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Challenges of implementing nano-specific safety and safe-by-design principles in academia

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ABSTRACT

Safe-by-design is an essential component for creating awareness of the potential novel risks associated with the introduction of sophisticated nanomaterials (NMs) with novel properties. SbD is also a useful tool for meeting EU policy ambitions such as the European Green Deal which includes circular economy and moving towards a zero pollution (pollution-free) environment. Unidentified risks are a growing concern with the rapid and exponential advances of nanotechnology innovation, and the increase in fundamental research on NMs and their potential applications. Therefore, addressing nano-specific safety issues early in the innovation process is vital for reducing the uncertainties of novel NMs. The challenge is that many innovators and material scientists are not toxicologist and are not aware on how to assess the safety of their innovations and novel materials. Safe-by-design is a concept that aims at reducing uncertainties and risks for humans and the environment, starting at an early phase of the innovation process and covering the whole innovation value chain, including research. This perspective tries to get a better understanding on the role of safe-by-design within engineered nanomaterial research to create awareness on the importance on assessing the safety early in research. A method was developed that integrates SbD with a set of questions to aid material scientists assess the safety of their materials (nano-specific safety aspects) and Risk Analysis and Technology Assessment (RATA). Here we present the results of a workshop for material scientists (PhD students) with limited toxicology knowledge at the Debye Institute for Nanomaterials Science (Utrecht University, The Netherlands) with the main goals to create awareness with regard to basic NM safety and to explore the possibilities for applying safe-by-design principles in academia. The approach presented here can be applied by researchers and innovators to assess the safety of NMs at an early stage of the innovation process, and this work is framed in the context of Responsible Research and Innovation using RATA.

1. Introduction

Responsible research and innovation (RRI) is considered a cross-cutting issue in Europe for research funding and technology development and is highly promoted in EUs Horizon 2020 research programs. The main themes associated with RRI include the integration of technology assessment, risk analysis and life cycle assessment (Forsberg et al., 2016; van Wezel et al., 2018). These concepts are captured in one way or another in the Safe Innovation Approach (SIA) under development within the EU project NanoReg2 (www.nanoreg2.eu), where SIA enhances the ability of all stakeholders to create robust, yet flexible processes for integrating the safety evaluation already from the early phases of the innovation process. SIA is an approach that combines a) the Safe-by-design (SbD) concept, and b) the Regulatory Preparedness (RP) concept. The SbD concept aims at reducing uncertainties and risks for humans and the environment, starting at an early phase of the innovation process and covering the whole innovation value chain, including research. The RP concept encompasses the development and application of a set of tools and procedures for regulators to prepare for innovations to minimize the time gap between appearance and (provisional) approval of innovations and appropriate legislation (Soeteman-Hernandez et al., 2019).

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Bringing a concept such as SIA and SbD to practical applicability is challenging, yet significant steps have been made in nanotechnology in the last decade. For instance, the Nanosafety Cluster has a working group 'Innovation and Safer by Design',[1] the OECD has an ad hoc group 'Safer Innovation Approach for More Sustainable Nanomaterials and Nano-enabled Products: Overview of existing risk assessment tools and frameworks, and their applicability in industrial innovations',[2] and the European program Horizon 2020 has invested in several projects (NANoReg, NanoReg2, ProSafe, SabyNA) to bring SbD to practical application by industry. Other developments include the proposed SbD project under CEN which will facilitate SbD industrial application. SbD is also an important strategy for achieving policy ambitions such as the European Green Deal[3] where it is being proposed as one of the tools to help achieving its goals such as circular economy (EU Action Plan for Circular Economy[4]) and moving towards a zero pollution (pollution-free) environment. In addition, SbD is also an important tool in the Horizon Europe European Partnership on Assessment of Risk of Chemicals (PARC). These initiatives aid in bringing the SbD concept closer to practical applicability yet after decades since its introduction, creating awareness about the importance of SbD and guidance on how to assess the safety of NMs early in the innovation is key to bringing this concept further. This perspective provides an approach for creating awareness among stakeholders such as material scientists with limited toxicology background and guidance on how to perform a risk analysis early in the innovation process.

Research and development occurs in many sectors of industry, from start-ups and small and medium sized enterprises (SMEs) to large companies and academia. Material scientists in academia are a significant stakeholder group in innovation and creating awareness about nano-specific safety and possible applications of SbD are desired for achieving RRI and for practical applicability of SbD. The objective of this paper is not only to create awareness about SbD and RRI among young scientist in academia but also to ensure that the innovations developed in academia (many funded by industry) with an application, are developed with SbD principles in mind. From a regulatory perspective (regulatory preparedness), the objective is to gain knowledge with regards to novel materials and techniques that are being developed in academia to help regulatory risk assessors keep up with upcoming developments and translate them into possible harmonized guidelines for safety testing if applicable. Experiences in projects such as NanoNextNL (a large scale Dutch national research and technology program for micro- and nanotechnology; www.nanonextnl.nl) showed that Risk Analysis and Technology Assessment (RATA) is a good method to put responsible innovation in practice as an integrated part of a research program to increase awareness of RATA, and to help technology developers perform and use RATA to move towards safer and more sustainable innovations (van Wezel et al., 2018). RATA provides a basis to assess human, environmental, and societal risks of new technological developments during the various stages of technological development (van Wezel et al., 2018). Several questions were developed by van Wezel et al. (2018) to check RATA awareness but assessing the nano-specific safety aspects of NMs is challenging and to ease this process, we developed sets of questions that can help innovators to assess nano-specific safety aspects of their product or material along the various stages of the innovation process (Dekkers et al., 2020). Addressing these questions will aid innovators to identify which type of information may support decisions on how to address potential human and environmental health risks in the innovation process. SbD can be better applied when material scientists know which type of information is needed to assess nano-specific human and environmental health risks early in the innovation process. This enables the elimination or reduction of potential human and environmental health risks from an early phase of the innovation process onwards, maximising use of resources, and expediting the development of novel NMs and products. The questions were used to complement the RATA. Here we describe an approach which integrates the identification of safety aspects needed early in the innovation process with SbD (Dekkers et al., 2020) and the RATA awareness questions developed by van Wezel et al. (2018). This adapted approach can be utilized to create awareness among material scientists and principal investigators of nanosafety and possible SbD implementation to support RRI and European policy ambitions such as the European Green Deal[3].

2. Method

2.1. Approach development

An information package (Appendix A: Supplemental data) was developed to support the collection of safety information for NMs and to create nano-specific safety awareness at the early phase of the innovation process (Dekkers et al., 2020) with the aim to complement the RATA (van Wezel et al., 2018) and to facilitate SbD applicability. These questions are a result from work from NANoREG[5] where six risk potentials (solubility/dissolution rate, stability of the particle coating, accumulation, genotoxicity, immunotoxicity, ecotoxicity) were identified for a safety screening strategy within the risk assessment of NMs, and from NanoReg2 where the most important questions and issues in the area of regulatory toxicology and risk assessment of NMs have been identified (Dekkers et al., 2020). These questions are only a supportive tool given that many material scientist have a limited toxicology background. These questions are not meant to be an extensive overview of the state-of-the-art in the safety assessment of NMs but rather a simplified overview for material scientist to have an indication on parameters that may be associated with a potential hazard. PhD students were asked to read Dekkers et al. (2016), Park et al. (2017) and van de Poel and Robaey (2017). To better assist the information gathering with regards to hazard and exposure, PhD students were asked to gather safety information for their case study from the public literature, the European Chemicals Agency (ECHA) website (https://echa.europa.eu/), and ToxNet (https://www.nlm.nih.gov/toxnet/index.html).

2.2. Workshop: risk analysis

The RIVM hosted the workshop and its aims where to learn about SbD, to become aware of tools and guidance documents used to assess nano-specific safety, to learn how to incorporate nano-specific safety in the core of research design, and to reflect on the challenges of incorporating SbD in research.

Four case studies from PhD projects were considered: hollow silica nanocubes, ferrrofluids, noble metal clusters and nanocapillary electrokinetic stage (nanoCET) technology. During the workshop, RIVM colleagues went through the collected basic information on the risk potentials in classification and labelling information from ECHA alongside the literature search for the safety issues related to each of the four case studies. The workshop was attended by PhD students from Debye Institute for Nanomaterials Science (Utrecht University, The Netherlands). The questions that were discussed with the PhD students during the workshop included: what is known about the safety of the innovation? What are the uncertainties? And what actions that can be

[1] https://www.nanosafetycluster.eu/nsc-overview/nsc-structure/working-groups/wge/.

[2] https://www.nanotec.or.th/en/?p=11656.

[3] https://www.climatechangenews.com/2019/12/12/eu-releases-green-deal-key-points/; https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:52019DC0640&from=EN.

[4] https://ec.europa.eu/environment/circular-economy/.

[5] https://www.rivm.nl/sites/default/files/201811/NANoREG%20WP6%20Task%206.2%20Safe%20by%20Design%20concept.pdf.

Table 1
Risk Analysis and Technology Assessment questions to check RATA awareness (adapted from van Wezel et al. (2018)) The Risk Analysis questions in *italics* were replaced by the safety aspects questions developed by Dekkers et al. (2020) whose specific overview is in the Appendix A: Supplemental data.

Risk analysis	Technology assessment
Is product less risky than existing products? What are new aspects, related to already authorized products? What is the 'nano' aspects of your development? What is the legislative framework for market introduction? *Are there any discussions on 'nano' within this legislative framework?* *What do you already know on the safety aspects?* *Do you have information on the intrinsic hazardous aspects?* *Do you have information on the environmental fate and behaviour?* *Can material be released in significant quantities during the production, use, or waste phase?* *Could you minimize emissions?*	Which other stakeholders, besides suppliers could you imagine? How will these stakeholders be affected, in both positive and negative ways? How does this new technology influence stakeholders' responsibility and liabilities? How does this new technology influence the relationship between stakeholders? What is society missing out on, both positive and negative effects if your ideas do not reach the market? Which different possible futures could you imagine with your development?

taken to make innovation less hazardous or reduce exposure (SbD actions)? The PhD students filled out their responses to the questions as outlined in Tables 2, 4, 6 and 8.

2.3. Post-workshop reflection: technology assessment and SbD applicability reflection

After the workshop, PhD students were asked to write a small description of each case study and a reflection of the workshop and SbD applicability in their research. The results in Tables 2, 4, 6 and 8 were then put in the context of RATA along with the reflection on SbD applicability in the different case studies from the PhD students. The RATA questions were derived from NanoNextNL (www.nanonextnl.nl) and from van Wezel et al. (2018) (Table 1).

3. Results

For each case study, a Risk Analysis was performed to assess the nanosafety of the NM or process by identifying uncertainties and action perspectives (SbD implementation) that could be taken to make the NM or process less toxic or to reduce exposure (Tables 2, 4, 6 and 8). A post-workshop reflection led to a brief Technology Assessment and a reflection on SbD applicability for each case study.

3.1. Hollow silica nanocubes

Shape is an important parameter for the interactions between NMs. Simulations predict that different shapes form different solid phases (Damasceno et al., 2012). Experiments on cuboidal colloids (Rossi et al., 2015), for instance, show that the exact shape of the particle has an influence on the properties of the solid phase that forms. Hollow silica nanocubes are good model particles to study the influence shape has on colloidal interactions.

Cubic nanoparticles can be prepared in a number of ways. A well-known method is the preparation of cuprous oxide cubes (Park et al., 2009; Gou and Murphy, 2003) because these nanocubes have a specific size (50–200 nm) and can be produced in significant yields. Although copper is toxic for aquatic animals (CDH, 2018), switching to less toxic heamatite cubes would put severe restraints on research.

Another important step is the coating of cuprous oxide nanocubes with stöber silica (Dekker et al., 2018) for the preparation of hollow silica nanocubes. Silica is a commonly used material in colloid science and its properties are well understood. Furthermore, the coating step allows control of the final particle shape.

3.1.1. Risk analysis for hollow silica nanocubes

3.1.1.1. Hazard. A preliminary safety assessment of hollow silica

Table 2
Risk analysis for hollow silica nanocubes and SbD considerations.

Case study	What is known about the safety of your innovation?	What are the uncertainties?	SbD implementation: Are there any actions that can be taken to make your innovation less hazardous or to reduce exposure?
Hollow silica nanocubes Size: 75–150 nm Shape: cube Functionality: optical properties of silica Possible application: solar panels and coatings	*Hazard*: Quartz (SiO_2, CAS 14808-60-7) Solubility: insoluble HARN: no No harmonized classification, self-classification[a]: STOT RE 1 and 2 (lung, inhalation); Carc. 1A and 2 (inhalation); Acute Tox 4 (inhalation); STOT SE 1 (inhalation); Muta 2; Eye irrit 1 SiO_2 is bioaccumulative and toxic Cu_2O (CAS 1317-39-1) Solubility: insoluble Hazard: harmonized classification[a]; Aquatic Acute 1; Aquatic Chronic 1; Acute Tox 4 (oral and inhalation); Eye dam. 1. *Exposure*: Expected route of exposure: inhalation, dermal Worker: when NMs are dispersed in liquid. Environment: waste	*Hazard*: Extent of which the insolubility of SiO_2 and Cu_2O affect environmental and health toxicity Possible carcinogenicity of SiO_2 Possible environmental toxicity of 40 ppm of Cu_2O left in solution. -Minor concentrations may play a major role on safety during industrial upscale. Long-term stability of hollow silica nanocubes is not known (aggregation?). *Exposure*: Possible risk to: Workers which drain drums of silica nanocubes; consumers which may come in contact with NM as final product and to environment though waste.	Find methods to recycle Cu_2O to reduce environmental exposure and waste. Change process to get rid of all the Cu_2O from solution. Search for alternatives to Cu_2O that are less toxic.

[a] Classification and Labelling information: STO RE, specific target organ toxicity with repeated exposure; Carc, carcinogen; Muta, mutagen; STOT SE, specific target organ toxicity with single exposure; Eye irrit., eye irritant; Skin irrit. Skin irritant; Acute tox; toxicity after acute exposure; Aquatic acute, representative of environmental toxicity, particularly to aquatic organisms; Skin sens., Skin sensitizer.

nanocubes (Table 2) showed that potential human health and environmental hazards include the insolubility of SiO_2 and Cu_2O, in combination with the possible human carcinogenic and mutagenic properties, lung specific toxicity when inhaled and eye irritation of SiO_2. In addition, SiO_2 is bioaccumulative and toxic to the environment. Cu_2O is hazardous to aquatic environment (acute and chronic) and may cause acute human toxicity via oral and inhalation exposure.

In general, NMs may have a different hazard profile than bulk materials of the same chemical composition. They are of higher concern because NMs may be more reactive due to their relatively higher surface area and their small size may enable them to reach (parts of) organisms that are out of reach for bulk chemicals. Rigid, persistent, fibre-like materials with high aspect ratios (> 1:5), are of high concern because of their resemblance to asbestos. On the other hand, if a NM has a very fast dissolution rate (i.e. close to instantly dissolved), the NM will probably convert into its molecular or ionic form before it reaches its potential target. For this situation, the NM can be evaluated using the information on the chemical composition(s) of the non-NM (Dekkers et al., 2016, 2020). The hollow silica nanocubes may be more reactive than the bulk chemicals of the same chemical composition, due to their small size and they may accumulate due to their slow dissolution rate. However, they do not resemble asbestos, as they are not rigid or fibre-like.

3.1.1.2. Exposure. The (anticipated) route of exposure gives information on which hazard data needs to be collected. For example, information on dermal sensitization is only relevant if exposure to the skin is expected. Aspects such as the potential for splashes from colloidal solutions, or aerosolization leading to respirable particles, that could lead to dermal exposure or inhalation exposure need to be kept in mind as risk potentials (Geiser et al., 2017; Riediker et al., 2019).

Potential inhalation and dermal exposure to aerosolized SiO_2 and Cu_2O might occur to workers which drain drums of silica nanocubes. Consumers may have limited dermal exposure as the NM is incorporated in the solid matrix of the final product (solar panels or coatings). Environmental exposure may occur though waste. Special attention is needed to avoid inhalation exposure given the lung specific toxicity when inhaled.

3.1.2. Integrating risk analysis with technology assessment and reflection on SbD applicability for hollow silica nanocubes

The RATA for hollow silica nanocubes is presented in Table 3 which combines the RA from Table 2 and TA derived as a post-workshop reflection.

3.1.2.1. Reflection on SbD applicability. At this early point in the innovation process, the development of a product is not in sight with the silica nanocubes and focus is on obtaining a particular shape and surface functionality. Changing the preparation method for safety or SbD considerations is therefore not envisioned because the procedure does not pose significant safety hazards for the researcher and the waste is disposed properly, resulting in minimal environmental exposure. Putting restrictions on the innovation process limits research options. It is, however important to be aware of nano-specific safety issues regarding NMs, both for the safety of the researcher and consumer as well as the safety of possible applications of the studied NM. This information can later be communicated if NM is later used in a novel product such as a solar panel or coating.

3.2. Ferrofluids

Ferrofluids are concentrated suspensions of magnetic nanoparticles. The key feature of ferrofluids is their behaviour as both liquid and magnet (Rosensweig, 1985). The research at Debye Institute for Nanomaterials Science (the Van't Hoff laboratory) focusses on the fundamental aspects of ferrofluid stability (Bob et al., 2012a,b). In the fundamental research, safety is considered only where it comes to the personal safety of the researchers involved in the experiments.

The ferrofluids that are investigated consist of aqueous dispersions of iron oxide nanoparticles. The data on toxicity of these NMs is still inconclusive (Singh et al., 2010), but iron oxide nanoparticles have many applications (Odenbach, 2009) and even have an E-number, although the European Food Safety Authority (EFSA) panel recently concluded that no adequate safety assessment could be carried out (EFSA, 2015). As with many other NMs, the surface chemistry and the coating of the particles has a significant effect on the toxicity (Abakumov et al., 2018).

3.2.1. Risk analysis for ferrofluids

3.2.1.1. Hazard. A preliminary safety assessment of ferrofluids (Table 4) showed that potential human health and environmental hazards include the insolubility of Magnetite (Fe_3O_4) and Maghemite (Fe_2O_3) and the possible human oral acute toxicity, lung specific toxicity when inhaled and eye and skin irritation. Special attention is needed in surfactant selection to ensure it is non-toxic.

Again, solubility/dissolution rate is an important risk potential because if a NM has a very fast dissolution rate (i.e. close to instantly dissolved), the NM will probably convert into its molecular or ionic form before it reaches its potential target. For this situation, the NM can be evaluated using the information on the chemical composition(s) of the non-NM (Dekkers et al., 2016, 2020). However, Magnetite (Fe_3O_4) and Maghemite (Fe_2O_3) do not dissolve very fast and are not expected to convert to their molecular or ionic form before reaching their target.

3.2.1.2. Exposure. Potential dermal exposure to Magnetite (Fe_3O_4) and Maghemite (Fe_2O_3) might occur during production or use, due to splashes from the suspensions. Special attention is needed to avoid inhalation exposure given the lung specific toxicity when inhaled. However, exposure via inhalation is less likely since the NMs are dispersed in suspensions, making aerosolization less likely.

3.2.2. Integrating risk analysis with technology assessment and reflection on SbD applicability for ferrofluids

The RATA for ferrofluids (iron oxide) is presented in Table 5, which combines the RA from Table 4 and TA derived as a post-workshop reflection.

3.2.2.1. Reflection on SbD applicability. The workshop created awareness that in the early-design phase of a project, nano-specific safety is one of the aspects that is easily overlooked. Creating awareness of the nano-specific safety for NMs among researchers can be a very potent way of improving health and environmental safety of NMs, leading to safer innovations.

3.3. Noble metal clusters

Noble metal nanoclusters (with size < 2 nm) have unique properties that are not present in larger nanoparticles. Certain sizes are significantly more stable than others, so that they form preferentially during synthesis. This means that it is possible to synthesise atomically monodisperse nanoclusters rather than a mixture of sizes as is common for large nanoparticles. The monodispersity allows one to correlate the properties of the cluster with its size and structure (Jin et al., 2016a,b).

The possibility to explain differences in properties at the single-atom level is an important reason why researchers study these NMs. When choosing which nanocluster to use for a particular experiment, the main consideration is its suitability: the stability under the experimental conditions, a particular functionality of interest (e.g. luminescence, catalytic activity), preparation and handling, and can it be obtained in sufficient quantity? Safety is not considered to be of great importance at this stage.

Table 3
RATA for hollow silica nanocubes.

Risk analysis		Technology assessment Post-workshop reflection	
Is product less risky than existing products?	Difficult to assess: possible application for solar panels and coatings	Which other stakeholders, besides suppliers could you imagine?	Customers (users)
What are new aspects, related to already authorized products?	Single particle dispersion for magnetic separation	How will these stakeholders be affected, in both positive and negative ways?	Better solar panels and coatings
What is the 'nano' aspects of your development?	Hollow silica nanocube (75–150 nm)	How does this new technology influence stakeholders' responsibility and liabilities?	Not certain
What is the legislative framework for market introduction?	REACH	How does this new technology influence the relationship between stakeholders?	Not certain
Are there any discussions on 'nano' within this legislative framework?	*Hazard*: Extent of which the insolubility of SiO_2 and Cu_2O affect environmental and health toxicity	What is society missing out on, both positive and negative effects if your ideas do not reach the market?	Not very much
What do you already know on the safety aspects?	Possible carcinogenicity of SiO_2 Possible environmental toxicity of 40 ppm of Cu_2O left in solution. -Minor concentrations may play a major role on safety during industrial upscale. Long-term stability of hollow silica nanocubes is not known (aggregation?).	Which different possible futures could you imagine with your development?	Innovation to market: possible better solar panels and coatings. Innovation not to market: current solar panels and coatings.
Do you have information on the intrinsic hazardous aspects?	*Exposure*: Expected routes of exposure: inhalation and dermal		
Do you have information on the environmental fate and behaviour?	Possible risk to: Workers which drain drums of silica nanocubes;		
Can material be released in significant quantities during the production, use, or waste phase?	consumers which may come in contact with NM as final product and to environment though waste.		
Could you minimize emissions? *Or are there any SbD actions that can be taken?*	Find methods to recycle Cu_2O to reduce environmental exposure and waste. Change process to get rid of all the Cu_2O from solution. Search for alternatives to Cu_2O that are less toxic.		

Table 4
Risk analysis for ferrofluids and SbD considerations.

Case study	What is known about the safety of your innovation?	What are the uncertainties?	SbD implementation: Are there any actions that can be taken to make your innovation less hazardous or to reduce exposure?
Ferrofluids (iron oxide) Size: 5–10 nm Shape: roughly spherical Functionality: single particle dispersion for magnetic separation Application: magnetic density separation for recycling plastics	*Hazard*: Magnetite (Fe_3O_4) (CAS 1309-38-2) Solubility: insoluble HARN: no No harmonized classification, self-classification[a]: Acute tox. 4 (oral); STOT RE 2 (lung, inhalation); STOT SE 3 (lung, inhalation); Eye irrit. 2 and Skin irrit. 2 Maghemite (Fe_2O_3) (CAS 12134-66-6) Solubility: insoluble HARN: no Not classified *Exposure*: Expected route of exposure: dermal Generally a closed system. Workers: exposed during production or use.	*Hazard*: Although effects to health and environment are being investigated, results are still inconclusive. Effects are very dependent on surface chemistry and surfactants. *Exposure*: Waste, environmental effects are still not fully known.	Select a safe option for surfactant.

[a] Classification and Labelling information: STO RE, specific target organ toxicity with repeated exposure; Carc, carcinogen; Muta, mutagen; STOT SE, specific target organ toxicity with single exposure; Eye irrit., eye irritant; Skin irrit. Skin irritant; Acute tox; toxicity after acute exposure; Aquatic acute, representative of environmental toxicity, particularly to aquatic organisms; Skin sens., Skin sensitizer.

This is also the case when developing new synthesis protocols to obtain different nanocluster sizes. The most important factor is the success of the synthesis. Is the synthesis easy? Can some parameter be tuned to easily obtain different sizes? Is it possible to prepare new sizes or structures that have never been made before? The toxicity of the starting materials, solvents, intermediates and final products is not so important.

3.3.1. Risk analysis for noble metal clusters

3.3.1.1. Hazard. A preliminary safety assessment of noble metal clusters (Table 6) showed that potential human health and environmental hazards for LA include possible human acute toxicity with all exposure routes (oral, dermal, inhalation), possible human skin irritation, skin sensitization and eye irritation, and lung specific organ toxicity when inhaled. LA is also hazardous to aquatic environments. For Ag, potential human health and environmental hazards include the possible human carcinogenic effects, lung specific toxicity when inhaled, eye and skin irritation and it is also hazardous to aquatic environments. For $AgNO_3$, potential human health and environmental hazards include its corrosive properties and hazardous to aquatic environments. These hazards can be expected in the nano metal cluster Ag_{29}.

3.3.1.2. Exposure. Potential dermal exposure to LA, Ag, $AgNO_3$, and

Table 5
RATA for ferrofluids.

Risk analysis		Technology assessment Post-workshop reflection	
Is product less risky than existing products?	Difficult to assess due to new process: magnetic density separation for recycling plastics	Which other stakeholders, besides suppliers could you imagine?	Customers (users)
What are new aspects, related to already authorized products?	New method: Single particle dispersion for magnetic separation	How will these stakeholders be affected, in both positive and negative ways?	Novel method for plastic separation and recycling. This is good for the environment.
What is the 'nano' aspects of your development?	Ferrofluids (iron oxide) 5–10 nm	How does this new technology influence stakeholders' responsibility and liabilities?	Not certain; more options to recycle plastics
What is the legislative framework for market introduction?	REACH	How does this new technology influence the relationship between stakeholders?	Not certain
Are there any discussions on 'nano' within this legislative framework? What do you already know on the safety aspects?	*Hazard*: Although effects to health and environment are being investigated, results are still inconclusive. Effects are very dependent on surface chemistry and surfactants. *Exposure*: Expected route of exposure: dermal Waste, environmental effects are still not fully known.	What is society missing out on, both positive and negative effects if your ideas do not reach the market? Which different possible futures could you imagine with your development?	Society gets a cleaner environment because these methods help to recycle plastics. Innovation to market: a better method for plastic recycling. Innovation not in market: plastic problem still huge environmental issue.
Do you have information on the intrinsic hazardous aspects?			
Do you have information on the environmental fate and behaviour?			
Can material be released in significant quantities during the production, use, or waste phase?			
Could you minimize emissions? *Or are there any SbD actions that can be taken?*	Select a safe option for surfactant. Further assess the safety of ferrofluids and in magnetic separation method to reduce uncertainties.		

Table 6
Risk analysis for noble metal clusters and SbD considerations.

Case study	What is known about the safety of your innovation?	What are the uncertainties?	SbD implementation: Are there any actions that can be taken to make your innovation less hazardous or to reduce exposure?
Nobel metal clusters (Ag_{29}) Size: ~1 nm (core) and 2.5–3 nm with (±)-α-lipoic acid (LA, a disulfide) ligand $Ag_{29}LA_{12}^{3-}$ Shape: pseudo-spherical Functionality: Luminescence Application: possible bioimaging and labelling applications	*Hazard*: LA (CAS 1077-28-7): No harmonized classification, self-classification[a]: Acute Tox.4 (oral, dermal, inhalation), Aquatic Chronic 2 & 3, Skin Irrit. 2, Skin Sens. 1, Eye Irrit. 2., STOT SE 3 (inhalation, respiratory system); (the ligand is chiral and we use the racemic mixture). Solubility: soluble HARN: no Ag (CAS 7440-22-4): No harmonized classification, self-classification[a]: Aquatic Acute 1, Aquatic Chronic 1, Eye Irrit. 2, STOT RE 1 (inhalation, respiratory system), Skin Irrit. 2, STOT SE 3 (inhalation, respiratory system), Carc. 2 Solubility: soluble HARN: no $AgNO_3$ (CAS 7761-88-8): Harmonized classification[a]: Aquatic acute 1, Aquatic Chronic 1, Skin Corr. 1B, Ox. Sol. 2. Solubility: soluble HARN: no *Exposure*: Expected route of exposure: dermal Worker exposure when in contact with cluster solution and when disposing waste.	*Hazard*: Unknown risks of what happens to the Ag_{29} particles in the human body (cell uptake, ligand exchange with proteins or DNA, reactions with Ag^+ ions and possible ROS generation and possible removal via kidneys or liver). *Exposure*: Waste and environmental effects given the aquatic toxicity of LA, Ag and $AgNO_3$.	Engineer ligand shell that is stable and facilitates fast elimination. If Ag is too toxic then Au can be a possible alternative. Higher QY to reduce exposure.

[a] Classification and Labelling information: STO RE, specific target organ toxicity with repeated exposure; Carc, carcinogen; Muta, mutagen; STOT SE, specific target organ toxicity with single exposure; Eye irrit., eye irritant; Skin irrit. Skin irritant; Acute tox; toxicity after acute exposure; Aquatic acute, representative of environmental toxicity, particularly to aquatic organisms; Skin sens., Skin sensitizer.

Table 7
RATA for noble metal clusters (Ag_{29}).

Risk analysis		Technology assessment Post-workshop reflection	
Is product less risky than existing products?	Noble metal clusters can be a less risky alternative to some bioimaging and labelling applications	Which other stakeholders, besides suppliers could you imagine?	Patients needing bioimaging, medical doctors performing scans
What are new aspects, related to already authorized products?	Noble metal clusters have luminescent properties.	How will these stakeholders be affected, in both positive and negative ways?	Novel method for bioimaging
What is the 'nano' aspects of your development?	~1 nm (core) and 2.5–3 nm with (±)-α-lipoic acid (LA, a disulfide) ligand $Ag_{29}LA_{12}^{3-}$	How does this new technology influence stakeholders' responsibility and liabilities?	Better bioimaging technology
What is the legislative framework for market introduction?	REACH & EMA (medical devices)	How does this new technology influence the relationship between stakeholders?	Not certain
Are there any discussions on 'nano' within this legislative framework?	*Hazard*: Unknown risks of what happens to the Ag_{29} particles in the human body (cell uptake, ligand exchange with proteins or DNA, reactions with Ag^{+} ions and possible ROS generation and possible removal via kidneys or liver). *Exposure*: Expected route of exposure: dermal	What is society missing out on, both positive and negative effects if your ideas do not reach the market?	Society gets a possible new technique for bioimaging (early disease detection?).
What do you already know on the safety aspects?		Which different possible futures could you imagine with your development?	Innovation to market: a better method for bioimaging. Innovation not in market: rely on current bioimaging methods.
Do you have information on the intrinsic hazardous aspects?	Waste and environmental effects given the aquatic toxicity of LA, Ag and $AgNO_3$.		
Do you have information on the environmental fate and behaviour?			
Can material be released in significant quantities during the production, use, or waste phase?			
Could you minimize emissions? *Or are there any SbD actions that can be taken?*	Engineer ligand shell that is stable and facilitates fast elimination. If Ag is too toxic then Au can be a possible alternative. Higher QY to reduce exposure.		

the nano metal cluster Ag_{29} might occur when workers come in contact with the metal nano cluster solution and when disposing waste. Extra caution needs to be taken if Ag_{29} is to be used for bioimaging given the above mentioned hazards for LA, Ag and $AgNO_3$. Special attention is also needed to avoid inhalation exposure to LA and Ag given the lung specific toxicity when inhaled.

3.3.2. Integrating risk analysis with technology assessment and reflection on SbD applicability for noble metal clusters

The RATA for noble metal clusters is presented in Table 7, which combines the RA from Table 6 and TA derived as a post-workshop reflection.

3.3.2.1. Reflection on SbD applicability. Fundamental researchers consider safety to be important, but this is mostly related to personal safety. A particular experiment might be hazardous but still worthwhile to do because knowledge can be gained about the material or phenomenon. Changing the experiment for safety reasons might not be desired so early in the research stage. The main goal of the research on noble metal clusters is to better understand their behaviour and not to apply innovation to a technology per se. Some concrete examples from literature on the field of noble metal clusters:

Noble metal nanoclusters are usually capped by thiols, which bind as thiolates. Some research groups also study selenolate ligands, for instance to see how this affects cluster stability, or to see how ligand exchange rates vary for thiolates vs selenolates (Kurashige et al., 2012). Nanoparticles with selenium are very toxic, but that is not due to the nanoparticles but because of the selenium ligands that might decompose.

It is interesting to see how the cluster properties change with increasing size. To do this, one obviously needs many different sizes, for instance from 25 Au atoms to 940 Au atoms (Zhou et al., 2016). Perhaps the toxicity of the clusters is also strongly size-dependent, but if some sizes are omitted from the study because of safety concerns, the study would be incomplete.

Some nanoclusters are luminescent, and the origin of this luminescence is still not clear. The electron donating capability of the ligand seems to play a role (Zhou et al., 2016). For this study, Au_{25} clusters with different ligands were studied. This size was chosen because it is easy to prepare with different ligands.

Research on noble metal clusters has grown since the first atomically monodisperse clusters were prepared and their structures determined. There are a number of research groups who are doing more applied research. Clusters are not only studied to determine what their properties are and why, but also how they could be used for something else - for instance for sensing applications or for biomedical imaging. Many of these applications are still in the "proof of principle" stage. The application could even be in fundamental research – for instance using noble metal clusters as model catalysts to understand the catalytic process better (Yuan et al., 2015). With a growing number of applications of noble metal clusters from energy to biomedicine (Mathew and Pradeep, 2014; Luo et al., 2014; Liu et al., 2017), knowledge about nanosafety is warranted.

3.4. Nanocapillary electrokinetic stage (nanoCET) technology

Accurately measuring the size distribution of nano objects is challenging. The nanoCET technology is able to characterize single particles and to accurately measure the distribution of sizes in a sample. Hollow optical fibers are employed where the samples are loaded and light is guided. The scattering signal is then monitored through a microscope equipped with a camera. Studying single-particles allows for the study heterogeneous samples where average properties are not necessarily representative of the sample.

By tracking the position of single nanoparticles over time, it is possible to calculate their diffusion coefficient and therefore their

Table 8
Risk analysis for nanoCET and SbD considerations.

Case study	What is known about the safety of your innovation?	What are the uncertainties?	SbD implementation: Are there any actions that can be taken to make your innovation less hazardous or to reduce exposure?
Technique Nanocapillary electrokinetic tracking stage (nanoCET) technology Functionality: facilitates NM characterization by simultaneously measuring the size, concentration, and charge.		*Hazard*: Risk of life-cycle of cartridges (waste disposal) is unknown. *Exposure*: Expected route of exposure: dermal Workers exposed to nanoCET and NMs while using device.	Generate a closed system to minimize exposure to NMs. Disposable cartridges with proper waste classification aiming to have low hazard waste and recyclable materials.

diameter. The main advantage of the technique is that thanks to the confinement produced by the fibre, the same particle is tracked over extended periods of time (minutes) and with very low background levels. Metallic particles with diameters below 20 nm at frame rates above 1 kHz have been tracked successfully (Faez et al., 2015).

The nanoCET technology can be employed to characterize samples in which different populations of particles are present, or in which a precise representation of size distribution is needed. This opens possibilities not only in quality assurance, but also in monitoring the exposure to NM both by workers and consumers. A correct sample characterization is the first step to designing safer processes and products.

3.4.1. Risk analysis for nanoCET

3.4.1.1. Hazard. A preliminary safety assessment of nanoCET (Table 8) indicated a special attention to ensure the cartridges used to characterize NMs did not pose an environmental or human hazard and to ensure that these cartridges can be recyclable and non-toxic.

3.4.1.2. Exposure. Workers might be dermally exposed to nanoCET and NMs while using device. Special attention is needed to avoid inhalation exposure to NMs as this is a risk potential for NMs (Dekkers et al., 2020), however exposure via inhalation is less likely, since aerosolization is unlikely because the nanoCET works with NMs suspensions.

3.4.2. Integrating risk analysis with technology assessment and reflection on SbD applicability for nanoCET

The RATA for nanoCET is presented in Table 9, which combines the RA from Table 8 and TA derived as a post-workshop reflection.

3.4.2.1. Reflection on SbD applicability. The workshop provided valuable tools and insight to help in the identification of areas in which nanoparticle characterization can lead to safer product development and SbD applicability. Awareness was gained on the design principles that can be followed in order to develop methods that are safer both for the users and for the environment. After the workshop, a better understanding was obtained on how to include nano-specific safety as a principle in future projects.

3.5. General workshop reflection

Fundamental research is primarily focused on trying to understand the functionality and behaviour of NMs. One aspect of the workshop that was striking was the different interpretation of ‘safety’: academics practice lab safety but do not always consider other nano-specific safety aspects past using the correct waste container for disposal (mostly based on solvent or chemical composition, not on nano-specific aspects). Nano-specific safety or thinking about the safety of novel NMs, does not generally play a significant role in design and it is often considered to hinder innovation. Examples of this included: the ferrofluid case study where the first part of the research project was aimed at gaining knowledge on the behaviour of ferrofluids; the hollow silica nanocube case study where the focus was on creating a process which resulted in a particular cube shape; or in the noble metal cluster case study where the primary focus was to gain knowledge on cluster generation.

Nano-specific safety is generally considered only when an application for the novel NM is defined. A distinction therefore needs to be made between fundamental research and research with possible industrial or consumer applications where nano-specific safety can be included in an early phase in the innovation process. Examples for this include the ferrofluids' second part of the research project where a process needs to be designed for plastic recycling or for the application of nobel metal clusters for imaging. Thus, safety as defined as lab safety is generally strictly enforced but not the full life-cycle safety approach required by SbD.

The exchange of knowledge between material scientists (PhD students) with limited toxicology knowledge and regulatory risk assessors was successful. Students learned about some basic nano-specific safety aspects and issues surrounding their innovation and regulatory risk assessors learned about the novel NMs and their possible applications, thus supporting regulatory preparedness. Awareness is therefore needed to include nano-specific safety in the research plan or proposal to protect the student's safety, for adequate waste disposal and to obtain safer NMs when the research leads to industrial or consumer applications. Another opportunity for further collaborations is the techniques used to characterize NMs (as in the nanoCET case study) in order for regulatory risk assessors to keep up with upcoming developments and translate them into possible harmonized guidelines for safety testing.

With regard to taking actions for SbD implementation, PhD students had very limited input into the planning of their project, at least at the early stage; it will thus be important to address the principal investigators and the funding agencies to encourage and support RRI and SbD.

4. Conclusions and recommendations

Several challenges are foreseen in the implementation of nano-specific safety and SbD principles in academia. A key challenge is creating awareness of the importance of addressing nanosafety aspects in research among Deans and principal investigators in technical universities. One way to create this awareness is if major funding agencies added a section for the early assessment of the safety of materials in research and for encouraging the application of SbD. The inclusion of the RATA framework adds the societal assessment and impact of the innovation. By including the early assessment of human, environment and societal risks to research on material science facilitates the transition towards RRI and provides research that support EU policy ambitions such as the European Green Deal. By educating early investigators, a new mind-set of interdisciplinary researchers and material scientists is created that takes into account human, environmental and societal well-being. Incentives such as more opportunities

Table 9
RATA for nanoCET.

Risk analysis		Technology assessment Post-workshop reflection	
Is product less risky than existing products?	Difficult to assess given that this is a novel method to characterize single particles and to accurately measure the distribution of sizes in a sample	Which other stakeholders, besides suppliers could you imagine?	Researchers, NM innovators, risk assessors
What are new aspects, related to already authorized products?	No methods available that can characterize NMs by simultaneously measuring the size, concentration, and charge.	How will these stakeholders be affected, in both positive and negative ways?	Stakeholders will have a technique to characterize NMs by simultaneously measuring the size, concentration, and charge.
What is the 'nano' aspects of your development?	Used to characterize NMs	How does this new technology influence stakeholders' responsibility and liabilities?	Not certain
What is the legislative framework for market introduction?	REACH	How does this new technology influence the relationship between stakeholders?	Not certain
Are there any discussions on 'nano' within this legislative framework?	*Hazard*: Risk of life-cycle of cartridges (waste disposal) is unknown.	What is society missing out on, both positive and negative effects if your ideas do not reach the market?	Not certain
What do you already know on the safety aspects?	*Exposure*: Expected route of exposure: dermal Workers exposed to nanoCET and NMs while using device.	Which different possible futures could you imagine with your development?	Innovation to market: a better NM characterization method. Innovation not in market: rely on separate methods for characterization of NMs.
Do you have information on the intrinsic hazardous aspects?			
Do you have information on the environmental fate and behaviour?			
Can material be released in significant quantities during the production, use, or waste phase?			
Could you minimize emissions? *Or are there any SbD actions that can be taken?*	Generate a closed system to minimize exposure to NMs. Disposable cartridges with proper waste classification aiming to have low hazard waste and recyclable materials.		

for funding, increase probability to publish work in high impact journals, and monetary awards by material science associations need to be created to motivate principal investigators to embark in this transition.

Assessing the safety of NMs is very challenging and a field on its own. For this reason, closer collaboration and interaction is necessary among nano-toxicologist and material scientists. During the workshop, there was a high threshold of knowledge that had to be bridged. Approaches like the one presented here, helps to bridge this threshold and it creates awareness of the importance of thinking about safety aspects of novel materials early in the innovation process.

An additional challenge that was mentioned by PhD students was time. PhD students have so many academic obligations with courses, research and conferences that there is limited time for additional activities such as the workshop that was provided. To alleviate this pressure, we propose that a mandatory course is embedded in the curriculum of all technical universities addressing material sciences in order for PhD students to have the time to not only become aware of SbD and RRI but also to apply these principles in their own research. The success of the workshop was due to the fact that students applied these principles to their own research and were able to make important reflections.

The overall aim of this perspective was to get a better understanding on the role of safe-by-design within engineered nanomaterial research and to create awareness on the importance on assessing the safety early in research. An integrated method was developed that combines nanosafety aspects, SbD and RATA which can be used by innovators or material scientists with limited toxicological background. The nanosafety aspects were identified through a set of questions which guide material scientists and provide a basis to assess human, environmental safety while the technology assessment helped to address possible societal risks of new technological developments. The collaboration between regulatory risk assessors and academia helps regulators to keep up with novel materials and techniques and support regulatory preparedness. The approach presented here can be applied by researchers and innovators to not only assess the human and environmental safety of NMs at an early stage of the innovation process, but also to assess possible societal risks to support RRI and European policy ambitions such as the European Green Deal[3].

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CRediT authorship contribution statement

Lya G. Soeteman-Hernández:Conceptualization, Methodology, Writing - original draft.**Gerhard A. Blab**:Investigation, Writing - review & editing.**Aquiles Carattino**:Writing - original draft.**Frans Dekker**:Writing - original draft.**Susan Dekkers**:Methodology, Writing - review & editing.**Marte van der Linden**:Writing - original draft.**Alex van Silfhout**:Writing - original draft.**Cornelle W. Noorlander**:Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

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