Risk assessment nano project – IGEM

Contact information

Principal investigators:	Prof. Dr. Dennis Claessen
Researchers:	Tino Hoeksma, Jennifer Adami, Zinab Rashid
Institute:	Institute Biology Leiden (IBL) – Sylvius Building
Project:	IGEM
Safety officers:	dr. Mark Begieneman & Marc Fluttert

Introduction

Nanomaterials are defined as materials and structures smaller than 100 nanometers and consist of nanoplates (nano object with one external dimension on the nanoscale), nanofibers (object with two external dimension on the nanoscale) and nanoparticles (object with all three external dimensions on the nanoscale). When it comes to the hazards of nanoparticles, the 4S rule is often used: Size (1-100 nm), Shape (i.e. fibers or spheres), Structure (chemical composition) and Surface (coating). These 4 factors combined determine the toxic effect of the nanoparticle.
Not a lot is known about health effects. However, research has shown that inhalation of nanoparticles can have negative effects on long function and are taken up easily. The large surface/volume ratio is an important factor herein, as it increases catalytic activity that may result in reactive oxygen species that damages cells and tissues. Also, nanoparticles are known to be able to accumulate, to a higher degree, in cells and organs disrupting cellular and/or organ function. Finally, some bio persistent particles can also interfere with biological processes in the body.

Project background and safety question

For the IGEM project, genetically modified E.coli bacteria k12 are used with 3 types of vectors: pET-16b (list A2 RGGO), pCA24N (https://www.addgene.org/87741/) and pETDuet-1 (https://www.addgene.org/vector-database/2659/) to create silver nano rocks in solution. By adding gold ions, urchin-like bimetallic nanoparticles with a silver core and gold 'spikes' (size 20-50 nm) can be created.

Little is known about the biological synthesis of the shape of nanoparticles using E.coli. To create a layer of gold around the silver nanoparticle a broad range of parameters (pH, temperature, silver stressor) are tested to determine how a correct batch of nanoparticles can be created.
To ensure safety and health and that regulations* are followed, a risk assessment and evaluation was performed of this project.

* Occupational health and safety law & regulation genetically modified organisms environmental management 2013 (see reference 2).

Method

An interview was taken with Tino Hoeksma, Jeniffer Adami and Zainab Rashid to discuss the details of the project and nanoparticles and the lab has been visited.
Risk assessment was performed using the control banding tool, as described in 'werken met synthetische nanomaterialen – aanvullende risico inventarisatie en evaluatie (2011)' (see reference 3). The control banding tool analysis the severity of the nanoparticles and probability of exposure to determine the risk level. Severity is based on characteristics of the nanoparticles such as: shape, size, toxicity, solubility, carcinogenic, mutagenic and reprotoxic properties. The probability is based on: amount of nanoparticles used, dustiness/mistiness, frequency of operations, duration of operations

and number of employees exposed.
When combined a risk score is given resulting in a risk level varying from 1 to 4 (see below).

Risk levels		
Risk level	**Description**	**Action**
1	Low	No further action required
2	Medium	Control measures required
3	High	Extensive control measures required
4	Very high	Experiment should not be performed / work cannot be done

To determine the hazard class of the E.coli strain k12 (list A1 RGGO 2013) and classified as ML-I. BW 25113 (risk group 1 according to DSMZ, TRBA classification). Because the E-coli BW 25113 is not mentioned on list A1, a request 2.13 has to be made to the office GMO which will take about 40 days for acceptation on classification ML-I.

Results

The nano RIE control banding for silver, which will be created by E-coli has been executed. Also the nano RIE control banding for the addition of gold ions to the silver particles has been performed. For every task/step in the experiment an assessment has been performed. For the details of the risk assessment, see excel file: control banding Masterfile_v4_23062022.xls (tab: IGEM).

Risk level category of all task performed (for both the creation of silver particles and addition of gold) was found to category: ***2 – medium – control measures required***.

Hazards of the silver nitrate and gold ions are given below

Silver nitrate		**Gold ions**	
H sentence	**Description**	**H sentence**	**Description**
H272	May intensify fire; oxidizer	H290	May be corrosive to metals
H290	May be corrosive to metals	H302	Harmful if swallowed
H314	Causes severe skin burns and eye damage	H314	Causes severe skin burns and eye damage
H410	Very toxic to aquatic life with long lasting effects	H373	May cause damage to organs through prolonged or repeated exposure
		H411	Toxic to aquatic life with long lasting effects

Risk level category of the ***microorganisms*** used was found to be ***ML-I*** . Importantly, for using the E-coli BBW 25113 a 2.13 request to Office GMO has to be started.

Advice

In general, it is important to understand that nanoparticles spread like gasses and most harm is done when inhaled. Therefore, it is important to prevent nanomaterials from becoming airborne and

spread into the environment. An important safe working method is to keep the nanomaterials either in solution or bound to a matrix and to work in a closed system as much as possible, or at least in a well ventilated system (i.e. fumehood or biosafety cabinet class 2 (HEPA filters, filter nanomaterials out too). Also, pay attention to processes in which solutions are heated and/or centrifuged/mixed/resuspended as this often creates aerosols that can increase possible exposure. Also, formation or use of (fine) powders should be prevented as much as possible.

Control measures

Ventilation and personal protection

- When working with GMO's only (E.coli, ML-1). Work can be performed on the lab table using safe microbiological working methods.
- When working with the nanoparticles only, work in a fumehood or biosafety cabinet class 2 with HEPA filter*.
- When working with both nanoparticles and GMO's, work in a biosafety cabinet class 2*. Even better is to use a biosafety cabinet that ventilates to the outdoors.
- As the nanoparticles are potentially toxic to the skin and might cause skin burns (H314 sentence), use of nitril gloves (preferably long sleeve) is recommended till over the sleeve of the labcoat.
- Always where a labcoat and good fitting protective glasses (this because both the silver nitrate and gold ions can cause severe skin burns and eye damage, see H314 sentence).
- Prevent formation of aerosols. If solutions are mixed and/or centrifuged wait for at least 30 minutes to let the aerosols formed, precipitate. When resuspending, do it slowly.
- Keep the number of actions performed as low as possible. The less actions, the less chance of nanomaterials escaping into the environment and thus exposure.

* It is important that the safety cabinets and fume hoods used, are periodically maintained and checked for proper functioning! Also, check up front that systems are properly working (i.e. check with a piece of paper if a flow of air can be detected).

Waste & Spills

- Waste with GMO's can first be treated with Ethanol 70%, bleach or autoclaved for liquids waste to kill the bacteria. Then liquid waste can be thrown away in the liquid waste cans (white cans – check sticker for the right disposal route).
- Liquid (small) nanoparticle spills can be collected with a tissue in a local plastic bag and the bag can be discarded in the solid chemical waste containers. If the spill contains also GMO bacteria, ethanol 70% has to be used.
- Nanoparticle spills/powders can be collected with a wet tissue and thrown away into the solid chemical waste containers (make sure you move slowly, to prevent particles from getting airborne).
- As both the silver nitrate and gold ions are very toxic to aquatic life (H410 and H411), it must be prevented that these materials are discarded to the environment.

Transport

- When transporting samples with nanoparticles outside the lab, always use a double containment system (i.e. Eppendorf tube in sealed plastic bag). This to prevent the solution from spreading (into the environment) if, in case of an incident, the tube is broken.

Cleaning

- Area's in which nanomaterials are used, should properly and regularly be cleaned after use. Only do this by means of 'wet cleaning'. Surfaces should be scrubbed as nanomaterials tend to 'stick' to surfaces.

Lab access

- Labs that are used for working with nanoparticles should not be accessible for everybody. Limit the accessibility of work spaces were nanoparticles are used.
- Warning signs can be used for signaling (see picture below).

Nano hazard

Instructions & supervision

- Make sure that everybody who will be working on this project and perform these experiments, know about the risk and control measures of working with nanoparticles combined with GMO's (as described in this advice).
- All students and employees who work with GMO have to follow the GMO training which will be organized every month by the BSO.
- Also, make sure that proper supervision is arranged so that periodically is checked if control measures and safe working methods are followed and personal protection is used and used correctly (i.e. lab coat, gloves, protective glasses) at all times.

Literature / references

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