Computational and Structural Biotechnology Journal 25 (2024) 127–142

Contents lists available at ScienceDirect

Computational and Structural Biotechnology Journal

journal homepage: www.elsevier.com/locate/csbj

Innovation report

A roadmap towards safe and sustainable by design nanotechnology: Implementation for nano-silver-based antimicrobial textile coatings production by ASINA project

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ARTICLE INFO

Keywords:
Safe and sustainable by design
Silver nanomaterials
Spray coating
Textiles
New approach methodologies
Roadmap

ABSTRACT

This report demonstrates a case study within the ASINA project, aimed at instantiating a roadmap with quantitative metrics for Safe(r) and (more) Sustainable by Design (SSbD) options. We begin with a description of ASINA's methodology across the product lifecycle, outlining the quantitative elements within: Physical-Chemical Features (PCFs), Key Decision Factors (KDFs), and Key Performance Indicators (KPIs). Subsequently, we delve in a proposed decision support tool for implementing the SSbD objectives across various dimensions—functionality, cost, environment, and human health safety—within a broader European context. We then provide an overview of the technical processes involved, including design rationales, experimental procedures, and tools/models developed within ASINA in delivering nano-silver-based antimicrobial textile coatings. The result is pragmatic, actionable metrics intended to be estimated and assessed in future SSbD applications and to be adopted in a common SSbD roadmap aligned with the EU's Green Deal objectives. The methodological approach is transparently and thoroughly described to inform similar projects through the integration of KPIs into SSbD and foster data-driven decision-making. Specific results and project data are beyond this work's scope, which is to demonstrate the ASINA roadmap and thus foster SSbD-oriented innovation in nanotechnology.

1. Introduction

In the evolving landscape of nanotechnology, the Safe(r) and (more) Sustainable by Design (SSbD) concept has emerged as a pivotal framework, guiding researchers, innovators, industry and policymakers towards the creation of advanced (nano)-materials (NMs),[1] (nano-enabled) products (NEPs) or processes that not only push the boundaries of innovation but also prioritize human and environmental health safety

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[1] https://www.ami2030.eu/wp-content/uploads/2023/04/Ami2030-Dossier-2.pdf

https://doi.org/10.1016/j.csbj.2024.06.013
Received 5 June 2024; Received in revised form 11 June 2024; Accepted 12 June 2024

and sustainability. Such a concept is being intensively explored in the field of nanotechnology that has laid the foundation for the design of various NMs that surpass the capacities of bulk materials due to their unique chemical, physical, electrical, and mechanical properties [1–3]. Despite being a relatively new concept in the nanotechnology domain, SSbD has become an important component of many European (EU) nanosafety projects. A detailed overview of all the projects related to SSbD exploration under various industrial case studies and related fields, such as innovative methods, advanced characterization methodologies and integrated approaches, can be found here.[2] Current research efforts in nanotechnology are increasingly aligned, reflecting a collective attempt to define and realize SSbD principles [4]. As we stand on the tip of technological innovations that promise to redefine industries ranging from the healthcare, energy, construction, mobility to environmental remediation [5], the imperative for integrating safety and sustainability into the very starting point of the NM design is paramount. The journey towards actualizing the SSbD concept in nanotechnology is marked by both advancements and challenges [6–9]. For example, SSbD's adoption reflects a shift towards integrating various disciplines—safety, environmental science, computer science, economics, and social sciences—to ensure that all aspects are considered. Initiatives like the IRISS project[3] demonstrate the importance of collaborative efforts among various stakeholders, including regulatory bodies, industry, and academic institutions. In addition, the establishment of entities such as a nano-risk governance council (Gov4Nano, RiskGONE,[4] and NanoRIGO[5]) underscores the progress in creating structured frameworks that support the SSbD concept to ensure that the technological benefits can be safely and ethically realized [10]. This required collaboration across disciplines is crucial for applying SSbD principles practically and effectively in research and beyond [11]. One of the most significant challenges is converting research outcomes into practical insights that can guide decision-making processes. **Defining quantifiable metrics to measure how much safe(r) or (more) sustainable a material, product, process is, remains a priority albeit difficult goal** [8,12,13]. In addition, developing universal criteria that can be applied across different sectors remains problematic due to the diversity of NMs and their intended applications, meaning each sector may require tailored approaches that can then be generalized. As the field continues to mature, the quest for comprehensive quantitative metrics, approaches and methodologies that encapsulate the SSbD ethos are ongoing. This report seeks to contribute to this dynamic discourse, by elucidating the conceptual underpinnings of the SSbD approach, and charting a course for future research and application.

2. Project description

The ASINA project introduces and implements an SSbD Management Methodology (ASINA-SMM), an approach designed to enhance quality, safety, and sustainability throughout the lifecycle of NMs [14]. This methodology is applied to two major categories of NEPs that are prevalent in the market: self-cleaning/air-purifying/antimicrobial coatings and nano-structured capsules for active delivery in cosmetics. It integrates and addresses critical pillars at the early stages of NEP development: environmental impact, techno-economic performance, functionality, and human and environmental safety. The goal of implementing this methodology is to ensure industrial relevance, and readiness for regulatory changes. This integrative strategy is supported by a Decision Support Tool (DST) that leverages quality data collected across the NMs' lifecycle from the ASINA-SMM implementation. The aim is to identify design options maximizing functionality and nano-safety, while minimizing costs and environmental impacts, thereby meeting stakeholder needs, regulatory obligations, and sustainability goals. Additionally, ASINA investigated the potential of digitalization, such as the use of Digital Twins (DT), to accelerate the nano-industry's transition towards comprehensive sustainability in economic, environmental, and safety dimensions.

2.1. Project's objectives

ASINA project was designed to elevate the nanomanufacturing industry's approach to safety and sustainability through its SSbD methodology. The project's objectives, aimed at integrating scientific and technical excellence into practical tools that optimize the balance between speed to market, costs, and implementation barriers for SSbD products and processes. For this, the following objectives were set:

- Deliver material SSbD solutions by linking NMs physicochemical (pchem) factors to their techno-economic performances, enhancing both functionality and cost-efficiency and ensuring sustainability (chapter 3.2).
- Extrapolate hazard criteria through mechanistic toxicity data, paving the way for the creation of less hazardous NMs (chapter 3.3).
- Provide process SSbD solutions, using monitoring campaigns, Life Cycle Assessment (LCA) and modelling tools to link process decision factors with environmental and safety dimensions and cost metrics (chapter 3.4).
- Integrate hazard and exposure data to establish comprehensive safety profiles for end-users (chapter 3.5).
- Develop a DST, i.e. the MultiOptimal360™ IT platform,[6] to identify the most efficient SSbD solutions (chapter 3.7).

Collectively, these objectives demonstrate ASINA's commitment to advancing nanomanufacturing towards greater safety, sustainability, and economic viability. The final objective was the creation of a roadmap to guide stakeholders in adopting these practices, to communicate this knowledge and to support the information exchange between stakeholders, which is achieved through this innovation report.

2.2. ASINA roadmap

2.2.1. Overview of ASINA-SMM

ASINA proposes a methodology for implementing the SSbD objectives throughout the product lifecycle enabling a holistic perspective and supporting human-centric informed decisions [14]. A comprehensive SSbD case-study implies addressing the lifecycle of pristine NMs and related NEPs. To allow a modular approach and a stage-gate decision process, the NMs lifecycle is segmented into four Life-Cycle Stages (LCSs): (1) synthesis of NMs, (2) NM surface or bulk incorporation into intermediate (nanocomposite) materials and finally into NEPs, (3) NEPs (and implied NMs) use phase, (4) NMs and NEPs End of Life (EoL). In this manner, a design case-study may focus on one or more LCSs and may be combined with other partial design case-studies towards attaining proposed SSbD solutions across its entire lifecycle. SSbD solutions aim to achieve an optimal balance across multiple dimensions that guarantee functionality, cost-effectiveness, human and environmental safety and sustainability, i.e. the multi-performance hyperspace (referred simply as "hyperspace") that defines the NMs' multi-performance profile across the entire lifecycle. Those solutions provide a comprehensive vision of the effects associated with the implementation of the addressed solutions on both the anthroposphere and the ecosphere. More dimensions may be included in the hyperspace, such as societal aspects, although social impacts were outside the ASINAs' scope. Each dimension of the

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[2] https://www.nanosafetycluster.eu/nsc-overview/spg/#1706188945110-faa40a78-da72

[3] https://iriss-ssbd.eu/

[4] https://riskgone.eu/2022/

[5] https://nanorigo.eu/

[6] https://www.projecthub360.com/multioptimal360/

hyperspace has criteria that must be defined quantitatively. From the proposed methodology, three quantitative elements, defined from the data, models and tools generated within LCSs and dimensions, are suggested to guide decision-making: the Key Decision Factors (KDFs), Key Performance Indicators (KPIs) and Physical-Chemical Features (PCFs) associated to NMs, NEPs and processes.

-KDFs: Each case-study involves various design options that define the possible SSbD solutions, thereby allowing the differentiation of the intended multi-performance profile. These solutions are collectively referred to as the Decision Space (DS) and can encompass both discrete choices—such as different materials, synthesis methods, or EoL strategies— and continuous variables like processing parameter values, that independently influence KPIs. Essentially, **KDFs define the design options within the DS, while KPIs quantitatively assess each dimension for the applicable LCSs.** One of the principles of the proposed DST is the significant dependency of KPIs on KDFs, selectable within the DS. This selection allows for the modulation of KPIs, enabling a differentiation between SSbD solutions. This is exemplified in Section ***3.7***, which illustrates a practical application of these concepts. By adjusting KDFs, *designers* have the flexibility to explore configurations and their impact on KPIs, embodying the "by-design" principle at the core of the SSbD approach which applies across various LCSs, including molecular redesign (LCS-1, materials synthesis), process redesign (LCS-2, incorporation phase), product redesign (LCS-3, use phase), and designing EoL options (LCS-4). This approach ensures that the solutions are deliberate and aligned with the SSbD objectives.

-KPIs: they quantitatively reflect the dimensions designed to assess health, environmental, economic, and functional aspects related to NMs and related NEPs at various LCSs. Collectively, the KPIs reflect the multi-performance profile within the hyperspace. The intention of KPIs is to "*map complex systems behaviour to single numbers for scaling, rating and ranking systems or system components*" [15]. Historically, KPIs have been applied across sectors including construction project management [16], the defence industry [17], institutional organization management [18], and workplace safety [19], to name a few. **This work marks the first comprehensive summary of SSbD-KPIs used within the context of nanotechnology, establishing a foundational reference for comparative analyses and future research**. KPIs may be either quantitative or semi-quantitative, and they include: i) simple KPIs directly measured representing straightforward indicators; ii) composite KPIs as more intricate, formulated by amalgamating multiple KPIs to capture complex objectives when no single KPI can represent the multifaceted goals of an evaluation [20].

-PCFs: serve as critical quantifiers of both i) intrinsic properties of pristine NMs, such as the molecular structure, general morphology, particle size distribution, reactivity etc., and ii) extrinsic properties that characterize composition/structure with respect to surrounding conditions (system-dependent) such as when NMs are in biological or environmental compartments media [21–23]. These characteristics are pivotal[7] in defining the functional, environmental, and safety profiles of NMs and play an essential role in shaping the final KPIs.

2.2.2. *Structure of ASINA-DST*

The proposed DST follows the scheme of international standards (such as EN ISO14040–44 for LCA) where each case-study is organised in four phases: (I) definition of Goal and Scope, (II) data generation, and (III) impact assessment followed by (IV) interpretation and the identification of SSbD-solutions.

I. In the Goal phase, the objective(s) are stated. These may involve the creation of a new NM or NEP that represents a breakthrough with no pre-existing benchmarks, or the redesign of an entire lifecycle of a NM to enhance their multi-performance profile. This objective could be aimed at achieving better safety, environmental sustainability, functional performance while maintaining the same levels at a competitive cost, or a combination of these objectives. The objectives are set to target the maximization or minimization of KPIs within the dimensions, either by surpassing existing benchmarks or maintaining levels below predefined thresholds. The Scope is delineated to cover specific or multiple LCSs, potentially encompassing the entire lifecycle of a NM. This defines the level of detail and the extent of the SSbD assessment. During the case study's Goal and Scope definition, the functional unit of the NM/NEP is specified (a fixed point of reference where all comparisons are anchored throughout a study), and the system boundaries are established through expert judgment, along with assumptions, approximations, cut-offs, and data requirements. The hyperspace is structured around a set of dimensions (φ-functionality, γ-cost, ε-environment, σ-human health safety, λ-social), as depicted in Fig. 1. These dimensions contain the KPIs and are based on adaptations from the EU-based framework [24]. The diagram shows how KDFs are identified and incorporated into the Design of Experiment (DoE) to generate data for the definition of the KPIs for each dimension.

II. Data generation involves collecting data under the ASINA-SMM in a harmonised manner which corresponds to different design options outlined in a DoE matrix [25–27]. The DoE is used to plan experiments ensuring that all potential combinations of KDFs are considered, and is designed to establish clear links between KPI levels and its corresponding set of KDFs facilitating interpretations. This approach also enables the application of grouping and read-across approaches [28,29] by providing diversity in data and by reducing the likelihood of missing data since all experiments are executed according to the matrix. Data are generated by experimental monitoring campaigns, human- and eco-toxicity endpoint assessments, pchem and functional characterisations of NM/NEPs, measuring and modelling NMs emission and exposure in environmental compartments, and the assessment of economic impacts and ecological mid-points impacts (Fig. 1). Data generation workflow for providing input for KPIs comparison and for SSbD decision process is corroborated by the MultiOptimal360™ IT platform,[8] which identifies the best KDFs values combinations corresponding to select the SSbD solutions.

III. Impact assessment is achieved by analysing KPIs and how specific KDFs combinations impact their achievement identifying correlations between the quantitative elements (KPIs, KDFs, PCFs) through response surface modelling or machine learning (ML) techniques [30,31].

IV. Interpretation and identification of SSbD solutions involves a i) comparison of KPIs and may involve ranking the solutions to assess which KDF configurations most effectively meet the SSbD objectives; ii) benchmarking KPIs against predefined thresholds/target values, drawn from external standards or best practices.

For ideal design options where KDFs are continuous variables, it is possible to correlate KDFs with KPIs across dimensions. This correlation enables the identification of KDF values that fulfil objectives simultaneously. The process of identifying these optimal combinations involves the use of Multi-Criteria Decision Analysis (MCDA) algorithms, such as those implemented through the MultiOptimal360™ IT platform.[9] This platform facilitates solving the Multi-Objective Optimization Problem (MOOP) within the SSbD approach. An example of this application is demonstrated in the NM synthesis case study, shown in Section ***3.7***, where different design options are identified by a combination of KDF levels that simultaneously satisfy the objectives of maximizing functional performance and safety, minimizing environmental impacts while ensuring cost-effectiveness.

2.3. *ASINA's consortium*

The ASINA project brought together a diverse expert consortium

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[7] https://one.oecd.org/document/env/jm/mono (2019)13/en/pdf

[8] https://www.projecthub360.com/multioptimal360/)

[9] https://www.projecthub360.com/multioptimal360/

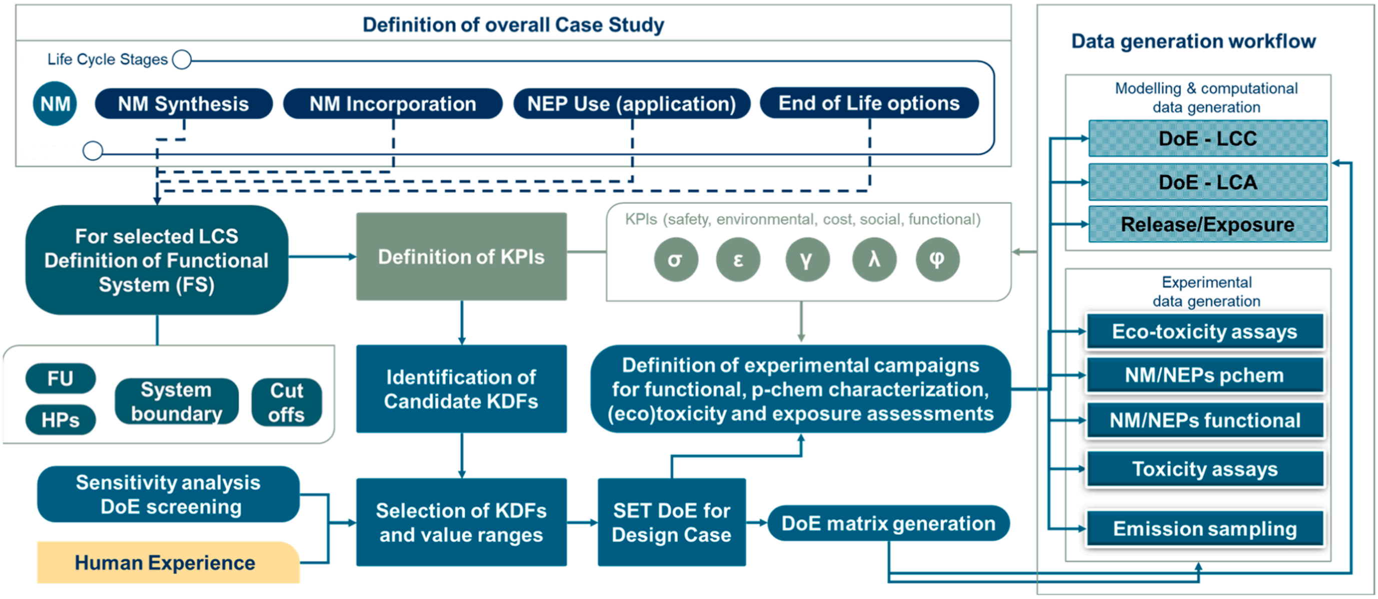

Fig. 1. Case-study goal and scope definition alongside the structured Design of Experiment (DoE) matrix. KPIs definition involves the σ, ε, γ, φ and λ dimensions (λ—the latter not addressed within the project).

composed of research organizations, academic institutions, SMEs, and industry, each bringing knowledge and capabilities to tackle the project's objectives. CNR lead the consortium playing a pivotal role in the synthesis and characterization of NMs (CNR-ISSMC), as well as managing occupational exposure measurement campaigns. UNIMIB was responsible for the hazard aspects, defining safety criteria. TECNALIA specializes in nanomanufacturing and digitalization of manufacturing processes and focused on computational modelling and innovative control measures. LEITAT was the leader of NM release and risk assessment. UNILI, CEA, UKCEH brought expertise in nanoscale characterization, in vitro toxicology, and environmental risk assessment, supporting safety aspects. HUB was responsible of the multi-criteria DST to identify efficient SSbD solutions, enhancing technology transfer and innovation. Industrial partners like WITEK manufactured NEPs, focusing on spray coatings. SMEs like APM and AC offered support for the exposure assessment and the definition of regulatory requirements. TGO was engaged in utilizing ML models to predict SSbD profiles of NMs and NEPs. Finally, CENTI were involved into a pilot action plan to demonstrate the practicality of the SSbD solutions in near real-world industrial settings. The team members can be found on the project website: https://www.asina-project.eu/partners/.

2.4. Funding sources

ASINA project was supported under EU Union's Horizon 2020 research and innovation programme under grant agreement N. 862444. The financial support was provided in the form of grants and was applied through the submission of a proposal. The EU Union's Horizon 2020 research and innovation programme, was designed to financially support research and innovation activities across various sectors and disciplines. The primary goal was to ensure EU's global competitiveness and to drive economic growth by offering support for innovation, including investment in key technologies like nanotechnology.

3. Impact - results and outcomes

In the following chapters, we provide a high-level overview of the technical aspects involved in defining the quantitative elements (KPIs, KDFs, and PCFs) including design rationales, experimental procedures, and tools/models used across the entire NMs lifecycle, addressing all relevant dimensions.

3.1. Introduction to case study addressed in ASINA

Textiles can provide a conducive environment for the growth of microorganisms, such as bacteria and fungi that might pose health risks to end-users [32]. To combat these challenges, there has been a significant shift towards leveraging nanotechnology to functionalize textiles, enhancing their antimicrobial properties. Our case study regards the application of silver NMs (AgNMs) in this purpose. AgNMs are at the forefront of this innovation, due to their effective antimicrobial properties [33–35]. Despite the advantages, the use of AgNMs raises concerns regarding their potential environmental and health impacts [36]. The SSbD approach is designed to address these concerns by incorporating functional, cost effective, safety and sustainability assessments throughout the lifecycle of the NEPs. Antimicrobial textiles, due to their widespread use in various applications [37–39] presented a valuable case-study for investigating SSbD principles.

3.2. LCS-1: synthesis phase

(Re) design options rationale: The focus was on engineered AgNMs categorized into three levels to facilitate comparison of SSbD assessment and implementation process: Baseline: benchmarking NMs characterised in other EU nano-safety projects such as uncoated AgNMs 484059 Sigma-Aldrich and PVP coated 576832 Sigma-Aldrich [40,41]. Tier 1: AgHEC, patented[10] green synthesis of AgNMs synthesized by nucleating silver within hydroxyethyl cellulose at HEC/Ag and NaOH/Ag molar ratios of 5.5 and 2.8; Tier 2: tier 1 modifications by employing a design of experiments (DoE) approach to explore KDF synthesis variations of tier 1 AgHEC (different HEC/Ag and NaOH/Ag molar ratios), aimed at identifying points in the DS that maximize the antimicrobial functionality objective. A DoE matrix comprising six NM design options (including tier 1) were synthesized according to the KDF values that defined the corresponding DoE (Section ***3.3***). The NMs

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[10] This synthesis process of tier 1 AgHEC is patented by ISSMC-CNR as a green and sustainable process, offering a SSbD alternative to uncoated AgNMs (Technology Readiness Level, TRL 4–6. Patent number granted in USA: US10525432B2)).

portfolio underwent pchem characterization to ensure quality (batch-to-batch reproducibility, stability under various storage over time and handling conditions) and to provide PCFs in LCS-1. In this phase, the NMs functionality, sustainability and cost effectiveness KPIs of the synthesis process are defined (see in Fig. 2).

3.2.1. *φ-functionality dimension*

The acceptance of NMs intended for industrial applications and commercialisation cannot disregard their intended functionality levels.

-NMs Functionality KPI: In Table S1 *(*Supplementary material*)* we present a detailed outline of the KPI including the measurements methods and units, thresholds, objectives and the associated KDFs and PCFs. Doak, Clift [42] followed a structured similar manner representing the PATROLS testing strategies. The KPI assesses the functionality of pristine NMs in solution following EN 1040:2006 protocol assessing the abatement against *Escherichia coli* (E. Coli) and *Staphylococcus aureus* (S. Aureus) at various concentrations over a 24-hour period. The thresholds/target value for the acceptance of NMs was set at > 90 %.

3.2.2. *ε-environment dimension*

While the primary focus of the project was on Safe-bD approaches, elements of sustainability were integrated into due to rapid advancements in research, driving the transition from SbD to SSbD.

-NMs synthesis sustainability KPI: In Table S2 a composite KPI that assesses synthesis process contributions to global warming through greenhouse gas emissions is shown. The assessment was based on cradle to gate modelling following EN ISO 14044:2006 for the amount of 30 g AgHEC hydrogel product obtained by the synthesis process (functional unit). This composite KPI includes the subsequent KPIs (kg CO_2 eq) of fossil CO_2 emissions per unit product, CO_2 eq from land transformation, Biogenic CO_2 eq and CO_2 uptake.

3.2.3. *γ-cost dimension*

Life Cycle Costing (LCC) is defined according to EN ISO 15686–5[11] as an: *"economic assessment considering all agreed projected significant and relevant cost flows over a period of analysis expressed in monetary value"*. This standard although applying to the building and construction sector, provides a general definition of LCC applicable to other systems.

-NMs synthesis cost effectiveness KPI: In our case, costs related to transport of resources, raw materials, human labour cost etc. which would be common to the different design cases were not considered. In this dimension the composite KPI (Table S3) captures synthesis cost changes as a function of KDFs to satisfy economic requirements at laboratory scale based on energy consumption and raw materials cost.

3.3. *Lifecycle inherent safety: NMs intrinsic hazard properties*

The objective is to identify KPIs that can be incorporated into the lifecycle to ensure inherent safety. This step does not focus on a specific LCS but rather evaluates the potential human and eco-toxicological hazard of pristine NMs on the basis of their potential release during the NMs' lifecycle, causing potential exposure to workers, end-users and the environment. Pristine NMs are initially encountered in LCS-1 where are involved in the synthesis process. In LCS-2, these NMs are incorporated into intermediate materials and ultimately into NEPs. While pristine NMs may be released from products during LCS-3, it is important to recognize that they likely have undergone transformations due to exposure to environmental factors such as air, which could lead to oxidation, or solvents and washing procedures, which could result in partial dissolution. Similarly, in LCS-4, depending on the disposal method, while emissions can occur through leaching in landfills or airborne emissions from incineration processes, the NMs are unlikely to remain in their pristine state. As a first approximation, we consider NMs as pristine across LCS for simplicity, although it is acknowledged that they may have been transformed during the processing and EoL stages. A preliminary emission assessment was performed within the project for the NMs lifecycle to identify release and exposure hot-spots. For this step, the different KPIs explored are shown in Fig. 3.

3.3.1. *σ-human health safety dimension*

A tiered approach utilizing in vitro testing based on Adverse Outcome Pathways (AOPs) was employed to choose KPIs [42,43]. The AOP framework identified main key events governing AgNMs-toxicity including oxidative stress, inflammation, DNA damage, and apoptosis, that were selected as subsequent KPIs. The in vitro models represent target-organs such as pulmonary model (alveolar lung cells, A549) and intestinal model (HCT-116) at a cellular level of biological representation [44,45]. The cell lines represent different exposure routes i.e. inhalation and ingestion.

-NMs Intrinsic Inhalation Hazard KPI: Inhalation is a major route of human exposure to airborne NMs and it may occur at workplaces and to end users after abrasion of the textiles [46]. The composite KPI (Table S4) contains i) cell viability (%) using assays such as MTT, Alamar blue, and LDH; ii) oxidative stress (fold-change) under DCFH2-DA assay; iii) Genotoxicity (fold-change) under γ-H2AX assay; v) apoptosis (%) annexin V/PI analysis and vi) inflammatory potential (pg/mL or fold change) using ELISA assay to measure markers such as IL-8. As PCFs, the extrinsic properties of NMs are influenced by the media and are dependent on the dispersing cell medium (Dulbecco's Modified Eagle Medium DMEM 1 % fetal bovine serum FBS with pH=7,2–7,4). Moreover, size distribution could change during time [47]. To account for alterations of properties in time measurements (hydrodynamic size and PdIs) performed at t_0 and t_{24} were considered.

-NMs Intrinsic Ingestion Hazard KPI: Ingestion is another important route of NMs exposure to both workers and end-users [48]. Regarding intestinal exposure, NMs pass through various biological environments such as saliva, gastric and intestinal fluids before coming into contact with intestinal cells [49,50]. To better reflect the potential hazard the intestinal model was exposed to artificially-digested NMs (tier 2) [45]. Similar subsequent KPIs were utilised for this composite KPI (Table S4). For PCFs, due to the complex nature of gastrointestinal fluids such as acidic conditions, presence of salts and biomolecules, the NMs pchem were significantly altered before, during, and after passing through the gastrointestinal tract (GIT) [45], thereby affecting their final form, bioavailability, and bioactivity [51,52]. *In vitro* parameters capturing (tissue)-specific characteristics are considered constant variables and were not part of DS. Assay related parameters used to measure each KPI represent factors influencing the interpretation of each KPI and could be either constant variables or KDFs [53,54]. If assay measurements are deliberately varied to assess their impact on the output, they would be considered KDFs. The dermal hazard KPI was placed in the LSC-3, where the potential hazard from the NEPs is assessed considering modelling emissions for realistic exposure dose.

3.3.2. *ε-environment health safety dimension*

The NMs hazard was assessed using different species representing two compartments for the environment.

-NMs Intrinsic Aquatic Hazard KPI: Zebrafish (*Danio rerio*) in freshwater were employed to investigate late adverse effects at the organ/organism level [55] following OECD TG 236 for fish embryo acute toxicity (FET) test (Table S5***)***. The composite KPI contains endpoints such as i) embryotoxic effects (hpf-LC50 and EC50, mg/L) determined based on mortality (%) and rates of malformed embryos; ii) teratogenicity (Teratogenic index= LC50/EC50); iii) early embryonic development; v) chondrogenesis in the head and jaws for craniofacial malformations; vi) Morphometric parameters and vii) hatching ability (HT_{50}).

-NMs Intrinsic Soil Hazard KPI: Soil invertebrate species, the

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[11] ISO 15686–5:2017 Buildings and constructed assets — Service life planning — Part 5: Life-cycle costing

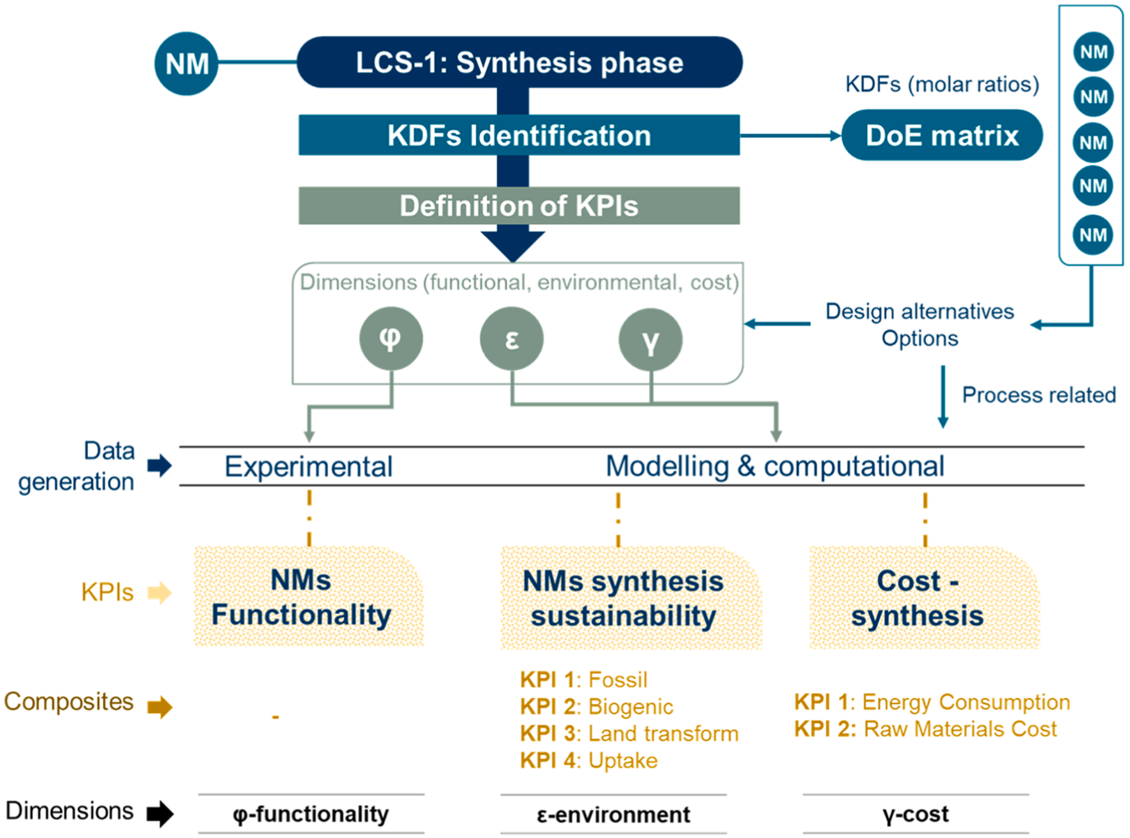

Fig. 2. Overview of the (re)design strategy in LCS-1 initiating by a hypothesis rational based on a DoE action. This phase encompasses three dimensions: φ-Functionality, ε-environment, and γ-cost.

nematode *Caenorhabditis elegans* and potworms *Enchytraeus crypticus* were utilised and exposed to NMs in synthetic soil pore water (SSPW) and/or natural soil pore water (LPW–Lufa 2.2) following a laboratory analysis under a ISO10872 modified protocol [56] in SSPW and LPW. Endpoints of the KPI assessed included survival, reproduction and key events at an organismal level (Table S5). It is important to note, this approach does not imply that the species used are the only suitable species which could be employed. These are representative species for the environmental compartments selected based on their sensitivity and utility for testing small quantities of NMs which will be relevant at innovation stages for products. Species selection is based on expert judgment taking into account the specific circumstances of each case-study, including knowledge of the species relevance to the release and exposure pathway for the NM and the species sensitivity of the NMs under investigation alongside their regulatory relevance [57,58]. This flexible, informed approach allows for the most scientifically valid and practically feasible testing to be employed given the goals of research and any constraints.

For this LCS, KDFs captured the exposure conditions (includes parameters such as the concentration in μg/mL followed by the duration of exposure in h or hpf in case of ecotoxicological endpoints). PCFs include the extrinsic pchem properties of NMs influencing their behaviour in biological species or related media (i.e. FET, dissolution rates in SSPW and LPW).

3.4. LCS-2: NMs incorporation phase

AgNMs can be applied to textiles using various methods aimed at embedding particles within fibers or dispersing them as coatings. These methods include processes such as impregnation and dyeing, spray coating, printing technologies, spinning processes etc. The choice of method influences the AgNM adherence (the amount of NMs deposited and its leaching potential) and textile's functionality [59,60]. The incorporation phase in this study regards the deposition of AgNMs-based coatings on polyester textiles applying a spray coating finishing technique [61] to generate NEPs and involves several dimensions (Fig. 4).

In the manufacturing of antimicrobial textiles, the deposition technique significantly influences the process relevant KDFs. Each technique, whether spray coating, dip padding, or others requires a unique set of KDFs tailored such as the conveyor belt speed, dipping time, curing time, squeeze pressure etc, to optimize the functionality and safety. For spray coating, the KDFs focus are shown in Table S6*,* containing suspension flow rate (ML/min), number of spraying nozzles, suspension concentration (%) of NM in suspension sprayed to the fabric, substrate matrix composition which can affect the adhesion of NMs and the spraying time duration per each session (min).

3.4.1. *φ-functionality dimension*

-NEPs Functionality KPI: The most important indicator of functionality at LCS-2 is the minimum amount of NM deposited on NEPs providing the maximum level of antimicrobial activity (assessed in LCS-3, section ***3.2.4***) in order to minimise the waste of sprayed material assessed by elemental analysis of coated fabrics by inductively coupled plasma - optical emission spectrometry (ICP-OES), mineralised by acidic microwave digestion (Table S6*)*.

3.4.2. *σ-human health safety dimension*

-Occupational Exposure KPI: The occupational safety is of utmost importance and compliance with regulatory standards, such as Directive 98/24/EC,[12] is essential to workers' health. Koivisto, Spinazzè [62] presented a tiered approach based on emission monitoring campaigns

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[12] https://eur-lex.europa.eu/eli/dir/1998/24/oj

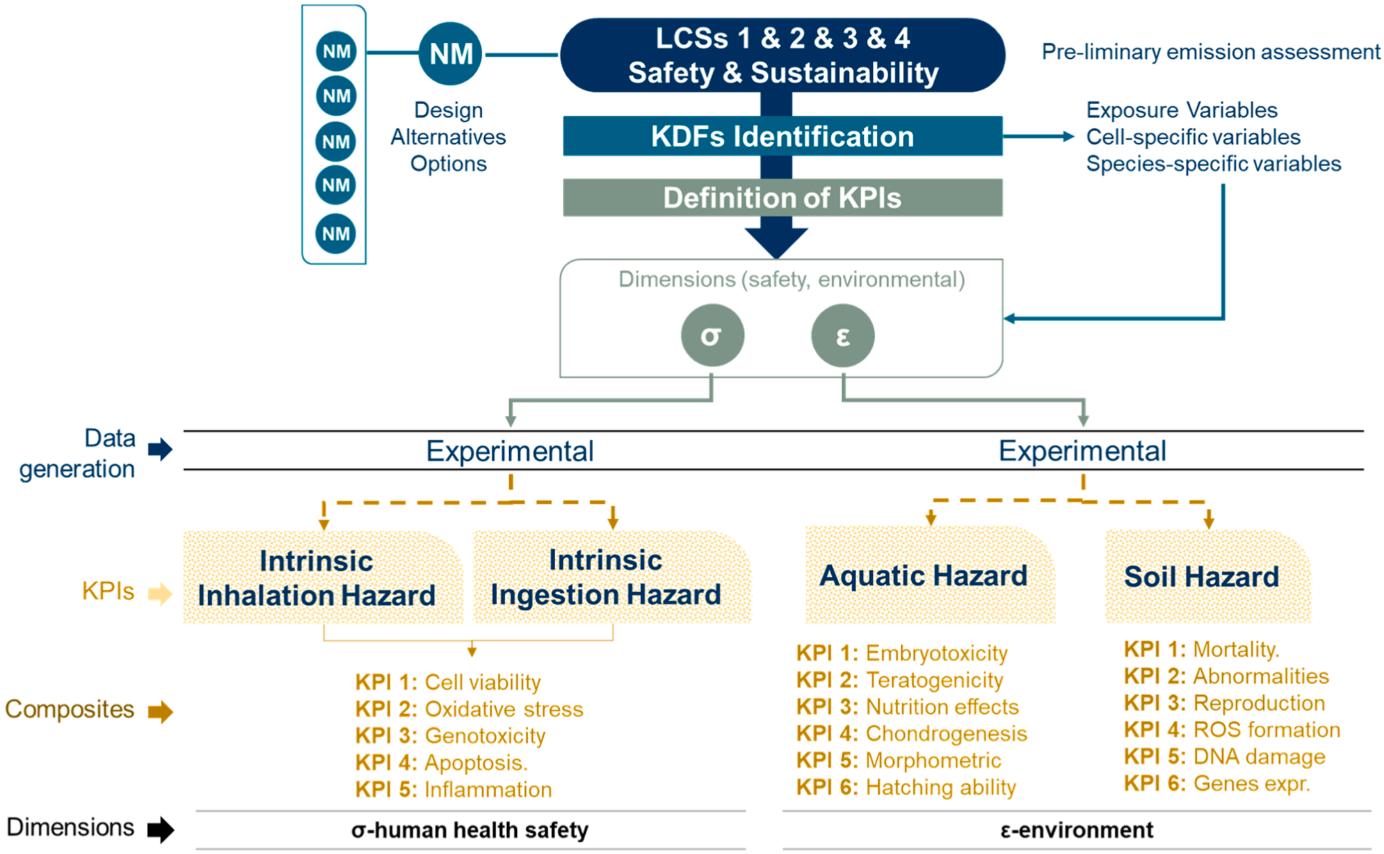

Fig. 3. Overview of intrinsic hazard properties initiating by a pre-liminary emission assessment. This phase encompasses two dimensions: σ-human health safety and ε-environment.

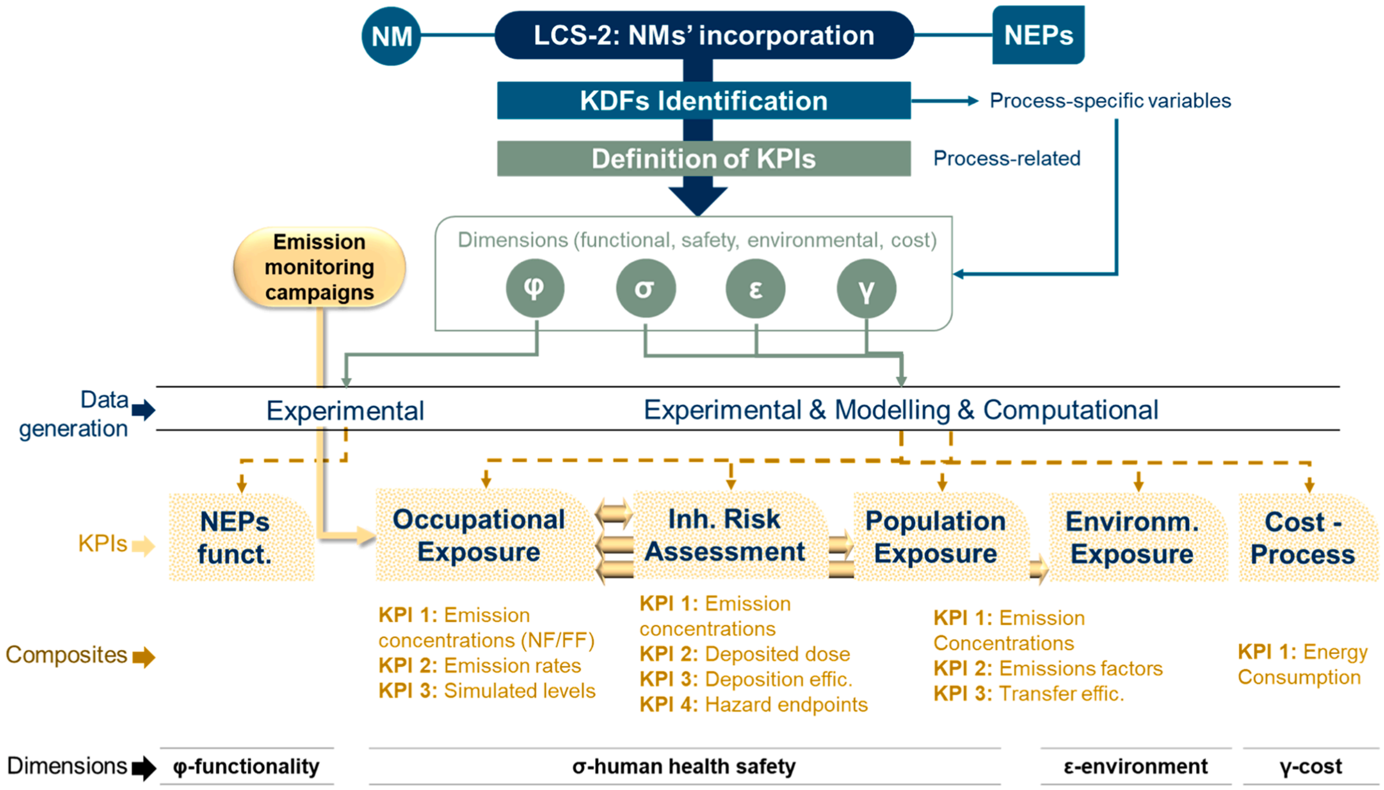

Fig. 4. Overview of the NMs incorporation phase (LCS-2). This phase encompasses all dimensions: φ-functionality, ε-environment, σ-human health safety and γ-cost.

[61,63] and probabilistic modelling to establish Conditions of Use (CoU) for exposure scenarios. The methodology tackles the challenge of managing a number of exposure assessments due to changes in Operational Conditions (OC). By quantifying OC concentrations and identifying Reasonable Worst-Case (RWC) scenarios through probabilistic modelling, it offers a solution for risk evaluation that complements the need for individual assessments complying with European Chemicals Agency (ECHA) guidelines and EN 689 exposure assessment[13] strategy. From the above approach, a composite KPI (Table S7*)* provides estimates of worker inhalation exposure levels targeting the 95th percentile of the lognormal distribution of 8-hour exposure to AgNMs. The threshold contains a comparison with occupational exposure limit (OEL), in our case the NIOSH Recommended Exposure Limit (REL) values given as respirable fraction is 0.9 μg/m3 for Ag as 8-h total weight average. CoU is considered adequate when the 95th percentile of the lognormal distribution of 8-h exposure is below 0.1 ×REL. Additionally, the personal breathing zone concentrations and exposure modelling helped ensure that exposure levels remain within acceptable limits. The regulated strategy for evaluating exposure to agents by inhalation according to EN 689 was used, by determining the concentration of AgNMs in the worker's breathing zone. The samples of respirable fraction were captured using a cyclone and a mixed cellulose membrane filter. The concentration of Ag in the filters was determined with inductively coupled plasma mass spectrometry (ICP-MS).

Finally, a study was performed to optimize the deposition efficiency (the ratio of deposited NM to the atomized suspension sprayed) thereby minimizing occupational exposure [64].

-Inhalation risk assessment KPI: Motta, Gualtieri [65] described a New Approach Methodology (NAM) of inhalation toxicology to assess potential hazards associated with the incorporation of NMs. The integration of monitoring data with laboratory-based exposures using appropriate in vitro model and air-liquid interface equipment's, exemplifies a holistic approach, defining an KPI (Table S7*)*. The fate and internal lung deposited dose were quantifying via the Multiple-Path Particle Dosimetry (MPPD) model and replicated in vitro, providing a direct link between production processes and potential health impacts. The alveolar retained doses are translated in real occupational monthly or yearly exposure doses and tested in an in vitro co-culture model representative of the alveolar space. Exposures were performed at the air liquid interface, to mimic actual inhalation and deposition.

-Population Inhalation Exposure KPI: Beyond occupational safety, attention was also directed towards safeguarding the general population from potential inhalation exposure to AgNMs. A multi-tier air emission assessment based on monitoring campaigns and a bi-Gaussian plume model IMPACT (Immission Prognosis Air Concentration Tool) to calculate the industrial, residential, traffic, and agricultural emissions impact on the air concentrations and depositions on a local scale (grid set as 2 × 2 km) was developed. The assessment was performed under RWC conditions assuming highest production volumes and material usage in full capacity production where machine is assumed operates 5d/w through a year and 8 h/d [66,67]. Regarding the thresholds of this KPI **(**Table S7*)* ECHA reports a AgNM DNEL 2 $\mu g/m^3$ for general population inhalation exposure.[14] Exposure was compared with REL specified for 8-h TWA occupational exposure extrapolated for the general population as 24 -h continuous exposure. Proposed OEL vary for AgNM from 0.098 to 10 $\mu g\text{-}Ag/m^3$ when given in different size fractions and under different experimental conditions. We assumed OEL value can be extrapolated for the population by scaling 40 h work week exposure to continuous weekly exposure by a factor of 4 and applying an assessment factor of 2 to describe children and senior sensitivity. The lower range of the proposed OELs using assignment factor of 8 results in general population limits of 0.01 $\mu g\text{-}Ag/m^3$. These limit values are indicative values used in this KPI.

3.4.3. ε-environment dimension

-Environmental Exposure KPI: It encompasses a tiered approach that uses data from monitoring campaigns and captures i) spray coatings' environmental emissions and ii) environmental impacts of AgNMs during LCS-2 (Table S8). For the first part, Koivisto, Del Secco [67] using monitoring emission data, established a quantitative foundation for assessing exposure outdoor air levels with a mechanistic model analysing mass flows per spraying nozzle to the LEV, assuming 50 % filtration efficiency, after defining the transfer efficiency which is used to calculate environmental emissions, according to the material use. For the second part, Koivisto, Altin [66] coupled the environmental emissions directly with environmental exposure models, estimating the accumulation concentrations of NMs in the soil top layer (μg-Ag/kg ww) considering long-term environmental process emissions. The accumulation was estimated by employing a single compartment model (bi-Gaussian plume model- IMPACT) and using default soil parametrization based on ECHA Chapter R.16. By comparing the accumulations to measured elemental concentrations (threshold of.01 μg-Ag/m3 derived from OEL to respective elemental Ag concentrations in EU soils) this approach can provide evidence of environmental impact, contributing to an understanding of the process's environmental safety.

3.4.4. γ-cost dimension

Keeping production and operational costs low while maintaining functionality and safety is essential for competitive pricing. In addition, efficient use of materials and energy reduces waste and operational costs, contributing to overall sustainability.

-NMs incorporation cost effectiveness KPI: LCC method specific to economic evaluation in industrial processes according to ISO 15686 was used (Table S9) with representative costs the ones associated to: reagents, energy consumption, and infrastructure use per NEP unit quantity (cost required to treat 1 square meter of fabric). The cost ratio is calculated by normalizing the specific cost by the amount of AgNMs deposited and dividing it by the maximum normalized cost across all tests.

3.5. LCS-3: NEPs use phase

The assessment in the use phase aims to address related impacts associated with direct exposure and considers both ε-environment and σ-human health safety dimensions. Ensuring the functionality of NEPs is a paramount concern in product development, marketability and for meeting consumer expectations (Fig. 5):

3.5.1. φ-functionality dimension

Following functionality, ensuring the quality and durability of NEPs becomes imperative ensuring long-term reliability and consumer confidence.

-NEPs Functionality KPI: A composite KPI reflecting the antimicrobial performance of NEPs and the technical quality and durability of NEPs is proposed (Table S10). Laboratory analysis according to ASTM E 2149–2013 standard protocols against *E. Coli* and S. *Aureus* to assess the functionality, EN ISO 12947–2, EN ISO 12947–3 for abrasion resistance, and EN ISO 105-C06 A1S for washing stability. The percentage of Ag amount remaining on the substrate determines the functionality, evaluated by Inductively ICP-MS. The NEPs undergoes quality and stability tests if bacterial reduction is > 90 %. KDFs in this stage present the number of washing and abrasion cycles along with the quantity of AgNMs deposited and remaining on the fabric per unit mass or surface area.

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[13] https://echa.europa.eu/documents/10162/17224/information_requirements_r14_en.pdf

[14] https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/16155/7/1/?documentUUID= 0e882fc5–9383-4a60-a78a-457fce9245be

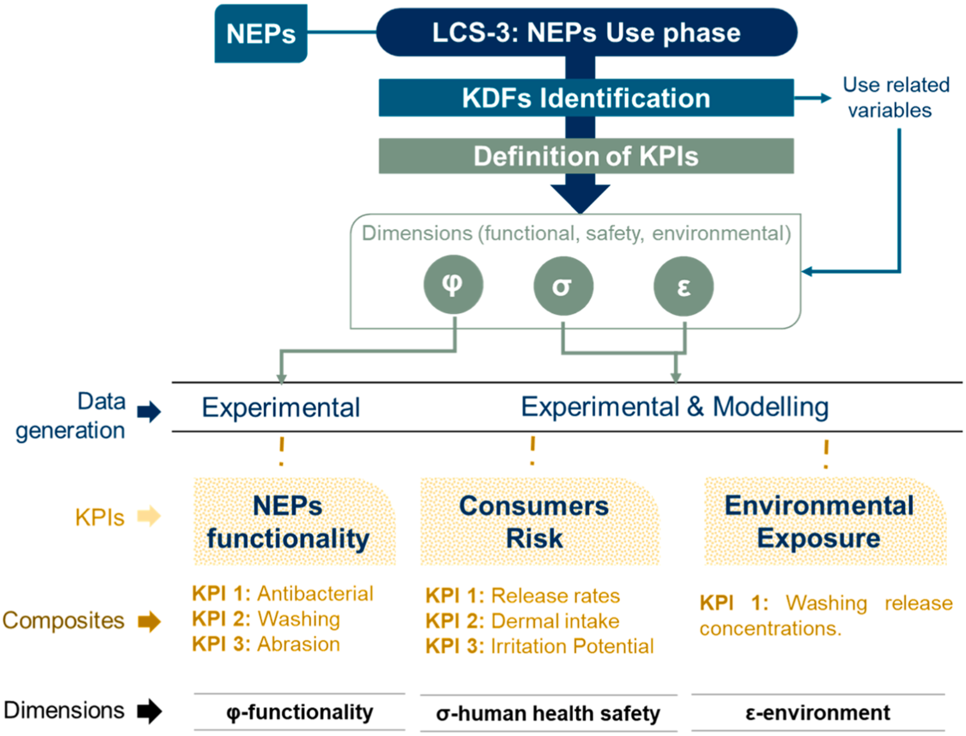

Fig. 5. Overview of the NEPs use phase (LCS-3). This phase encompasses three dimensions: φ-functionality, σ-human health safety and ε-environment.

3.5.2. *σ-human health safety dimension*

Human exposure due to migration or releases from the textiles during the direct use of textiles might occur via dermal contact [68,69]. Indirect exposure pathways are for example hand-to-object contact followed by finger mouthing or object-to-mouth contact, for example when wearing a face mask, resulting in ingestion or inhalation exposure.

-Consumers Risk KPI: A tiered approach comprising three steps was used for the proposed KPI (Table S11) i) Laboratory abrasion measurements to quantify release from textiles under artificial sweat immersion and mechanical stress using a crock-meter for dermal contact simulations based on an adaptation of BS EN ISO 105-X12:2016; ii) a simplified permeability modelling to estimate intake mass into the skin via percutaneous absorption and inadvertent (peri-)oral for risk characterization purposes using release rate constants, mass transfers and intake simulation quantifying the fraction of NMs that penetrate into the stratum corneum [71]; iii) skin hazard posed by NEPs assessed through laboratory analysis where NMs from NEPs are extracted in artificial sweat following ISO 10993–23:2021 and OECD TG 439 for skin irritation test for ISO/TC 194/WG 8 Medical Device extracts utilizing a reconstructed human epidermis model. The threshold for this KPI contain the systemic DNELs for the general population range from 0.01 to 0.0375 mg/kg-bw/day, and 0.02 to 0.075 mg/kg-bw/day for workers (also relevant in LCS-2). For the in vitro part, thresholds are based on mean tissue viability after 18 h of exposure according to Global Harmonized System criteria: Irritant if the mean tissue viability is ≤ 50 % or Non-Irritant if > 50 %.

3.5.3. *ε-environment dimension*

NMs releases to wastewater and wastewater treatment (WWT) facilities can occur as a result of textile washing [70,71]. WWTP technologies have proven effective in removing AgNMs from wastewater [72]. Studies have shown that after preliminary and secondary treatments, approximately 85 % of AgNMs are entrapped in sludges, leaving 10 % in the wastewater, of which half remains unaltered and the other half degrades into silver sulfide (Ag_2S) [73,74]. Based on the literature, it can be concluded that the environmental disposal of AgNMs does not pose substantial risks [75,76]. Transitioning to a circular economy, AgNMs would benefit from developing methods to recover silver-containing compounds from sludges. Currently, there is a notable gap in the literature regarding effective recovery techniques for these materials, indicating a critical area for future research. The waste products from WWT (effluents and sludges) will ultimately be released to water and soil, through effluent release or biosolid application to land.

-Environment Exposure KPI: To estimate the composite KPI, we followed a tiered modelling exposure assessment, incorporating release rate data (EN ISO 105-C06 (A1S) and ICP-MS) into exposure models to estimate Predicted Environmental Concentrations (PECs) using SEAT (Simple Environmental Exposure Assessment Tool) a simplistic model, SimpleBox4nano, a screening-level multimedia box model, and NanoFASE (Nanomaterial Fate and Speciation in the Environment), a spatiotemporal model [77] (Table S12*)*. Exposure scenarios assumed releases occurred during the first 10 washing cycles with pre-determined release rates; partitioning assumed 83 % of releases go to soil and 17 % to surface waters. In SEAT, all releases were considered without partitioning. Two geographical scenarios - catchments, Thames, UK, and Ebro, Spain, were used to demonstrate different climatic environments; In addition, PCFs containing biological fate and uptake kinetics for NMs (OECD 317) considering exposure relevant forms (Ag_2S) was performed as the application of biosolids to land is expected to be the main release pathway to the soil. *Eisenia fetida* were introduced for a 14 days elimination phase and Ag concentration in organisms measured by ICP-MS. Dissolution rates in SSPW were measured in line with OECD GD 318.

Finally, diverse tools such as SUNDs (low-tier) and GUIDEnano (high-tier), can be used for assessing environmental exposure [78]. The GUIDEnano allowed RCR calculations by utilizing the PECs for environmental compartments (freshwater, soil, and sediment), associated with the washing of textiles. The values were < 1, meaning that the scenarios associated with the washing of the textile and release to the environment is considered as safe based on the current accepted methodology for such evaluation. This was an example that showcase the utility of tools when data becomes available.

3.6. LCS-4: NEPs EoL

3.6.1. ε-environment dimension

In considering the EoL phase for textiles incorporating NMs, it's crucial to define KPIs that address environmental impact, sustainability, and safe disposal practices to minimizes their ecological footprint. EoL options may be diverse: materials landfilling, incineration of textile wastes and recycling of fibers. These define different potential NMs emissions to the environment due to leaching when fabric materials are exposed to meteorological agents or to abrasion in recycling operations.

-NEPs Leaching Potential KPI: Due to technical limitations for simulating incineration, the release hotspot investigated was evaluated by landfilling tests through leaching experiments and abrasion of fibres due to mechanical operations considered in mechanical recycling processes under standardized protocol EPA Standard Method 1311: Toxicity Characteristic Leaching Procedure (1992) (***Table 13***) The total amount of elemental Ag mass was quantified by ICP-MS.

3.7. MultiOptimal360™ applied to AgNMs synthesis phase

The goal of the DST applied to the synthesis of AgHEC is to identify the combination of KDFs levels that simultaneously fulfil the design objectives by minimizing environmental impacts (represented by associated CO_2 emissions), maximizing NMs antibacterial functionality with respect to two bacteria strains (*E. Coli, S. Aureus*), and reducing AgHEC synthesis costs (Table 1).

To achieve this objective, we deployed a MCDA which operates on a harmonized dataset derived from various inputs associated with the design options specified in the DoE matrix (Fig. 6, left). The MultiOptimal360™ IT platform[15] was employed for the algorithm to (a) investigate the implication of the synthesis parameters on resulting AgHEC PCFs, (b) assess the functionality of AgHEC associated different design options, (c) assess the toxicological and environmental impacts associated to the synthesis process and design option. The human centric decision process starts by considering the set of multi-optimal design options (Fig. 6, right). MultiOptimal360™ allowed also correlating the set of multi-optimal AgHEC synthesis KDFs values to the corresponding NMs expected pchem attributes, and the PCFs to the corresponding expected KPIs values, providing a comprehensive scenario quantitatively described with a selected set of SSbD optional solutions.

4. Discussion

In this report, we have outlined the multifaceted methodological underpinnings followed in ASINA roadmap, aimed at advancing the definition of SSbD criteria within the realm of nanotechnology research. We provided a comprehensive overview of PCFs, KDFs, KPIs across all dimensions establishing a blueprint for evaluating the safety and sustainability of NMs lifecycle, following the ASINA-SMM approach. This roadmap not only establishes a scientifically basis for future SSbD implementations but also serves as a foundation for future guidelines in this critical field. Furthermore, we aimed to foster a collaborative environment that supports the efforts towards a common SSbD roadmap, ultimately aligning with the EU Union's ambitious Green Deal objectives.

4.1. Impact of ASINA's structured KPIs, KDFs, and PCFs on enhancing SSbD integration in nanotechnology through AI

Table 1
KPIs-KDFs-PCFs to obtain the set of candidates multi-optimal SSbD solutions.

Case study	AgHEC: LCS-1 synthesis process
Goal	Identify the conditions for AgHEC synthesis (find values of KDF1 and KDF2) that simultaneously comply with the following design criteria: 1. Synthesize AgHEC NMs displaying highest antibacterial functionality towards the following bacterial strains: *E. coli, S. aureus* 2. Minimize synthesis process costs 3. Minimise CO_2 emissions per unit synthesized NM 4. Minimize human toxicity potential (mid-point indicator) associated to synthesis process
Scope (system description)	Analysis of the synthesis process in a "cradle to gate" approach. **System**: AgHEC lab scale synthesis. **System boundaries**: from raw materials and energy sources to the delivery of product unit. **Functional Unit**: 30 ML of hydrogel containing the synthesized NMs.
KPIs	**KPI 1:** Antibacterial Activity (AA) against E. Coli **KPI 2:** Antibacterial Activity (AA) against S. Aureus **KPI 3**: process yield **KPI 4:** functional unit cost. **KPI 5:** global warming potential per functional unit **KPI 6**: human toxicity potential per functional unit
composite KPIs	**cKPI1**: <AA> =1/2 * [(AA) against *E. coli*+ (AA) against *S. aureus*], averaging results of functionality against *E. coli* and *S. aureus* strains, process yields and costing assessment. This was done through the definition of a compound antibacterial activity (cAA) KPI by averaging the AA level for the bacteria strains obtained for 10 mg/L concentration. **cKPI2**:=cKPI1/(KPI4/KPI3) representing the cost-effectiveness of antibacterial functionality per process yield.
KDFs	**KDF 1**: Concentration of HEC, **KDF 2**: Concentration of NaOH.
PCFs	PCF1: NM hydrodynamic diameter (nm), PCF2: Zeta potential (mV), PCF3: pH
Data generation and assessment criteria (objectives)	**-Measurement method:** -Antibacterial Activity (AA): Log count reduction for a 10 mg/L concentration of hydrogel in bacterial culture (end-point assessment). -Costing: Life cycle costing of the synthesis process including reagents and energy use. -Environmental Impact: Life cycle assessment for global warming potential and human toxicity potential (mid-point assessment). -Process Yield: Experimental assessment. -PCFs: Measurements using TEM and Zeta potential analyser. **-Objectives:** Maximisation of cKP I2, Minimisation of KPI 5, KPI 6

The organization of ASINA methodology into KPIs, KDFs, and PCFs enhances the quantitative elements, facilitating the integration of SSbD-KPIs in nanotechnology. This structuring also strategically positions the study within the evolving digital landscape in the realms of Artificial Intelligence (AI) and ML. **We aim to facilitate the integration of SSbD concepts into digital frameworks that leverage AI and lay the groundwork for the digitization of the SSbD**. As digitization continues to transform research and development, the clear delineation of KPIs, KDFs, and PCFs can enable AI systems to generate meaningful outputs [79,80]. The KPIs provide clear outputs for AI algorithms, optimizing for safety and sustainability in NMs design and application. KDFs, on the other hand, present decision-making input variables, while PCFs offer important factors, enriching the AI's decision matrix with contextual insights. NAMs, including modelling and ML methods, play a crucial role in bridging data with real-world applications [81], in

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[15] https://www.projecthub360.com/multioptimal360/

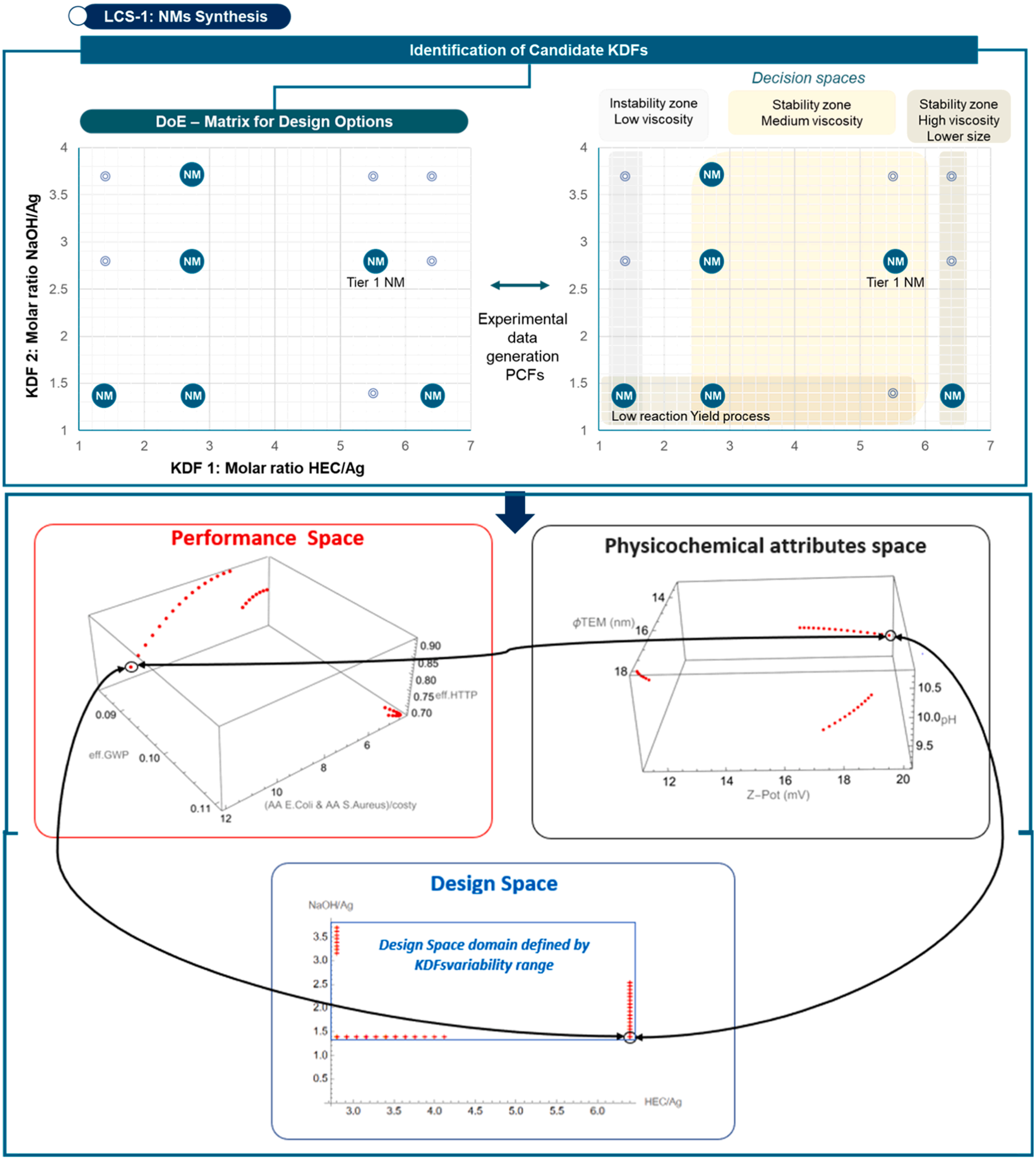

Fig. 6. Top left: the DoE matrix with the KDFs as axes and the depiction of design spaces according to synthesis parameters influence on the PCFs and four zones of the DSs sharing similar behavior in terms of colloidal stability, viscosity, reaction yield, and particle size distribution. Top right: each point belongs to the set of multi-optimal synthesis design cases that simultaneously achieves multiple KPIs. Bottom: each point of the performance space is associated with one point to the design space specifying the synthesis variables and the associated pchem attributes. Based on the results, the safest and most sustainable AgHEC complying with addressed product functionality is the one corresponding to the circled point in design space, corresponding to molar ratio HEC/Ag= 6.4 wt/wt, NaOH/Ag= 1.4 wt/wt.

alignment with the principles of the 3Rs (replacement, reduction, and refinement) of conventional animal experiments (Directive 2010/63/EU[16]). In a recent work, NAMs are reported and categorized in eight groups that represent the most commonly adopted strategies for the assessment of NM safety during their whole lifecycle [82]. ML algorithms offer significant potential for identifying patterns and offer predictive insights associated with KPIs assessments and can streamline decision-making processes, although it requires careful validation and interpretation to ensure relevance [83,84].

4.2. Integrating predictive modelling in SSbD principles across NMs' LCSs

During the construction of the ASINA roadmap, various ML have been developed that target KPIs (data-driven KPIs) within dimensions, utilising KDFs and PCFs as input features. For example, a random forest algorithm was trained using data from literature, enabling the prediction of the simple NMs functionality KPI in LCS-1, expressed as zone of inhibition (mm). The algorithm utilizes inputs including: primary particle size, ζ-potential (PCF1 and PCF3 respectively in Table S1), experimental conditions (KDF4), and bacterial species (KDF5) [85]. In another ML application [86], regression models were developed to predict the KPI 1 of the composite *NEPs functionality KPI* in LCS-3 (% bacterial reduction). These models leverage a range of inputs including subsequent KPIs such as KPI 2, which tracks the number of washing cycles and the retained functionality of NEPs (Table S10*)*. This approach demonstrates how ML can be used as a tool, trained using subsequent KPIs, to enable predictions of composite KPIs. A Bayesian Network was constructed to predict the KPI 1 in σ-human health safety dimension related to intrinsic hazard properties (lifecycle inherent safety) utilizing data from both lung and intestinal human tissues (Table S4*)*, while incorporating KDF1 (exposure conditions) and PCFs (intrinsic and extrinsic) [87]. This illustrates how disparate KPIs, associated with different potential exposure routes, can be integrated to provide a unified prediction model for enhanced hazard evaluations. In addition, interpretable KPI1 rules were derived from the structure of the network that can guide material scientists in the synthesis process. Supplementary algorithms have been implemented to predict KPI1 merging data from the literature [88]. By leveraging regression predictive models, it is feasible to forecast PCFs from synthesis parameters (such as synthesis duration, scale, and the choice of capping agent), thus enabling the rational design of NMs [89]. It is outside the scope of this study to outline the various models available in the literature for each dimension. However, we acknowledge that conducting such a comprehensive review would be highly beneficial. A detailed examination and comparison of existing models could provide valuable insights for the proposed roadmap.

The DoE approach followed within the re-design phase, serves as a bridge between laboratory experimentation and predictive modelling, offering a systematic tool for exploring the effects of predefined KDF on KPIs. The use of a DoE matrix ensures that data is collected in a structured and systematic manner, covering a comprehensive range of combinations of KDFs. This diversity in data helps in training more robust ML models. The DoE data generation reduces the likelihood of missing data by ensuring that all experimental setups are and executed according to the matrix, as the surrounding data provides context for what the missing data should likely entail. The selection of the KDFs for the DoE approach is a complex task. Jankovic, Chaudhary [90] stressed the lack of a systematic approach for selecting the most effective DoE among a plethora of potential options. The authors evaluated over thirty DoEs, nearly half a million simulated experimental runs, aimed at assessing the thermal behaviour of double skin facades, with the full factorial design serving as a 'ground truth'. From this analysis, the study formulated actionable recommendations and a generalized decision tree chart, aiding in the selection of the most suitable DoE based on the degree of nonlinearity and interaction among the KDFs involved. The human-centric element followed within this study tackled the plethora of options.

4.3. Implementing Digital Twins for textile coating processes: a case study in LCS-2

DT technology was explored for the optimization of the textile coating process in LCS-2 [91]. In this sense, various KPIs have been designed to monitor process optimization, such as e.g. NM (mL/m^2) and energy (kWh/m^2) consumptions, or process emissions (PM4, μg/m^3) using EN ISO 22400–2[17] standard as a guide for the management of manufacturing operations. The real-time monitoring and predictive modelling would facilitate the management of environmental and occupational risks associated with the process. Within the hierarchy of SSbD strategies for process (re)design, DT technology can be considered as an active protection strategy. However, as the operation of the DT focuses on process optimization, it also provides inherent safety strategies for risk reduction in real time, based for example on the minimization of the consumption of hazardous substances and energy, or on moderation of the process parameters. Therefore, DT technology offers a promising strategic path to incorporate SSbD principles throughout the lifecycle of manufacturing processes, which has been highlighted by the EC (2022)[18] as a research priority. Lopez de Ipiña, Lopez [92] developed and tested a low-cost particulate matter sensor technology to monitor the concentration of airborne NMs, with the purpose of integrating this information within the data collection layer of the DT for manufacturing. As part of this project's internal strategy to validate SSbD approaches, a pilot action was implemented for this specific study design into LCS-2, and involved spray coating test beds and pilot plants focused on NEP production and exposure assessment, to assess the feasibility and effectiveness of integrating safety and sustainability principles into a manufacturing process and validating the methodology hypothesis in a (close to) industrial environment. The first critical points listed above (functionality, quality and occupational safety), were explored in a realistic scenario, and considering spray coating parameters as product and process-specific KDFs.

4.4. Towards defined KPIs: the main challenges

KPIs represent a useful tool for measuring progress towards SSbD goals, allowing for continuous monitoring and evaluation of progress and identification of areas for improvement [93]. The presence of subsequent KPIs within main KPIs illustrates the complex cascading nature of assessment criteria. For example, in the manufacturing stage, monitoring field campaigns results were used in the hierarchical development of composite KPIs such as occupational exposure, environmental accumulation in topsoils, population exposure and realistic in vitro doses (4 main KPIs utilising emission concentrations as subsequent KPIs). In another example, within the sustainability-synthesis KPI, the subcategories of fossil CO_2 emissions, CO_2 eq from land transformation, and biogenic CO_2 eq collectively contribute to the overall assessment of environmental impact. This structure enriches the evaluation by allowing for detailed analyses of specific components of a broader indicator. However, it also complicates the assessment process, requiring a comprehensive understanding of the interrelations and potential

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[16] https://eur-lex.europa.eu/eli/dir/2010/63/2019–06-26

[17] ISO 22400–2:2014 Automation systems and integration. Key performance indicators (KPIs) for manufacturing operations management. Part 2: Definitions and descriptions

[18] European Commission, Directorate-General for Research and Innovation, Strategic research and innovation plan for safe and sustainable chemicals and materials, Publications Office of the European Union, 2022, https://data.europa.eu/doi/10.2777/876851

trade-offs between different KPIs. Understanding the interplay between these subcategories allows for a comprehensive evaluation of sustainability performance. Pandian, N [94] presented a model based on a pair wise comparison method for the establishment of Composite Performance Index (composite KPI in our case) for sustainability in line with generalized multi- criteria evaluation mode through a questionnaire from industrial respondents. The criteria associated with this study were the KPIs of economic and social sustainability dimensions. In our proposed DST, MCDA and MOOP solutions, are proposed. Merging subsequent KPIs into singular, comprehensive criteria for NMs SSbD assessment metric presents the main challenge for two main reasons: **heterogeneity of metrics and interpretation of results**. Consider, for example, a scenario where NMs toxicity, outside regulatory frameworks i.e., REACH, is being evaluated. In the assessment it is common to encounter subsequent KPIs that utilize different metrics for measurement. For instance, one KPI may be expressed as a percentage while another may be in parts per million, and yet another in absolute numerical values or fold-change. Each of these metrics provides valuable insights into different aspects of the phenomenon being studied but integrating them to form a cohesive assessment poses significant challenges. The primary challenge lies in the inherent incompatibility of different measurement scales, making direct comparisons and aggregations difficult. Attempting to merge these metrics requires careful consideration of scaling methods, conversion factors, and normalization techniques to bring them onto a common scale. The challenge lies not just in the heterogeneity of metrics **but also in the absence of target values across all of these domains**. **Another challenge is the interpretation of results** which may be relative to a control rather than absolute and may vary **depending on the context and the specific objectives of the assessment**. Given the varying significance of different hazard endpoints (e.g., genotoxicity might be weighted more heavily than oxidative stress due to its long-term implications), assigning weights to each KPI is essential. These weights reflect the relative importance or potential severity associated with each hazard, informed by regulatory guidelines, expert consensus, and scientific evidence. As a result, expert judgment (sector specific and the regulatory context which they have to comply with) becomes crucial in interpreting results and making informed decisions. The necessity of expert judgment makes the process inherently flexible but also somewhat subjective. This flexibility allows for adaptability to different contexts and case studies, although it introduces challenges in standardization and consistency across assessments. Another example of context interpretation, the toxicity of zinc oxide NMs to earthworms, has been shown to decrease in soils with a higher pH (e.g., >7) [95]. This highlights how environmental conditions can modulate the behaviour and toxicity of NMs. Furthermore, the selection of test species is critical, as differential species sensitivity can influence the outcome of a hazard assessment for a NM. Where data are available, utilizing species sensitivity distributions based on different NM PCFs could provide insights into which species are most likely to be sensitive to a particular NM parameter. Within the ASINA roadmap, data were compiled for various soil organisms to establish species sensitivity distributions based on different PCFs of NMs to provide a probabilistic estimation of a toxicity threshold. The 5th percentile of this distribution, the Hazard Concentration for 5 % of the species (HC_5), represents the concentration that is protective of 95 % of species in the species sensitivity distribution. **Defining KPIs constitutes a cumbersome task not only within the SSbD concept, but prevalent across diverse disciplines** [96]. Saroglou, Selvan [97] developed a conceptual framework to rigorously define and align objective-KPI relationships among multiple stakeholders within the context of multi-species urban architecture. This approach not only facilitates human-centric interactions but also enriches urban environments in ways that benefit both ecological and human constituents. The methodology highlights the utilization of shared KPIs among stakeholders—humans, plants, animals, and microbiota—to computationally optimize design outcomes. In a similar manner, the same approach could be used within SSbD.

4.5. Dissemination of ASINA's results

ASINA results have been disseminated across 68 conferences participations in total, 16 workshops and 17 networking/participation in activities organized jointly with other H2020 projects. Among those, the ASINA Exploitation Workshop (https://www.asina-project.eu/asina-exploitation-workshop/), organized within NanoInnovation Conference, served as a platform for discussing the industrial application of the SSbD approach. The workshop showcased experiences from across EU in advancing materials technology under the SSbD framework. Participants explored case studies and results from EU projects like SABYDOMA, SAbyNA, and SbD4Nano. The fourth webinar in the ASINA series focused on the architecture and implementation of the ASINA's DST for validating materials design options (https://www.asina-project.eu/asina-webinar-on-safe-by-design-asina-decisional-tool-for-validation/). This event outlined the main objectives and features of the DST, highlighting its architecture and practical implementation aspects, emphasizing its requirements, potential benefits, and limitations. The ASINA's DST was also showcased at MECSPE (https://www.mecspe.com/en/), the international fair held in Bologna, Italy recognized as the key reference event for the manufacturing industry around the pivotal themes of training, digitalization, and sustainability, reflecting the essential pillars for advancement in a 4.0 industrial context. This event was not only a chance to showcase the advancements made in the ASINA project but also an invaluable opportunity to engage directly with industry leaders. Additionally, the interactive environment at MECSPE allowed our team to gather critical feedback, enhancing our understanding of industry needs and expectations in terms of digitalization and sustainable practices in manufacturing.

4.6. Future work and further funding

The complexity of integrating KPIs into a cohesive metric is not a small task. However, this report successfully navigates these challenges, providing a comprehensive outline for the development quantitative KPIs within the SSbD paradigm. Despite the inherent challenges associated with the KPIs, this report represents a significant stride forward in the field of nanotechnology. The roadmap demonstrates the utility of KPIs in transforming subjective decision-making into a data-driven process. In addition, the structured approach outlined in this study lays a solid foundation for the effective dissemination of future results. By establishing KPIs, PCFs and KDFs, the framework ensures consistency and transparency in reporting outcomes, making it easier for subsequent studies to build upon our work. It provides a common language and set of metrics that can be applied, facilitating the communication of complex technical information to a broader audience, including policymakers, and the scientific community [13,42]. A common understanding of SSbD is fundamental for the development and successful implementation of SSbD itself as well as related specific guidelines and all communication activities in this field. Following the successful completion of the ASINA, further fundings were acquired for its sustainability and to continue refining this approach (https://www.integrano.eu/). INTEGRANO proposes a general assessment approach based on the quantitative evidences derived also from ASINA to be applied in practice for new specific NMs design cases. The HORIZON MSCA-2022-PF-01–01 program, part of the Marie Skłodowska-Curie Actions (MSCA) Postdoctoral Fellowships under Horizon EU, aims to financially support postdoctoral researchers which are currently working on the data generated within ASINA. The objectives of MSCA program are to enhance the creative and innovative potential of researchers holding a PhD and to foster their careers by providing funding for training and interdisciplinary, and intersectoral mobility.

5. Conclusions

This report presents the enabling procedures developed in ASINA to facilitate the SSbD adaptation in the field of nanotechnology.

- Incorporating multifaceted dimensions of SSbD—spanning functionality, quality, occupational consumer and environmental safety—fosters a holistic lifecycle quantitative approach to their assessments, which also leads to developing novel approaches.
- ASINA provides a detailed roadmap with quantitative metrics and comprehensive instructions. The suggested approach establishes a generic basis for future SSbD implementations but also serves as a consensus prototype towards a common SSbD roadmap, ultimately aligning with the EU Union's ambitious Green Deal objectives.
- Each KPI in use across LCSs is defined and documented in detailed in published articles of the approaches developed within ASINA.
- Findings, successes and failures of the approaches tested and followed, lay a blueprint for future research, policy formulation, and industrial practices in nanotechnology.
- Development of a DST, i.e. the MultiOptimal360™ IT platform, to identify the most efficient SSbD solutions.
- The application of DT to optimize the design and manufacturing processes of NEPs is highlighted in the discussion. DT technology acts as a transformative tool in LCS-2 for coating processes, enhancing real-time process optimization and risk management.
- ML models, enables prediction of various KPIs using KDFs and PCFs as essential inputs across LCSs.
- The work reported invites the research and industrial communities to engage with, critique, and further develop and refine the SSbD KPIs approach.
- Refining and validating the proposed KPIs, ensuring they effectively address sustainability and safety challenges, is a collaboration act of multi-disciplinary experts and multilevel stakeholders.
- Collective efforts and continuous implementations of the SSbD approach, lead transitioning SSbD from a theoretical framework to a practical and impactful approach in product design and development.

In conclusion, the ASINA project has catalysed advancements in nanotechnology through its strategic application of SSbD principles. The project has successfully implemented a comprehensive SSbD framework, characterized by the definition of PCFs, KDFs, and KPIs, across various stages of the NMs lifecycle and developed a DST for the implementation of the SSbD concept. The innovations developed through the ASINA project have yielded multiple scholarly articles, contributing knowledge to the scientific community and establishing a methodology that can be employed by other researchers in the field. As we continue to build on the foundations laid by the ASINA project, the ongoing collaboration among academia, industry, and regulatory bodies will be pivotal in refining these methodologies.

CRediT authorship contribution statement

Magda Blosi: Writing – review & editing, Supervision, Conceptualization. **Jesús Lopez de Ipina:** Writing – review & editing, Supervision, Methodology, Investigation. **Irini Furxhi:** Writing – original draft, Visualization, Validation, Methodology, Data curation, Conceptualization. **Alessia Nicosia:** Writing – review & editing, Methodology, Investigation. **David Burrueco-Subirà:** Writing – review & editing, Methodology, Investigation. **Socorro Vázquez-Campos:** Writing – review & editing, Supervision. **Elma Lahive:** Writing – review & editing, Methodology, Investigation. **Marie Carriere:** Writing – review & editing, Methodology, Investigation. **Massimo Perucca:** Writing – review & editing, Visualization, Supervision, Methodology, Data curation, Conceptualization. **Claudia Vineis:** Writing – review & editing, Methodology, Investigation. **Antti Joonas Koivisto:** Writing – review & editing, Methodology, Investigation. **Anna Luisa Costa:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Rossella Bengalli:** Writing – review & editing, Methodology, Investigation, Data curation. **Paride Mantecca:** Writing – review & editing, Supervision, Methodology, Investigation. **Juliana Oliveira:** Writing – review & editing, Methodology, Investigation.

Declaration of Competing Interest

Massimo Perucca is the owner of the MultiOptimal360TM IT platform (https://www.projecthub360.com/) who developed the DST as an integral part of the project. No potential conflict of interest was reported by the authors.

Acknowledgements

First, we would like to acknowledge the financial support from the European Union's Horizon 2020 research and innovation programme, which was instrumental in funding and guiding the progress of this project. We extend our thanks to all project partners and collaborators for their dedication and expertise that greatly enriched our research. The authors would like to thank Franco Belosi (CNR-ISAC) for his feedback related to LCS-2 and the extensive work on emission monitoring campaigns performed within and Maurizio Gualtieri, Giulia Motta (UNIMIB) and Ozge Kose (CEA) for their work and feedback on the lifecycle inherent safety section.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.csbj.2024.06.013.

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