forms or physical states when evaluating available information for a classification (CLP Art 9(5)). A substance with different particle size or forms can

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have different classification. If both nano form and bulk form are placed on the market, a separate classification may be required if available data on intrinsic properties indicates a difference in hazard classification between the different forms [11].

A supplier of a substance or a mixture must deliver a safety data sheet when the substance in its own or in a mixture meets the criteria for classification as hazardous in accordance with the CLP regulation or any other of the requirements to provide safety data sheets in accordance with the REACH Regulation (Art. 31 and 32).

Occupational regulation

In addition to the obligations in REACH exposure to chemicals at the workplace is regulated by framework Directive 89/391/EEC in the European Union [2]. The directive places several obligations on employers to take measures for worker safety and health protection. Nanomaterials are not specifically addressed in the directive; however, they are fully covered. In short, employers must carry out risk assessments; and if a risk is identified, take measures to handle the risk. A later directive specifically addresses exposure to chemicals in the workplace [12]. This directive aims at protecting the health and safety of workers, among others by introducing Occupational Exposure Limit values (OELs). OELs are 8-hour time weighted averages of the concentration of the chemical agent in the air of a work place within the breathing zone of the worker. The 8 hours corresponds to a working day of 8 hours. Regardless of the OELs, exposure should be reduced to a minimum with respect to both duration and intensity. At present, the OELs for the bulk form of the materials do also apply to the nano forms of the same material. This is because specific OEL-values for the nano forms of the materials have not been established within the EU [13]. Common EU initiatives towards nano-specific OELs are not expected within the nearest future, but there are initiatives in some EU-countries towards the establishment of such values at national level, e.g., in Denmark [14].

Environmental regulation

There is no specific environmental regulation of nanomaterials, which are subject to the general environmental legislation. A review of the EU environmental legislation on waste and water initiated by DG Environment identified gaps in the coverage of nanomaterials under the EU legislation [15]. The reviewed environmental legislations address nanomaterials in principle, but there were several limitations mainly due to lack of knowledge and technical capacity. In some cases, also due to inapplicability of existing legal mechanisms, such as concentrations thresholds to control the presence of pollutants.

The environmental legislation is, in several cases, based on information from other legislations, such as the CLP and REACH regulations. In the absence of available nano-specific data under these regulations, nanomaterials will, in principal be categorised according to the bulk form and in some cases hazardous properties of the nano form may not be recognised. Given the limitations in scientific understanding of the environmental risk of nanomaterials, it is relevant to apply the precautionary principle to regulate the potential risks of nanomaterials [15].

Food Contact Material regulation

Legislation of food contact materials relates to the safety of the consumer when consuming food, which was in contact with the material, as packaging or other article or material, which comes into contact with the food stuff. The main requirements for all food contact materials in the European Union are laid down in the Framework Regulation (EC) No. 1935/2004 [16]. In Article 3 is required:

1. *Materials and articles, including active and intelligent materials and articles, shall be manufactured in compliance with good manufacturing practice so that, under normal or foreseeable conditions of use,*

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they do not transfer their constituents to food in quantities which could:

a. endanger human health; or

b. bring about an unacceptable change in the composition of the food; or

c. bring about a deterioration in the organoleptic characteristics thereof.

2. *The labelling, advertising and presentation of a material or article shall not mislead the consumers.*

Specific rules for plastic food contact materials are laid down in Regulation (EU) No. 10/2011 and its amendments [17]. Specific rules for active and intelligent materials are set in Regulation (EC) No. 450/2009 [18]. This Regulation distinguishes between the active part (e.g. antimicrobially effective part) and the passive part of an active system. The active part is within the scope of Regulation (EC) No. 450/2009, whereas the passive part must comply with the respective food contact material regulation, in NanoPack case with the Plastics Regulation (EU) No. 10/2011. Both regulations require that only approved substances will be used. The 'Plastics Regulation' has a positive list for monomers, other starting substances, additives and polymerisation preparation aids. The positive list which contains the approved active components according to the 'Active and Intelligent Materials Regulation' is not yet implemented by the EU Commission. Active components, which are released to the food and have a technical effect in or on the surface of the food are exempted from listing within the 'Active and Intelligent Materials Regulation' if they are approved as food additives in the respective food regulation. According to the guidance document [19] to that regulation, it is considered as a general rule that all released active components shall be approved as food additives.

The halloysite nanotubes (HNTs) are related to the passive part of the active system. The halloysite mineral is a component of the mineral kaolin, which is approved as an additive in the EU Plastics regulation but not as a nanomaterial. Furthermore, it is intended to modify the mineral with organic components in order to improve the properties. The final HNTs (modified or unmodified) will need to be approved as food contact plastic additive.

The active components of the NanoPack system are the essential oils, which act antimicrobially. In Europe, the candidate essential oils are listed and approved as flavouring substances, but not for their antimicrobial application and will need specific approvals as food additives for this use. In the USA, the candidate essential oils are either classified as generally recognized as safe (GRAS) or approved as synthetic flavouring substances. If used in concentrations not exceeding the concentrations in the applications as flavouring, approvals as flavouring components are considered as a good basis for approval as active components.

Details on the regulations and the evaluation of the candidate substances for the NanoPack system are described in the NanoPack Deliverable D6.1 [20].

4. Ethics

The European Commission adopted a recommendation on a Code of Conduct (CoC) for responsible nanosciences and nanotechnology on 7th February 2007 [3]. The CoC is a supplement to the regulation. The aim is to promote safe and responsible nanosciences and nanotechnologies research in Europe for the benefit of the society as a whole. It was the intention to revise the CoC every two years. Public consultations were launched between 2007 and 2011 [21], but it has not led to any revision yet.

An ethics assessment of nanotechnologies as a case of an emerging technology showed that most of the ethical concern related to technology apply to the various applications of nanotechnologies. An elaboration of Environmental Health and Safety (EHS) and Ethical, Legal and Society Aspects (ELSA) from different sources led to deriving of lists of specific EHS and ELSA for different uses [22].

For nanomaterials in consumer products such as cosmetics, foods and textiles, etc. the following specific EHS and ELSA could be applied [22]:

- Claims in the use of nanotechnologies i.e. transparency and gaining the trust of citizens
- Labelling of nano-related products
- Proportionality of risks and benefit
- Regulation and control of nano-related products entering the market, to ensure safety and transparency along the value chain

EHS and ELSA for nanomaterials and nanotechnologies for industrial use could be:

- Regulation and control of nano-related products entering the market i.e. precaution, safety of nanomaterials in any exposure scenario and life cycle of nanomaterials and end of life impacts
- Information and transparency along the supply chain e.g. material safety data sheets for nanomaterials.

5. Safety requirements for handling of HNTs

Collection of information on use scenarios

For obtaining data regarding usage scenarios we sent out a questionnaire on exposure and handling of HNTs to NanoPack partners with activities, which involve handling of, or contact with materials containing HNTs. From the answers of this questionnaire, working processes with a high risk of exposure to HNTs should be identified in order to ensure safe handling of nanomaterials by minimizing the risk of exposure. The data gathered in the survey will also form the backbone for human and environmental risk assessment of HNT during manufacturing, uses and disposal.

The questionnaire was based on guidance and recommendations on safe handling of carbon nanotubes and nanofibers in laboratory and pilot scale manufacturing issued by NIOSH [23, 24] and the general safety rules for handling nanomaterials described by NRCWE (Annex 2)

Only processes posing a significant risk of HNTs exposure in the laboratory or pilot scale activities during the NanoPack project were included in the questionnaire. The elaborated questionnaire can be found in Annex 1 and include the following topics:

- Processes identified with exposure hazard
- Amount of HNTs handled
- Time and frequency of exposure
- Measures of engineering control and operating personnel.

In addition, questions on storage and transport considerations, waste handling and disposal, cleaning procedures, safety instruction and procedures and training of personnel were included in the survey. Each partner will keep their data on processes and measures updated during the project.

Results from questionnaire answers, summary of uses

We have collected eight questionnaires, which were returned from the seven NanoPack partners with activities handling pristine or functionalized HNTs. In general, the processes were well described, although not specifically detailed. Results from the questionnaire are shown in Figure 1. All seven partners used processes of receive and store, and weighing of HNTs. The amount of HNTs used in these processes ranged from 50 µg in small-scale settings and up to 50 kg in large-scale settings. Overall the quantity of HNTs used in the individual processes was smaller among small-scale users compared with larger-scale users.

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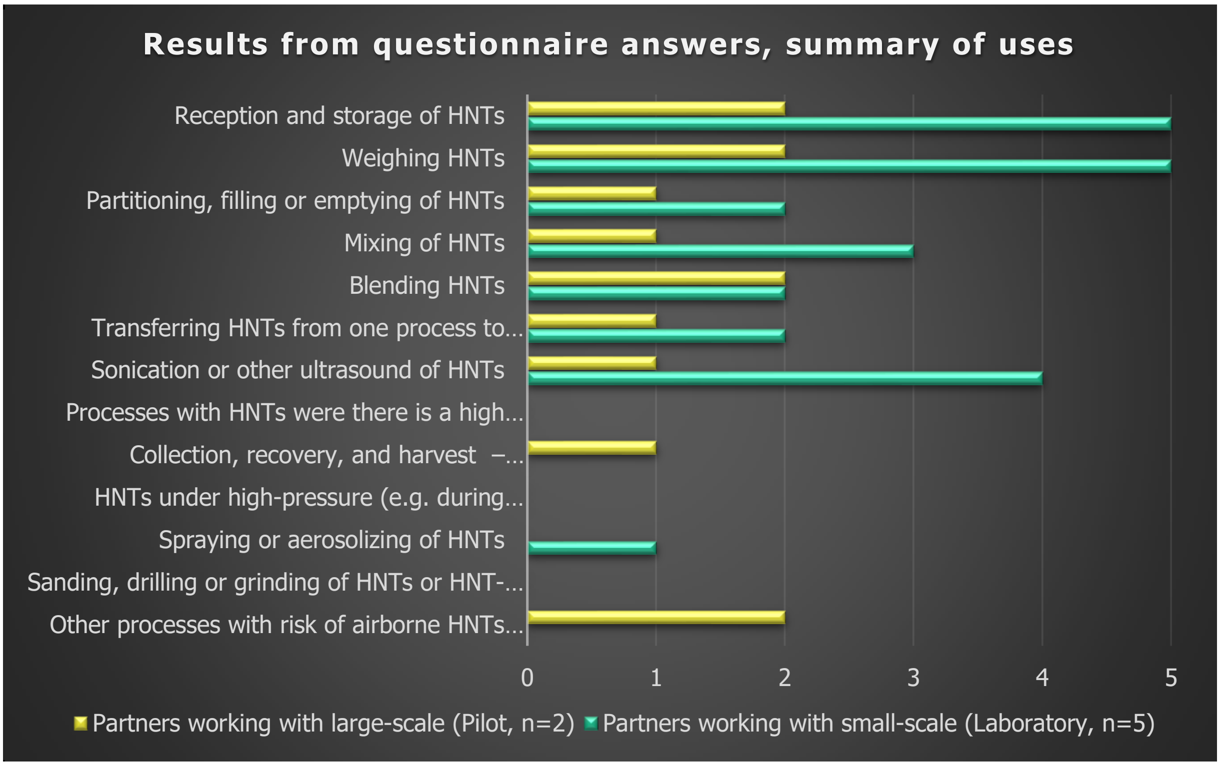

FIGURE 1 *RESULTS FROM QUESTIONNAIRE ANSWERS, SUMMARY OF USES*. PROCESSES IDENTIFIED AS A POTENTIAL RISK OF EXPOSURE TO HNTS ARE LISTED ON THE LEFT SIDE. ON THE RIGHT-HAND SIDE, THE ANSWERS FROM THE SEVEN PARTNERS ARE SHOWN. YELLOW BARS REPRESENTS THE TWO PARTNERS OPERATING IN PILOT SCALE SETTINGS, WHERE THE FIVE PARTNERS OPERATING IN LABORATORY SETTINGS ARE SHOWN IN GREEN BARS. IF NO BARS SHOWN THE PROCESS WAS NOT IDENTIFIED TO BE USED BY ANY OF THE PARTNERS.

Two small-scale users and one larger-scale user used processes involving partitioning, filling or emptying of HNTs. Further, three small-scale users and one larger-scale user used processes of mixing HNTs, where two small-scale users and two large-scale user used processes involving blending of HNTs. These processes were typically performed with 10 – 1000 g of HNTs. Two small-scale users and one large-scale user used processes where HNTs were transferred from one process to another, and the HNTs were typically transferred in closed containers. Furthermore, sonication or ultrasound treatment of HNTs were used in the processes at four small-scale and one large-scale user(s). There was only one large-scale user using a process involving recovery and collection of HNTs, here the process was evaporation where the HNTs was not dried to a powdery form. A process involving spraying or aerosolizing of HNTs was reported by one small-scale user, who performed a dustiness test under strictly controlled conditions. Moreover, two large-scale users used other processes with high risk of airborne HNTs including, spill of HNTs from the bag to the feeder of the compounder, and transportation of HNTs on aluminum trays from the fume hood to the oven for drying. Finally, none of the seven partners used processes involving high risk of explosion, HNTs under high-pressure or sanding, drilling or grinding of HNTs or HNT-composite material.

Risk management for handling of nanomaterials (HNT) in the occupational setting

Physical-chemical characteristics and toxicological aspects of HNTs

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Halloysite nanotubes (HNTs) consist of kaolinite, a clay mineral with the chemical composition of $Al_2Si_2O_5(OH)_4$. Their inner diameter range from 10 to 70 nm, the outer diameter from 20 to 200 nm, and lengths in range from 50 nm to 5 μm [25]. The MSDS for Dragonite HNTs states that approximately 90% of the particles are below 10 μm in length, and by far the majority is below 2 μm. In this size range a large proportion will be able to be inhaled and settle in the deepest parts of the lungs, the alveoles. There is no evidence that they will be solubilized in the lung after inhalation (pH 7) or after cellular uptake (pH 4-5), and from the alveoles, HNTs will be only slowly removed. HNTs are high aspect ratio nanomaterials (HARN), i.e. their length is many times that of their width, and therefore, HNTs should be regarded as inhalable, insoluble fibres [26] HNTs are however likely too short to adhere to the "fibre paradigm", that applies to biopersistent fibres that are longer than 15-20 μm with diameters less than 3 μm, and are associated with an increased risk of developing cancer [27, 28]. They would therefore probably not hold asbestos-like properties. They will however, very likely be potent inducers of inflammation in the lungs and will thus likely increase the risk of, e.g., cardiovascular disease. Apart from a few *in vitro* cell studies, HNTs have not been studied for their toxicity. The cell studies indicate low cytotoxicity but also that the HNTs induce inflammation [26]. Their lung inflammatory potential *in vivo* will be assessed in WP6.3.

Occupational exposure limits

According to Danish regulations, the occupational exposure limit (OEL) for the analogue bulk material, kaolinite is 2 mg/m^3. In Europe, kaolinite OELs generally vary between 2 and 10 mg/m^3 (respirable fraction). Mineral fibers that have properties resembling those of HNTs, but are confirmed to adhere to the fibre paradigm, are regulated with the following, and hold much lower OELs [29, 30]:

Erionite fibers $(Na_2,K_2,Ca)_2Al_4Si_{14}O_{36}\cdot 15H_2O$):	0.5 fibre/cm^3
Wollastonite fibers ($CaSiO_3$):	1 fiber/cm^3
Ceramic fibers	1 fiber/cm^3
(Asbestos, chrysodolite)	0.3 fiber/cm^3)

Risk management by use of the control banding tool, NanoSafer

Currently, we have no information on the propensity of HNTs to generate airborne dust during handling. (The "dustiness" of the HNTs powders will however be assessed at a later time point, in WP6.2). The lack of toxicity and dustiness data precludes a detailed risk assessment. However, in order to cope with these limitations/uncertainties and for further development of a risk management strategy an assessment using the control banding tool of NanoSafer (http://www.nanosafer.org/), a freeware tool from NRCWE, has been performed.

Scenarios and exposure estimates were made for use of the HNTs in a small laboratory setting (4x4x2.3 m), where 1 g of material was handled by a person for 1 minute, assuming that the material had a very high dustiness (worst case scenario). The full NanoSafer reports are available in Annex 3:

- Scenario 1 (Dragonite HP): Pristine HNTs (under the assumption that there was no surface coating and that the OEL of 2 mg/m^3 for the analogue bulk material, kaolinite, was correct, i.e. that the HNTs are not a potentially hazardous high aspect ratio nanomaterial).
- Scenario 2 (Halloysite – Dragonite-HP:KT, treated): Functionalized HNTs (i.e., with surface coating, otherwise similar assumptions as for scenario 1.

NanoSafer predicted that the functionalized variant constituted the worst case (Scenario 2) due to precautionary risk management for unknown effects of functionalized halloysite. If handling 1 g of material takes place in a room, only with general ventilation, this would imply a need for handling in a fume-hood, or highly efficient local exhaust ventilation. Although the NanoSafer tool is considered conservative, the current assessment assumed that the OEL for kaolinite (2 mg/m^3) is valid for nanoparticles/-fibres, which may not be the case when taking into account the physico-chemical characteristics of the HNTs. The least risk was predicted in the case of handling of 1 g of the pristine HNT (Scenario 1).

Risk management measures as described in the survey

Review of occupational procedures and risk management measures reported in questionnaires returned from seven partner institutions found that the procedures were generally well described, although not specifically detailed:

- Glove exhaust box was not used commonly, when the powdered nanomaterials were handled during weighing and particles transferred between containers, and most institutions operated in fume hoods. In some instances where weighing/blending of up to 100 g of HNTs performed in an exhaust chemical fume hood without indication of the level of ventilation and enclosure. This was however somewhat compensated for using a full-face mask with P3 filter. Handling of particles outside of glove box, fume hood etc. does however imply a risk for contamination of the surroundings and thus exposure of other workers not using this equipment.
- A double layer of gloves was also only used by few institutions – this has the advantage, that the outer glove can be changed when contaminated, while the inner glove protects against skin contact during the change.
- Transport of nanomaterials was performed in double containers (one container inside another) only at some places. Transportation of HNTs was in one institution performed on aluminum trays from the fume hood to the oven for drying, implicating a risk for contamination of the surroundings.

Risk management

Overall recommendations:

Based on the available information and the questionnaire answers, the overall focus in relation to processes and handling of HNTs should be to avoid inhalation of the HNTs, thus, **it is strongly recommended to avoid all inhalation exposure to HNTs.** In general, the answers from the questionnaire would not indicate that oral exposure or skin exposure constitute any risk due to minimal uptake via these routes [31, 32]. It is suggested to adhere to the following (the NRCWE SOP for safe handling of nanomaterials is included in Annex 2) and also to consult the European Commission Guidance on protecting the health of workers from the potential risks related to nanomaterials at work [28] and working safely with manufactured nanomaterials [33]. The overall focus in relation to processes and handling is to avoid inhalation and inadvertent ingestion of HNTs:

1) At small-scale use, such as in laboratories, all handling of dry powders of HNTs should take place in a glove box or in an efficient and correctly operated fume hood with appropriate exhaust filtration. If handling of powder in open rooms cannot be avoided, all persons in the working area should wear high-level PPE (personal protective equipment) suitable for prevention towards dermal exposure and inhalation exposure to dust with fibres. Be aware of potential contamination of the room air and dust build-up on surfaces. Maintain a clean working environment. Use cleaning principles, which have low risk of re-suspending dust powders and fibres, such as wet-wipes. Special vacuum-cleaning with, e.g., a central suction system may be applicable for removing deposited dust and powder.
2) If handling of HNTs occurs in a large room, we recommend separation or encapsulation in a smaller ventilated room with efficient process ventilation. PPE suitable for prevention towards

dermal exposure and inhalation exposure to potentially hazardous dust and fibres should be used when being in a room where HNTs are handled. Be aware of potential contamination of the room air and dust build-up on surfaces. Maintain a clean working environment. Use cleaning principles, which have low risk of re-suspending of dust powders and fibres. Special vacuum-cleaning with, e.g., a central suction system may be applicable for removing deposited dust and powder.

3) Once HNTs are suspended in liquid, paste, slurry or as 'wet sand', the potential for airborne dust release is greatly reduced. However, due to risk of splashes and sprays during handling, it is recommended to conduct the work under a local exhaust ventilation and using PPE suitable to prevent against dermal exposure. Care should be taken that spills are cleaned up before drying. Follow cleaning instructions as mentioned above.
4) All HNTs should be disposed of as a compound containing a fraction of potentially hazardous fibres.

Below the safety measures are described in more detail, based on the Standard Operating Procedure from the National Research Centre for the Working Environment, Denmark. Do however keep in mind the hierarchy of risk management controls, when establishing proper measures to eliminate exposure (see box below).

Box: Hierarchy of Controls Options [28, 33].
Modify process: Change the process to reduce the extent of concern by, for example: • Handling HNTs in liquid media or binding HNTs in solid media • Reducing the amount of MNMs handled, on any given occasion or **Isolate or Enclose:** Operations which involve the likely release of HNTs into the air should be performed in contained installations or in facilities that can be operated remotely from a protected area **Engineering Control:** Processes where there is a potential for creating dust or aerosols with HNTs should be carried out in areas with efficient local exhaust or extraction ventilation. **Administrative Control:** Working procedures and assignment to tasks should be developed so as to ensure safe handling of HNTs: -Adequate training and information should be provided to individual workers; -An Emergency Management Plan should be established. **Personal Protective Equipment (PPE):** PPE should be regarded as a 'last resort' control measure or a supplement to with other measures.

Safety measures

Instruct personnel
Individuals who handle nanomaterials should be instructed in how the materials should be handled.

Avoid dispersal
Spreading of the material and contaminating the laboratory should be avoided by all means.

- During transport, nanomaterials should be stored and transported in a tightly closed container, enclosed in a larger closed container.
- Containers with HNTs should be opened only with an adequate exhaust, preferably a glove box .
- Consider the material to behave similarly to gas, rather than viewing it as traditional, larger particles, as nanomaterials released into air may remain suspended for a long period of time. Whenever possible, use HNTs in suspension.

Cleaning

Workplaces including laboratories, fume hoods and glove boxes where HNTs will be handled should be designed for this purpose. Workplaces should be equipped with as few things as possible, easing frequent cleaning. Equipment that has been in contact with the nanomaterials must be cleaned thoroughly before being taken out of the enclosure (fume hood, glove box), e.g. by washing with soap and water, followed by wiping with alcohol before transfer to ordinary dishwashing.

Waste

Nanomaterials and everything that has been in contact with the nanomaterials, for example, used for cleaning or used to collect waste is considered waste and should be handled accordingly. Dry waste should be wrapped and sealed effectively, and allocated to the type of waste to which the nanomaterial belongs.

Personal protective equipment to be used when HNTs are handled in a closed system/containment

Since nanoparticles are so small, ordinary protective equipment, such as lab coats and gloves, are not expected to protect the individuals effectively. It is therefore important to consider which personal protective equipment will protect against exposure, when the particles are handled in a fume hood.

Lab coat: Plain long sleeved coat should be used and should be changed frequently. The long sleeves are to ensure that no air goes into the gloves. After use, place it in a water soluble bag, so material is not dispersed during transport for washing.

Gloves: Use gloves that are documented to protect against transfer of particles. It is recommended to use an inner long and an outer short glove. Multiple layers protect better than a single layer, and the outer glove can be changed when contaminated. The selection of the type of gloves depends on the individual, type of work load and the nature of the nanomaterials used.

Disposable spare sleeves: These can be used outside the lab coat sleeves if the individual needs to work with the arms deep into the fume hood, e.g. when cleaning or operating equipment.

Risk management related to the environment

The kaolin group of clay minerals includes kaolinite and its hydrated form halloysite, which has a tubular shape. In the nano form halloysite is referred as halloysite nanotubes (HNTs) and belongs to the same group of high-aspect nanomaterials (HARNs) as CNTs as explained in the section above. However, chemically it belongs to the group of nanoclays, which are organized in classes depending of their morphology: montmorillonite, bentonite, kaolinite, hectorite, and halloysite [34].

Uptake in organisms

The uptake of HNTs in aquatic and terrestrial organisms has only been limited investigated, whereas data from studies on CNTs are emerging with great pace [35]. It has been shown that CNTs are actively taken up by filter feeders or stuck on the gills of fish, however there is no or little evidence of passive uptake of CNTs by aquatic organisms [35]. Ingestion of both SWCNTs and MWCNTs was

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reported in studies in *Daphnia magna* [36] and other aquatic organisms without evidence of cellular uptake [37, 38], whereas a few studies reported cellular uptake of CNTs [39, 40]. In addition, in rainbow trout SWCNTs stuck on the gills after exposure to 0.1 – 0.5 mg/L causing irritation and increased mucus production [41]. Summarized, the available data supports the plausibility of CNTs accumulating in organisms and the food chain.

Ecotoxicity of Nanoclays and CNTs

To our knowledge, there are only two ecotoxicity studies on HNTs (HNT- supported palladium nanoparticles) and HNT nanoclay investigating the effect on plant seed growth [42] and toxicity to the protozoan *Paramecium caudatum* (*P. caudatum*) [43]. A few OECD test guideline (TG) studies are available on Nanoclay NM-600 Bentonite (hereafter called NM-600) that has a platy structure and not a tubular shape such as nanotubes. Even though, their differences in morphology we have included the available OECD TG studies here to illustrate the ecotoxicity of Nanoclays to aquatic organisms well knowing that it is not suitable for read-across to HNTs based on structure. However, most data on aquatic and terrestrial ecotoxicity originates from studies using CNTs. Data from short- and long-term ecotoxicity studies of nanotubes to aquatic and terrestrial organisms are presented in tables in Annex 4.

Aquatic organisms

Algae and protozoa

In an OECD TG 201 study 72 hours of exposure to NM-600 in green algae caused inhibition of the growth rate with an EC50 of 248.5 mg/L and biomass accumulation with an EC50 of 39.2 mg/L [44]. In two other OECD TG 201 studies in green algae exposure to SWCNTs showed inhibition in the growth rate 72h after exposure with an EC50 > 10 mg/L [46]. A similar OECD TG 201 study with MWCNTS showed growth inhibition and inhibition of biomass incensement with EC50 values of 120 mg/L and >10 mg/L, respectively [45]. Further, a recent study investigated the toxicity of a panel of nanoclays (montmorillonite, bentonite, kaolinite, and halloysite) in the protozoan model organism *P. caudatum* [43]. The overall conclusion was that nanoclays showed low toxicity in terms of survival and markers of oxidative stress compared to graphene oxide and silica nanospheres, furthermore halloysite was the nanoclay with the lowest toxicity [43].

Crustaceans

In a 48h OECD TG 202 study, the short-term toxicity of NM-600 was tested in *Daphnia magna* with concentrations ranging from 1.2 – 100 mg/L. None of the concentrations caused toxicity in terms of immobilization and no lethal concentration (LC) could be determined [44]. Moreover, two short-term toxicity studies (OECD TG 202) of MWCNTs in *Daphnia magna* showed immobilization after 48h with an EC50 >10 mg/L and 100 mg/L, respectively [45]. In addition, three other OECD TG 202 studies of SWCNTs in *Daphnia magna* reported immobilization with EC50 values between 1.3 - >10 mg/L [46]. Chronic studies on the toxicity of MWCNTs in Daphnia magna reported EC50 values of >0.3 mg/L and >100 mg/L [45], whereas similar guideline studies with SWCNTs reported EC50 values of >0.3 mg/L and >1.0 mg/L [46].

Fish

In a 96h OECD TG 203 study the short-term toxicity of NM-600 was tested in zebrafish *Danio rerio* with a nominal concentration of 1.0, 10, and 100 mg/L. No fish died or showed abnormal behaviour, the no observed effect level (NOEC) was determined to >100 mg/L [44]. Two similar short-term toxicity studies (OECD TG 203) with MWCNTs in zebrafish showed no mortality with concentration up to 100 mg/L and the 96h LC50 was determined to be >10 mg/L and >100 mg/L [45]. However, a 14 days (OECD TG 203) study with MWCNTS in Japanese Medaka fish showed a 14 days NOEC: 3.2

mg/L, a 14 days Lowest observed effect concentration (LOEC): 10 mg/L and a 14 days LC50 >10 mg/L [45]. Several short- and long-term OECD TG studies with SWCNTs showed LOEC and LC50 values >10 mg/L [46].

Terrestrial organisms

There are no OECD TG studies on the terrestrial effect of NM-600 exposure. One study on halloysite nanotube-supported palladium nanoparticles showed that exposure to 1500 mg/L of HNTs, functionalized-HNTs, and HNT-PdNPs had no significant influence on germination, seedling development, xylem differentiation, or mitotic index. However, functionalized-HNTs significantly increased the number of aberrations in low-vigor seeds [42]. In a 28 days OECD TG 216 study on MWCNT exposure to soil micro-organisms a 28-day EC50 based on the inhibition of nitric acid synthesis was determined to be >100 mg/kg soil dw [45]. In addition, two OECD TG 216 studies on SWCNTs showed an EC50 >1000 mg/kg soil dw [46].

In summary, the literature on the environmental fate and toxicity of HNTs is non-existing or at best, very premature. Studies on CNTs may be used for read-across to HNTs when it comes to evaluating the nanotoxicity of HNTs. However, read-across is strictly based on size and structural similarity (tubular morphology), not considering other important factors such as the differences in chemistry that may have great impact on the ecotoxicity. Overall data assessed here, suggest that there is a potential risk for bioaccumulation of HNTs in the aquatic environment. However, this assumption is somewhat weak since it is based on read-across from CNTs. The ecotoxicity data presented here suggests that platy nanoclays are less toxic to aquatic species than tubular-shaped CNTs and show no or low toxicity towards aquatic invertebrates and fish. Moreover, since HNTs is a nanoclay and not a carbon-based nanotube it tempting to speculate that pristine HNTs are also less toxic than CNTs; however, studies are needed to assert this hypothesis. Whether the same holds true for functionalized HNTs is beyond the scope of data used in this assessment. Surface functionalization of nanotubes (e.g. carboxylation) is known to modulate the intrinsic properties of the tubes, which could affect the toxicity.

Risk management measures as described in the survey

The environmental part of the survey reported by seven partners of NanoPack showed the following overall procedures for handling of wastes with a possible content of HNTs:

- Exhaust effluent air was treated by HEPA filter by the main part of the partners, one used scrubbing of the air and a single partner had no treatment
- Dry waste was mainly wrapped in sealed plastic bags while wet waste was either placed in sealed plastic bags or plastic waste containers
- Wastewater with HNTs was mainly collected as chemical waste. However, two partners had direct discharge to the sewer.

Risk management measures recommended

When managing recycling or disposal of wastes containing emerging and new technologies, as HNTs, with unknown impact on the environment and population, one should bear in mind the precautionary principle to foster a safe and healthy environment [15]. We propose the following precautionary handling of the HNT-waste:

- *Exhaust air*: The air should be either HEPA filtered or scrubbed to remove particles
- *Waste disposal*: Dry waste should be wrapped and sealed effectively, and normally put in the specific chemical group to which the nanomaterial belongs. The waste container should be

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labeled clearly with what the waste consists.
Wet waste should be collected as other fluid chemicals in plastic waste containers and marked by the waste group in which it belongs. If there is biological material in the waste, this together with the nanomaterial should be disposed as "hospital waste" and incinerated.

- *Effluent water*: HNT-contaminated wastewater should not be led directly into the sewer or water systems, regardless of production scale. We recommend engineering control e.g. filtering or sedimentation of HNT in contaminated wastewater before drainage to the sewer, or collecting wastewater for recycling or disposal by professionals.

6. Conclusion

Knowledge on safety regulation is highly relevant for NanoPack. This applies in the development and the implementation of the production processes as well as bringing the final product – the food material (FCM) – to the market. Both the production processes and the application of the FCM itself are covered by the general EU regulations. This includes the EU chemical regulation REACH, the EU Council Directive on safety and health of works at work, the EU environmental legislation and additional regulations directed specifically to food contact materials. There is no separate legislation for nanomaterials but specific guidelines on nanomaterials requirements have been developed in some instances.

The Code of Conduct (CoC) for responsible nanoscience and nanotechnologies adopted by the European Commission in 2007 supplement the regulation. When the human and environmental risk related to a nano-related product or nanotechnology is limited or unknown, a precautionary approach should be applied to regulate the potential risk of nanomaterials.

From our Nanopack survey we identified ten processes of use with possible risk of exposure from airborne HNTs from the NanoPack partners with activities involving handling of HNTs in either laboratory or pilot scale. The processes covered among others: reception and storage of HNTs; weighing, partitioning, fillings and emptying of HNTs; mixing; transferring from one process to another; spraying and aerosolizing.

HNTs belong to the category of high aspect ratio nanomaterials (HARN) and should be regarded as inhalable, insoluble fibres. They are not expected to hold asbestos-like properties but will very likely be potent inducers of inflammation in lungs. Results of the few *in-vitro* cell studies of HNTs presented in the literature indicates that HNTs induce inflammation. Prediction of exposure for use of the HNTs in small laboratory settings using the control banding tool NanoSafer showed that functionalised HNTs constituted the worst-case scenario. This was mainly due to applying precautionary risk management for unknown toxicological effects of HNTs.

The risk management measures for occupational safety as described in the survey included in most instances safety measures such as fume hoods, face mask, gloves and transport of NMs in double containers, while glove exhaust box and double layer of gloves were not commonly used. Based on the available information and the results of the survey among the NanoPack partners, it can be concluded the overall risk management for the identified processes has to focus on avoiding inhalation of HNTs, which should be strongly recommended. Guidance on suitable risk management are given for: small-scale use of HNTs; handling of HNTs in large rooms; handling of HNTs in suspension and for disposal. Further, a range of safety measures are described in more details.

The environmental risk management measures for handling of wastes with a possible content of HNTs were mainly: treatment of exhaust effluent air by HEPA filtering; wrapping of waste in sealed plastic bags or plastic containers and collection of wastewater as chemical waste.

Two available studies of the environmental effects of HNTs showed no or low ecotoxicity of HNTs. Assessment of results of OECD TG ecotoxicological studies on platy nanoclays and CNTs led to the suggestion that nanoclays including pristine HNTs in general are less toxic than CNTs. However, studies are needed to test this hypothesis.

Due to the unknown impact on the environment and population of HNTs, we proposed a set of precautionary risk management measures for handling of the HNTs-wastes.

The occupational and environmental exposure and hazard assessment of HNTs will be improved thorough the NanoPack project as new results occur either within the NanoPack (WP 6 tasks 6.1 to 6.4) or the public literature. This again will be reflected in the recommendation of risk managements.

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8. Annexes

Annex 1

Questionnaires: Exposure and handling assessment

NANOPACK WP6.6
Exposure and handling assessment

Introduction:

Halloysite NanoTube (HNT) is a high-aspect ratio nanomaterial with similarity to carbon nanotubes and therefore potentially harmful when inhaled or exposed in other ways. According to the European Chemicals Agency (ECHA) Classification and labeling (non-harmonised) Halloysite clay - synonym Kaolin - (CAS No. 1332-58-7) is potentially a skin- and eye irritant and investigated for causing cancer, lung damage, allergy or asthma when inhaled. Nonetheless, these effects are without consideration of the effects caused by nanotubes themselves. Hence, to be in compliance with the ethics of Horizon 2020 and to ensure the health and safety for people exposed to HNTs during this project, targeted guidelines on safe handling of HNTs will be developed.

Scope:

The purpose of this survey is to identify working processes with a high risk of exposure to HNTs and to ensure safe handling of nanomaterials. Data obtained from the survey will form the basis for making targeted guidelines for working with HNTs and will be issued to all project members. Furthermore, the data will form the basis for the development of a proposal for human and environmental risk assessment of HNT during manufacturing, uses and disposal (Task 6.6).

To increase the validity of these upcoming guidelines, please fill in as much information about the working procedures as possible. In case of both running, a laboratory and a production facility please fill in one questionnaire for each.

The survey can be filled in online or by hand and then emailed (as PDF) to dvch@dhigroup.com (with a copy to mwn@dhigroup.com). Please be aware of the deadline 2017.03.31.

Organisation:	
WP-contributor:	
Filled in by:	
Date:	

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Storage considerations

Where is the HNTs stored (e.g. chemical cabinet, fume hood, fridge, shelve or other)?
Is the HNTs (stock or diluted) labeled with hazard and precautionary statements and pictograms in accordance with regulations? ☐ Yes ☐ No
Are there any installations in adjacent areas that could create a hazard (e.g. risk of explosion or fire, etc.)? ☐ Yes (details) ☐ No

Transport considerations

Are HNT powder physically transported internally in the organization or externally to collaborators? ☐ Yes ☐ No
If yes – specify what controls are in place to minimize the exposure (e.g. sealed container, trolley, emergency procedure, hazard labeling, etc.)?

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Handling and exposure hazards

Do any of the following processes with high-risk of airborne HNTs take place in the organization?

For each process, please indicate whether the process is relevant for the organization (Yes or No). If Yes – please give a brief description of the process and fill in the details about the amount, ventilation, PPE, duration, frequency, and operation personnel. (An example of how to fill in this part of the survey is given at the end of the document).

Process	**Amount or concentration of HNTs** (µg, mg, kg or mg/L, of pristine HNTs, functionalized HNTs or loaded HNTs)	**Ventilation is used as a measure of control** (e.g., laboratory fume hood with HEPA filter, biological safety cabinet, glove box, dedicated ventilated room or another kind of ventilation)	**Personal protection equipment (PPE) is used** (e.g., Respirator, lab coat, chemical protection suit, safety glasses, disposable rubber gloves (nitrile, latex, short or long-sleeved), hairnet, shoe covers)	**Duration of the activity** (Estimated time in minutes)	**Frequency of the process** (Times per day, week or month)	**Involved operation personal** (e.g., technical staff, ground and facility staff, academic staff, students or other)	**A brief description of the process** (if yes, please briefly describe the process)
Reception and storage of HNTs ☐ Yes ☐ No							
Weighing ☐ Yes ☐ No							
Partitioning, filling or emptying of HNTs ☐ Yes ☐ No							

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Process	Amount or concentration of HNTs (µg, mg, kg or mg/L, of pristine HNTs, functionalized HNTs or loaded HNTs)	Ventilation is used as a measure of control (e.g., laboratory fume hood with HEPA filter, biological safety cabinet, glove box, dedicated ventilated room or another kind of ventilation)	Personal protection equipment (PPE) is used (e.g., Respirator, lab coat, chemical protection suit, safety glasses, disposable rubber gloves (nitrile, latex, short or long-sleeved), hairnet, shoe covers)	Duration of the activity (Estimated time in minutes)	Frequency of the process (Times per day, week or month)	Involved operation personal (e.g., technical staff, ground and facility staff, academic staff, students or other)	A brief description of the process (if yes, please briefly describe the process)
Mixing of HNTs ☐ Yes ☐ No							
Blending HNTs ☐ Yes ☐ No							
Transferring HNTs from one process to another ☐ Yes ☐ No							
Sonication or other ultrasound of HNTs ☐ Yes ☐ No							

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Process	**Amount or concentration of HNTs** (μg, mg, kg or mg/L, of pristine HNTs, functionalized HNTs or loaded HNTs)	**Ventilation is used as a measure of control** (e.g., laboratory fume hood with HEPA filter, biological safety cabinet, glove box, dedicated ventilated room or another kind of ventilation)	**Personal protection equipment (PPE) is used** (e.g., Respirator, lab coat, chemical protection suit, safety glasses, disposable rubber gloves (nitrile, latex, short or long-sleeved), hairnet, shoe covers)	**Duration of the activity** (Estimated time in minutes)	**Frequency of the process** (Times per day, week or month)	**Involved operation personal** (e.g., technical staff, ground and facility staff, academic staff, students or other)	**A brief description of the process** (if yes, please briefly describe the process)
Processes with HNTs were there is a high risk of explosion ☐ Yes ☐ No							
Collection, recovery, and harvest – including, evaporation and freeze drying – of HNTs ☐ Yes ☐ No							

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Process	Amount or concentration of HNTs (µg, mg, kg or mg/L, of pristine HNTs, functionalized HNTs or loaded HNTs)	Ventilation is used as a measure of control (e.g., laboratory fume hood with HEPA filter, biological safety cabinet, glove box, dedicated ventilated room or another kind of ventilation)	Personal protection equipment (PPE) is used (e.g., Respirator, lab coat, chemical protection suit, safety glasses, disposable rubber gloves (nitrile, latex, short or long-sleeved), hairnet, shoe covers)	Duration of the activity (Estimated time in minutes)	Frequency of the process (Times per day, week or month)	Involved operation personal (e.g., technical staff, ground and facility staff, academic staff, students or other)	A brief description of the process (if yes, please briefly describe the process)
HNTs under high-pressure (e.g. during functionalization, loading, or production) ☐ Yes ☐ No							
Spraying or aerosolizing of HNTs ☐ Yes ☐ No							
Sanding, drilling or grinding of HNTs or HNT-composite material ☐ Yes ☐ No							

4

DHI - 2017.03.09

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Process	Amount or concentration of HNTs (µg, mg, kg or mg/L, of pristine HNTs, functionalized HNTs or loaded HNTs)	Ventilation is used as a measure of control (e.g., laboratory fume hood with HEPA filter, biological safety cabinet, glove box, dedicated ventilated room or another kind of ventilation)	Personal protection equipment (PPE) is used (e.g., Respirator, lab coat, chemical protection suit, safety glasses, disposable rubber gloves (nitrile, latex, short or long-sleeved), hairnet, shoe covers)	Duration of the activity (Estimated time in minutes)	Frequency of the process (Times per day, week or month)	Involved operation personal (e.g., technical staff, ground and facility staff, academic staff, students or other)	A brief description of the process (if yes, please briefly describe the process)
Another process with risk of airborne HNTs not listed ☐ Yes ☐ No							

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Waste air

How is HNT exhaust effluent air treated? ☐ HEPA filter ☐ Scrub ☐ Other (details):
Is the HNT concentration in the exhaust effluent air known (i.e. amount in µg-, mg- or kg/m^3)? ☐ Yes (details) ☐ No

Waste disposal

How is HNT dry waste (powder) collected and disposed? ☐ Open recycle bin ☐ Sealed plastic bag ☐ Other (details):
How much dry HNT waste is typically produced per week (i.e. amount in µg, mg or kg)?
How is HNT wet waste (in solution) collected and disposed? ☐ Open recycle bin ☐ Sealed plastic bag ☐ Other (details):
How much wet HNT waste is typically produced per week (i.e. amount in µL, mL or L, and mg/L)?
Is the HNT concentration of the wet waste known (i.e. µg/ml or mg/L) ☐ Yes (details) ☐ No
How is HNT-contaminated wastewater treated? ☐ Collected as chemical waste ☐ Led into the sewer ☐ Other (details):
How much HNT-contaminated wastewater is typically produced per week (i.e. amount in L or m^3)?

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Is the HNT concentration of the wastewater known (i.e. in mg/L)? ☐ Yes (details) ☐ No
Is the HNT waste disposed and labeled according to the local regulations on waste classification? ☐ Yes ☐ No ☐ Other (details)

Cleaning procedures

What kind of laboratory or production equipment is in contact with HNTs and afterward cleaned (Please, specify)   The equipment belongs to a facility in: ☐ Laboratory scale ☐ Pilot scale ☐ Large production scale
Briefly, describe the cleaning procedure, and what controls are in place to minimize contamination of the surroundings (e.g. ventilation or wetting of dry material to avoid the material getting airborne)

Safety instructions and procedures

Are there any Standard Operation Procedure (SOP) or work instructions on handling nanomaterials – including nano-clay, fibers and nanotubes – available for those working with nanomaterials? ☐ Yes ☐ No
If yes – Are all people working with or in contact with nanomaterials aware of the present and content of the SOP or work instructions? ☐ Yes

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☐ No ☐ Do not know
Are there any guidelines or SOP on handling accidental spills and contamination with HNTs? ☐ Yes ☐ No

Training

Are people handling the nanomaterials trained herein? ☐ Yes ☐ No
Have the personal responsible for cleaning of nanomaterial-contaminated equipment been trained in handling nanomaterials and nanomaterial waste? ☐ Yes ☐ No
Have the personal responsible for the daily cleaning in rooms where HNTs have been used (i.e. floors and tables, recycle bin emptying etc.) received trained in cleaning rooms with risk of nanomaterial contamination? ☐ Yes ☐ No

Monitoring

Are any nanomaterial air-monitor equipment available in the organization (e.g. Fast Mobility Particle Sizer, Condensation Particle Counter or other)? ☐ Yes ☐ No
If Yes – is there any procedure for monitoring nanomaterials in the workspace (details)?
Are people handling nanomaterials enrolled in a health-monitoring program? ☐ Yes ☐ No

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An example of how detailed an answer to the exposure part of the survey should be (blue text):

Process	Amount or concentration of HNTs (µg, mg, kg or mg/L, of pristine HNTs, functionalized HNTs or loaded HNTs)	Ventilation is used as a measure of control (e.g., laboratory fume hood with HEPA filter, biological safety cabinet, glove box, dedicated ventilated room or another kind of ventilation)	Personal protection equipment (PPE) is used (e.g., Respirator, lab coat, chemical protection suit, safety glasses, disposable rubber gloves (nitrile, latex, short or long-sleeved), hairnet, shoe covers)	Duration of the activity (Estimated time in minutes)	Frequency of the process (Times per day, week or month)	Involved operation personal (e.g., technical staff, ground and facility staff, academic staff, students or other)	A brief description of the process (if yes, please briefly describe the process)
Weighing X Yes ☐ No	50-100 µg	Laboratory fume hood with HEPA filter, biological	-Respirator -Lab coat -Safety glasses -Disposable rubber gloves (long sleeved nitrile)	60 – 180 minutes/day	One time/month	Technical and Academic staff	The HNT container opened and closed inside the fume hood. The balance is permanently placed inside the fume hood. All material that has been inside the fume hood (e.g. container, vials, scale, markers, plastic bags, etc.) are carefully cleaned using ethanol and water before leaving the fume hood. All waste is double-bagged in a

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Process	Amount or concentration of HNTs (µg, mg, kg or mg/L, of pristine HNTs, functionalized HNTs or loaded HNTs)	Ventilation is used as a measure of control (e.g., laboratory fume hood with HEPA filter, biological safety cabinet, glove box, dedicated ventilated room or another kind of ventilation)	Personal protection equipment (PPE) is used (e.g., Respirator, lab coat, chemical protection suit, safety glasses, disposable rubber gloves (nitrile, latex, short or long-sleeved), hairnet, shoe covers)	Duration of the activity (Estimated time in minutes)	Frequency of the process (Times per day, week or month)	Involved operation personal (e.g., technical staff, ground and facility staff, academic staff, students or other)	A brief description of the process (if yes, please briefly describe the process)
							disposable plastic bag. Outside the fume hood, the waste is labeled "hazardous Nanomaterial waste - toxic" and disposed according to with local regulations

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Annex 2

NRCWE SOP for safe handling of nanomaterials

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Standard Operating Procedure (SOP)

General safety rules for handling nanomaterials	Page 1 of 5
Person in charge: SHN	15. marts 2007
File: C:\Users\mwn\AppData\Local\Microsoft\Windows\Temporary Internet Files\Content.Outlook\6W4590QU\NanomaterialsGeneralSOP_NRCWE (002).doc	

Internal safety requirements for handling of nanomaterials at the National Research

Standard Operating Procedure (SOP)

General safety rules for handling nanomaterials	Page 5 of 5
Person in charge: SHN	15. marts 2007
File: C:\Users\mwn\AppData\Local\Microsoft\Windows\Temporary Internet Files\Content.Outlook\6W4590QU\NanomaterialsGeneralSOP_NRCWE (002).doc	

Annex 3

NanoSafer reports

ack_EU

NanoSafer Control Banding Report for Airborne Occupational Exposure Assessment

_EU

Near-field Acute	Near-field Daily	Far-field Acute	Far-field Daily
0.0643	0.0150	0.0280	0.0122
EB1: Very low exposure potential	EB1: Very low exposure potential	EB1: Very low exposure potential	EB1: Very low exposure potential

Disclaimer: It should be noted that the NanoSafer 1.1 output consist of an automated risk assessment and risk management recommendations considering user-dependent input. The National Research Centre for the Working Environment and other contributers as well as the program developers are not liable for any damage to humans or material or loss of income that would arise as a result of the assessments provided using NanoSafer 1.1. The outcome must be considered as a guide, but the final responsibility belongs to the safety managers using the results.

Near-field Acute	Near-field Daily	Far-field Acute	Far-field Daily
Near-field Acute 0.0643 EB1: Very low exposure potential	**Near-field Daily** 0.0150 EB1: Very low exposure potential	**Far-field Acute** 0.0280 EB1: Very low exposure potential	**Far-field Daily** 0.0122 EB1: Very low exposure potential
RL3: Intermediate toxicity suspected and/or moderate exposure potential. The work should be conducted in a fume-hood or with highly efficient local exhaust ventilation in combination with use of respiratory protection equipment (PP3 or higher quality) depending on the work situation. Make sure to have the personal respiratory protection equipment available in case of accidents.	RL3: Intermediate toxicity suspected and/or moderate exposure potential. The work should be conducted in a fume-hood or with highly efficient local exhaust ventilation in combination with use of respiratory protection equipment (PP3 or higher quality) depending on the work situation. Make sure to have the personal respiratory protection equipment available in case of accidents.	RL3: Intermediate toxicity suspected and/or moderate exposure potential. The work should be conducted in a fume-hood or with highly efficient local exhaust ventilation in combination with use of respiratory protection equipment (PP3 or higher quality) depending on the work situation. Make sure to have the personal respiratory protection equipment available in case of accidents.	RL3: Intermediate toxicity suspected and/or moderate exposure potential. The work should be conducted in a fume-hood or with highly efficient local exhaust ventilation in combination with use of respiratory protection equipment (PP3 or higher quality) depending on the work situation. Make sure to have the personal respiratory protection equipment available in case of accidents.

Based on the estimated hazard and exposure potential it is recommended to apply engineered protection equipment with a protection factor of 1 or higher
If the estimated risk level is low with exposure ratios lower than 0.1, it is recommended to consider working under at least local exhaust ventilation or in a fume hood as possible depending on the work situation.

Elaborated description of work situation assessed
Semi-worst case assessment of handling 1 g functionalized halloysite powder in the laboratory; assumimng that 2 mg/m3 is a valid OEL

Material, safety and contextual information used in the assessment

Material and safety data entered	Exposure situation data entered
Manufacturer: Applied Minerals	**Process type:** Powder handling
Relevance: No	**Energy level:** H5 (1.00) : Very high energy (eg. Drop height > 100 cm; dry mixture, dry cleaning with a brush or compressed air, accidents)
Coated: Yes	**Cyclus volume:** 0.001 kg
Known shape: Yes	**Cyclus duration:** 1 min
Morphology: Tube	**Cyclus pause:** 0 min
Shortest dimension: 70 nm	**Cyclus repeated daily:** 1 times
Longest dimension: 2000 nm	**Mass handled per cycle:** 0.001 kg
Size is known: No	**Time required per cycle:** 1 min
Average size: No	**Length room:** 4 meters
Size range known: No	**Width room:** 4 meters
Surface area: 75 m2/g	**Height room:** 2.7 meters
Relative density: 2.65 g/cm3	**Air exchange room:** 1 meters
Solubility: Insoluble (< 1 g/L)	**Activity level room:** Low quiet
Respirable dustiness: 937.5 mg/kg	

Further information and guidance on exposure management to MNM: e-Learning tool.

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Annex 4

Ecotoxicity data for nanoclays and –tubes

Table 1 Ecotoxicity of nanoclays and -tubes **to algae and protozoa**

Test substance	Species	Method/exposure	Result	Reference
NM-600	Green alga: *Pseudokirchneriella subcapitata*	OECD TG 201 Conc. 11 – 100 mg/L. 72h exposure. Static	Concentration dependent inhibition of: Growth rate 72-h-EC_{50}: 248.5 mg/L, and biomass 72-h-EC_{50}: 39.2 mg/L.	[44]
Nanoclays: (montmorillonite, bentonite, kaolinite, and halloysite) and silica nanospheres and graphene oxide	Protozoan: *Paramecium caudatum, (P. caudatum)*	Nanoparticles added to growth media at conc. 0.625, 1.25, 2.5, 5, and 10 mg/mL. For graphene oxide toxicity was studied at 0.0625, 0.125, 0.25, 1.5, 2 and 4 mg/mL.	Concentration depended decrease in survival and growth rate for all nanoclays Nanoclays induced lower levels of malondialdehyde and catalase activity than graphene oxide.	[43]
Arkema Graphistren gth C100, MWCNT	Green alga: *Pseudokirchneriella subcapitata*	OECD TG 201 Conc. 10, 22, 48, 105, 230, 500, and 1000 mg/L. Static	Inhibition of: Growth rate 72-h-EC_{50}: 150 mg/L, and biomass 72-h-EC_{50}: <10 mg/L.	[45]
Nikkiso SWCNT	Green alga: *Pseudokirchneriella subcapitata*	OECD TG 201	72-h EC50 >10 mg/L 72-h NOEC: 0.32 mg/L.	[46]
Super Growth SWCNT	Green alga: *Pseudokirchneriella subcapitata*	OECD TG 201 Conc. 0, 0.10, 0.32, 1.0, and 10 mg/L	72-h EC50 >10 mg/L 72-h NOEC: 0.32 mg/L.	[46]

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TABLE 2 ECOTOXICITY OF NANOCLAYS AND -TUBES TO AQUATIC CRUSTACEANS

Test substance	Species	Method/exposure	Result	Reference
NM-600 (Bentonite)	*Daphnia Magna*	OECD TG 202 Conc. 1.2, 3.7, 33.0, and 100 mg/L. Static	48-h NOEC ≥ 100 mg/L. No ECx-values could be determined.	[44]
Nikkiso MWCNT	*Daphnia Magna*	OECD TG 202 Conc. 10 mg/L Static	48-h EC50 >10 mg/L, immobilization	[45]
Arkema Graphistrength C100, MWCNT	*Daphnia Magna*	OECD TG 202 Conc. 100 mg/L.	48-h EC50 >100 mg/L, immobilization	[45]
Nikkiso SWCNT	*Daphnia Magna*	OECD TG 202 Conc. 0, 0.42, 0.94, 2.1, 4.5, and 10 mg/L	48-h EC50 >10 mg/L, immobilization	[46]
Super Growth SWCNT	*Daphnia Magna*	OECD TG 202 Conc. 0, 0.42, 0.94, 2.1, 4.5 and 10 mg/L Static	48-h EC50 >10 mg/L, immobilization	[46]
SWCNTs, Shenzhen Nanotech Port Co., Ltd.	*Daphnia Magna*	Modified OECD TG 202 Conc. 0.1, 0.5, 1, 5, 10, 50, and 100 mg/L	48-h EC50 >1.306 mg/L, immobilization	[46]

TABLE 3 ECOTOXICITY OF NANOCLAYS AND -TUBES TO FISH

Test substance	Species	Method/exposure	Result	Reference
NM-600 (Bentonite)	*Danio rerio*	OECD TG 203 Conc. 1.0, 10.0, and 100 mg/L. Static	96-h NOEC ≥ 100 mg/L. No EC-values could be determined.	[44]
Nikkiso MWCNT	*Oryzias latipes*	OECD TG 203 Conc. 10 mg/L Static	96-h LC50 >10 mg/L No mortality observed at 10 mg/L after 96h	[45]
Arkema Graphistrength C100, MWCNT	*Danio rerio*	OECD TG 203 Conc. 1.0, 35, 50, and 100 mg/L. Static	96-h EC50 >100 mg/L	[45]
Nikkiso MWCNT	*Oryzias latipes*	OECD TG 204 Conc. 0.10, 0.32, 1.0, 3.2, and 10 mg/L Semi-static	14 days EC50 >10 mg/L. 14 days NOEC: 3.2 mg/L. 14 days LOEC: 10 mg/L.	[45]
Nikkiso SWCNT	*Oryzias latipes*	OECD TG 203 Conc. 10 mg/L Static	96-h EC50 >10 mg/L, No mortality observed	[46]
Super Growth SWCNT	*Oryzias latipes*	OECD TG 203 Conc. 10 mg/L Static	96-h EC50 >10 mg/L No mortality observed	[46]
Nikkiso SWCNT	*Oryzias latipes*	OECD TG 204 under flow conditions Conc. 0.10 to 10 mg/L	14 days EC50 >10 mg/L 14 days NOEC: 10 mg/L 14 days LOEC >10 mg/L	[46]
Super Growth SWCNT	*Oryzias latipes*	OECD TG 204 under flow conditions	14 days EC50 >10 mg/L	[46]

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Test substance	Species	Method/exposure	Result	Reference
		Conc. 0.10 to 10 mg/L	14 days NOEC: 10 mg/L 14 days LOEC >10 mg/L	
Cheap tubes SWCNT	Rainbow Trout: *Salmo gairdnerii*	10 days of exposure Conc. 0, 0.1, 0.25, or 0.5 mg/L. SWCNTs dissolved in sodium dodecyl sulfate (SDS) and sonication.	Dose dependent increase in ventilation rate for SWCNTs versus SDS controls Increased mucus production and SWCNTs accumulation on the gills	[46]

Table 4 Terrestrial ecotoxicity of nanotubes

Test substance	Species	Method/exposure	Result	Reference
Halloysite nanotube-supported palladium nanoparticles	Seed of *R. sativus*	Conc. up to 1500 mg/L. 3 h, 6 h, 9 h, 24 h, 48 h, or 72 h exposure.	No influence on seed germination physiology, seedling development and growth, mitotic index, or chromosomal figures. Functionalized HNTs significantly increased the number of aberrations in low-vigor seeds.	[42]
Nikkiso MWCNT	Micro-organisms in soil	OECD TG 216. Conc. 1,000 mg/dry-kg. 28 days exposure.	28-day EC50: > 100 mg/L, inhibition of nitric acid synthesis	[45]
Nikkiso SWCNT	Micro-organisms in soil	OECD TG 216. Conc. 1,000 mg/dry-kg. 28 days exposure.	EC50 >1000 mg/kg soil dw	[46]

Super Growth SWCNT	Micro-organisms in soil	OECD TG 216 Conc. 1,000 mg/dry-kg. 28 days exposure.	EC50>1000 mg/kg soil dw	[46]

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