

Implementation of the NANoREG Safe-by-Design approach for different nanomaterial applications

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Abstract. The Safe-by-Design (SbD) concept is already in use in different industrial sectors as an integral part of the innovation process management. However, the adopted approach is often limited to design solutions aiming at hazard reduction. Safety is not always considered during the innovation process, mainly due to the lack of knowledge (e.g. in small and medium companies, SMEs) and the lack of dialogue between actors along the innovation chain. The net result is that safety is considered only at the end of the innovation process at the market authorization phase, with potential loss of time and money. This is especially valid for manufactured nanomaterials (MNM) for which the regulatory context is not completely developed, and the safety knowledge is not readily available. In order to contribute to a sustainable innovation process in the nanotechnology field by maximising both benefits and safety, the NANoREG project developed a Safe Innovation approach, based on two elements: the Safe-by-Design approach which aims at including risk assessment into all innovation stages; and the Regulatory Preparedness, focused on the dialogue with stakeholders along the innovation chain. In this work we present some examples about the implementation in our Laboratory of this approach for different MNM applications, covering different steps of the innovation chain. The case studies include: the feasibility study of a medical device including substances, for topical application; the testing of two potential nanotech solutions for the consolidation of cultural heritage artifacts; the testing of coatings already on the market for other uses, which was tested as food contact materials (FCM) to evaluate the conformity to food applications. These three examples represent a good opportunity to show the importance of NANoREG SbD and Safe Innovation Approach in general, for developing new nanotechnology-based products, also highlighting the crucial role of EU ProSafe project in promoting this concept to industries and interested stakeholders.

1. Introduction

The European Commission, together with National Authorities is working toward a research agenda supporting the regulatory implementation of nanosafety. This effort is included in the European innovation agenda, including the creation of a sustainable job environment, re-industrialization of Europe, the digital single market, and the energy union. All these goals may benefit from the implementation of nanotechnology. Regulatory approach to nanosafety was pursued in a systematic way starting with the FP7 project NANoREG, which involved a huge number of partners from inside and outside Europe. The scope of NANoREG was to develop tools supporting the generation of robust data for regulatory use, and concepts allowing a rational and faster regulatory assessment of nanomaterials.



Among the concepts developed in NANoREG, the Safe Innovation Approach (SIA) is a systematic framework supporting pre-regulatory and regulatory safety assessment as well as the dialogue between regulators and decision makers, researchers, and especially industry. The SIA is designed to be used mainly by small and medium companies (SMEs), since they are lacking the means to properly tackle the regulatory and sometimes the technical challenges posed by the implementation of nanotechnology in products and processes. This scenario calls for the intervention of private organizations acting as intermediaries between the European and national authorities and companies. These organizations concentrate the technical and regulatory knowledge to be transferred to companies in a context of Responsible Research and Innovation (RRI). Safe Innovation is a mean to implement the RRI in SMEs, and in this work it was evaluated through three case studies the applicability of SIA by an excellence center, discussing what was possible and where were the hurdles and limitations in relation to: efficacy of innovation, nanosafety assessment, and the dialogue with regulators.

2. Safe Innovation approach

The SIA approach, as developed by RIVM and TEMAS in FP7 NANoREG project, disseminated in the H2020 ProSafe project, and under implementation in H2020 NANOREG² project, is described in Figure 1. It includes two parts, a Safe-by-Design (SbD) approach, which aims at reducing uncertainties and risks as early as possible in the innovation chain, and the Regulatory Preparedness concept aiming at building a structured and integrated dialogue between decision makers and industry to allow the risk governance of nanotech-based innovation.

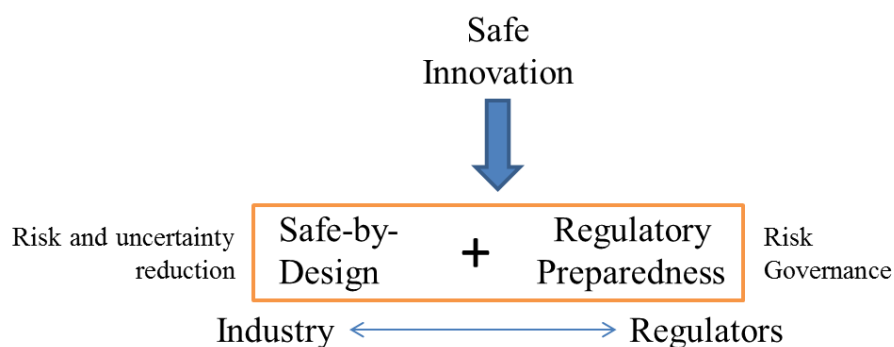


Figure 1: Overview of Safe Innovation Approach elements

SbD is built on the stage-gate approach (Figure 2), which is used by companies to manage innovation. Stage-gate model is structured as a set of steps (stages) where some activity is carried out, from the idea stage to the prototyping and placing to the market. Stages are interlaced by decision points (gates) where the decision makers decide whether the innovation project has to be cancelled, to be modified, or it can go on to the next stage. For each stage, a set of tools can be used to assess safety of the novel product/process for human health, environment, and consumers. The information is organized following the current vertical and horizontal regulatory framework, allowing a pre-regulatory and regulatory dossier compilation, and also avoiding the collection of unnecessary information in relation to the specific stage.

Regulatory Preparedness is based on the definition of “trusted environments”, where industry, regulators, and researchers generating the regulatory safety data can exchange views, anticipate information (also sensitive information), and decide upon a course of action to demonstrate safety of the product/process in a robust way. This approach can work both at specific level to deal with specific company needs, and at general level to establish EU-wide policies, leading to improvement of the regulatory safety assessment process and the implementation of a innovation risk governance.

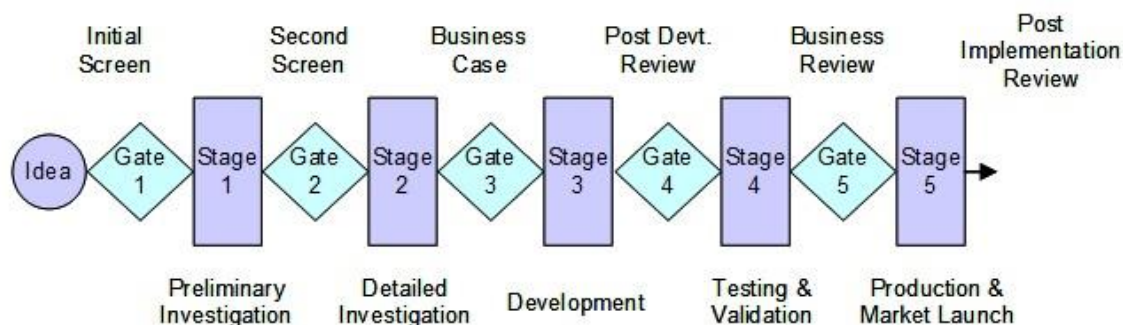


Figure 2. Stage-gate model. Adapted from [1]

3. Case studies

The applicability of the SIA to SMEs by an excellence center was evaluated considering three case studies on the basis of three main criteria: i) efficacy; ii) safety; iii) dialogue with regulators.

Efficacy is essential for the innovation process, and something that companies would like to assess as early as possible in the innovation process. Therefore, while not an inherent safety issue, it is an important component that needs to be considered in the SIA application. Safety is the core element of the SIA, and in this context what is considered is the availability of tools and data to assess exposure and hazard of nanomaterials. Finally, the dialogue with regulators is essential in the nanotech innovation field, since there is lack of experience in the regulatory world, and the legislation is not always clear. While technical guidance is available in some cases, the dialogue with regulators may give indications to companies about necessary information, and allow regulators to use robust information for their decision-making.

3.1. Topical medical device

The first case study is about a medical device (MD) and it is located at the beginning of the innovation chain, since it is a feasibility study. The idea at the basis of the new product is to develop a nano-enabled topical medical device, including botanical substances with both non-pharmacological and pharmacological mode of action, to support treatment of health conditions. In this case study were considered two related aspects: identification of suitable substances and regulatory feasibility. Both aspects are relevant for the development of the final product, due to specific regulatory requirements. Addressing safety data and needs at this stage would save resources for the next stages of the product development, but the regulatory context under development is posing issues of innovation governance.

The selection of suitable substances was based on both efficacy and safety, and also in terms of mode of action (in reference to the regulatory context). At this stage of innovation it was verified that published literature was sufficient to identify the suitable substances among a large panel of possibilities. Mode of action, efficacy, and potential toxicity were all well described, and supported the expert selection of the best candidates with examples of potential nano formulations. For some applications, such as botanicals, existing data are often sufficient to properly define the prototype. This may not be always the case, and computational models may be useful to fill some relevant data gaps. However, these models are completely missing right now for nanomaterials.

Concerning the regulatory feasibility, the regulatory framework of MD based on substances is complicated, since this kind of products fall between Medical Device directive [2] and Medicinal Products directive [3]. In addition, there is a proposal for a European Medical Device Regulation [4] which is posing stricter requirements on safety assessment of substances and of nanomaterials used in MDs. Therefore, a dialogue with national authorities was initiated, leading to a good relationship with national Notified Body which supported the enquiries about the feasibility as MD of the proposed product. However, the regulatory development with the new European Regulation is posing a challenge to this dialogue, since industry would need timely guidance from competent authorities

about the implementation of the new legislation requirements, which in absence of the official technical documentation can be difficult to get.

3.2. Cultural heritage restoration

Cultural heritage has a great socio-economic importance, both representing the history and culture of a country, but also representing one of the main drivers of tourism. Therefore, its conservation and restoration is paramount, especially in Italy. Cultural heritage is often exposed to environmental stressors, both outdoors and indoors, and there is the need of more effective and respectful treatment approaches. Nanotechnology, as key enabling technology, is already providing solutions for cleaning and conservation of paintings, frescos, and stone manufactures. Consolidation is a physical stabilization of the work of art aiming at binding constituting materials, and ensuring both any further treatment procedure and its stability over time. This case study is focused on the comparison of efficacy and toxicity of two different SiO₂ nanoparticles used in consolidation: unmodified silica and chemically modified silica. This case study is an example of chemical composition modification of a product to reduce impacts and increase efficacy, with nanotechnology playing a positive role. The modification of the chemical composition is one of the common strategies to address SbD, and here it is demonstrated that there are tools to apply this strategy for nanomaterials in a SIA context.

The substitution of solvent-based consolidants in favor of solvent free nanoparticle-based products is already a trend in the market. However, for the same nanoparticle different formulations are possible, and the nano-specific assessment of toxicity and safety for both professional users and the environment may lead to the selection of the best product. This case study is located at the end of the innovation chain, because these products are already on the market, and it is focusing on the efficacy and safety assessment [5]. Also, some considerations about the regulatory context in relation to the testing requirements are addressed.

The nano-product efficacy was measured taking into account the consolidation properties (Figure 3) and the antibacterial activity, since bacteria can cause the works of art degradation. Antibacterial efficacy of the two formulations was compared against both gram+ and gram- bacteria usually present on paintings and monuments. At professional use concentrations, both types of nanoparticles inhibit bacterial growth, with some difference in terms of bacterial species.

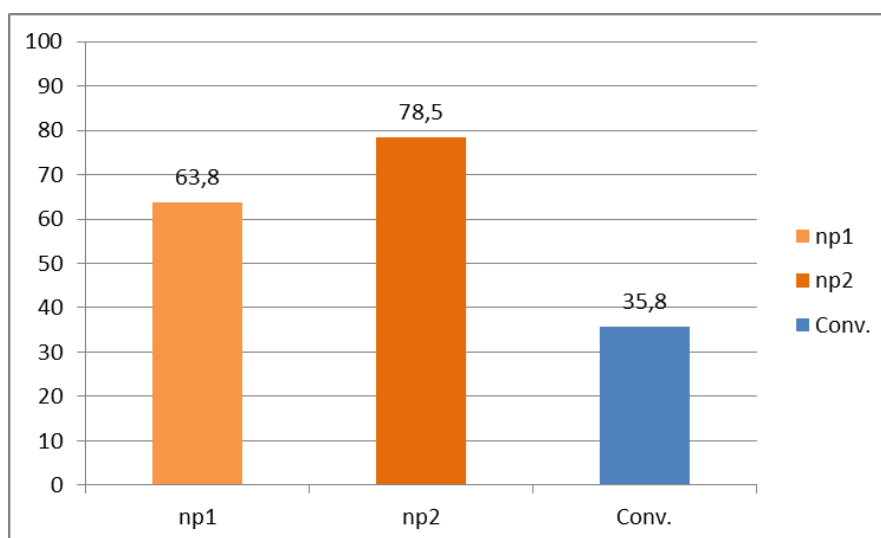


Figure 3. Cohesion factor of different products. np1 and np2 are the two silica nanoparticles, compared to the conventional product (Conv.)

After a proper pchem characterization, measuring shape, particle size distribution, and surface charge, *in vitro* toxicity tests were used to test for potential exposure routes and internal target organs

(i.e. lungs, skin, and kidney). The results showed that, while chemically modified SiO_2 did not induce toxicity, unmodified SiO_2 showed toxic effects at realistic concentrations on all *in vitro* models. An example is shown in Figure 4.

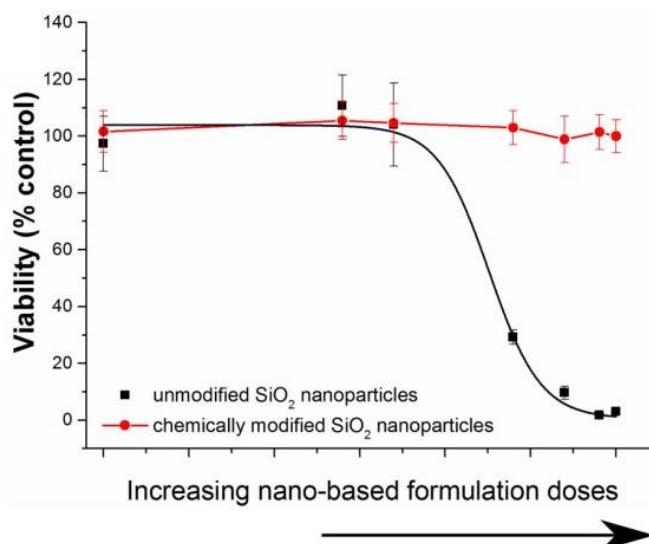


Figure 4. Example of effects on cell viability of the two nano formulations

The results of the study highlighted that it is possible to develop more effective and safer products at the same time, taking into account that small changes in nanoparticle features, like surface chemical modification, deeply affect biological behavior of nanomaterials. *In vitro* tools provide answers that can support the design of safer nanoparticles at early stages, but also to support the marketing strategy of a company, focusing on the best product and advertising the advantages in terms of sustainability.

From a regulatory point of view, this material is covered by REACH. However, nano SiO_2 is only one of the many forms of the substance SiO_2 as registered in REACH, but the dossier cannot realistically cover all the possible existing nanoforms. Therefore, the safety of a nanoform is not necessarily adequately covered by the REACH Chemical Safety Report. This underlines the importance of risk governance also in terms of industry voluntary actions, where the innovation is based also on social responsibility. SIA can support this approach in SMEs, optimizing the use of resources besides or in addition to the regulatory requirements.

3.3. Food contact material

Food industry is one of the sectors in which nanotechnology is increasingly employed. While silica dioxide (SiO_2) is one of the most used additive in food, its use as food contact material (FCM) is not so common.

This case study is dealing with the request to evaluate the compliance of a SiO_2 coating to the Italian Food Contact Material legislation. The product was already on the market for other uses, and therefore in this case we are covering the last part of the innovation chain. To assess the applicability of this coating as FCM entails the need to characterize the coating assuring that it is not nanostructured, and measuring (detecting and quantifying) the release of SiO_2 as chemical, but also as nanomaterial. This approach is a key requisite for improving its safe use and to protect consumer's health [6]. Therefore the case study is focused on the dialogue with regulators and the assessment of potential exposure.

The first step was to contact regulators to collect information on the national provisions about the use of SiO_2 in coatings, and what was the orientation of the Italian Ministry with regard to

nanomaterials. The dialogue was fruitful, and all necessary and pertinent clarifications were provided in time. Therefore, in this case, regulatory preparedness dialogue worked very well, allowing the excellence center to provide the services to the client.

Concerning the exposure assessment, in this case study we explored the potential of Single particle Inductively Coupled Plasma-Mass Spectrometry (spICP-MS) for detecting and characterizing SiO₂ NPs in food simulating media. Determination of Si in biological matrices by standard ICP-MS results in a very high background level. On the contrary, the spICP-MS with the adoption of a very short dwell time allowed to significantly increase the signal-to-background ratio (S/B) for SiO₂ nanoparticles. The low limit of detection (LOD) for number concentration (~104 particles per mL are sufficient for robust statistics) allowed high sample dilution, with further reduction of matrix effects. The estimation of nanoparticles' size by spICP-MS is indeed concentration independent. Simultaneous determination of dissolved Si (not possible by AF4-MALS, DLS and TEM) within a single analysis was achieved by implementing a statistical procedure [7] conceived to separate the signals generated by dissolved species and nanoparticles, the latter being detected individually.

Validation and direct comparison to TEM, DLS and AF4-MALLS was performed by analyzing standard suspensions prepared in ethanol 95%. All techniques except TEM effectively detected nanoparticles with consistent size distribution (Figure 5). Beside the unique information of dissolved Si level, spICP-MS provided the most narrow particle size distribution (70-120 nm) with respect to AF4-MALLS (40-200 nm) and DLS (60-400 nm considering the number fraction). Since direct measurement of individual particles mass can be achieved by spICP-MS, this technique is not affected by dominating signals of large particles like in DLS, or by shape effects like can occur in AF4-MALLS. Overall, its integration in multi-technique approaches was established as an efficient strategy to achieve the most robust assessment of nanoparticles in complex media within a regulatory framework.

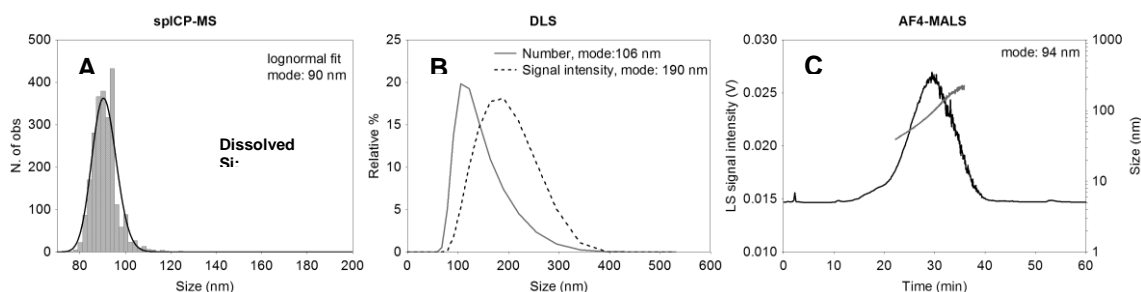


Figure 5. Size distribution of SiO₂ nanoparticles in pre-concentrated food simulating medium (ethanol 95%) after migration test from a Si-coated food-contact inox support, as obtained by spICP-MS (A), DLS (B) and AF4-MALS (C)

Real-case testing of such an approach was carried out to assess the potential migration of SiO₂ nanoparticles from a silica-coated stainless steel support into food simulating media. Migrations were prepared in acetic acid 3% w/v, ethanol 10 and 95% v/v according to the UNI EN 1186-3,14 standard procedures. Preliminary determination of global and specific migration revealed significant (still, well below the limits of Reg. CE 10/2011) results only for ethanol 95%, but no significant signals attributable to nanoparticles were detected by TEM, DLS or spICP-MS.

4. Conclusions

The application of Safe-by-Design and Safe Innovation Approach to three case studies involving the development of products for small companies led to the identification of positive aspects and issues still to be resolved. As a positive note, the case studies showed that SbD and SIA can be effectively integrated into the innovation processes of SMEs, and there is no additional burden but instead the

rationalization of the whole innovation approach. In fact, SIA can very well work as an innovation safety management system, identifying development phases, responsibilities, regulatory requirements, data needs, and data providers. Robust methods and tools for the SIA application on nanomaterials are available, allowing the measurement of all risk assessment components: from physicochemical characterization (e.g. spICP-MS) to hazard assessment (e.g. *in vitro* tests). In fact, the available knowledge and instruments are sufficient to answer the requests of the companies, from pre-regulatory assessment to the preparation of the dossier for the authorization to place on the market the product. However, acceptability of these methods by regulators is not granted. While standardized approaches and SOPs for nanomaterials are increasingly available, not all endpoints can be measured with e.g. OECD methods, and anyway adaptations could be necessary. While interaction with companies and technical partners from the beginning is useful to develop a sound experimental plan, more structured exchanges with technical authorities to define an agreed experimental approach could be useful to generate regulatory acceptable data. Part of the experimental approach could include *in silico* approaches, such as QSAR, grouping, and read across, that could reduce the need of testing in the early stages of the innovation process. However, these approaches are still in their infancy with regard to nanomaterials, and their development is expected to be slow despite the global efforts.

Regarding the regulatory preparedness, often competent authorities are not prepared to deal with a proactive approach toward safety, adhering strictly to regulations timing and requirements. In addition, communication is not always fruitful, especially in borderline cases, where different regulations may apply to the same product or regulations are changing. There is the need to define specific moments at different stages of the innovation process when industry can consult regulators, to be able to share information and come to an agreed market authorization process which is satisfactory for all parties. For example, the Food and Drug Administration in the United States strongly advice companies that want to put nano foods on the market to consult the agency before starting the actual development. An interactive system like this would improve the communication between competent authorities, industry, and researchers, streamlining the effectiveness of regulatory interpretations and implementation, and accelerating the transfer of the innovations benefits to the society.

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