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Review Article

EU Research in Nanotoxicology 2000 - 2020 and its Applications for Ensuring "Safe by Design" Nanotechnology Products for Medicine

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Abstract

The first two decades of the 21st century has seen a rapid increase, from a near zero base, for European nanosafety and nanomedicine research. This has been achieved by dramatically increasing funding and establishing effective networks of experts in both disciplines to drive forward science and technology for both and inform EU Research and Innovation planning. Initially, both disciplines focused on their respective areas, but from 2010 onwards they increasingly co-operated to better drive forward their respective disciplines and achieve safe and ethical solutions for future industrial and clinical beneficiaries of their respective science areas. At the junction of the 2nd and 3rd decades and the transition from Horizon 2020 and Horizon Europe Programs, it is possible to describe general Safe-by-Design principles for nanoenabled pharmaceuticals and implanted medical devices that are heavily dependent on the new science created by nanosafety and nanomedicine science to meet the demanding clinical requirements of the 3rd decade.

Keywords: *Nanomedicine; Nanoenabled Medical Devices; Nanosafety; Hazard; Exposure; Nano-EHS; Safe-by-Design*

Evolution of EU Nanotechnology R&I Policy

The European Union's policy for nanotechnology research and innovation (R&I) has evolved over 2 decades. In response to the US National Nanotechnology Initiative (NNI) established in 2002, the European Commission's Framework programme-6 (FP6) Industrial Technologies' Expert Advisory Group (EAG), conducted an economic analysis [1] of the market potential for nanotechnology products over the period 2001 - 2015. Concerned that the EU's planned R&I investment was significantly lower than those for the US and Japan, in 2002 [1] the EAG recommended that the investment in nanotechnology R&I programme for 2007 - 2013 (FP7) should be increased by a factor of 3x deployed for 2002 - 2006 (FP6) to ensure the EU gained a dominant share of the rapidly growing nanotechnology market. Conscious of growing public concerns for then unknown potential environment, health and safety (EHS) risks for nanotechnology, the EAG also recommended a 5x increase in funding be made in FP7 for nano-EHS research, to grow a strong independent community formed from: a) academia; b) independent research and technology organisations (RTOs) and c) industry, both large and SME companies, to develop the science and technology to investigate and quantitate the possible toxicity effects of these new materials.

Two Decades of Nano-EHS R&I in the EU.

The expenditure on nano-EHS R&U from FP5-H2020 is shown in figure 1. The effect of the policy change for FP7 relative to FP6 is clear. At the end H2020, the total expenditure will be €440 million, whereas the US will have spent $920 million on nano-EHS research.

The nature of EU research has changed over the 2 decades, guided by the Nanosafety Cluster (NSC) [2]. The latter is an interdisciplinary democratic "club" of researchers from academia, independent RTOs and industry drawn from across Europe and sponsored by the European Commission, to guide the evolution of nanosafety science and R&I policy. Initially, as powerful new methods e.g. electron mi-

croscopy and particle counting, became widely available, the focus was on developing characterisation, reference methods and standards. These were used to study both the effects of nanoparticle hazard, together toxicity mechanisms and how they might be modulated by protein corona formation *in vivo* or *in vitro* tests [3]. By the end of the first decade, the research community was evolving from a focus on basic research to translational R&I as shown by the bibliometric analysis of Scopus data of nano-EHS publications [5] from 2002 - 2015. The drivers of this change in focus is illustrated in the analysis of 84 FP7 projects carried out in 2013 (Figure 2). Two projects NanoReg [4] and ProSafe [5], were established to collate the data bases and learning from all the other projects to create Intelligent Testing Strategies (ITS) and Risk Management Frameworks (RMF) for guidance to industry, researchers, regulators, standards bodies and the public for best practice to minimise or eliminate risks from nanotechnology products from cradle to grave. From 2008 onwards,-quantitative structure - activity relationships (QSARs) [6-9] became increasingly important tools for predicting the severity of hazards of nanomaterials and eliminating the use of animal testing. Public concerns regarding the safety of nanotechnology were high in first decade. By 2012, it was beginning to change [10] as evidenced by CEFIC's biannual survey [11] in 2014 finding that nanotechnology's public acceptance was favourable for 91% of citizens across Europe.

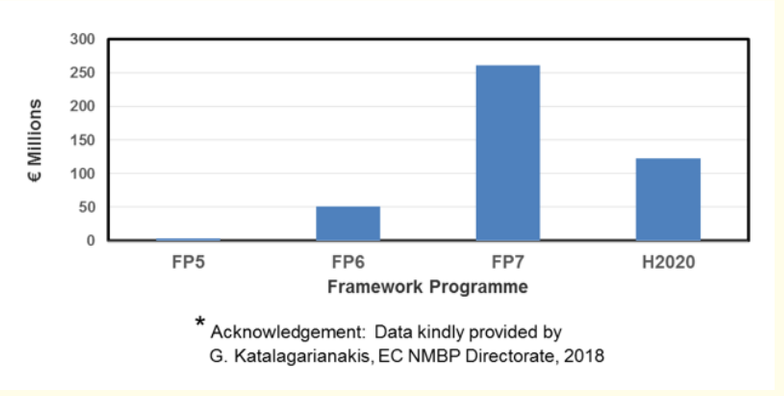

Figure 1: *EU nano-EHS project funding (1998 - 2020).*

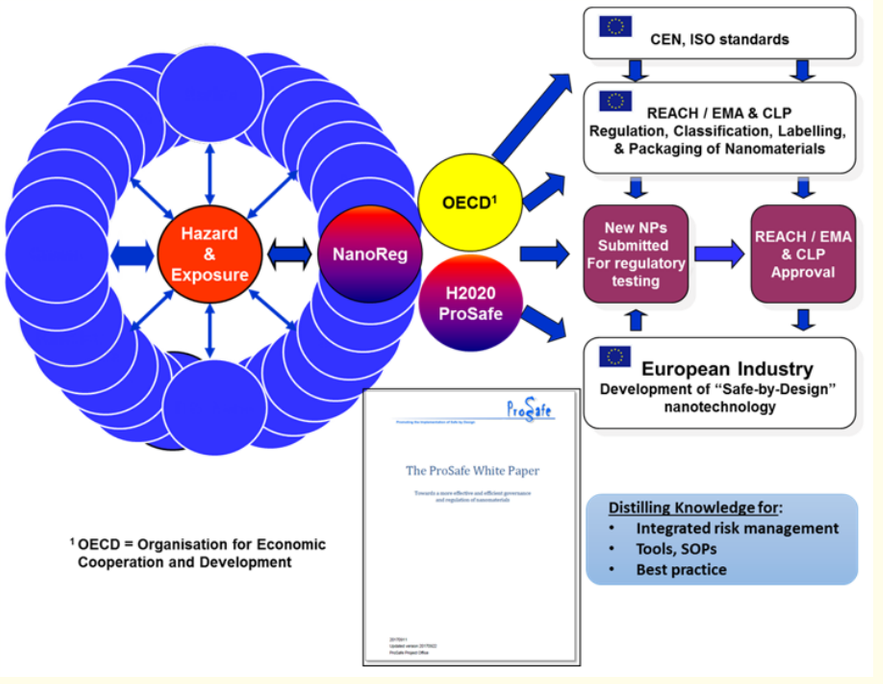

Figure 2: *Analysis of 84 FP7 and H2020 nano-EHS projects.*

Merging nano EHS with nanomedicine R&I

The European Technology Platform Nanomedicine [12] was formed in 2005 as forum for researchers from Universities, RTOs and industry**.** Its purpose was to share best practice in nanomedicine R&I, identify future trends in this field and to advise both the EU and Member States of future R&I priorities. During the first decade, interactions between the nanomedicine and nanosafety R&I communities were low. From 2010, onwards, the two fields began to come together. The nano-EHS databases and nano-QSAR methods became useful to the nanomedicine community for materials selection and product design. This sharing of knowledge has the additional potential for further reduction in animal testing for new products. The BIORIMA project [13,14] is an example of such a collaboration, where both ITS and RMF approaches are adapted into Safe-by-Design strategies for use in the development of *in vivo* nano-enabled targeted drug delivery pharmaceuticals and implanted medical devices. The latter incudes artificial joints and dental applications. The nanomaterials being studied in BIORIMA are classified as 1) Bioinert; 2) Bioactive and 3) Biomimetic/Bioresorbable/Stimulating specific cellular responses at the molecular level.

In general, biopharma active pharmaceutical ingredients (APIs), such as nucleic acid, aptamers or monoclonal antibody fragments delivered *in vivo* by targeting nano-encapsulating agents, are generally manufactured by materials processing methods. Artificial joints, dental implants and replacement bone implants are made by fabrication processes. Generic Safe-by-Design (SbyD) approaches are reported for the first time in this editorial, for both types of medical products are described below.

(SbyD) principles for nanobiopharmaceuticals

The principle of multivariate process analytical control was initially developed in the chemical industry for Quality-by-Design (QbD) of nano-TiO_2 manufacturing on 100 ktonne/year scale by Wilkins., *et al* [15]. This approach was adapted for small molecule active pharmaceutical ingredients (APIs) manufacturing by Afnan [16], who incorporated it into US FDA guidance in 2004. By adding nanoparticle characterisation, nano-EHS testing and nano-QSAR methods plus NanoReg and ProSafe risk management framework approaches to the product design, process development and manufacturing workflow processes, Wilkins., *et al*. [8] have upgraded QbyD to a generic SbyD process for nanomedicine APIs and drug delivery agents (Figure 3).

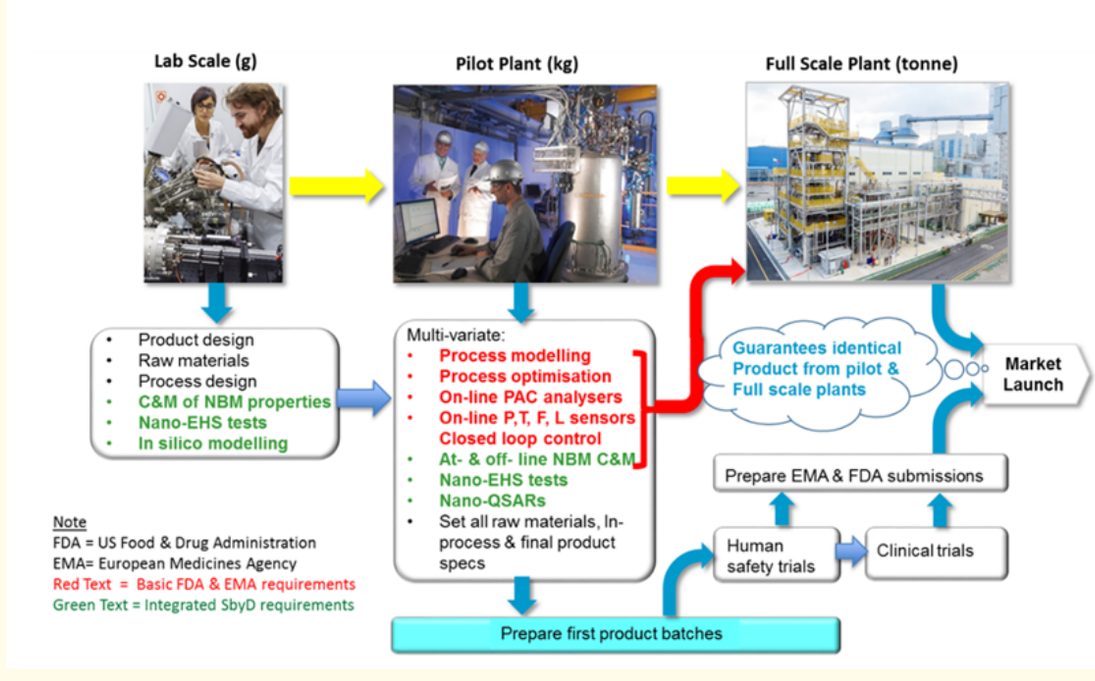

Figure 3: *SbyD principles integrated into nanobiopharmaceutical product design, process design and manufacturing workflow.*

This generic approach ensures SbyD objectives are met from product conception to market application at the same time as providing a rapid route to clinical applications.

(SbyD) principles for nanoenabled medical devices

Hitherto, the safety of metals, polymers and ceramic materials for use in replacement joints has been assessed only on the bulk materials. Whilst, the bulk metals can release toxic ions, the major causes of morbidity and mortality from these devices results from inflammatory effects arising from wear particles. To eliminate these risks, surgical revision is used. Hip and knee revisions annually are 6% and 10% [17]. But revisions can be a source of infections. To overcome these risks a new design of replacement joint has recently been developed to last a whole life *in vivo*. This was achieved by adapting the SbyD principles above for Nanobiopharmaceuticals [1,18] to the design and manufacturing of a new generation of replacement hip joints with the metal ball, metal shaft and ceramic socket components plasma coated with nanostructured Si_3N_4. This new design of hip joints was tested under load in robotic rigs over months. The joints were encased in plastic bags containing artificial synovial fluid containing human serum albumin and lubricin. The Si_3N_4 wear nanoparticles (mean diameter 47 nm) were isolated and using serum from human volunteers, reactive oxygen stress (ROS), cytokine production, cytotoxicity and genotoxicity (Comet assay) tests were undertaken (Figure 4).

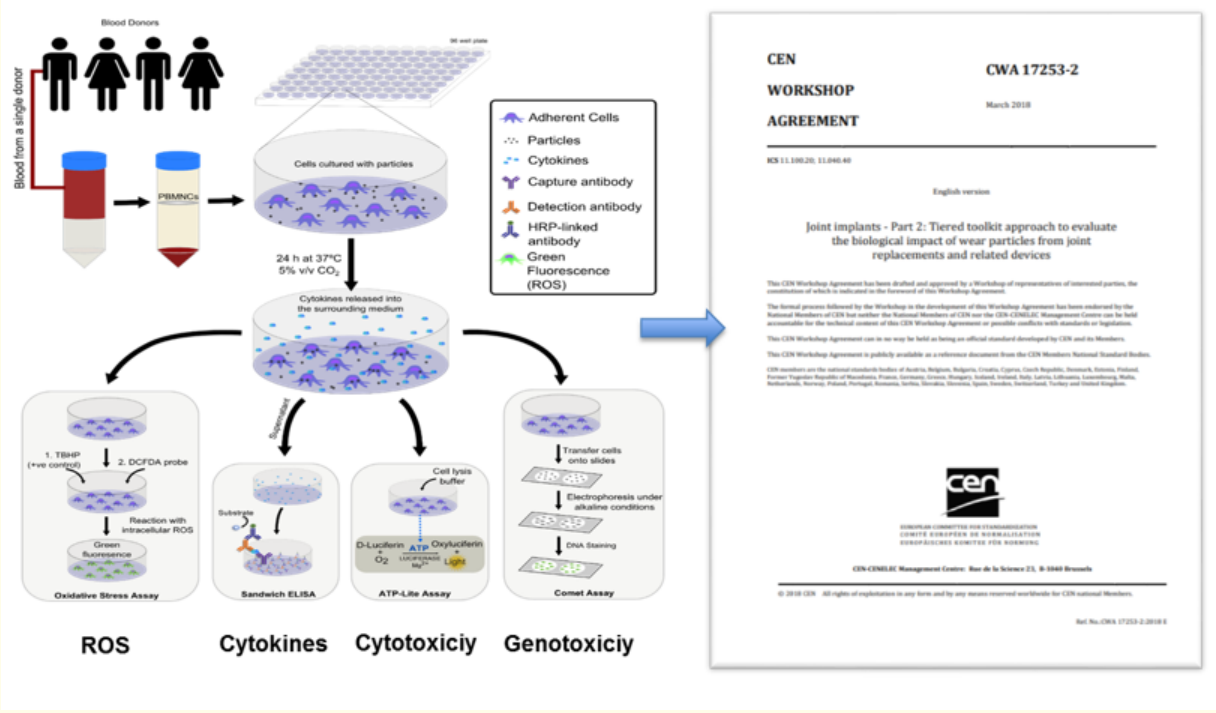

Figure 4: *Joint implant Si3N4 wear nanoparticle toxicology evaluations.*

These studies demonstrated that the numbers of wear nanoparticles produced by this new SbD replacement hip joint were significantly lower than for all other designs. They were also considerably less toxic than all other designs. In addition, the Si_3N_4 wear nanoparticles are biodegradable *in vivo*.

Together with lower friction coefficient characteristics, this design is likely to lead to a substantial reduction in the numbers of revisions. Historically, biocompatibility of such devices has been tested on bulk materials against CEN and ISO standards. As part of this development new generally applicable CEN Workshop Agreements, CWA 17253-1 and 17253 have been obtained for the first time to evaluate wear nanoparticles and guarantee Safe-by-Design of implanted devices. These new standards raise the bar for safety for all replacement hip joints and demonstrate the value of close collaboration of the nanomedicine and nanosafety research and innovation communities.

Conclusion

In conclusion, the first two decades of the 21st Century have seen a period of rapid exponential growth for nanosafety and nanomedicine research, judged by R&I investment and numbers of publications generated for both. In the first decade, the two disciplines were focusing on their respective needs for creating new research tools and exploring new scientific concepts. As a result of interdisciplinary collaborations between the two communities were low. The second decade has seen increasing collaborations between the two research communities wherein each discipline has informed the other. The latter has led to productive cooperation and cross-fertilisation of ideas. The nanomedicine community has been able to benefit from the high sensitivity hazard and exposure techniques developed by the nanosafety community to quantitate the risks in nanomedicines and nanoenabled medical devices. At the end of the decade the two communities are now collaborating in moving the focus of research away from a reactive stance towards an active approach using the learning from nanosafety, to build Safe-by-Design principles bottom up from clinical design to product to process to patient. Examples of general principles have been illustrated for: a) nanobiopharmaceuticals and *in vivo* imaging agents and b) medical devices such artificial hip and knee joints.

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