Capacity building in safe nanotechnologies

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Abstract. In all places where engineered Nanoparticles (ENPs) are produced, used or handled, adequate workplace safety precautions should be implemented due to the protection of workers and the surrounding environment. Any possible accidental release of ENPs should be evaluated. Thereby detected potential risks have to be eliminated as far as possible. An implemented reasonable safety culture in each ENP-related company will help to meet this challenge. Different infrastructures and workplace design can help to reduce the risk of an accidentally contact of the workers with ENPs: Transferable examples will be shown from the semiconductor and life-science Industry. These systems like clean rooms, glove boxes, fume cupboards, filter and suction systems and other restricted area barrier access systems (RABS) are mainly being developed to protect sensitive products, but they can also be used to protect working personnel. Clean environments regarding airborne particulate contaminations can be classified according to ISO 14644-1. A short insight into this ISO-classification will be given. But overall, a simple and reasonable workplace and workflow organization will reduce the risk of an accidental release of ENPs largely. This may lead to a therefore necessary adaption of existing workflow patterns. The workers have to get aware about the potential risks! This can be done with appropriate education materials, leaflets, posters and brochures. These are some of the later outcomes from the NanoDevice dissemination and handbook work package.

1. Workplace safety
In this article, the focus will be on the production and handling of airborne engineered nanoparticles (ENP). Looking at different exposure scenarios, several infrastructures and equipment can help to fulfill a necessary workplace safety to protect workers from accidentally contact with these potentially harmful substances. Starting from using gloves and safety goggles in nearly all laboratory settings from using different kind of breathing protections working with harmful aerosols up to the full set of clothing and breathing apparatus used by firefighters, a whole set of commercially available devices can be used for worker protection.

Another issue is the protection of the environment from any accidental release of harmful airborne substances. This can be achieved using air filters or air washers based in the air conditioning system of a laboratory setup. Also an useful laboratory layout design itself can help to reduce any risk of an accidental release of ENPs. Differential pressure from the environment with atmospheric pressure down to the laboratory settings or production plants with reduced pressures minimizes the accidental release of ENPs even if there is only a minor leak in the system. By constant monitoring the actual differential pressure, any fault in the system can for example lead to an instant emergency stop and/or the activation of other safety systems like for example additional fume extraction.
2. Existing examples of safety systems focused on workplace and product safety

Different already long established disciplines developed specific safety precautions to protect either the worker or the handled product. From each branch, some systems can be well used for the set-up of a laboratory setting or production plant with an appropriate workplace safety level regarding ENPs. Therefore, a short introduction will be given to each controlled environment from the different disciplines/industries.

2.1. Biological safety labs

Biological safety labs have to meet special criteria for each necessary safety level. These safety levels are set up to protect the worker and as well to protect environment and therefore human population from accidental release.

2.1.1. Classification. Biological labs are classified according to their biosafety level from S1 (lowest) to S4 (highest). The necessary level depends on the used organisms worked with. A biosafety level is the level of the biocontainment precautions required to isolate dangerous biological agents in an enclosed facility. In the United States, the Centers for Disease Control and Prevention (CDC) have specified these levels [1]. In the European Union, the same biosafety levels are defined in a directive [2].

Organisms generally regarded as safe (GRAS) like *Saccharomyces cerevisiae*, *Lactobacillus plantarum* or *Escherichia coli* can be handled in a S1 setting. S2-settings are suitable for work involving agents of moderate potential hazard to personnel and the environment, like Salmonella sp., Pseudomonas aeruginosa or HIV virus. S3-level is necessary for agents which may cause serious or potentially lethal disease after inhalation. It includes various bacteria, parasites and viruses that can cause severe to fatal disease in humans, but for which vaccines or other treatment exist, such as *Leishmania donovani*, *Mycobacterium tuberculosis*, *Bacillus anthracis* and *yellow fever virus*.

The safety level S4 is required for work with dangerous agents that pose a high individual risk of aerosol-transmitted laboratory infections, agents which cause severe to fatal disease in humans for which vaccines or other treatments are not available, such as Bolivian and Argentine hemorrhagic fevers, Marburg virus and Ebola virus.

2.1.1. Necessary facilities and precautions for each safety level. This incomplete list should give the reader only a very short overview about the main precautions. Some precautions can be easily adapted for a setting working with ENPs.

S2:

1. Laboratory personnel have specific training and are directed by scientists with advanced training
2. Access to the laboratory is limited when work is being conducted
3. Extreme precautions are taken with contaminated sharp items
4. Certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

S3: All S2 prerequisites plus

1. Double-door access zone and sealed penetrations
2. The filtered exhaust air from the laboratory room is discharged to the outdoors
3. The ventilation to the laboratory is balanced to provide directional airflow into the room
4. Access to the laboratory is restricted when work is in progress

S4: All S3 prerequisites plus

1. Self-contained oxygen supply is mandatory
2. The facility is either in a separate building or in a controlled area within a building, which is completely isolated from all other areas of the building
3. Multiple airlocks are employed and are electronically secured to prevent both doors opening at the same time.
4. All air and water going to and coming from a biosafety level 4 lab will undergo special decontamination procedures to eliminate the possibility of an accidental release.

2.1.2. Outcome. For handling potentially hazardous ENPs, it will be useful to have restricted access to the production plant or lab, handle open processes in physical containment equipment or restricted area barrier access systems (RABS) and use pressure drops from the environment to the area with possible ENP exposure.

2.2. GMP controlled environments
Environments controlled under the Good Manufacturing Guidelines (GMP) for the production of sterile or aseptic products are regulated in the EU-GMP Annex 1 [3]. There, the different GMP cleanliness classes are defined according to their particulate and microbiological level airborne and on surfaces. Additionally, the guideline claims as follows: «...in clean areas, all surfaces should be smooth, imperious and unbroken in order to minimize the shredding or accumulation of particles or microorganisms and to permit repeated application of cleaning agents and disinfectants where used...The manufacture of sterile products is subject to special requirements in order to minimize risks of microbiological contamination, and of particulate or pyrogen contamination...». This means, that for all tribological elements, like joints, bearings and other material pairings, the material selection has to be carefully considered regarding their particulate emission during operation. Some material pairings tend to produce an immense load of particulate contamination released into the surrounding air, they have to be avoided. Some plastics are so called “self-lubricant” and show a reduced particulate emission during operation, like Polytetrafluorethylene (PTFE) plastics. Used materials in GMP controlled environments have to be resistant to all applied chemicals present in cleaners and disinfectants. The biological inertness is another important issue. Materials which tend to be metabolized from fungi and bacteria can have a major negative impact if microbial colonies will establish due to poor hygienic precautions. All materials should be resistant to the action of microorganisms [4]. The chemical microbiological resistance has to be evaluated prior using the material in GMP controlled environments. Relevant test procedures are described in ISO 846 [5] and ISO 2812-1 [6]. A possible classification is outlined in the guideline VDI 2083-17 [7] and -18 [8].
2.2.1. **Classification.** The EU-GMP Annex 1 defines the cleanliness classification as follows:

**Table 1.** Limits of particulate contamination defined in EU-GMP Annex 1.

| Cleanliness Class | Maximum permissible particle count per m$^3$ in a resting state | Maximum permissible particle count per m$^3$ in an operating state |
|-------------------|---------------------------------------------------------------|---------------------------------------------------------------|
|                   | > 0.5 µm | > 5 µm | > 0.5 µm | > 5 µm |
| A                 | 3,520 | 20   | 3,520 | 20   |
| B                 | 3,520 | 29   | 352,000 | 2,900 |
| C                 | 352,000 | 2,900 | 3,520,000 | 29,000 |
| D                 | 3,520,000 | 29,000 | Not fixed | Not fixed |

2.2.2. **Outcome.** For handling potentially hazardous ENPs, it will be useful to have well cleanable surfaces being resistant to all applied chemicals. No corrosion should minimize the cleaning efficiency. The microbiological resistance is of minor importance for the safe handling of ENPs; nevertheless it should be regarded due to personnel worker safety in the corresponding workplace settings.

2.3. **Cleanrooms for semiconductor industry**

Cleanrooms for semiconductor industry are used to gain a controlled surrounding mainly regarding airborne particulate contamination. They have a very narrow operating window regarding climatic conditions of about 22 +/- 0.5 °C and 45 +/- 5 % relative humidity. ISO 14644-1 defines the airborne particulate cleanroom classification from ISO 9 to ISO 1, the best defined ISO-class [9]. ISO 5 equals the EU-GMP Annex 1 cleanliness class A regarding particles with 0.5 µm diameter or larger. Nanoscale relevance has the ISO 14644-1 classes 6 to 1 as there, the limits for nanoscale particles down to 100 nm are defined as well.

In an average urban surrounding, between 5 and 35 trillion particles with a diameter of > 0.5 µm are present in 1 m$^3$ air. With an immense particulate filtration effort using pre-filtration and laminar air flow from filter-fan units installed in the ceiling to the bottom with an airflow of 0.45 m/s, the particulate contamination can be reduced below 1 particle (size >0.5 µm) in 1 m$^3$ cleanroom air. This equals an ISO class 1 cleanroom according to ISO 14644-1.

Airborne molecular contaminations (AMC) as defined in ISO 14644-8 [10] play an increasing role in semiconductor industries. Some critical compounds are even completely forbidden in controlled environments during open process production steps, like organophosphates, phthalates, amines and siloxanes. Organophosphates condensed on a wafer can result in an incorrect random doping process; siloxanes condensed on electric conductive surfaces can reduce connectivity of these electrical contacts during wafer testing. Volatile organic compounds (VOC) are another major concern as they may condense on critical surfaces as the lenses from the lithography tool etc. [11]. Therefore, in a cleanroom setting for the semiconductor industries, only low VOC-emitting materials without the presence of critical compounds are allowed to be used. Prior application, materials should be tested according to the guideline VDI 2083-17 [7].
2.3.1. **Classification.** The air cleanliness classes according to ISO 14644-1, is defined as follows. For comparison reasons, the values defined in the EU-GMP Annex 1 and the withdrawn US Fed. Standard 209E are listed accordingly:

**Table 2.** Limits of particulate contamination defined in ISO 14644-1, EU-GMP Annex 1 and the withdrawn US Fed. Standard 209E.

| DIN EN ISO 14644-1 | EU-GMP “at rest” | EU-GMP “in operation” | US Fed. Standard 209E | 0.1 μm | 0.2 μm | 0.3 μm | 0.5 μm | 1.0 μm | 5.0 μm |
|-------------------|-----------------|------------------------|-----------------------|--------|--------|--------|--------|--------|--------|
| 1                 | 10              | 0.3                    | 2                     | 0.1    |        |        |        |        |        |
| 2                 | 100             | 3                      | 24                    | 10     | 0.3    | 9      | 0.1    |        |        |
| 3                 | 1,000           | 30                     | 224                   | 7      | 100    | 3      | 35     | 1      | 6      |
| 4                 | 1,240           | 30                      | 245                   | 8      | 106    | 3      | 39     | 1      | 6      |
| 5                 | 10,000          | 300                     | 2,370                 | 67     | 1,020  | 29     | 352    | 9      | 8      |
| 6                 | 100,000         | 3,000                   | 23,700                | 671    | 10,200 | 289    | 3,520  | 100    | 83     |
| 7                 | 10,000          | 300                     | 2,370                 | 67     | 1,020  | 29     | 352    | 9      | 8      |
| 8                 | 1,000,000       | 300,000                 | 23,700,000            | 671    | 10,200 | 289    | 3,520  | 100    | 83     |
| 9                 | 1,000,000       | 300,000                 | 23,700,000            | 671    | 10,200 | 289    | 3,520  | 100    | 83     |

2.3.2. **Outcome.** For open process handling of potentially hazardous ENPs, it might be useful to have a cleanroom setting to protect the worker from potentially inhalable ENP dust. There is already an existing standard ISO 14644-1 defining nanoscale particulate reduction for some cleanroom classes down to 100 nm particles which eventually can be useful in the future for safe ENP handling. A laminar flow cleanroom can guide the contaminated air directly to the bottom. The necessary filtration system will retain the ENPs in the filter media. But mostly, the usage of a restricted area barrier access system (RABS), a mini-environment or a glovebox is adequate and there is no need to install a complete cleanroom system. Possibly useful additional safety equipment can be an emergency fume extractor for the whole lab activated through a specially labelled emergency switch in the laboratory setting itself. An important issue is the filtration of the outlet air prior release into the environment!

3. **Safety equipment for workers**

A lot of different safety equipment for workers - single or multiple usages - is already on the market and well established in different laboratory and industrial settings. Most of them can be useful to be applied in all handling operations regarding ENPs. The relevant safety equipment with focus to ENP handling is outlined in brief below.

3.1. Gloves

Single use gloves are widespread used in different laboratory settings. Standard latex gloves, powder-free latex gloves, nitrile gloves and gloves with reduced particulate contamination for cleanroom applications as well as gloves for heat protection and other purposes are used to protect the worker from the individual contamination sources. It is reported that nanoparticles can penetrate human skin based on minor scratches and injuries. Thus, it is advisable to use at minimum standard latex gloves when handling ENPs all the time. After usage, they have to be discharged accordingly.
3.2. Breathing protection

Single use breathing protections can be used to protect the worker as well as to protect the equipment or hospital patients. They are classified according to their particulate retention ability from filtering face piece (FFP) FFP 1 to FFP 3 according to EN 149 [12]. Class FFP 3 masks filter at least 99 % of airborne particles and the inward leakage between mask and face is less than 2 % (ten human subjects perform five exercises each and for eight of these individuals the average measured inward leakage listed above must not be exceeded). Other standards describing the retention ability of human face masks are EN 143 defining classes P1 to P3 for human face masks particle filters [13]. P3 masks filter at least 99.95 % of airborne particles. The US-American National Institute for Occupational Safety and Health (NIOSH) announced its own classification system. Face masks class 100 according to NIOSH filter at least 99.97 % of airborne particles. In general, the usage of the highest particle retention ability is advisable all the time. As basic rule for handling ENPs, the usage of a FFP3 mask is advisable.

3.3. Lab coat

Entering a laboratory setting, wearing a lab coat is mostly mandatory. Depending on the safety level of the different labs, it can start from standard multiple use cotton lab coats up to a full protection with internal breathing device used in a biological safety lab S4. For an ENP laboratory setting, the necessary safety level and its definitions still needs to be defined. From the actual point of view, if only an accidental release of ENPs is expected, a standard multiple use lab coat might be enough. It has to be made sure that the cleaning of these lab coats is done in a controlled manner. The used coats have to be collected in specially labelled containers to make following personnel aware of the potential risk dealing with used clothing.

4. Workplace and workflow organization

A sensible workflow organization can play a vital role during controlled handling of potentially dangerous goods such as ENPs. Some basic rules, adapted from the workflow advises for semiconductor fabs, are outlined below:

- Reduce the necessary handling steps and avoid unnecessary double handling steps
- Keep the potentially risky substances as far away as possible from human workers
- If handling ENPs in an open process, use fume extractors, minienvironments or RABS systems as far as possible
- A logical workflow setup from clean to dirty areas minimizes possible cross contamination
- Label all containers filled with ENP-substances clearly with substance, filling date and name of the worker
- Storage areas should be labeled accordingly
- The whole lab area should be restricted access only with an appropriate warning sign at the entrance doors for “nanorisk” (see warning signs for biological risk or radiation as possible basis of a ENP warning sign).
- All basic and mandatory lab requirements need to be addressed as well: Emergency shower? Eye cleaning devices? First aid kit? Evacuation plan? Alert plan? Regularly training of personnel? Lab area and workers visible from outside? Emergency phone numbers? Remind that an ENP lab is mainly an extension to a standard chemical lab with the necessarily of fume extractors and all mentioned bullet points above.
- Single usage disposable cleaning cloths and mops should be preferred.

5. Waste treatment – an open topic about the life cycle of ENPs?

As the potential risk from ENP is still in discussion and has to be either neglected or confirmed. This is an actually widely discussed topic with open outcome depending on the defined risk potential of
ENPs. Following questions will arise if the existence of a potential health risk from ENPs is proved from ongoing projects and projects in the future:

- How should lab or industrial wastewater with possible ENP contamination be treated?
- How should the exhaust air from air conditioning systems and fume extractors be filtered?
- Which filter system has to be used to retrain the focused ENPs?
- What about the whole life cycle of a product containing ENPs?
- What happens during thermal treatment in a waste disposal plant?
- How will ENPs being introduced in the environment behave after release? Will accumulation or even bioaccumulation occur inside microorganisms or higher living organisms?
- Which other organisms can be negatively influenced from ENP introduction into the environment (plants, vertebrates, insects, …)?
- Will biodegradation or chemical degradation occur to some ENP groups, like carbon nano tubes (CNT)?

If the later result from the ongoing toxicological experiments also done during the NanoDevice project states that ENPs are generally regarded as safe (GRAS), the long-term question about the life cycle of ENPs is still not solved. Therefore, it is mostly advisable to deal with all ENP-relating topics in a responsible and sustainable way.

6. Get aware of the potential risks!
One major issue in risk management is the constant education and training of the employees. This may already start during the education period at schools and universities and other education bodies. Knowing about a potential risk is the first step in controlling and reducing it. It does not need to be taught in depth, but a first insight into the subject of potential risks from ENPs and its risk management helps to remind the worker even after years of what to think about if handling ENPs.

6.1. NanoDevice dissemination
In the project NanoDevice, different dissemination activities will support the nano-related community regarding safe handling of ENPs. First, the scientific community will keep informed about the outcome of the project using the public available internet source www.nano-device.eu. This page gives an overview about all project partners, their main tasks during the project and involved personnel. All project-relevant conferences, seminars and workshops are addressed accordingly. The link to the restricted access project domain disseminates all relevant information to the project partners. The presence at different conferences together with a project leaflet will help to get the project known in the scientific community. It is planned to establish a joint workshop between different existing nanoscale-relevant other EU FP7 projects and platforms in spring 2011, as EUMINAfab, Minam (www.micromanomanufacturing.eu), NanoCom (www.nanocom-eu.org) and NanoFutures (www.nanofutures2010.eu).

A first draft of an internationally recognized warning sign similar to the biorisk sign was designed and was open for discussion in the scientific community [14].

Figure 1. First draft of a possible sign “nanorisk”
7. Summary
Dealing with ENPs, the still unsure potential danger if inhaled or get into contact with these particles has to be addressed in a responsible manner. Standard precautions already used in other industrial areas can reduce the risk of getting accidentally into contact with ENPs intensively. RABS systems or other mini environments for open process handling seem to be the major safety precaution dealing with nanorisk. Usually, the worker should wear gloves, a lab coat kept in the laboratory setting and a Class FFP3 breathing protection. Fume extractors may help to raise the nano-related safety to an even higher level. All areas with possible ENP release should be labeled with a well recognized warning sign as well as all containments with ENPs inside. Restricted access is mandatory. Well organized workflows design replenishes the actual necessary precautions dealing with ENPs. All exhaust air from the nanorisk-controlled areas has to be filtered with appropriate filtration systems to keep the environment free from ENPs as far as possible. Last but not least a personnel training plays a crucial role in controlling and reducing nano-related exposure risks. Please visit www.nano-device.eu for further information.

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