This is a postprint version of an article published in NanoImpact in 2020. The published version can be accessed here: http://dx.doi.org/10.1016/j.impact.2020.100267

Safe by Design implementation in the nanotechnology industry

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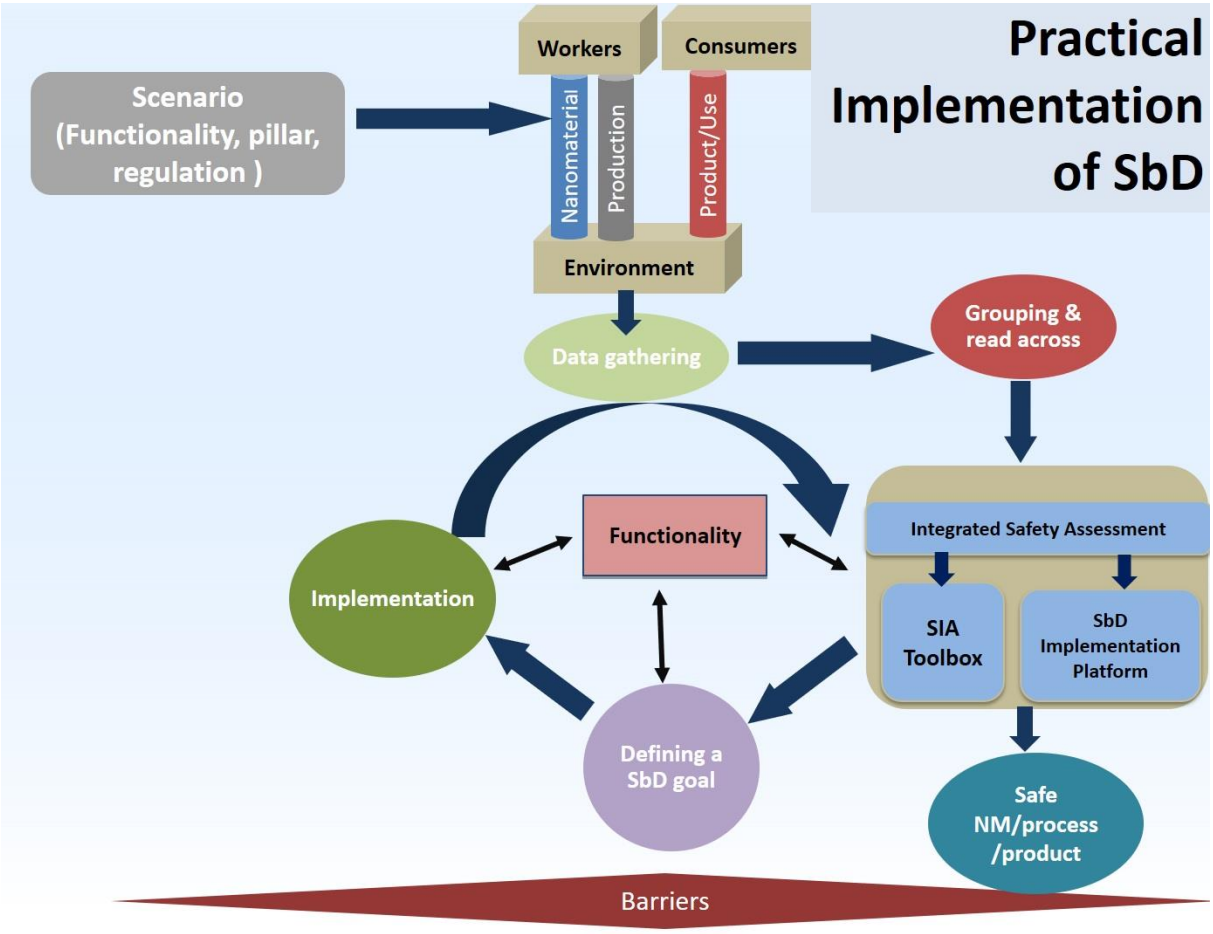
Highlights

- SbD implementation in real-life industrial case studies in the nanotechnology sector
- Risk Assessment, Life-cycle Assessment and Socio-Economic Assessment of nanomaterials

Graphical Abstract



Practical Implementation of Safer by design (SbD). SIA: Safe Innovation Approach

Abstract

The implementation of Safe(r) by Design (SbD) in industrial innovations requires an integrated approach where the human, environmental and economic impact of the SbD measures is evaluated across and throughout the nanomaterial (NM) life cycle. SbD was implemented in six industrial companies where SbD measures were applied to NMs, nano-enabled products (NEP) and NM/NEP manufacturing processes.

The approach considers human and environmental risks, functionality of the NM/NEP and costs as early as possible in the innovation process, continuing throughout the innovation progresses. Based on the results of the evaluation, a decision has to be made on whether to continue, stop or re-design the NM/NEP/process or to carry out further tests / obtain further data in cases where the uncertainty of the human and environmental risks is too large. However, SbD can also be implemented at later stages when there is already a prototype product or process available, as demonstrated in some of the cases.

The SbD measures implemented in some of the case studies did not result in a viable solution. For example the coating of silicon nanoparticles with amorphous carbon increased the conductivity, the stability and reduced the dustiness of the particles and therefore the risk of explosion and the exposure to workers. However the socioeconomic assessment for their use in lithium-ion batteries for cars, when compared to the used of graphite, showed that the increase in performance did not overcome the higher production costs.

This work illustrates the complexities of selecting the most appropriate SbD measures and highlights that SbD cannot be solely based on a hazard and exposure assessment but must include other impacts that any SbD measures may have on sustainability including energy consumption and waste generation as well as all associated monetary costs.

Keywords: nanomaterials, safe by design, risk assessment, life cycle assessment, nano-enabled products, nanotechnology

1 Introduction

The rapid rate at which novel materials are generated requires an agile process to effectively assess and regulate the risks associated to those materials. The development of materials that are safe to humans and the environment from the beginning of the innovation process offers tremendous advantages in a variety of ways (e.g. lower uncertainty of the risks, higher value increased stakeholder confidence, preparedness for future regulation, etc).

While safe by design (SbD) or similar concepts such as green chemistry (Anastas and Warner, 2000), prevention through design (Cowley, 2000, NIOSH 2011) and inherently safe(r) (Dir. 2006/42/EC, Kletz, 2003) are well defined and have been used for decades in various fields (e.g. occupational health and safety, pharmaceutical industry) there is presently no agreed consensus on what SbD encompasses in the nanotechnology sector. Morose et al. 2009 concept design for safer nanotechnology was based on five design principles 1) Size, surface and structure; 2) Alternative materials; 3) Functionalisation, 4) Encapsulation and 5) Quantity reduction) that aim to make safer nano-enabled products. Geraci et al. 2015 reported the views from a discussion on the applicability of principles of "Prevention through Design" (PtD) developed for health and safety (i.e. elimination, substitution, engineering controls, administrative controls and personal protective equipment) for to design safer nanomaterials (NMs), manufacturing processes and nano-enabled products (NEP). The participants agreed that PtD further serves a platform to identify opportunities for a risk-focused dialogue up and down the life cycle.

Cobaleda-Siles et al. (2016), in line with Monrose's approach, advocates for to establish SbD selection rules and synthetic approaches that can be used for the reduction of hazard exposure and the reduction of NMs migration and release, taking into consideration all stages of the life cycle of the NEP.

In 2017, Hjorth et al. reviewed the current SbD concepts and acknowledged that the way SbD is currently communicated tends to treat safety as an inherent material property when it is not and can lead to unrealistic expectations. The authors concluded that SbD should be considered a starting point rather than an end, meaning that products will still need to progress thorough safety evaluations and regulation.

Within the European projects NANoREG and Prosafe (Prosafe, 2017) a new concept was developed where SbD aims at identifying, estimating and reducing uncertainties and risks for humans and the environment along the entire value chain, ideally starting at an early stage of the innovation process (Soeteman-Hernandez et al. 2018, Kraegeloh et al. 2018). This concept advocates that safety should be considered as an integral part of the design process (together with functionality and costs), rather than at a later stage once the process is already well advanced. SbD must thereby also include a life cycle sustainability assessment of the long-term ecological and economic impact (Salieri et al. 2020).

This manuscript describes the implementation of the NANoREG and Prosafe concept (referred to as the NanoReg2 concept) in six industrial case studies. The NanoReg2 project built around the challenge of coupling SbD to the regulatory process, to demonstrate new principles and ideas based on data from value chain implementation studies to establish SbD as a fundamental pillar in the validation of a novel manufactured nanomaterials (NMs). The companies applied SbD measures to the NMs they commercialised to reduce the hazard (HIQ-nano, Group Antolin and Nanomakers), to reduce the exposure to workers (Avanzare, Group

Antolin, Nanomakers), and to reduce the waste and protect the environment (NanoGap) and to the product to protect consumers (nanoComposix).

2 Methods

The implementation of SbD was industry-led with the assistance of technical experts in toxicology, exposure and risk assessment. A task force was created who visited the companies and discussed potential case study proposals with them. Companies then, after considering their capabilities and time-scale of the project presented their SbD innovation plan to the task force who, upon further discussion, approved it. The selection criteria considered whether the implementation was focused on SbD and cover at least one of the pillars explained below the innovation plan objectives were achievable and the timescale realistic (within the confines of the overall project timescales). The case studies involved different stages of the innovation chain (1) idea; 2) concept 3) prototype 4) pilot production; 5) market entry) and a variety of NMs (graphene, carbon nanofibers (CNF), dye doped SiO_2, silver nanowires, silver nanoparticles, silicon based NMs.

Companies had to achieve one or more of the NanoReg2 SbD pillars:

Pillar 1 : safer materials and products by design: This refers to identifying less hazardous NMs for humans and the environment and designing NEPs that, under normal and unforeseeable conditions, do not release free NMs (unless that is a requirement for their performance) to the environment and where the NMs can be recycled at the end of life.

Pillar 2: safer use of products: This consists of evaluating the risks during all uses throughout the product lifecycle in order to optimize defined acceptable uses. Building on the first SbD pillar, when a product has been made as safe as is possible, this second pillar will facilitate an evaluation and determine any potential restrictions on the use of a specific NEP.

Pillar 3: safer industrial production: This pillar aims to enable a better control on the industrial processes along the production chain. The aim is to design processes that eliminate/reduce release of NMs to the workplace and outdoor environment, do not use hazardous chemicals, reduce NM-waste, do not pose a safety hazard (e.g. explosion) and optimize energy consumption.

Before the implementation was started, training on SbD was provided to the six companies in the form of a face-to-face workshop and a technical partner was allocated to each company to advise them during the implementation. Companies were not given a specific protocol, they applied SbD adapting it to their existing decision making processes. Overall the SbD implementation implied the following steps:

1) Scenario Identification: identify the pillar(s) that will be the focus of the implementation, the functionality of the NM and the stage of the innovation process, as this is relevant in terms of the information available on the NM and the adequacy of the risk assessment tools to be used.

2) Preliminary risk assessment for those companies that already had a prototype NM / NEP or set up a process and the type of risk was not clear.

3) Setting up SbD goals, this refers to the ambition of the company.

4) Identify SbD measures to achieve the desired explicitly stated goals.

5) Post SbD measure implementation risk evaluation and sustainability assessment to demonstrate the safety of the NM / NEPs or process and evaluate the impact of the SbD measures.

However, given the timescale of the project we did not follow the companies throughout the entire innovation process. Some companies (Group Antolin, HIQ-nano, Nanomakers) had already identified SbD measures and therefore not all the decisions made for each step is described for every case study.

All the toxicity, exposure and risk assessments (RA), lifecycle assessments (LCA) as well as socio-economic analysis (SEA) to facilitate the SbD process were undertaken by external experts as most companies did not have the human resources to conduct such assessments. The specific methods for these assessments have been reported separately in Jacobsen et al. 2020; Rodríguez-Llopis et al. 2020 and Salieri et al. 2020, but are briefly summarised as follows.

For the human RA, NanoSafer (Kristensen et al. 2010), the Swiss Precautionary Matrix (SPM, Höck et al. 2008), Stoffenamanger-Nano (van Duuren-Stuurman et al. 2012), NanoRiskCat (Hansen et al. 2011), the Weight of Evidence Approach (WoE, Hristozov et al. 2014) and the Sustainable Nanotechnologies Project Decision Support System (SUNDS)[1] were considered. Within each case study, we used the most relevant tool considering the domain of interest (exposure, human hazard or overall risk), the SbD measures taken and the availability of information.

The likelihood of occupational exposure was assessed following the exposure assessment strategy and criteria for classification of exposure in EN17058:2018.

The criteria for the assessment of the human toxicity was done following the method developed as part of the FP7 project "Nanosolutions" and adapted for NanoReg2 (Suarez-Merino et al. 2018). Details of the toxicity assessments are reported in Jacobsen et al. 2020.

For the ecotoxicity assessment we followed the criteria in the European Regulation (EC) No. 1272/2008 on classification, labelling and packaging of substances and mixtures (CLP, 2008). Details of the cytotoxicity in fish cell lines have been published in Kalman et al. 2019 and Barrick et al. 2019.

The LCA was carried out to evaluate all the potential impact of the SbD measures, as before embarking in any changes affecting the production, the company has to check their potential influence not only in terms of hazard and exposure, but also in terms of energy efficiency, resource depletion, emission of substances that contribute to different environmental impact categories, like climate change (*i.e*. global warming potential, GWP), ozone depletion, etc. The LCA was carried out using the software Simapro 8, the Ecoinvent v3.4 database as background database, and the ReCiPe method (at Midpoint level) for the impact assessment (Pennington et al 2004; Goedkoop et al. 2008).

For the Nanomakers case study we carried out a SEA where the base scenario was the use of an electric vehicle battery without NMs.

[1] https://sunds.gd/

3 Results

Table 1 summarises the SbD pillar each company addressed, the situation before the SbD implementation, the SbD measures adopted and the overall result.

205 **Table 1 shows a summary of the companies involved, NMs considered, their application, innovation stage and the SbD measures and results.**

NM/Company Country	Market Sector Stage Gate	SbD Pillar	Before SbD	SbD Measure	SbD Result	Conclusions/Benefits
Graphene AVANZARE (Spain)	Electric coatings & paints (Stage 2 Concept)	Safer process (minimal waste) (upscale production)	No previous prototype for comparison	SbD principles applied: - wet synthesis in water - recycle of waste into new batches Semi-automatic packing for dry product with LEV	Lower exposure as graphene is commercialized in wet form. Reduced handling of dry graphene No liquid waste & very low solid waste.	Significant improvement in product sustainability compared to other synthesis
CNF GRUPO ANTOLIN (Spain)	Automotive (Stage 5 Market entry)	Safer NM (lower toxicity) Safer process (upscale production)	Exposure risk in production & surface treatment stages High hazard potential due to HARN. Impact driven by high energy resources in production. Emission of greenhouse gases	Three candidate CNFs with different degree of impurities & crystallinity (GAtam, GANF, GANFg) Automated pneumatic transport Improve production process	Workers exposure reduced. Comparable for the 3 NMs ≠ CNT Environmental Impact reduced due to reduced emissions.	GATam toxicity comparable to GANF. GAtam production more efficient than GANF. Significant energy savings. Healthier working environment
Fluorescence NMs HIQ-NANO (Italy)	Biosensors (Stage 3 Prototype)	Safer NM (lower toxicity)	QD doped SiO_2 High ecotoxicity due to the presence of Cd	Substitution of QD for a dye doped SiO_2	Lower toxicity. Slightly higher exposure Similar process for both NMs. Changes driven by composition (elimination of Cd).	Similar risk. Reduction in all environmental Impact categories: 5% (Ecotoxicity) to 75% (Ozone Depletion) lower impact per kg of material.
Ag nanowires NANOGAP (Spain)	Photovoltaic panels (Stage 5 Market entry)	Sustainable process	High Ag waste Impact driven by energy demand per Kg AgNF & generated waste Risk of exposure	Change synthesis parameters Automated filtration	Higher process efficiency: contribution to impact categories decrease up to 90%. Reduced exposure but high risk due to the HARN nature of the NM.	Significant improvement in process sustainability
Ag nanoparticles nanoComposix (Czech Republic)	Antibacterial coatings (Stage 3 Prototype)	Safer product (minimum release during use)	Potential consumer exposure to Ag ions	Design solution that limits release of Ag+ during use preserving functionality for longer. Selection of low exposure coating method Selection of purification method with low waste	Low release of Ag+ from trolley coating Low exposure: dip-coating method High energy consumption	Safer product as release of Ag+ during use is insignificant Sustainable product in terms of releases to the environment. Impact due to high electricity consumption & waste generation
Si based NMs NANOMAKERS (France)	Batteries for electric vehicles (Stage 3 Prototype)	Safer NM (lower flammability)	High dustiness High flammability Moderate toxicity	Carbon Coating Increase particle size Si@40nm, Si@C40nm & Si@C75nm	Reduced dustiness Reduced flammability Si@C40nm slightly more toxic Comparable environmental impact SEA: High uncertainty in these results due to lack of data	Considerable lower risk of ATEX for coated NMs. Comparable impact for the three NMs. Higher impact compared to using graphite (without NMs) but better performance A 10% increase in battery capacity generates more costs than benefits.

206 CNT: Carbon Nanotubes; CNFs: Carbon Nanofibers; GANF, GAtam, GANFg: Group Antolin CNFs with with graphitization degrees of 60, 70 and 90% respectively; HARN: High Aspect Ratio
207 Nanomaterials; NPs: nanoparticles; NMs: nanomaterials; ATEX: Explosive Atmosphere

3.1 Avanzare

The main goal of the Avanzare case study was the upscaling of graphene production using a new pilot plant designed within the SbD concept (Table 1). The focus was on developing a safer process with lower energy consumption and minimal workplace and wider environment emissions. This was achieved by developing a wet synthesis method to minimise exposures in the workplace, using graphite, a catalyser and water as main solvent for the exfoliation process. Water is used in a continuous loop where at the end of the batch production process the remaining water is used in new batches, thus eliminating liquid waste. Solid waste from cleaning & maintenance operations is minimal. When the process is finished the graphene slurry is filtered by gravity. The filter retains the solid material as a compact wet graphene dispersion which is immediately packed in plastic bags and then aluminium bags. The graphene properties are shown in Table 2.

Table 2 Graphene properties.

Property	Value
Shape	Platelets
Size (bulk, nm)	Thickness: 1-10 Lateral size: 100-200
Surface area ($m^2\ g^{-1}$)	>200 m^2g^{-1}
Density (kg m^{-3})	2-2.1

Graphene in a water medium is not compatible with all the intended applications and for some uses the dry form is required. This dry graphene is dried in an oven after synthesis. Before the SbD implementation graphene packing was done manually. An exposure assessment was carried out where drying and packing were identified as having a high exposure. Two different SbD options were considered: 1) packing within a fume-hood, 2) a semi-automatic system with local exhaust ventilation (LEV). Option 2 was chosen as it reduced the manual handling and transport to the fume hood. .Task-based personal exposures (of 90 min) of elemental carbon were reduced from 4.2 µg m^{-3} to 1.2 µg m^{-3} (average of the two operators involved in the task) thus demonstrating the efficacy of the SbD measure.

The graphene in the dispersion and the dry graphene showed similar human toxicity except for the *in vitro* inflammation where the graphene in dispersion showed a higher increase in cytokines (Table 3). This might have been due to the presence of endotoxin in the samples, or because of a protective effect of the BSA solution used with the dry graphene. A similar effect was observed with the CNF of Grupo Antolin and this hypothesis is discussed in further detail in Jacobsen et al. 2020.

Table 3 Human toxicity assessment of graphene (Avanzare).

Assay	Graphene in liquid dispersion	Dry graphene	Conclusion
Cytotoxicity (24, 48, 72 hrs, 0.6,32,64 µg cm^{-2} A549, Impedance)	Non toxic	Slightly toxic (after 72 hours)	Dry seems to be slightly more toxic
ROS production (24 hrs, 25&50 µg cm^{-2} A549 & 3T3)	Low	Low	Comparable
In vitro Inflammation (24 hrs,32,64 µg cm^{-2} ,THP-1)	High (IL-8, TNF-α, & IL-1β)	High (IL-8)	Graphene in dispersion > dry graphene
In vivo instillation lung toxicity (rats, repeated exposure over 10 days, recovery up to 28 days)	NA	• Acute inflammation returning to basal level 28 days after exposure • No genotoxicity in lung & liver tissue (Comet assay). • No genetic mutation in blood (Pig-A gene mutation assay) • Alveolar & bronchi alterations.	Comparable
Skin irritation (OECD 439)	Non-irritant	Non-irritant	Comparable
Ocular irritation (OECD 492)	Non-irritant	Non-irritant	Comparable

IC 50: half maximal Inhibitory concentration; ROS: Reactive Oxygen Species; NA: not available

In vivo data suggest that the dry form induces some acute inflammation response which returns to basal level 28 days after exposure. Nevertheless, lung histopathological analysis showed alveolar and bronchi alterations: hyperplasia associated with inflammation leading to bronchial obstruction (bronchiolitis obliterans).

To estimate the overall risk to workers we used NanoSafer, the SPM and the WoE. There were differences in the tools outputs. After the implementation of LEV, the WoE tool indicated, that the risk of exposure was low. In contrast, NanoSafer still showed that in the near field, exposure was still high which was not supported by the measurements. The SPM was not sensitive to the differences before and after the implementation of the exposure control measures but indicated a need for precautionary measures due to the intrinsic characteristics of the NM (reactivity and tendency to form aerosols < 10 µm).

Graphene was also tested for its aquatic ecotoxicity. Representative organisms from multiple trophic levels and ecosystems were selected in the present study to better establish a holistic environmental hazard assessment: *in vivo* on microalgae by studying the growth inhibition, on microinvertebrates by studying the acute toxicity on *Daphnia magna*, and *in vitro* on fish cell lines and mussel cells (cytotoxicity) (Table 4).

Table 4 Environmental toxicity assessment (Avanzare).

Ecotoxicity	Graphene in liquid dispersion	Dry graphene	Conclusion
Algal growth inhibition test (IC50) (OECD 201)	8.5 mg L^{-1} [8.0 - 9.2}	> 10 mg L^{-1}	Dry form slightly less toxic
Daphnia magna acute immobilisation test (EC50) (OECD 202)	60 mg L^{-1} [37.9 – 89.7]	85 mg L^{-1} [54.8 – 136.2]	Dry form slightly less toxic
Cytotoxicity in fish cell lines (72 hrs, 128 µg ml^{-1}) (IC50)			Liquid form non toxic Test interferences in dry form didn't allow to test higher concentrations.
Lysosomal function (Neutral Red)	>256	>128	
Mitochondrial activity (Alamar Blue)	> 128	> 16	
Membrane integrity (CFDA-AM)	>256	>31	
Cytotoxicity in hemocytes cells from mussels			
Mitochondrial activity (Alamar Blue)	>16	> 16	Non Toxic
Membrane integrity (CFDA-AM)	>256	58	Dry form more toxic

IC50: half maximal inhibitory concentration. ; CI95% are given in square brackets

The *in vivo* results showed the liquid dispersion form may be more toxic than the dry from. The *In vitro* test on fish cell lines and mussel cells showed no toxicity whatever the graphene form, dry or in liquid suspension. A slight effect was observed on membrane integrity after exposure to the dry form. However, some interferences with the test were identified.

The production of 50% graphene as a slurry (by eliminating the drying process) reduced the environmental impact by 18% mainly due to lower energy consumption.

3.2 Grupo Antolin

The company Grupo Antolin wanted to upscale production of CNFs focussing on safer and more sustainable CNFs as well as optimization of the production process to reduce emissions to the workplace and the environment.

The company uses two manufacturing processes, both based on CVD (Chemical Vapour Deposition) to make two types of CNFs (GANF and GATam). A third CNF (GANFg) was included in the study for comparison purposes. GANFg is synthesised as GANF but has a higher level of graphitization (Table 5). While the GANF and GATam materials are synthesised using different furnaces and operating times, they are oxidised (to clean the CNF surface) in the same way. The production process involves the following steps: synthesis in a furnace, collection and transport to the oxidation furnace, surface treatment through oxidation, collection, weighing, packing and preparing of dispersions.

The SbD measures included selection of the safer CNF (from three CNFs with different surface functionalization, degree of impurities and crystallinity (Table 5); automatization of the collection steps by pneumatic transport of the GATam, and an automated gravimetric dosing system for the dispersion and packaging stages of all the CNFs. A semi-automatic process with LEV as that applied in Avanzare to collect the dry graphene from the oven was not considered in this case since collection takes place from the furnace and a plume is generated when the furnace is opened. Therefore a fully automated system was considered safer.

285 **Table 5 Physical characteristics of the CNF (Grupo Antolin).**

Physical Characteristics	GANFg	GANF	GATam
Diameter (nm)	20-80	20-80	20-80
Length (nm)	200-10,000	200-20,000	100-10,000
Bulk density (g cm^{-3})	0.08	0.06	0.08
Crystallinity (degree of graphitization, XRD)	≈ 99 %	≈ 70 %	≈ 60 %
Specific surface area (BET m^2 g^{-1})	80-120	100-170	70-140
Carbon purity (TGA in N_2)	>99%	>85%	>80%
Oxygen content (CHNS-O)		≈ 5 %	≈ 10 %
Electrical resistivity (Ohm m)	$1*10^{-4}$	$1*10^{-3}$	$1*10^{-3}$

286
287 Adapted from the Grupo Antolin Carbon Nanofibres technical data sheet.
288
289 The results from the RA showed a risk reduction mostly driven by the reduction in the exposure due
290 to the automatization of the GATAm collection (results shown in Rodríguez-Llopis et al. 2020), having
291 the three fibre types comparable toxicities (Table 6).
292
293 The selected assays were based on the HARN nature of the NM, being inhalation the main
294 occupational exposure route (Table 6).
295

Table 6 Human toxicity assessment of CNFs (Grupo Antolin).

Endpoint		GANFg	GANF	GATam	Conclusion
Cytotoxicity (IC50) (24, 48, 72 hrs, 0.6,32,64 μg $cm^{-2,}$ A549, Impedance)		NA	No effect	No effect	Comparable
ROS generation (24hrs, 25&50 μg cm^{-2} A549)					
	In BSA*	No evidence	Evidence	Evidence	GANF = GATam > GANFg
	In Water#	Low	Moderate	Moderate	
In vitro Inflammation (24 hrs, 32, 64 μg cm^{-2}, THP-1)					
	In BSA*	Low (IL-1β)	No evidence	No evidence	GANFg> GAtam=GANF
	In water#	Low (IL-1β)	No evidence	No evidence	GANFg> GAtam=GANF
Genotoxicity (A549)					
Comet assay with & without Fpg		Negative	Negative	Negative	
Micronucleous (OECD 487)	in BSA*	Equivocal	Positive	Positive	GANF=GAtam > GANFg
	In water#	Negative	Equivocal	Equivocal	
In vivo instillation lung toxicity (mice)					
Genotoxicity (Comet assay)					
	BAL cells	Positive	Positive	Positive	GANFg > GAtam=GANF
	Lung	Positive	Negative	Negative	
	Liver	Negative	Negative	Negative	
	Inflammation	All materials were inflamogenic with a response similar to to Carbon Black Printex 90 (14 nm)			Comparable (GANFg show a faster return to baseline)
In vivo instillation lung toxicity (rats)					
Genotoxicity (Comet assay)					
	BAL cells	Negative	NA	Negative	Comparable
	Lung	Negative		Negative	
	Liver	Negative		Negative	
	Pig-A gene mutation	Negative	NA	Negative	Comparable
	Inflammation	Temporary	NA	Lasted over 3 days but return to baseline level after 28 days except for TNF-α	Higher for GATam
	Hystopathology	Bronchiolitis obliterans that tend to decrease over time		Bronchiolitis obliterans that tend to decrease over time	More frequent for GATam
Skin irritation (OECD 439)		Non-irritant	Non-irritant	Non-irritant	Comparable
Ocular irritation (OECD 492)		Non-irritant	Non-irritant		Comparable

IC50: half maximal inhibitory concentration; ROS: reactive oxygen species; NA: not available. BSA: bovine serum albumin. Fpg; formamidopyrimidin glycosylase *High energy sonication process (Nanogenotox dispersion protocol), which led to significant fibre shortening. #Dispersion in water with very mild or no sonication. Rats and mice were exposed to equivalent doses. Analysis were performed 1, 3, and 28 days after exposure. Full details are reported in Rodríguez-Llopis et al. 2020.

Overall GANF and GAtam showed a similar toxicological profile and the slight differences in crystallinity and impurity content (Table 4) did not affect their hazard response. The comparison with the GANFg fibres (with a higher crystallinity but without impurities) yield different results in the *in vitro* and *in vivo* tests. The results from the *in vitro* inflammation showed a higher response for GANFg, but in the *in vivo* study in mice the response was similar for the three materials whilst for the *in vivo* study in rats GAtam showed a higher inflammation response than the GANFg.

The lower effect on ROS production with the use of BSA was attributed to the formation of a BSA protein corona around the fibres which affects their interaction with the cells decreasing their biological response. Previous studies have reported this effect with carbon materials (Sengupta et al. 20015; Bai et al. 2016). Further details on the toxicity assessment are reported in Rodríguez-Llopis et al. 2020.

The CNFs were also tested for their environmental toxicity in algae, daphia magna, fish cells and mussels (Table 7).

Table 7 Environmental toxicity assessment of CNF (Grupo Antolin).

Endpoint	GANFg	GANF	GATam	Conclusion
Algal growth inhibition test (IC50) (OECD 201)	8.5 mg L^{-1} [7.4 – 12.1]	3.1 mg L^{-1} [2.4 - 3.3]	2.1 mg L^{-1} [1.9 - 2.3]	GAtam ≈ GANF > GANFg
Daphnia magna acute immobilisation test (EC50) (OECD 202)	5.8 mg L^{-1} [4.8 - 7.8]	9.9 mg L^{-1} [8.1 – 12.0]	8.9 mg L^{-1} [7.2 - 10.6]	Comparable
Daphnia magna chronic toxicity test (EC50) (OECD 211)	6.2 mg L^{-1} [4.7 - 8.2]	1.6 mg L^{-1} [1.4 - 1,8]	0.32 mg L^{-1} [0.1 - 0.4]	GATam> GANF > GANFg
Cytotoxicity in fish cell lines (72 hrs) (IC50)				
Neutral Red	> 256 mg L^{-1}	256 mg L^{-1}	165 mg L^{-1}	GAtam > GANF > GANFgGANF > GANFg and GATam GANF > GATam > GANFgNon Toxic
Alamar Blue	32.0 mg L^{-1}	18.9 mg L^{-1}	46.7 mg L^{-1}	
CFDA-AM	252 mg L^{-1}	37.6 mg L^{-1}	89.9 mg L^{-1}	
In vivo test on mussels	No mortality after 1 day of exposure Mussels extremely efficiency filtering CNF			Non Toxic

IC50: half maximal inhibitory concentration; EC50: half maximal effect concentration. CI95% are given in square brackets. Further details on the ecotoxicity are reported in Barrick et al. 2019.

The data obtained *in vivo* show that the CNFs are ecotoxic toward the freshwater organisms. GANF and GAtam have a comparable ecotoxicity as observed in the human toxicity assessment while GANFg appears to be less toxic.. Further details on the ecotoxicty have been published in Barrick et al. 2019 and Kalma et al. 2019.

Regarding the exposure the WoE model indicates, in accordance with the experimental results, that the potential exposure after the SbD implementation, is low, NanoSafer indicated that for some stages, storage and dispersion in water of GAtam, exposure is still high. The SPM was not sensitive to the differences before and after the SbD indicating a need for precautionary measures due to intrinsic characteristics of the NM (reactivity and stability).

The LCA showed that the impact of the emission during production has limited impact on the global environmental impact. Nevertheless, the reduction of the emissions due to the SbD implementation was noticeable. The method for producing GATam CNF was more energy efficient, due to a higher yield of the CVD reaction, and presented a lower environmental impact than the GANF method. A full description of the case study is reported in Barrutuetabeña et al. 2020.

3.3 HIQ-nano

HIQ-nano manufactured Quantum Dots (CdSe) doped silica nanoparticles used a tracers in vitro biological testing. The SbD goal was to develop a new particle with a lower toxicity to reduce the risk to humans and the environment but maintaining the fluoresce properties. The SbD measure was the substitution of CdSe for an organic pigment. HIQ-Nano came up to the decision for this specific SbD option base on safety considerations of the pigment that appeared better than the known toxicity of Cadmiun and their good performance as cellular marker in vitro .

Therefore, the case study focused on the comparative functionality and risk assessment of both particles. The dye doped SiO_2 showed higher fluorescence properties compared to the QDs. QDs-doped particles exhibited a much more varied appearance, as well as an increased background compared to the dye doped SiO_2. Both particles were synthesised in an enclosed system with a larger duration for the dye doped SiO_2 NPs. The production process in terms of likelihood of exposure was low for both NPs. The overall risk was estimated using the CB Nanotool, the Swiss SPM and WoE. The LCA was only applied to the production stage as the particles are used in the human body and therefore a full LCA is not appropriate. The toxicity characterization is shown in Tables 8 (human toxicity) and Table 9 (environmental toxicity).

Table 8 Human toxicity assessment of fluorescence NPs (HIQ-nano).

Endpoint	QDs doped SiO_2 NP	Dye doped SiO_2 NP	Conclusion
Cytotoxicity (24, 48, 72 hrs, 0.3, 3, 16, 33 µg cm^{-2}, A549, Caco-2, HFF-1)	No effect	No effect	Comparable
ROS (5 min, 30min, 1h, 3h, 8h 24h, 3, 33, µg cm^{-2} Caco-2,)	Evidence; 30 min to 3h	Evidence; 30 min to 8h	Comparable
ROS (96 hrs, 0.3, 3, 16, 33, µg cm^{-2} Caco-2)	No evidence	No evidence	Comparable
In vitro inflammation (24 hrs, 10, 104 µg ml^{-1}, RT-PCR in HFF-1 cells)	Slight upregulation of gene expression at 8-30h exposures. Strongest effect to induce IL6 and IL8 at 24-30 h	A significant upregulation of gene expression at 8-30h exposures. Strongest effect to induce IL6 and IL8 at 24-30 h	Dye > QD SiO_2 NPs.
Genotoxicity (A549) Micronucleus assay (OECD 487)	Equivocal although a statistically significant increase with conc 1 & 5 nM	Equivocal although statistically significant increase with conc of 5 nM	QDs doped SiO_2 slightly > dye doped SiO_2

Table 9 Environmental toxicity assessment of fluorescence NPs (HIQ-nano).

Endpoint	QDs doped SiO_2 NP	Dye doped SiO_2 NP	Conclusion
Cytotoxicity in fish cell lines (IC50) 0.2-256 ug/mL^{-1}			
Neutral Red	> 256 mgL^{-1}	119 mgL^{-1}	Dye doped SiO_2> QDs doped SiO_2
Alamar Blue	> 256 mgL^{-1}	88.8 mgL^{-1}	
CFDA-AM	> 256 mgL^{-1}	156 mgL^{-1}	

The results from NanoSafer and the SPM showed an increased risk after the SbD implementation (substitution of QDs by a dye) driven by the longer exposure periods and frequency of the production process. However, this potential risk was not confirmed in the exposure measurement campaign (results not shown).

The WoE was sensitive to the reduction in the hazard and as the hazard score due to the presence of toxic substances for the dye doped SiO_2 NP was lower (medium, compared to high for QDs due to the higher toxicity of the Cadmium present in the latter) the overall risk was reduced.

The LCA showed the major impact was due to the waste reduction during the production of the dye doped SiO_2 NPs due to the smaller amount of water required. Ozone depletion and fresh water ecotoxicity was also reduced with the introduction of the dye doped SiO_2 NPs.

3.4 NanoGap

NanoGap produced silver nanofibers for electrical applications. This case study was focused on the safer production pillar. The goal was to investigate potential exposure scenarios and introduced SbD measures to optimize the production process so as to reduce the amount of waste (silver nanofibers, unreacted silver and solvent) and the environmental impact.

To optimize the production process the different forms of silver during the purification process and in the final waste were characterized. Eighty percent of the silver mass in the waste was in the shaped of pseudo-spherical silver particles and the rest as nanowires. A first approach was to design a recycling process for the silver in the waste. However, the LCA results for the production process showed this had a high impact in terms of the energy and resources needed. Instead the production process (duration, temperature and pressure) was modified to reduce the amount of waste generated.

The process characteristics before and after the SbD implementation are shown in Table 10.

Table 10 Waste generation before and after the SbD implementation (NanoGap).

Parameter	Before SbD	After SbD
Ag nanowires dimensions	120 nm & > 20000 nm long	70 nm & >25000
Reaction rate (%)	41	90
Handling time (hrs)	28	1.7
Volume of solvent waste (L)	256	22
Ag waste (gL^{-1})	1.40	0.2

The new process resulted in a 50% reduction of the silver waste. The nanofibers were however slightly shorter (70 nm smallest dimension compared to 120 nm). The conductivity was within the desired range.

The exposure assessment revealed the presence of airborne nanofibers. Despite the entire process was wet, any splashes during product collection or items contaminated with the solution can give raise to suspended nanofibers once the solvent evaporates (Figure 1).

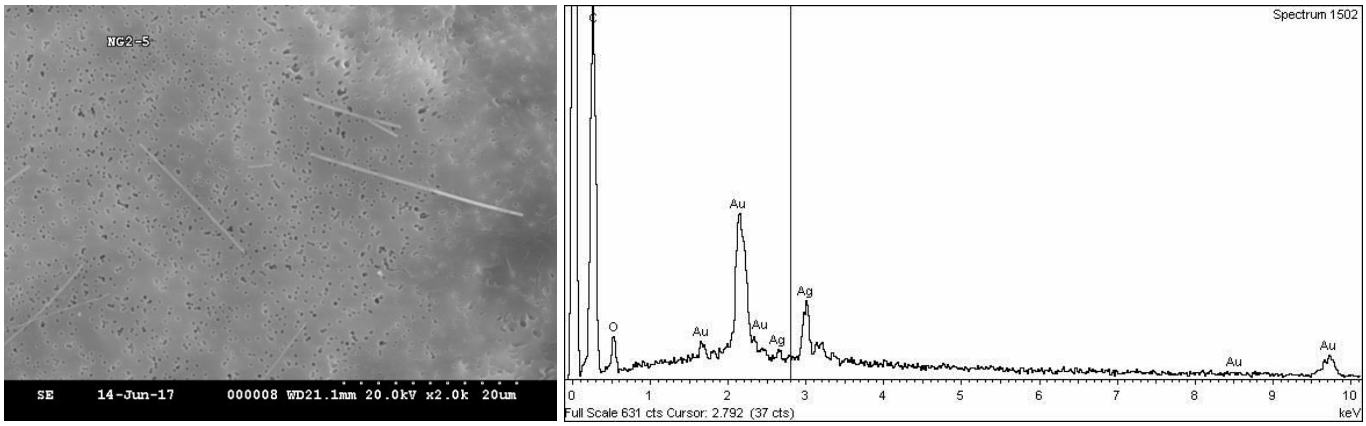


Figure 1 SEM (left) and EDX (right) of silver nanofibers collected during the production process.

A protocol for collection and cleaning was established to minimize the splashes and to clean any devices and surfaces in contact with the solution.

The exposure to workers before and after the SbD measures was also evaluated using several control banding tools. The SPM indicated the need of a precautionary need mostly driven by the hazard properties of the rigid Ag nanofibers. No differences were found before and after the implementation since good hygiene practices is not a parameter considered in the tool. The NanoSafer classified the risk after the SbD implementation as higher due to the smaller diameter of the nanofibers that results in a higher aspect ratio of the NM.

3.5 nanoComposix

The nanoComposix case study dealt with the development of antibacterial coatings for trolleys using silver nanoparticles (AgNPs). The overall goal of the case study was to determine if AgNPs used in a solution to coat trolleys were safe for workers and consumers and suitable for market application before moving on to pilot production. The trolleys were made of zinc-coated unvarnished material, and the AgNP were added to the coating solutions. Two different methods for applications of AgNPs were taken into consideration: a) dip coating (30 nm AgNPs); b) spraying (75 nm AgNPs). Table 11 shows the particle characteristics.

Table 11 Physico-chemical characteristics of AgNPs (nanoComposix).

Parameters	Ag NP-1	Ag NP-2
Matrix	NPs in lacquer	NPs is spray
Primary particle size (nm) (TEM, crystallite size)	30	75
Shape	Spherical	Spherical
Surface area (TEM)	19.4 $m^2 g^{-1}$	7.4 $m^2 g^{-1}$
Silver concentration	30 mg L^{-1}	30 mg L^{-1}

AgNPs are well established to be an antibacterial effective method for the treatment of skin bacterial infections. The Ag ions are released from the particle surface, with higher releases for larger surface area and smaller AgNP. Therefore the 30 nm Ag NPs might lead to a worst exposure scenario compared with larger particles. Also, edges and damaged particle surfaces may release more ions than necessary leading to a reduction in product life time and an increase in potential hazard as an excess of AgNP on the skin can cause irritation or argyria (grey-black staining of the skin and mucous membranes) in damaged skin (Hadrup et al. 2018). The released of silver from the trolley following incubation with artificial human sweat (Midlander et al. 2016), was determined by ICP-OES following ISO 11885:2009 (Table 12).

The antibacterial properties of the trolley's handle samples were tested by Agar diffusion test using Escherichia coli (K−12) and Bacillus subtilis (NCIB 3610) as reference strains for Gram-negative and Gram-positive bacteria, respectively.

The release of Ag NPs due to abrasion of the trolley parts was studied using the TABER© abrasion test (ASTM, 1996). The stress applied with the abrader simulates the typical applied in a domestic solicitation and addresses the question of possible long-term exposure (Vorbau et al., 2009; Hassan et al 2010). Ag NPs were not identified in the filter samples of the aerosols analysed by Transmission Electron Microscopy-Energy Dispersive X-ray Spectroscopy (TEM/EDS) for either the coated or the sprayed trolley parts.

Cytotoxicity, genotoxicity and ROS experiments were carried in human epidermal cells (HaCat) as the main exposure path was dermal (Table 12). Cells were incubated in the artificial sweat samples in contact with the coated parts of the trolley.

Table 12 Human toxicity assessment for Ag NPs (nanoComposix).

Endpoint	30 nm AgNP Coated technology	75 nm AgNP "Sprayed " technology	Conclusion
Release of Ag+ (ISO 11885:2009)	11 ± 3 ppm	10 ± 2 ppm	Comparable
Antibacterial activity against E.coli*, 1 year after production (diffusion test: 2, 4, and 24 h)	13, 17, and 51 %	24, 37, and 45%	Comparable Higher bactericidal effect at prolonged contact
Cytotoxicity (15 and 30 min, 1h , HaCat cells,)	No significant difference in viability compared to the uncoated control trolley.	No significant difference in viability compared to the uncoated control trolley.	Comparable
Cytotoxicity (15, 30 mins, 1h, 3D cell model (NHEK))	Moderate to Low	Low	Comparable
ROS generation 15, 30 mins, 1h,HaCat cells)	No significant difference from control	No significant difference from control	Comparable
Genotoxicity (HaCat cells) Micronucleus assay (OECD 487)	Equivocal	Equivocal	Comparable
Skin irritation, at 15 min, 30 min, 1h (OECD 439)	Non-irritant	Non-irritant	Comparable

NHEK: normal, human-derived epidermal keratinocytes

For the RA screening, NanoRiskCat and Swiss Precautionary Matrix were selected as control banding tools to assess the risk of consumers for both applications a) dip coating (30 nm AgNPs); b) spraying (75 nm AgNPs). This was accompanied by a complete RA based on scientific literature.

The control banding tools suggests possible exposure to workers and possible hazard, for both materials and processes (coating and spraying).

The LCA included the production of the 30 nm AgNPs, production of the lacquer containing the AgNPs and the actual dip coating process. At the level of production of AgNPs the functional unit of 1 kg of nanoparticle was applied. The LCA results revealed that the electricity consumption -used during the actual NP manufacturing process was the main contributor (with 75-92%) to all the impact categories investigated, followed by the consumption of chemicals (5-45%). The production of the Ag containing solution is a simple process in which a liquid solution is produced by mixing AgNPs and water. In this process no energy is consumed and no emissions are released. Therefore, the potential impacts are attributed to the liquid solution in which AgNPs are dispersed. To account the potential impact of the "dip coating process" 1 m^2 of treated (trolley) surface was chosen as functional unit. The treatment of the waste water is the main contributor to the overall impacts, followed by the used nano-Ag solution (10-35%).

3.6 Nanomakers

The company Nanomakers wanted to modify anode nanoparticles to increase the performance of Lithium-ion batteries. The company used pure silicon NPs due to their higher electrochemical performance compared to graphite. The SbD goal was to achieve a NP with a higher stability and performance, lower risk of explosion and low human and ecotoxicity.

In order to achieve that goal the company increased the NP size from 40 to 75 nm (reducing the alveolar deposition of inhaled aerosols) and coated the NP with amorphous carbon which increased the conductivity, increased stability and reduced the dustiness (and the explosivity as a consequence).

Tables 13 and 14 show the results from the human and environmental hazard assessment respectively. For the RA we used control banding tools (i.e. NanoRiskCat and Precautionary Matrix), the SUNDS decision system tool and WoE. LCA and SEA were also carried out.

The carbon coating resulted in a reduction of the dustiness from 1,163 (Si, 40 nm) to 150 (Si, 40 nm carbon coated) and to 21 mg kg^{-1} (Si, 75 nm carbon coated) resulting in a reduction of the explosion severity to class 1 for Si 75 nm carbon coated (comparable to some bulk silicon powders and sugar).

Table 13 Human and environmental toxicity assessment for pure Si 40nm, Si 40 nm and Si 75nm coated with carbon referred to as Si@C40 nm and Si@ 75 nm respectively (Nanomakers).

Endpoint	Si 40 nm	Si@C40 nm	Si@C75 nm	Conclusion
Cytotoxicity (24 hrs, 1, 3, 10, 20 µg cm^{-2} A459/THP-1)	Slightly toxic	No effect	No effect	Si > Si@C40 = Si@C75
ROS generation (24 hrs, 1, 3, 10, 20 µg cm^{-2} A459/THP-1)	No effect	No effect	No effect	Comparable
In vitro Inflammation (24 hrs, 1, 3, 10 µg cm^{-2} A459/THP-1)	High (IL-1β, IL-6) Negative (IL-8 & TNF-α)	High (IL-1β ,IL-6) Low (TNF-α) Negative (IL-8)	High (IL-1β , IL-6) Negative (IL-8 & TNF-α)	Si = Si@C75 > Si@C40 Si@C40> Si = Si@C75
Genotoxicity (A549)				
Comet assay with & without Fpg	Negative	Negative	Negative	Comparable
Micronucleous (OECD 487)	Equivocal	Equivocal	Equivocal	Comparable
In vivo instillation lung toxicity (rats)*				
Genotoxicity (Comet assay)				Comparable
BAL cells	Negative	Negative	NA	
Lung	Negative	Negative		
Liver	Negative	Negative		
Pig-A gene mutation	Negative	Negative	NA	Comparable
Inflammation	Temporary	Persistent after 28 days exposure	NA	Si@C40nm > Si
Histophatology	Bronchiolitis obliterans but tend to decrease in time	Bronchiolitis obliterans but tend to decrease in time	NA	More intense lesions for SiΩC@40
Skin irritation (OECD 439)	Non irritant	Non irritant	Non irritant	Comparable
Ocular irritation (OECD 492)	Non irritant	Non irritant	Non irritant	Comparable

ROS: reactive oxygen species; Fpg; formamidopyrimidin glycosylase; * single exposure, recovery up to 28 days.

The *in vitro* data indicates Si has a higher toxicity than the coated NPs. However the *in vivo* data, which represents the toxicity in the long term (lungs were examined after 28 days of exposure), showed that the SiΩC@40 NPs were slightly more toxic than Si because of their persistent inflammation parameters (cytokine secretion and BAL cells influx).

Table 14 Environmental toxicity assessment of pure Si 40nm, Si 40 nm coated with carbon (Si@C40 nm), and Si 75 nm coated with carbon Si@C75 nm.

Endpoint	Si 40 nm	Si@C40 nm	Si@C75 nm	Conclusion
Algal growth inhibition test (IC50) (OECD 201)	No effect (max con. 5 mgL^{-1})	No effect (max con. 5 mgL^{-1})	No effect (max con. 5 mgL^{-1})	Comparable (no effect)
Daphnia magna acute immobilisation test (EC50) (OECD 202)	>100 mgL^{-1}	>100 mgL^{-1}	>100 mgL^{-1}	Comparable (no effect)
Daphnia magna chronic toxicity test (OECD 211)	No effect (max con. 25 mgL^{-1})	No effect (max con. 25 mgL^{-1})	No effect (max con. 25 mgL^{-1})	Comparable (no effect)
Cytotoxicity in fish cell lines (EC50) CFDA-AM	129 mgL^{-1}	123 mgL^{-1}	>256 mgL^{-1}	Comparable for Si@40nm & Si@C40nm. No effect for Si@C75nm

IC50: half maximal inhibitory concentration; EC50: half maximal effect concentration. CI95% are given in square brackets.

The three NMs had comparable ecotoxicities except for the cytotoxicity in fish cell lines where the 40 nm NMs (coated and uncoated) showed a higher toxicity than Si@C75nm.

The control banding tools NanoRiskCat and the Swiss Precautionary Matrix were not sensitive to the small differences between the materials and processes and provided the same risk band: medium exposure risk for consumers, workers and the environment and insufficient data to assess the hazard effects. The results of the Swiss Precautionary Matrix indicate the need of precautions for workers and the environment.

NanoSafer was thought to be the most appropriate tool in this case since the material was a powder and there was available data on the dustiness. However, the coated materials, which have medium (SiC@40nm) and low dustiness (Si@C75nm) compared to the high dustiness of the uncoated materials, resulted in a higher risk since coatings are a cause of concern in NanoSafer (by principle).

The characterization factors (fate factor, effect factor and an exposure factor) estimated as part of the LCA analysis for the three NPs were comparable, despite the Si carbon coated NPs required an extra production step and an additional raw material.

For the SEA analysis the base scenario for comparison was the use of graphite (without NMs). The SEA results showed that the increase in performance did not overcome the higher production costs. However, due to the lack of data several assumptions were made and the data uncertainty was high.

4 Discussion

This study demonstrated the implementation of the Nanoreg2 SbD concept which aims to identify, estimate and reduce uncertainties and risks for humans and the environment along the entire value chain, ideally starting at the earliest stage of the innovation process.

Although most companies carried out an assessment to comply with the CLP Regulation, a full and integrated process where functionality, human and environmental aspects are considered together was not followed in any of the companies. During the discussions with the task force to approve the SbD proposal it was evident that the approach required the involvement from different departments (R&I, production, finance) and expertise that in some cases was not available at the company especially in regard to the toxicity and risk assessment. The issue of how safe a NM/NEP and process had to be so as to be labelled SbD was also extensively discussed and the creation of a SbD label was suggested to incentive other companies. The main view was in line with Hjorth et al. 2017: SbD is a process rather than a “property”. SbD can be used to address concerns at an early stage and show

that a clear and defined process is used to address potential and actual hazards associated with NMs. Also, the process is not linear but iterative, if a reduction in risk is not achieved the cycle starts again until a compromise between risk, functionality and cost is reached.

Overall the companies were satisfied with the process and the results although the overall output diverged from the stated goal, as for example in HIQ-nano where the substitution of the QD for the dye did not result in a significant reduction of toxicity. Others where the SbD was based at the start like Avanzare and nanoComposix acknowledged the advantages of assessing the risk along the functionality as the innovation progresses.

The results from the case studies highlighted the need to better understand the extent of changes required in the NMs / NEPs properties to produce a significant change in the hazard and exposure. It is important to also understand the impact of such change on other properties during large scale production, since in some instances some safety aspects are improved at the cost of others. For example, in the Group Antolin case study the higher crystallinity was achieved by a higher temperature process, and the resulting CNFs had lower impurities. The differences in crystallinity did not result in significant differences in the inflammation response, possibly because of the higher content in impurities of the CNF with lower crystallinity. Also, further work is required on the relationships between physico-chemical properties, hazard and functionality.

In the case of Nanomakers, the results from the in vitro and in vivo assays did not agree, so it was difficult to conclude the amorphous carbon coating reduced the toxicity. The coating did reduce the dustiness of the particles and therefore the risk of explosion and the worker's exposure. The coating also increased the conductivity and stability of the particles. However, the socioeconomic assessment for their use in lithium-ion batteries for cars, when compared to the used of graphite, showed that the increase in performance did not overcome the higher production costs.

For the toxicity assessment, OECD guidelines were used when available or Standard operating procedures (SOP) developed in other projects (eg. NanoTEST, NANoREG, Nanosolutions). However, not all the assays have an SOP or guideline. Moreover, in some cases there is more than one assay suitable to reach the same endpoint. Different assays with one endpoint could also give different results, therefore several assays have been used in each case study for the same endpoint. The same could be said about the experimental systems, for the same assay different cell lines or primary cultures could be used and each one could respond in a different way to the exposure of NMs. In the case studies, cell lines that were closer to the exposure routes of the NMs were used. A standardisation of assays will facilitate the testing and the compliance with regulation. This standardisation is now being performed in some ongoing H2020 EU projects, such as PATROLS (https://www.patrols-h2020.eu/) or RiskGONE (https://riskgone.wp.nilu.no/) and this will overcome some of the limitations of the assays performed.

There is no single tool available that can estimate the overall risks, offer SbD solutions based on the risks and estimate the impacts of such solutions. The selection of the risk assessment tools will depend on the safety aspect being addressed (human hazard, environmental fate, human exposure) and the availability of the information needed. A preliminary risk assessment was performed for all the case studies using at least two different control banding tools. This exercise allowed to identify point of concern where SbD measures could be needed and also gaps in information that should be filled. For comparative purposes The Swiss Precautionary Matrix that covered different safety aspects (workers, environment, consumers) was used in all case studies It is also a simple tool that uses default values for unknown information. The other tool varied according to the case study. Nanosafer, Control Banding Nanotool or Nanoriskcat were used. Control banding tools are less data demanding but they give a qualitative result so they are more appropriate for risk screening for the early stages of the innovation process identifying points of concern. For the purposes of SbD, where small differences in the properties of NMs and production processes are compared, control banding tools are, in general, not sensitive enough. These tools were designed under the precautionary principle and, in general,

are too conservative. However, they are still useful for the purposes they were created as a screening tool to prioritize areas or NM of concern before implementing SbD.

To determine the effectiveness of the SbD measures semi-quantitative or quantitative although more data demanding tools such as SUNDS, GUIDEnano or WoE may be required for a comparative risk assessment. The disadvantage is that the required data is sometimes not available in the first stages of the innovation.

The use of grouping approaches (e.g. Giusti et al. 2019) to read-across hazard endpoints of unknown NMs, based on a source material would have helped to reduce the number of tests carried out and speed up the hazard assessment. However, this approach was not used due to the lack of sufficient data available on the studied NMs at the time of the implementation.

In general, smaller companies reckoned it would have been difficult to implement SbD without the assistance of the technical experts. The main barriers identified were the terminology around SbD at the start of the project, the lack of data available, the cost of the testing required to produce data, the time invested in the planning, data gathering and interpretation, a clear path to demonstrate the SbD result, and the lack of regulation. Despite these challenges most companies showed an interest to apply SbD in future innovations.

Based on the experience and knowledge gained through this study a guidance for the nanotechnology industry was developed and it is provided in Sánchez Jiménez et al. 2020. However to overcome the barriers identified during the implementation of a trusting environment as that described in Soeteman-Hernandez et al. 2018 is essential to share data, experiences and expertise. The risk assessment tools have to be more sensitive to changes in hazard and exposure, and toxicity assays have to be standardized so data is comparable.

5 Conclusion

This study has showed the complexities and barriers of the practical implementation of the NanoReg2 SbD concept as well as the benefits of reducing risk uncertainties along the innovation process instead of doing it at the end.

SbD, or similar concepts are implemented in other sectors. Whilst different contexts bring different challenges it is important to collate the shared experiences and knowledge to encourage and facilitate the application of the concept to all industrial sectors. Overall the nanotechnology companies that participated in this study found value on the application of SbD. Avanzare shifted to zero liquid waste and almost eliminated employee handling of graphene in powder form. Group Antolin reduced workers exposure and was able to select the most efficient method for the production of CNFs (the method used for GATam CNFs). HIQ-nano was able to compare the toxicity of the both materials and think of new solutions. NanoGap reduced in 50% the silver waste. Nanomakers reduced the risk of explosion, workers exposure and was able to assess the financial viability of the SbD measures.

The implementation of SbD in the nanotechnology sector requires expertise in material science, chemical engineering, toxicity, exposure and risk and considerable amounts of data. To streamline the implementation and make it affordable for companies data on physico-chemical properties, hazard and exposure should be shared through robust and reliable databases. Training on how to use the databases and risk assessment tools should be provided. This would facilitate the SbD implementation and will further progress the development of sustainable NEPs.

6 Acknowledgements

This work was performed within the EU project NanoReg2, funded by the Horizon 2020 Framework Programme of the European Union under Grant Agreement Number 646221. We gratefully acknowledge the contributions of all the industrial partners and the entire NanoReg2 consortium for their continuous support and advice on the case studies.

602 All the *in vivo* experiments were performed in compliance with EU Directive 2010/63/EU for animal
603 experiments and institutional guidelines.

604 7 Competing interests

605 All companies that participated in the study commercialised NMs. GAIKER and INERIS have worked as
606 consultants for Grupo Antolin and Nanomakers respectively. GAIKER and Safenano (IOM) have worked
607 as consultants for Avanzare.

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