FAST FACTS

Duration:
42 Months

Completion Date:
February 2019

Total Funding:
€10 Million

Partners:
42 across 15+ countries

Objective:
Establish Safe by Design as a fundamental pillar in the development of nanomaterials or nano-enabled products

Outcomes:

- Nanomaterial
- grouping strategy
- Associated
- integrated testing
- strategy

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HiQ-Nano Industrial Demonstrator

Safe by Design in Action

Goal: To reduce human & ecological toxicity through replacement of Quantum Dots

Safe by Design Measures: Comparison of the toxicity between two types of SiO_2 and substitution of the original nanomaterial while preserving functionality

Outcomes: The use of dye-doped silica was demonstrated to be less toxic than quantum dots - doped silica.

HiQ-Nano is a manufacturer of highly engineered nanoparticles, based in Italy. Within the NanoReg2 Industrial Demonstrator, it focused on fluorescent silica nanoparticles and was at the testing and validation stage of the value chain when NanoReg2 started. This Industrial Demonstrator was selected by HiQ-Nano to investigate how a Safe by Design approach could yield a safe multi-constituent nanosystem. HiQ-Nano was looking to replace the cadmium selenide quantum dots (CdSe@ZnS) with fluorescent dyes in its fluorescent silica nanoparticles to create a safer and more biocompatible product.

In the original HiQ-Nano product, fluorescence was generated from the quantum dots. The toxicity of quantum dots has been well-documented, with the mechanism for fluorescence found to be toxic to humans and the environment due to the release of cadmium ions when the quantum dots degrade. Replacing these quantum dots with a fluorescent dye was seen as a potential mechanism to create a safer product without compromising the physio-chemical properties of the nanoparticles.

There are also many cases where a dye will fluoresce more than a quantum dot, thus the project was also seen as way of improving product performance. Most applications that feature dye-doped silica nanoparticles are in the bioimaging and drug delivery space, thus the toxicity of the nanoparticles is a very important factor for commercial use.

hiq-nano
THE ART OF PRODUCING NANOPARTICLES

This project has received €10M in funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 646221

Aims for Implementing Safe by Design

The project for HiQ-Nano was primarily centred around the need to improve the sustainability of manufacturing whilst making the process greener and improving the safety of the working environment. To achieve this, HiQ-Nano had five main objectives:

- Development of the new manufacturing process
- Grouping the results across the different Safe by Design tools to reduce the number of experiments needed to characterise the nanoparticles
- Obtaining new data regarding the characterisation, toxicity profile, exposure in working environment and economic constraints of the nanoparticles to determine whether there are any major barriers with the process
- Identification of any new markets that HiQ-Nano could enter once the biocompatibility of the nanoparticles was validated and confirmed

With regard to HiQ-Nano's experience, it had been implementing Safe by Design approaches prior to the NanoReg2 project and wanted to turn its attention to its new target nanomaterial product. HiQ-Nano aimed to receive independent assessment of the fluorescent nanoparticle's biocompatibility and functionality through the Industrial Demonstrator and use this to make a product improvement with Safe by Design. HiQ-Nano also anticipated obtaining a better evaluation of potential exposure risks during production and greater visibility for their product. Finally, HiQ-Nano aimed to expand the market for dye-doped silica nanoparticles and to perform risk assessments for the handling and production of nanoparticles.

Industrial Demonstrator Activities within NanoReg2

HiQ-Nano focused the Industrial Demonstrator on the 'safe products' pillar of Safe by Design. The development of the new nanomaterial from its inception, applying a Safe by Design approach, was based on significant data available in the literature regarding the toxicity of quantum dots. HiQ-Nano started with this data and evaluated the possible alternatives resulting in the selection of a dye with good fluorescence properties. The synthesis pathway selected enabled the original physio-chemical properties of the quantum dot doped silica nanoparticles to remain unchanged e.g. the surface charges from exposed chemical groups, whilst obtaining the fluorescence and biocompatibility properties of the fluorescent dye.

Throughout the Industrial Demonstrator and as part of their selected approach towards SbD, HiQ-Nano chose to undertake hazard and exposure assessments, as well as a life cycle analysis (LCA). For the screening risk assessment two control banding tools were used: the Swiss Precautionary Matrix (SPM) and CB toolbox. The output of the screening Risk Assessment (RA) exercise identified several data gaps that were filled during the project and a more complete RA was performed after completion of data with those tools, plus the Weight of Evidence (WoE). With the intended use of the nanomaterials in the body, this study could only be performed during the production phase.

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Outcomes from Safe by Design Application

HiQ-Nano reported that all required data gaps were filled through the NanoReg2 Industrial Demonstrator. The results from the life cycle analysis demonstrated that Safe by Design had a positive impact towards reducing nanomaterial exposure to the environment. Across all the categories examined, the reduction in exposure ranged from 5% to 75% depending on the category, with the lowest reduction being for freshwater ecotoxicity and the highest reduction being for ozone depletion.

Results demonstrated that the quantum dot-doped and fluorescent dye-doped nanoparticles had similar physio-chemical characteristics, including size distribution and monodispersity, content of silicon in the nanoparticle and Zeta potential.

In a cell culture medium, the size of both nanoparticles increased due to formation of a protein corona around the particle, by 100-200 nm in diameter in quantum dot doped nanoparticles compared to 60 nm with the dye-doped nanoparticles. Similarities were found between the surface charges of both nanoparticles, however the dye-doped nanoparticles were less stable, demonstrated by the formation of agglomerates.

Within human digestive fluids, both nanoparticles increased by approximately the same amount through development of the protein corona. Both were stable in terms of size and colloidal stability when in water-based saliva fluids, with some agglomeration again seen for the dye-doped nanoparticles. However, when nanoparticles were exposed to harsher digestive fluids (such as stomach), both increased in diameter by between 13-18 times, suggesting that both are only stable in water-based digestive fluids.

Neither nanoparticle altered cellular viability after long exposure times, while they both had differential effects on the gene expression of pro-inflammatory cytokines. Dye-doped nanoparticles generated slightly more reactive oxygen species (ROS) in certain cells and greater concentration of dye doped nanoparticles was internalised by cells. Nanoparticles with quantum dots exhibited greater cytotoxicity than the dye-doped nanoparticles. Both nanoparticles had a negative Zeta potential, and this helped to confirm that the physio-chemical properties of the nanoparticle had not changed by the addition of the dye. The Zeta potential of both nanoparticles reduced over time, suggesting a low colloidal stability, while fluorescence properties were superior for the dye-doped nanoparticles which emitted a more stable spectrum of fluorescence. Finally, neither nanoparticle significantly changed cell viability compared to the control or demonstrated micronucleus induction.

Summary

The use of Safe by Design enabled HiQ-Nano to develop a new type of nanoparticle that possesses superior fluorescence properties than the nanoparticles it was previously producing. These new dye-doped, rather than quantum dot-doped nanoparticles offered significantly lower environmental impact.

HiQ-Nano worked with many NanoReg2 partners, however did not report any barriers to external collaboration. The company has stated that the use of Safe by Design in this project has yielded excellent results for the company and it will continue to use it in the future.

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